World Journal of Gastroenterology®

Volume 12 Number 1
January 7, 2006

Supported by NSFC
2005-2006

National Journal Award
2005

The WJG Press

The WJG Press, Apartment 1066 Yishou Garden, 58 North Langxinzhuang Road, PO Box 2345, Beijing 100023, China
Telephone: +86-10-85381901
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http: //www.wjgnet.com

ISSN 1007-9327 CN 14-1219/R Local Post Offices Code No. 82-261

World Journal of Gastroenterology www.wjgnet.com Volume 12 Number 01 Jan 07 2006

ISSN 1007-9327
CN 14-1219/R

WJG

World Journal of Gastroenterology®

Indexed and Abstracted in:
Index Medicus, MEDLINE, PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, Nature Clinical Practice Gastroenterology and Hepatology, CAB Abstracts and Global Health.

Volume 12 Number 1
January 7, 2006

World J Gastroenterol
2006 January 7; 12(1): 1-180
Online Submissions
www.wjgnet.com/wjg/index.jsp
www.wjgnet.com
Printed on Acid-free Paper

A Weekly Journal of Gastroenterology and Hepatology

World Journal of Gastroenterology®

Editorial Board

2004-2006

Published by The WJG Press, PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893 E-mail: wjg@wjgnet.com http://www.wjgnet.com

Canada

Matthew Bjerknes, *Toronto*
Michael F Byrne, *Vancouver*
Wang-Xue Chen, *Ottawa*
Hugh J Freeman, *Vancouver*
Chantal Guillemette , *Québec*
Samuel S Lee, *Calgary*
Gerald Y Minuk, *Manitoba*
Morris Sherman, *Toronto*
Alan BR Thomson, *Edmonton*
Eric M Yoshida, *Vancouver*

China

Henry LY Chan, *Hongkong*
Xiao-Ping Chen, *Wuhan*
Jun Cheng, *Beijing*
Chi-Hin Cho, *Hong Kong*
Zong-Jie Cui, *Beijing*
Da-Jun Deng, *Beijing*
Er-Dan Dong, *Beijng*
Sheung-Tat Fan, *Hong Kong*
Xue-Gong Fan, *Changsha*
Jin Gu, *Beijing*
De-Wu Han, *Taiyuan*
Ming-Liang He, *Hong Kong*
Fu-Lian Hu, *Beijing*
Wayne HC Hu, *Hong Kong*
Guang-Cun Huang, *Shanghai*
Xiao-Long Ji, *Beijing*
Ching Lung Lai, *Hong Kong*
Kam Chuen Lai, *Hong Kong*
Yuk Tong Lee, *Hong Kong*
Suet Yi Leung, *Hong Kong*
Wai-Keung Leung, *Hong Kong*
Zhi-Hua Liu, *Beijing*
Ai-Ping Lu, *Beijing*
Jing-Yun Ma, *Beijing*
Lun-Xiu Qin, *Shanghai*
Yu-Gang Song, *Guangzhou*
Qin Su, *Beijing*
Yuan Wang, *Shanghai*
Benjamin Chun-Yu Wong, *Hong Kong*
Wai-Man Wong, *Hong Kong*
Hong Xiao, *Shanghai*
Dong-Liang Yang, *Wuhan*
Yuan Yuan, *Shenyang*
Man-Fung Yuen, *Hong Kong*
Jian-Zhong Zhang, *Beijing*
Xin-Xin Zhang, *Shanghai*
Zhi-Rong Zhang, *Chengdu*
Shu Zheng, *Hangzhou*

Croatia

Tamara Cacev, *Zagreb*
Marko Duvnjak, *Zagreb*

Czech

Milan Jirsa, *Praha*

Denmark

Peter Bytzer, *Copenhagen*
Hans Gregersen, *Aalborg*
Jens H Henriksen, *Hvidovre*
Fin Stolze Larsen, *Copenhagen*
SØren MØller, *Hvidovre*

Egypt

Abdel-Rahman El-Zayadi, *Giza*
Sanaa Moharram Kamal, *Cairo*
Ayman Yosry, *Cairo*

Finland

Pentti Sipponen, *Espoo*

France

Corlu Anne, *Rennes*
Denis Ardid, *Clermont-Ferrand*
Charles Paul Balabaud, *Bordeaux*
Jacques Belghiti, *Clichy*
Pierre Brissot, *Rennes*
Patrice Philippe Cacoub, *Paris*
Franck Carbonnel, *Besancon*
Laurent Castera, *Pessac*
Bruno Clément, *Rennes*
Jacques Cosnes, *Paris*
Thomas Decaens, *Cedex*
Francoise Lunel Fabiani, *Angers*
Gérard Feldmann, *Paris*
Jean Fioramonti, *Toulouse*
Chantal Housset, *Paris*
Juan Lucio Iovanna, *Marseille*
Rene Lambert, *Lyon*
Francis Mégraud, *Bordeaux*
Richard Moreau, *Clichy*
Thierry Piche, *Nice*
Jean Rosenbaum, *Bordeaux*
Jose Sahel, *Marseille*
Jean-Yves Scoazec, *Lyon*
Khalid Ahnini Tazi, *Clichy*
MC Vozenin-brotons, *Villejuif*
Jean-Pierre Henri Zarski, *Grenoble*
Jessica Zucman-Rossi, *Paris*

Germany

HD Allescher, *Garmisch-Partenkirchen*
Martin Anlauf, *Kiel*
Rudolf Arnold, *Marburg*
Max G Bachem, *Ulm*
Thomas F Baumert ,*Freiburg*
Daniel C Baumgart, *Berlin*
Hubert Blum, *Freiburg*
Katja Breitkopf, *Mannheim*
Markus W Büchler, *Heidelberg*
Reinhard Buettner, *Bonn*
Elke Cario, *Essen*
Uta Dahmen, *Essen*
CF Dietrich, *Bad Mergentheim*
Paul Enck, *Tuebingen*
Fred Fändrich, *Kiel*
Ulrich Robert Fölsch, *Kiel*
Peter R Galle, *Mainz*
Andreas Geier, *Aache*
Dieter Glebe, *Giessen*
Burkhard Göke, *Munich*
Florian Graeple, *Tuebingen*
Axel M Gressner, *Aachen*
Veit Gülberg, *Munich*
Rainer Haas, *Munich*
Eckhart Georg Hahn, *Erlangen*
Stephan Hellmig, *Kiel*
ohannes Herkel, *Hamburg*
Eberhard Hildt, *Berlin*
Joerg C Hoffmann, *Berlin*
Werner Hohenberger, *Erlangen*
RG Jakobs, *Ludwigshafen*
Jutta Keller, *Hamburg*
Stefan Kubicka, *Hannover*
Joachim Labenz, *Siegen*
Michael Peter Manns, *Hannover*
Stephan Miehlke, *Dresden*
Sabine Mihm, *Göttingen*
Silvio Nadalin, *Essen*
Markus F Neurath, *Mainz*
Johann Ockenga, *Berlin*
Gustav Paumgartner, *Munich*
Ulrich Ks Peitz, *Magdeburg*
Steffen Rickes, *Magdeburg*
Gerhard Rogler, *Regensburg*
Tilman Sauerbruch, *Bonn*
Andreas Schäffler, *Regensburg*
Hans Scherubl, *Berlin*
Roland M Schmid, *München*
AG Schreyer, *Regensburg*
Tobias Schroeder, *Essen*
Hans Seifert, *Oldenburg*
J Ruediger Siewert, *Munich*
Manfred V Singer, *Mannheim*
Gisela Sparmann, *Rostock*
Jurgen M Stein, *Frankfurt*
Manfred Stolte, *Bayreuth*
Rainer Straub, *Regensburg*
WR Stremmel, *Heidelberg*
Harald F Teutsch, *Ulm*
HL Tillmann, *Leipzig*
Tung-Yu Tsui, *Regensburg*
Axel Ulsenheimer, *Munich*
Patrick Veit, *Essen*
Siegfried Wagner, *Deggendorf*
Henning Walczak, *Heidelberg*
Fritz von Weizsacker, *Berlin*
Jens Werner, *Heidelberg*
Bertram Wiedenmann, *Berlin*
Reiner Wiest, *Regensburg*
Stefan JP Zeuzem, *Homburg*

Greece

Elias A Kouroumalis, *Heraklion*

Hungary

Peter Laszlo Lakatos, *Budapest*

Iceland

H Gudjonsson, *Reykjavik*

India

Sujit K Bhattacharya, *Kolkata*
Yogesh K Chawla, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Sri Prakash Misra, *Allahabad*
ND Reddy, *Hyderabad*

Iran

Reza Malekzadeh, *Tehran*
Seyed Alireza Taghavi, *Shiraz*

Ireland

Anthony P Moran, *Galway*

Israel

Simon Bar-Meir, *Hashomer*
Abraham Rami Eliakim, *Haifa*
Yaron Ilan , *Jerusalem*
Yaron Niv, *Pardesia*
Ran Oren, *Tel Aviv*

Italy

Giovanni Addolorato, *Roma*
Domenico Alvaro, *Rome*
Annese V, *San Giovanni Rotond*
Adolfo Francesco Attili, *Roma*
Giovanni Barbara, *Bologna*
Gabrio Bassotti, *Perugia*
Franco Bazzoli, *Bologna*
Stefano Bellentani, *Carpi*
Antomio Benedetti, *Ancona*
Mauro Bernardi, *Bologna*
Luigi Bonavina, *Milano*
Giovanni Cammarota, *Roma*
Antonino Cavallari, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Massimo Conio, *Sanremo*
Dario Conte, *Milano*
Gino Roberto Corazza, *Pavia*
Francesco Costa, *Pisa*
Antonio Craxi, *Palermo*
Roberto De Giorgio, *Bologna*

Giovanni D De Palma, *Naples*
Fabio Farinati, *Padua*
Andrea Galli, *Firenze*
Valeria Ghisett , *Turin*
Edoardo G Giannini, *Genoa*
Paolo Gionchetti, *Bologna*
Mario Guslandi, *Milano*
Giacomo Laffi, *Firenze*
Giovanni Maconi, *Milan*
ED Mangoni, *Napoli*
Giulio Marchesini, *Bologna*
Giuseppe Montalto, *Palermo*
Giovanni Monteleone, *Rome*
Gerardo Nardone, *Napoli*
Luisi Pagliaro, *Palermo*
Fabrizio R Parente, *Milan*
F Perri, *San Giovanni Rotondo*
Raffaele Pezzilli, *Bologna*
A Pilotto, *San Giovanni Rotondo*
Paolo Del Poggio, *Treviglio*
Gabriele Bianchi Porro, *Milano*
Piero Portincasa, *Bari*
Bernardino Rampone, *Siena*
Claudio Romano, *Messina*
Mario Del Tacca, *Pisa*
Pier Alberto Testoni, *Milan*
Enrico Roda, *Bologna*
Vincenzo Savarino, *Genova*
Roberto Testa, *Genoa*
Dino Vaira, *Bologna*

Japan

Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Taiji Akamatsu, *Matsumoto*
Sk Md Fazle Akbar, *Ehime*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Taku Aoki, *Tokyo*
Masahiro Arai, *Tokyo*
Tetsuo Arakawa, *Osaka*
Yasuji Arase, *Tokyo*
Masahiro Asaka, *Sapporo*
Hitoshi Asakura, *Tokyo*
Takeshi Azuma, *Fukui*
Yoichi Chida, *Fukuoka*
Takahiro Fujimori, *Tochigi*
Jiro Fujimoto, *Hyogo*
Kazuma Fujimoto, *Saga*
Mitsuhiro Fujishiro, *Tokyo*
Yoshihide Fujiyama, *Otsu*
Hiroyuki Hanai, *Hamamatsu*
Kazuhiro Hanazaki, *Nagano*
Naohiko Harada, *Fukuoka*
Makoto Hashizume, *Fukuoka*
Tetsuo Hayakawa, *Nagoya*
Kazuhide Higuchi, *Osaka*
Keiji Hirata, *Kitakyushu*
Yuji Iimuro, *Nishinomiya*
Kenji Ikeda, *Tokyo*
Fumio Imazeki, *Chiba*
Yasuhiro Inokuchi, *Yokohama*
Haruhiro Inoue, *Yokohama*
Masayasu Inoue, *Osaka*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Kei Ito, *Sendai*
Masayoshi Ito, *Tokyo*
Hiroaki Itoh, *Akita*
Ryuichi Iwakiri, *Saga*
Hiroshi Kaneko, *Aichi-Gun*
Takashi Kanematsu, *Nagasaki*
Junji Kato, *Sapporo*
Mototsugu Kato, *Sapporo*
Shinzo Kato, *Tokyo*
Sunao Kawano, *Osaka*
Mitsuhiro Kida, *Kanagawa*
Yoshikazu Kinoshita, *Izumo*
Tsuneo Kitamura, *Chiba*
Seigo Kitano, *Oita*
Kazuhiko Koike, *Tokyo*
Norihiro Kokudo, *Tokyo*
Satoshi Kondo, *Sapporo*
Shoji Kubo, *Osaka*
Shigeki Kuriyama, *Kagawa*
Masato Kusunoki, *Tsu Mie*
Katsunori Iijima, *Sendai*
Shin Maeda, *Tokyo*
Masatoshi Makuuchi, *Tokyo*
Osamu Matsui, *Kanazawa*
Yasushi Matsuzaki, *Tsukuba*
Kiyoshi Migita ,*Omura*
Tetsuya Mine, *Kanagawa*
Hiroto Miwa, *Hyogo*
Masashi Mizokami, *Nagoya*
Motowo Mizuno, *Hiroshima*
Morito Monden, *Suita*
Hisataka S Moriwaki, *Gifu*
Yoshiharu Motoo, *Kanazawa*
Akihiro Munakata, *Hirosaki*
Kazunari Murakami, *Oita*
Kunihiko Murase, *Tusima*
Yujl Naito, *Kyoto*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Mikio Nishioka, *Niihama*
Susumu Ohmada, *Maebashi*
Masayuki Ohta, *Oita*
Tetsuo Ohta, *Kanazawa*
Kazuichi Okazaki, *Osaka*
Katsuhisa Omagari, *Nagasaki*
Saburo Onishi, *Nankoku*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Yutaka Inagaki , *Kanagawa*
Hiromitsu Saisho, *Chiba*
Isao Sakaida, *Yamaguchi*
Michiie Sakamoto, *Tokyo*
Yasushi Sano, *Chiba*
Iwao Sasaki, *Sendai*
Motoko Sasaki, *Kanazawa*
Chifumi Sato, *Tokyo*
Shuichi Seki, *Osaka*
Hiroshi Shimada, *Yokohama*
Mitsuo Shimada, *Tokushima*
Tomohiko Shimatan, *Hiroshima*
Hiroaki Shimizu, *Chiba*
Ichiro Shimizu, *Tokushima*
Tooru Shimosegawa, *Sendai*
Tadashi Shimoyama, *Hirosaki*
Ken Shirabe, *Iizuka City*
Yoshio Shirai, *Niigata*
Katsuya Shiraki, *Mie*
Yasushi Shiratori, *Okayama*
Yasuhiko Sugawara, *Tokyo*
Hidekazu Suzuki, *Tokyo*
Tadatoshi Takayama, *Tokyo*
Tadashi Takeda, *Osaka*
Kiichi Tamada, *Tochigi*
Akira Tanaka, *Kyoto*
Eiji Tanaka, *Matsumoto*
Noriaki Tanaka, *Okayama*
Shinji Tanaka, *Hiroshima*
Wei Tang, *Tokyo*
Kyuichi Tanikawa, *Kurume*
Akira Terano, *Shimotsugagun*
Hitoshi Togash, *Yamagata*
Kazunari Tominaga, *Osaka*
Minoru Toyota, *Sapporo*
Akihito Tsubota, *Chiba*
Shingo Tsuji, *Osaka*
Takato Ueno, *Kurume*
Shinichi Wada, *Tochigi*
Hiroyuki Watanabe, *Kanazawa*
Toshio Watanabe, *Osaka*
Yuji Watanabe, *Ehime*
Chun-Yang Wen, *Nagasaki*
Koji Yamaguchi, *Fukuoka*
Takayuki Yamamoto, *Yokkaichi*
Takashi Yao, *Fukuoka*
Masashi Yoneda, *Tochigi*
Hiroshi Yoshida, *Tokyo*
Masashi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Kentaro Yoshika, *Toyoake*
Masahide Yoshikawa, *Kashihara*

Lebanon

Ala I Sharara, *Beirut*
Joseph Daoud Boujaoude, *Beirut*

Lithuania

Sasa Markovic, *Japljeva*

Macedonia

Vladimir Cirko Serafimoski, *Skopje*

Malaysia

Andrew Seng Boon Chua, *Ipoh*
Khean-Lee Goh, *Kuala Lumpur*
Jayaram Menon, *Sabah*

Mexico

Saúl Villa-Trevio, *México*
JKY Furusho, *Mexico*

Monaco

Patrick Rampal, *Monaco*

Netherlands

Lee Bouwman, *Leiden*
Rick Greupink, *Groningen*
Janine K Kruit, *Groningen*
Ernst Johan Kuipers, *Rotterdam*
Yi Liu, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
Michael Müller, *Wageningen*
Amado Salvador Peña, *Amsterdam*
Robert J Porte, *Groningen*
Andreas Smout, *Utrecht*
RW Stockbrugger, *Maastricht*
Renate G Van der Molen, *Rotterdam*
Karel van Erpecum, *Utrecht*
GV Henegouwen, *Utrecht*

New Zealand

Ian David Wallace, *Auckland*

Nigeria

Samuel Babafemi Olaleye, *Ibadan*

Norway

Trond Berg, *Oslo*
Helge Lyder Waldum, *Trondheim*

Pakistan

Muhammad S Khokhar, *Lahore*

Poland

Tomasz Brzozowski, *Cracow*
Robert Flisiak, *Bialystok*
Hanna Gregorek, *Warsaw*
Hanna Gregorek, *Warsaw*
DM Lebensztejn, *Bialystok*
Wojciech G Polak ,*Wroclaw*

Portugal

Miguel Carneiro De Moura, *Lisbon*

Russia

Vladimir T Ivashkin, *Moscow*
Leonid Lazebnik, *Moscow*
Vasiliy I Reshetnyak, *Moscow*

Singapore

Bow Ho, *Kent Ridge*
Khek-Yu Ho, *Singapor*
Francis Seow-Choen, *Singapore*

Slovakia

Anton Vavrecka, *Bratislava*

South Africa

Michael C Kew, *Parktown*

South Korea

Byung Ihn Choi, *Seoul*
Ho Soon Choi, *Seoul*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Myung-Hwan Kim, *Seoul*
Jong Kyun Lee, *Seoul*
Eun-Yi Moon, *Taejeon City*
Jae-Gahb Park, *Seoul*
Dong Wan Seo, *Seoul*

Spain

Juan G Abraldes, *Barcelona*
Agustin Albillos, *Madrid*
Raul J Andrade, *Málaga*
Luis Aparisi, *Valencia*
Fernando Azpiroz, *Barcelona*
Ramon Bataller, *Barcelona*
Josep M Bordas, *Barcelona*
Xavier Calvet, *Sabadell*
Vicente Carreño, *Madrid*
Antoni Castells, *Barcelona*
Vicente Felipo, *Valencia*
Juan C Garcia-Pagán, *Barcelona*
Jaime Bosch Genover, *Barcelona*
Jaime Guardia, *Barcelona*
Angel Lanas, *Zaragoza*
María Isabel Torres López, *Jaén*
José M Mato, *Derio*
MAM Navas, *Pamplona*
Julian Panes, *Barcelona*
Miguel Minguez Perez, *Valencia*
Miguel Perez-Mateo, *Alicante*
Josep M Pique, *Barcelona*
Jesus M Prieto, *Pamplona*
Sabino Riestra, *Pola De Siero*
Luis Rodrigo, *Oviedo*
Manuel Romero-Gómez, *Sevilla*

Sweden

Curt Einarsson, *Huddinge*
Xupeng Ge, *Stockholm*
Hanns-Ulrich Marschall, *Stockholm*
Lars Christer Olbe, *Molndal*
Xiao-Feng Sun, *Linkoping*
Ervin Tóth, *Malmö*

Switzerland

Chrish Beglinger, *Basel*
Pierre A Clavien, Zurich
Jean-Francois Dufour, *Bern*
Franco Fortunato, *Zürich*
Jean Louis Frossard, *Geneva*
Gerd A Kullak-Ublick, *Zurich*
Bruno Stieger, *Zurich*
Arthur Zimmermann, *Berne*

Turkey

Yusuf Bayraktar, *Ankara*
Figen Gurakan, *Ankara*
Aydin Karabacakoglu, *Konya*
Serdar Karakose, *Konya*
Hizir Kurtel, *Istanbu*
Osman Cavit Ozdogan, *Istanbul*
Cihan Yurdaydin, *Ankara*

United Arab Emirates

Sherif M Karam, *Al-Ain*

United Kingdom

Anthony TR Axon, *Leeds*
Mairi Brittan, *London*
Andrew Kenneth Burroughs, *London*
Paul Jonathan Ciclitira, *London*
Amar Paul Dhillon, *London*
Elizabeth Furrie, *Dundee*
Daniel Richard Gaya, Edinburgh
Subrata Ghosh, *London*
William Greenhalf, *Liverpool*
Peter Clive Hayes, Edinburgh
Gwo-Tzer Ho, *Edinburgh*
Anthony R Hobson, *Salford*
David Paul Hurlstone, *Sheffield*
Brian T Johnston, *Belfast*
David EJ Jones, *Newcastle*
Michael A Kamm, *Harrow*
Patricia F Lalor, *Birmingham*
Hong-Xiang Liu, *Cambridge*
Dermot Patrick Mcgovern, *Oxford*
Giorgina Mieli-Vergani, *London*
Nikolai V Naoumov, *London*
John P Neoptolemos, *Liverpool*
James Neuberger, *Birmingham*
Mark S Pearce, *Newcastle Upon Tyne*
Marco Senzolo, *Padova*
Robert Sutton, *Liverpool*
Simon D Taylor-Robinson, *London*
Ulrich Thalheimer, *London*
Nick Paul Thompson, *Newcastle*
David Tosh, *Bath*
Frank Ivor Tovey, *Basingstoke*
Diego Vergani, *London*
Peter James Whorwell, *Manchester*
Karen Leslie Wright, *Bath*
Min Zhao, *Foresterhill*

United States

Christian Cormac Abnet, *Maryland*
Gary A Abrams, *Birmingham*
Golo Ahlenstiel, *Bethesda*
Gavin Edward Arteel, *Louisville*
Jasmohan Singh Bajaj, *Milwaukee*
Jamie S Barkin, *Miami Beach*
Kim Elaine Barrett, *San Diego*
Jennifer D Black, *Buffalo*
Alan Cahill, *Philadelphia*
David L Carr-Locke, *Boston*
Ravi S Chari, *Nashville*
Jiande Chen, *Galveston*
Xian-Ming Chen, *Rochester*
Parimal Chowdhury, *Arkansas*
Raymond T Chung,Boston
James M Church, *Cleveland*
Vincent Coghlan, *Beaverton*
John Cuppoletti, *Cincinnati*
Peter V Danenberg, *Los Angeles*
Kiron Moy Das, *New Brunswick*
Vincent Paul Doria-Rose, *Seattle*
Bijan Eghtesad, *Cleveland*
Hala El-Zimaity, *Houston*
Michelle Embree-Ku, *Providence*
Ronnie Fass, *Tucson*
Chris E Forsmark, *Gainesville*
Scott L Friedman, *New York*
John Geibel, *New Haven*
Ignacio Gil-Bazo, *New York*
David Y Graham, *Houston*
Anna S Gukovskaya, *Los Angeles*
Stephen B Hanauer, *Chicago*
Gavin Harewood, *Rochester*
Alan W Hemming, *Gainesville*
Jamal A Ibdah, *Columbia*
Atif Iqbal, *Omaha*
Hajime Isomoto, *Rochester*
Hartmut Jaeschke, *Tucson*
Dennis M Jensen, *Los Angeles*
Leonard R Johnson, *Memphis*
Peter James Kahrilas, *Chicago*
AN Kalloo, *Baltimore*
Neil Kaplowitz, Los Angeles
Ali Keshavarzian, *Chicago*
Joseph B Kirsner, *Chicago*
Burton I Korelitz, *New York*
Robert J Korst, *New York*
Richard A Kozarek, *Seattle*
Shiu-Ming Kuo, *Buffalo*
Daryl Tan Yeung Lau, *Galvesto*
Glen A Lehman, *Indianapolis*
Frederick H Leibach, *Augusta*
Alex B Lentsch, *Cincinnati*
Andreas Leodolter, *La Jolla*
Gene LeSage, *Houston*
Ming Li, *New Orleans*
LM Lichtenberger, *Houston*
GR Lichtenstein, *Philadelphia*
Martin Lipkin, *New York*
Josep M Llovet, *New York*
Edward V Loftus, *Rocheste*
Robin G Lorenz, *Birmingham*
JD Luketich, *Pittsburgh*
Henry Thomson Lynch, *Omaha*
John Frank Di Mari, *Texas*
John M Mariadason, *Bronx*
WM Mars, *Pittsburgh*
George W Meyer, *Sacramento*
G Michalopoulos, *Pittsburgh*
S Pal Singh S Monga, *Pittsburgh*
Timothy H Moran, *Baltmore*
Hiroshi Nakagawa, *Philadelphia*
Douglas B Neison, *Minneaplis*
Curtis T Okamoto, *Los Angeles*
Stephen J Pandol, *Los Angeles*
Pankaj Jay Pasricha, *Gaveston*
Zhiheng Pei, *New York*
Michael A Pezzone, *Pittsburgh*
CS Pitchumoni, *New Brunswiuc*
Jay Pravda, *Gainesville*
M Raimondo, *Jacksonville*
Adrian Reuben, *Charleston*
Victor E Reyes, *Galveston*
Richard Rippe, *Chapel Hill*
Marcos Rojkind, *Washington*
Hemant Kumar Roy, *Evanston*
Shawn David Safford, *Norfolk*
NJ Shaheen, *Chapel Hill*
Stuart Sherman, *Indianapolis*
Shivendra Shukla, *Columbia*
Alphonse E Sirica, *Virginia*
Michael Steer, *Boston*
Gary D Stoner, *Columbus*
Yvette Tache, *Los Angeles*
Jayant Talwalkar, *Rochester*
K-M Tchou-Wong, *New York*
PJ Thuluvath, *Baltimore*
Swan Nio Thung, *New York*
RA Travagli, *Baton Rouge*
G Triadafilopoulos, *Stanford*
Chung-Jyi Tsai, *Lexington*
Hugo E Vargas, *Scottsdale*
Jian-Ying Wang, *Baltimore*
Steven David Wexner, *Weston*
Keith Tucker Wilson, *Baltimore*
Jackie Wood, *Ohio*
George Y Wu, *Farmington*
Jian Wu, *Sacramento*

Samuel Wyllie, Houston
Wen Xie, *Pittsburgh*
Yoshio Yamaoka, *Texas*
Liqing Yu, *Winston-Salem*
David Yule, *Rochester*
Ruben Zamora, *Pittsburgh*
Michael Zenilman, *Brooklyn*
Zhi Zhong, *Chapel Hill*

Yugoslavia
DM Jovanovic, *Sremska Kamenica*

National Journal Award
2005

World Journal of Gastroenterology®

Volume 12 Number 1
January 7, 2006

Supported by NSFC
2005-2006

Contents

World Journal of Gastroenterology
Volume 12 **Number** 1 **January 7**, 2006

Contents

World Journal of Gastroenterology
Volume 12 **Number** 1 **January 7**, 2006

Contents

World Journal of Gastroenterology (*World J Gastroenterol* , *WJG*), a leading international journal in gastroenterology and hepatology, has an established reputation for publishing first class research on esophageal cancer, gastric cancer, liver cancer, viral hepatitis, colorectal cancer, and *Helicobacter pylori* infection, providing a forum for both clinicians and scientists, and has been indexed and abstracted in *Index Medicus*, MEDLINE, PubMed, Chemical Abstracts, EMBASE, Abstracts Journals, Nature Clinical Practice Gastroenterology and Hepatology, CAB Abstracts and Global Health. *WJG* is a weekly journal published jointly by The *WJG* Press. The publication date is on 7th, 14th, 21st, and 28th every month. The *WJG* is supported by The National Natural Science Foundation of China, No. 30224801 and No.30424812, which was founded with a name of *China National Journal of New Gastroenterology* on October 1,1995, and renamed as *WJG* on January 25, 1998.

PRINTING GROUP
Printed in Beijing on acid-free paper by Beijing Kexin Printing House

Print ISSN 1007-9327 CN 14-1219/R.

EDITORIAL OFFICE
Editor: *World Journal of Gastroenterology*,
The WJG Press, Apartment 1066 Yishou Garden, 58 North Langxinzhuang Road, PO Box 2345, Beijing 100023, China
Telephone: +86-10-85381901
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

SUBSCRIOTION AND AUTHOR REPRINTS

Jing Wang
The WJG Press, Apartment 1066 Yishou Garden, 58 North Langxinzhuang Road, PO Box 2345, Beijing 100023, China
Telephone: +86-0-85381901
Fax: +86-10-85381893
E-mail: j.wang@wjgnet.com
http://www.wjgnet.com

Institutional Rates Print
2006 rates: USD 1500.00

Personal Rates Print
2006 rates: USD 700.00

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/wjg/help/instructions.jsp If you do not have web access please contact the editorial office.

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 1-5
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Changing face of irritable bowel syndrome

Eamonn MM Quigley

Eamonn MM Quigley, MD, FACG, Professor of Medicine and Human Physiology, Head of the Medical School, National University of Ireland, Cork, Ireland
Correspondence to: Eamonn MM Quigley, Alimentary Pharmabiotic Centre, Department of Medicine, Clinical Sciences Building, Cork University Hospital, Ireland. e.quigley@ucc.ie
Telephone: +353-21-490-1228 Fax: +353-21-490-1289
Received: 2005-06-15 Accepted: 2005-07-18

Abstract

Recent years have witnessed tremendous progress in our understanding of irritable bowel syndrome (IBS). It is evident that this is a truly global disease associated with significant symptoms and impairments in personal and social functioning for afflicted individuals. Advances in our understanding of gut flora-mucosal interactions, the enteric nervous system and the brain-gut axis have led to substantial progress in the pathogenesis of symptoms in IBS and have provided some hints towards the basic etiology of this disorder, in some subpopulations, at the very least. We look forward to a time when therapy will be addressed to pathophysiology and perhaps, even to primary etiology. In the meantime, a model based on a primary role for intestinal inflammation serves to integrate the various strands, which contribute to the presentation of IBS

Key words: Irritable bowel syndrome; Functional gastrointestinal disease; Intestinal inflammation

Quigley EMM. Changing face of irritable bowel syndrome. *World J Gastroenterol* 2006; 12(1): 1-5

http://www.wjgnet.com/1007-9327/12/1.asp

EPIDEMIOLOGY

In recent years, the true prevalence of IBS has been documented in many parts of the world. What is truly remarkable is how common IBS is, no matter where you look! It is absolutely clear, for example, that IBS is a common disorder, not only in North America and Western Europe but throughout Asia and Latin America and even in parts of Africa[1]. However, caution needs to be exerted in the interpretation of such studies. Typically, community or hospital-based surveys of IBS prevalence have utilized some iteration of Rome or Manning criteria as their diagnostic instrument; whether these diagnostic tools, developed in the West, are equally valid in emerging nations, where confusion with symptoms related to chronic parasitic infestations, for example, may be an issue. Clearly, we have much to learn from the epidemiology and natural history of IBS or IBS-like symptoms in this context.

DIAGNOSIS

Diagnostic confusion has also emerged as an issue in the West. Here, still the debate continues regarding potential overlap between IBS, IBD and celiac sprue[2]. Do reported instances of celiac sprue among patients with "typical" IBS, or the occurrence of IBS-type symptoms among IBD patients in apparent remission, reflect a true association between these disorders and, thereby, the effects of low grade inflammation on enteric nerve and muscle function, or does such apparent overlap simply serve to emphasize the non-specificity of many gastrointestinal symptoms? Explaining it simply, the gut has a limited symptomatic repertoire which may not allow us to differentiate between those complaints which are consequent upon continuing (but otherwise undetected) inflammation in IBD or in the non-compliant celiac and those which arise from a functional disorder, *per se*. Progress in this contentious area must await readily applicable measures of disease activity which are sufficiently sensitive and accurate to provide a true definition of remission.

In the meantime, how should the clinician interpret these dilemmas? It is evident, that the majority of celiacs now present later in life and usually with vague and non-specific gastrointestinal symptomatology; celiac disease must, therefore, be considered in all new IBS patients, especially in areas of high prevalence and regardless of the nature of presenting symptoms[3].

ASSOCIATED DISEASES

Over the years, IBS has been associated with a wide variety of intestinal and extra-intestinal symptoms and syndromes. Recent community surveys have confirmed how frequently IBS, functional dyspepsia (FD) and gastroesophageal reflux disease (and non-erosive reflux disease (NERD), in particular) overlap; a phenomenon that may complicate clinical trials as well as diagnostic and therapeutic strategies. My own belief is that we should be "lumpers" and not "splitters" here; I contend that efforts to separate IBS from FD and NERD are clinically unrealistic and

unhelpful. IBS has also been associated with a variety of psychological disorders; here, in contrast, the evidence for a true association is less firm, more recent analyses suggest that the occurrence of such symptomatology in IBS is largely the preserve of those who seek further referral alone and is not a feature of IBS in the community. Psychopathology should be viewed, therefore, not as a fundamental prerequisite for the development of IBS, but, rather, as a co-factor which, if present, will modify the individual's response to IBS symptomatology. IBS patients commonly complain of fatigue and tiredness; these appear to be real entities in IBS, yet have been scarcely acknowledged in the assessment of IBS activity or response to therapy. Urinary and gynecological symptoms are also common; the basis for these associations is less clear. Aware of the prominence of smooth muscle hyper-reactivity in both conditions, parallels have been drawn between IBS and asthma; whether these conditions are linked remains to be defined.

PATHOPHYSIOLOGY

Genetic factors

While IBS patients commonly give a positive family history, the relative roles of "nature" and "nurture" in this intra-familial aggregation of functional disorders have received little attention. For example, in a recent community survey almost 20% of IBS sufferers reported abdominal symptoms in a first degree relative; a relative risk of 2.5[4]. Whether this association reflects reporting bias, shared environmental factors or a true genetic basis has been addressed in two recent twin studies which both identified a genetic component to IBS[5,6]. This is not the whole answer by any means; thus in the study by Levy and colleagues, while the concordance for IBS was twice as high in monozygotic than in dizygotic twins (15.2% *vs* 6.7%), a history of IBS in a parent was a more potent predictor of IBS in a twin than was the presence of IBS in the other twin[6]. These findings suggest a relatively minor role for genetic factors in the basic pathogenesis of IBS. Genetic factors may, however, influence disease expression and therapeutic response, as evidenced by recent studies of G-protein subunit, IL-10, CCK-1 receptor, alpha 2 adrenoceptor and serotonin transporter genotypes among IBS patients[7-11]. These are complex studies but may pave the way for real progress in our understanding of the true diversity of IBS [12].

Gastrointestinal motor dysfunction

Dysmotility has long been considered a major factor in the pathophysiology of IBS, as indicated by the use, in the past, of such terms as the "spastic colon" to describe what is now referred to as IBS. Accordingly, it was suggested that gut spasm or other abnormal contractile activities led to the development of symptoms in IBS. There are, indeed, several reports of abnormal motor patterns in many parts of the gastrointestinal tract in IBS. The specificity of many of these abnormalities for IBS is, however, unclear[13]. In contrast, and of particular interest, are very recent observations on the handling of gas by the intestine in IBS[14,15]. Whereas gas infused into the small intestine was rapidly evacuated through the gut in normal volunteers, a similar infusion resulted in gas retention, symptoms and an increase in abdominal girth in IBS patients[14]; all reversible by administration of a prokinetic agent[15]. Distension, often the most distressing "gas"-related symptom in IBS, has, until recently, been assumed to represent a disturbance of perception, as apparently objective tests of abdominal volume failed to detect any increase in IBS[16]. This assumption has now been questioned[17] and it may well come to pass that more detailed studies of changes in distension over time[18] may detect significant diurnal variations in girth in IBS. While the balance of evidence suggests that intestinal gas production is not abnormal in IBS, one can visualize how relatively local changes in the gas content could lead to symptoms, given the aforementioned intrinsic abnormality of gas transport[19] and the hypersensitivity to intraluminal gas that are known to occur in IBS[20].

Visceral hypersensitivity and hyperalgesia

Recently, there has been considerable interest in these phenomena, not only in IBS, but also in functional disorders, in general[7]. The phenomenon of visceral hypersensitivity, to distention and other intra-luminal stimuli, appears to be common in patients with non-cardiac pain, FD and the irritable bowel, alike. Recently, it has been suggested that visceral hyperalgesia, the phenomenon whereby stimuli normally not experienced as painful become so, is highly specific for IBS[21]. Visceral hypersensitivity, visceral hyperalgesia and viscero-somatic referral (the phenomenon whereby stimuli are referred over wide areas) have, indeed, been confirmed, in IBS, in more recent studies, using a variety of methodologies and under controlled experimental conditions[22]. While visceral hyperalgesia has been postulated as being highly specific for IBS, it alone or in association with other manifestations of hypersensitivity cannot explain all of IBS; even the most celebrated enthusiasts for the sensory hypothesis concede that sensation is normal in some patients.

There are several possible anatomical locations for sensory abnormalities in IBS, ranging from sensory receptors on the gut wall, primary sensory afferent neurons, to the spinal cord and the brain itself. Research in this area in man is notoriously difficult; however, advances in functional brain imaging provided by such techniques as cerebral evoked potentials (CEP), positron emission tomography (PET), magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI), have provided insights into the brain's response to visceral stimuli. These and other studies have advanced the concept of an abnormal (or hypervigilant) central nervous system (CNS), in IBS, which records an exaggerated, inappropriate or aberrant perception of visceral events[23]. Other pieces of evidence support this concept. These include the conscious perception, by IBS patients, of intestinal motor events which are usually sub-conscious and evidence of abnormal psycho-neuro-hormonal responses, often implicating an abnormal hypothalamico-pituitary axis (HPA).

Motility and sensation may not be the most fundamental

causes of IBS; it is clear, however, that these phenomena play a significant role in symptom generation.

Infection, inflammation, immunity and IBS

It may come as a real surprise to many to hear that infection and inflammation are now seen as potential factors in the etiology of IBS. With regard to infection, we are now beginning to see the real data to directly support the concept of post-infective or post-dysenteric IBS.

Infection and IBS First reported by McKendrick and Read[24], the occurrence of IBS following bacteriologically-confirmed gastroenteritis has now been documented in several studies[25-30]. The risk of developing IBS following an episode of gastroenteritis is in the order of 4%-23%, with females, those with a more severe initial illness and pre-morbid psychopathology being most at risk[25,26,28,30]. One of these studies went on to establish a direct link between prior exposure to an infectious agent, persisting low-grade inflammation and IBS[28]. In this study, an increase in the number of chronic inflammatory cells in the rectal mucosa was seen only among those exposed patients who had developed IBS. Others have demonstrated a persisting increase in rectal mucosal enteroendocrine cells, T lymphocytes and gut permeability in patients with post-dysenteric IBS[29,30]. Post-infectious IBS may explain only a minority of cases of IBS but does represent a clear link between exposure to an environmental agent, inflammation and IBS, in predisposed individuals[31].

Inflammation and IBS Direct and compelling evidence was first provided by Chadwick and colleagues for a role of inflammation in IBS, in general. They evaluated 77 IBS patients of whom 55% would be considered as diarrhea predominant; none had a confirmed infectious origin for their IBS[32]. All had colonic biopsies taken for conventional histology and immunohistology. Thirty-eight had normal histology, 31 demonstrated microscopic inflammation and 8 fulfilled the criteria for lymphocytic colitis. However, in the group with "normal" histology, immunohistology revealed increased intraepithelial lymphocytes as well as an increase in CD3+ and CD25+ cells in the lamina propria; all, therefore, showed evidence of immune activation. These features were even more evident in the microscopic inflammation group who, in addition, revealed increased neutrophils, mast cells and natural killer cells. All of these aforementioned immunopathological abnormalities were most evident in the lymphocytic colitis group who, alone, also demonstrated HLA-DR staining in crypts and increased CD8+ cells in the lamina propria. Interestingly, taking the group of IBS patients as a whole, CD3+ cell number was higher among those with diarrhea than among alternators or those with predominant constipation. In contrast, in the non-inflamed IBS group the presence of mast cells was a predictor of constipation. Surprisingly, given the aforementioned description of a direct relationship between symptoms and chronic inflammation among patients with post-infectious IBS, these authors did not find an association between either the nature of disease onset or disease duration and immunological findings. In an accompanying editorial, Collins suggested that the increased presence of CD25+ cells may have indicated "auto- or exogenous antigen challenge in these patients, and that the CD25+ cells are preventing the progression to a more florid inflammatory response"[33]. That IBS patients may be predisposed to an, *albeit* contained, inflammatory response to luminal triggers is also supported by the finding, of Gonsalkorale and colleagues, of a reduced frequency of the high-producer phenotype for the anti-inflammatory cytokine interleukin-10 (IL-10) among IBS patients[9]. A direct linkage between immune activation and symptoms has been provided by the work of Barbara and colleagues who demonstrated, not only an increased prevalence of mast cell degranulation in the colon in IBS, but also a direct correlation between the proximity of mast cells to neuronal elements and pain severity[34].

While the inflammatory hypothesis in IBS is in its infancy, there is already some evidence for the extension of the inflammatory process beyond the confines of the mucosal compartment. Tornblom and colleagues addressed this issue in ten patients with severe IBS by examining full-thickness jejunal biopsies obtained at laparoscopy[35]. In nine, they found low-grade infiltration of lymphocytes in the myenteric plexus; four of these had an associated increase in intraepithelial lymphocytes and six demonstrated evidence of neuronal degeneration. Nine patients had longitudinal muscle hypertrophy and seven had abnormalities in the number and size of interstitial cells of Cajal. Interestingly, three of their patients reported an acute onset of their IBS; in two, possibly precipitated by gastroenteritis. The finding of intraepithelial lymphocytosis is consistent with the reports of Chadwick and colleagues[32], in the colon and of Wahnschaffe and colleagues, in the duodenum[36]. Most recently, in a group of 78 unselected IBS patients, we demonstrated, in peripheral blood mononuclear cells, an alteration in the ratio between the cytokines IL10 and IL12 which became skewed towards a Th1, pro-inflammatory profile[37].

With regard to the pathophysiology of the mucosal inflammatory changes, Spiller proposed that these changes could represent a response to an initial bacterial infection among individuals who are rendered susceptible by a relative deficiency of anti-inflammatory cytokines[38]. Alternately, could this low-grade inflammation represent either an abnormal reaction to the normal flora or a contained response to qualitative or quantitative changes in the intrinsic flora? Whether IBS is accompanied by quantitative or qualitative changes in the bacterial flora of the small or large intestine remains a contentious issue; while some have described bacterial overgrowth in the small intestine[39,40] and qualitative alterations in the fecal flora[41,43] and increased bacterial fermentation[44], in IBS, others have failed to replicate these findings[45]. The description of efficacy for certain probiotics, and *bifidobacterium*, in particular, in IBS[37] could also support a role of gut flora-mucosal interaction in IBS[46]. Bacterial overgrowth could also explain some of the proposed overlap between IBS and celiac sprue[47].

MANAGEMENT

Many IBS patients relate the onset of symptoms to intake of food and often incriminate specific food items. However, the role of food intolerance or food allergy in

IBS has remained undefined. While most would agree that there is scant evidence for classical food allergy in IBS, Whorwell and colleagues suggest that testing for food intolerance, utilizing IgG antibodies, can lead to a successful dietary modification regime[48].

In recent years, much interest has been generated by serotonin and the potential role of serotonergic drugs in IBS[49]. Tegaserod, a $5HT_4$ agonist, is effective in the therapy of female patients with constipation-predominant IBS and has demonstrated efficacy against some previously "resistant" symptoms, such as bloating[50,51]. Alosetron, a $5HT_3$ agonist, is effective in females with diarrhea-predominant IBS, but its prescription is now limited due to reports on ischemic colitis[52]. Cilansetron, a $5HT_3$ agonist, is effective in both males and females with diarrhea-predominant IBS[53]; here the specter of ischemic colitis has again become an issue with regulators, in the US. Indeed, ischemic colitis has become an issue for all of these agents, though it appears that many reports of association probably reflect diagnostic confusion *ab initio* between IBS and ischemic colitis rather than an effect of serotonergic agents, *per se*[54].

Given the explosion that has occurred in our understanding of the enteric nervous system, and of the pathways that link it to the CNS, it should come as no surprise that many agonists and antagonists of other putative neurotransmitters and neuromodulators are under study in IBS and related disorders.

Given the potential role of infection and inflammation in at least some instances of IBS, efforts have been made to address this aspect of pathophysiology in IBS. In this regard, a probiotic, bifidobacterium infantis has proved to be a very successful agent in unselected IBS patients[37]. Clearly, this is an area of increasing interest.

CONCLUSIONS

Our understanding of IBS has come a long way. This is a global disease associated with significant symptoms and impairments in personal and social functioning for afflicted individuals. Advances in our understanding of gut flora-mucosal interactions, the enteric nervous system and the brain-gut axis have led to substantial progress in the pathogenesis of symptoms in IBS and have provided some hints towards the basic etiology of this disorder, in some subpopulations, at the very least. We look forward to a time when therapy will be addressed to pathophysiology and, perhaps, even to primary etiology. In the meantime, as illustrated in Figure 1, I would suggest that a model based on a primary role for intestinal inflammation serves to integrate the various strands which contribute to the presentation of IBS.

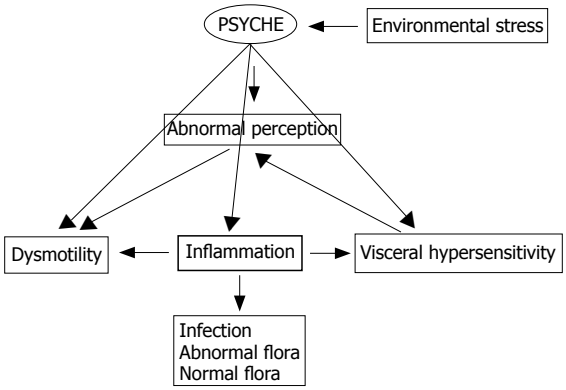


Figure 1 Interactions between the gut, the brain and the external and internal environments in IBS; the linflammation hypothesis (a personal view). Bacterial or viral infection, a disturbed flora or an abnormal response to a normal flora leads to mucosal linflammation which in turn can disrupt motility and augment visceral sensation. Centrally, perception is abnormal, thereby, contributing to symptom development. Central output can in turn influence motor events in the periphery. While not central to causation, psychological factors, either spontaneously or in response to environmental stressors, can influence motor and sensory events and immune activity in the gut.

REFERENCES

1 **Kang JY**. Systematic review: the influence of geography and ethnicity in irritable bowel syndrome. *Aliment Pharmacol Ther* 2005; **21**: 663-676
2 **Quigley EM**. Irritable bowel syndrome and inflammatory bowel disease: interrelated diseases? *Chin J Dig Dis* 2005; **6**: 122-132
3 **Spiegel BM**, DeRosa VP, Gralnek IM, Wang V, Dulai GS. Testing for celiac sprue in irritable bowel syndrome with predominant diarrhea: a cost-effectiveness analysis. *Gastroenterology* 2004; **126**: 1721-1732
4 **Locke GR**, Zinsmeister AR, Talley NJ, Fett SL, Melton LJ. Familial association in adults with functional gastrointestinal disorders. *Mayo Clin Proc* 2000; **75**: 907-912
5 **Morris-Yates A**, Talley NJ, Boyce PM, Nandurkar S, Andrews G. Evidence of a genetic contribution to functional bowel disorder. *Am J Gastroenterol* 1998; **93**: 1311-1317
6 **Levy RL**, Jones KR, Whitehead WE, Feld SI, Talley NJ, Corey LA. Irritable bowel syndrome in twins: heredity and social learning both contribute to etiology. *Gastroenterology* 2001; **121**: 799-804
7 **Holtmann G**, Siffert W, Haag S, Mueller N, Langkafel M, Senf W, Zotz R, Talley NJ. G-protein beta 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. *Gastroenterology* 2004; **126**: 971-979
8 **Gonsalkorale WM**, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? *Gut* 2003; **52**: 91-93
9 **Cremonini F**, Camilleri M, McKinzie S, Carlson P, Camilleri CE, Burton D, Thomforde G, Urrutia R, Zinsmeister AR. Effect of CCK-1 antagonist, dexloxiglumide, in female patients with irritable bowel syndrome: a pharmacodynamic and pharmacogenomic study. *Am J Gastroenterol* 2005; **100**: 652-663
10 **Kim HJ**, Camilleri M, Carlson PJ, Cremonini F, Ferber I, Stephens D, McKinzie S, Zinsmeister AR, Urrutia R. Association of distinct alpha(2) adrenoceptor and serotonin transporter polymorphisms with constipation and somatic symptoms in functional gastrointestinal disorders. *Gut* 2004; **53**: 829-837
11 **Camilleri M**, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE, McKinzie S, Urrutia R. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2002; **123**: 425-432
12 **Quigley EM**. Functional gastrointestinal disease: has the genomic era arrived? *Gastroenterology* 2004; **126**: 1193-1195
13 **Quigley EM**. Disturbances of motility and visceral hypersensitivity in irritable bowel syndrome: biological markers or epiphenomenon. *Gastroenterol Clin North Am* 2005; **34**: 221-33, vi
14 **Serra J**, Azpiroz F, Malagelada JR. Impaired transit and tolerance of intestinal gas in the irritable bowel syndrome. *Gut* 2001; **48**: 14-19

15 **Caldarella MP**, Serra J, Azpiroz F, Malagelada JR. Prokinetic effects in patients with intestinal gas retention. *Gastroenterology* 2002; **122**: 1748-1755
16 **Whorwell PJ**. The problem of gas in irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 1618-1619
17 **Koide A**, Yamaguchi T, Odaka T, Koyama H, Tsuyuguchi T, Kitahara H, Ohto M, Saisho H. Quantitative analysis of bowel gas using plain abdominal radiograph in patients with irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 1735-1741
18 **Lewis MJ**, Reilly B, Houghton LA, Whorwell PJ. Ambulatory abdominal inductance plethysmography: towards objective assessment of abdominal distension in irritable bowel syndrome. *Gut* 2001; **48**: 216-220
19 **Galati JS**, McKee DP, Quigley EM. Response to intraluminal gas in irritable bowel syndrome. Motility versus perception. *Dig Dis Sci* 1995; **40**: 1381-1387
20 **Quigley EM**. From comic relief to real understanding; how intestinal gas causes symptoms. *Gut* 2003; **52**: 1659-1661
21 **Mertz H**, Naliboff B, Munakata J, Niazi N, Mayer EA. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. *Gastroenterology* 1995; **109**: 40-52
22 **Lembo T**, Naliboff B, Munakata J, Fullerton S, Saba L, Tung S, Schmulson M, Mayer EA. Symptoms and visceral perception in patients with pain-predominant irritable bowel syndrome. *Am J Gastroenterol* 1999; **94**: 1320-1326
23 **Van Oudenhove L**, Demyttenaere K, Tack J, Aziz Q. Central nervous system involvement in functional gastrointestinal disorders. *Best Pract Res Clin Gastroenterol* 2004; **18**: 663-680
24 **McKendrick MW**, Read NW. Irritable bowel syndrome--post salmonella infection. *J Infect* 1994; **29**: 1-3
25 **Neal KR**, Hebden J, Spiller R. Prevalence of gastrointestinal symptoms six months after bacterial gastroenteritis and risk factors for development of the irritable bowel syndrome: postal survey of patients. *BMJ* 1997; **314**: 779-782
26 **Rodríguez LA**, Ruigómez A. Increased risk of irritable bowel syndrome after bacterial gastroenteritis: cohort study. *BMJ* 1999; **318**: 565-566
27 **Gwee KA**, Graham JC, McKendrick MW, Collins SM, Marshall JS, Walters SJ, Read NW. Psychometric scores and persistence of irritable bowel after infectious diarrhoea. *Lancet* 1996; **347**: 150-153
28 **Gwee KA**, Leong YL, Graham C, McKendrick MW, Collins SM, Walters SJ, Underwood JE, Read NW. The role of psychological and biological factors in postinfective gut dysfunction. *Gut* 1999; **44**: 400-406
29 **Spiller RC**, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000; **47**: 804-811
30 **Dunlop SP**, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; **125**: 1651-1659
31 **Spiller RC**. Postinfectious irritable bowel syndrome. *Gastroenterology* 2003; **124**: 1662-1671
32 **Chadwick VS**, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* 2002; **122**: 1778-1783
33 **Collins SM**. A case for an immunological basis for irritable bowel syndrome. *Gastroenterology* 2002; **122**: 2078-2080
34 **Barbara G**, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* 2004; **126**: 693-702
35 **Törnblom H**, Lindberg G, Nyberg B, Veress B. Full-thickness biopsy of the jejunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* 2002; **123**: 1972-1979
36 **Wahnschaffe U**, Ullrich R, Riecken EO, Schulzke JD. Celiac disease-like abnormalities in a subgroup of patients with irritable bowel syndrome. *Gastroenterology* 2001; **121**: 1329-1338
37 **O'Mahony L**, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; **128**: 541-551
38 **Spiller RC**. Role of nerves in enteric infection. *Gut* 2002; **51**: 759-762
39 **Pimentel M**, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. *Am J Gastroenterol* 2000; **95**: 3503-3506
40 **Pimentel M**, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2003; **98**: 412-419
41 **Bradley HK**, Wyatt GM, Bayliss CE, Hunter JO. Instability in the faecal flora of a patient suffering from food-related irritable bowel syndrome. *J Med Microbiol* 1987; **23**: 29-32
42 **Balsari A**, Ceccarelli A, Dubini F, Fesce E, Poli G. The fecal microbial population in the irritable bowel syndrome. *Microbiologica* 1982; **5**: 185-194
43 **Si JM**, Yu YC, Fan YJ, Chen SJ. Intestinal microecology and quality of life in irritable bowel syndrome patients. *World J Gastroenterol* 2004; **10**: 1802-1805
44 **King TS**, Elia M, Hunter JO. Abnormal colonic fermentation in irritable bowel syndrome. *Lancet* 1998; **352**: 1187-1189
45 **Quigley EM**. Current concepts of the irritable bowel syndrome. *Scand J Gastroenterol Suppl* 2003; : 1-8
46 **Quigley EM**. The use of probiotics in functional bowel disease. *Gastroenterol Clin North Am* 2005; **34**: 533-45, x
47 **O'Leary C**, Quigley EM. Small bowel bacterial overgrowth, celiac disease, and IBS: what are the real associations? *Am J Gastroenterol* 2003; **98**: 720-722
48 **Atkinson W**, Sheldon TA, Shaath N, Whorwell PJ. Food elimination based on IgG antibodies in irritable bowel syndrome: a randomised controlled trial. *Gut* 2004; **53**: 1459-1464
49 **Talley NJ**. Serotoninergic neuroenteric modulators. *Lancet* 2001; **358**: 2061-2068
50 **Müller-Lissner SA**, Fumagalli I, Bardhan KD, Pace F, Pecher E, Nault B, Rüegg P. Tegaserod, a 5-HT(4) receptor partial agonist, relieves symptoms in irritable bowel syndrome patients with abdominal pain, bloating and constipation. *Aliment Pharmacol Ther* 2001; **15**: 1655-1666
51 **Kellow J**, Lee OY, Chang FY, Thongsawat S, Mazlam MZ, Yuen H, Gwee KA, Bak YT, Jones J, Wagner A. An Asia-Pacific, double blind, placebo controlled, randomised study to evaluate the efficacy, safety, and tolerability of tegaserod in patients with irritable bowel syndrome. *Gut* 2003; **52**: 671-676
52 **Cremonini F**, Delgado-Aros S, Camilleri M. Efficacy of alosetron in irritable bowel syndrome: a meta-analysis of randomized controlled trials. *Neurogastroenterol Motil* 2003; **15**: 79-86
53 **Chey WD**, Cash BD. Cilansetron: a new serotonergic agent for the irritable bowel syndrome with diarrhoea. *Expert Opin Investig Drugs* 2005; **14**: 185-193
54 **Walker AM**, Bohn RL, Cali C, Cook SF, Ajene AN, Sands BE. Risk factors for colon ischemia. *Am J Gastroenterol* 2004; **99**: 1333-1337

S- Editor Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 6-9
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Helicobacter pylori virulence factors in duodenal ulceration: A primary cause or a secondary infection causing chronicity

Frank I Tovey, Michael Hobsley, John Holton

Frank I Tovey, Michael Hobsley, Department of Surgery, University College London, London, United Kingdom
John Holton, Department of Microbiology, University College London, London, United Kingdom
Correspondence to: Frank I Tovey, 5 Crossborough Hill, Basingstoke RG21 4AG,
United Kingdom. frank@tovey.fsnet.co.uk
Telephone: +44-1256-461521 Fax: +44-1256-323696
Received: 2005-05-30 Accepted: 2005-07-28

Key words: Duodenal ulceration; *Helicobacter pylori* virulence factors

Tovey FI, Hobsley M, Holton J. *Helicobacter pylori* virulence factors in duodenal ulceration: A primary cause or a secondary infection causing chronicity. *World J Gastroenterol* 2006; 12(1): 6-9

http://www.wjgnet.com/1007-9327/12/6.asp

Abstract

Reports from countries with a high prevalence of *Helicobacter pylori (H pylori)* infection do not show a proportionately high prevalence of duodenal ulceration, suggesting the possibility that *H pylori* cannot be a primary cause of duodenal ulceration. It has been mooted that this discrepancy might be explained by variations in the prevalence of virulence factors in different populations. The aim of this paper is to determine whether the published literature gives support to this possibility. The relevant literature was reviewed and analyzed separately for countries with a high and low prevalence of *H pylori* infection and virulence factors. Although virulent strains of *H pylori* were significantly more often present in patients with duodenal ulcer than without the disease in countries with a low prevalence of *H pylori* infection in the population, there was no difference in the prevalence of virulence factors between duodenal ulcer, non-ulcer dyspepsia or normal subjects in many countries, where the prevalence of both *H pylori* infection and of virulence factors was high. In these countries, the presence of virulence factors was not predictive the clinical outcome. To explain the association between virulence factors and duodenal ulcer in countries where *H pylori* prevalence is low, only two papers were found that give little support to the usual model proposed, namely that organisms with the virulence factors are more likely than those without them to initiate a duodenal ulcer. We offer an alternative hypothesis that suggests virulence factors are more likely to interfere with the healing of a previously produced ulcer. The presence of virulence factors only correlates with the prevalence of duodenal ulcer in countries where the prevalence of *H pylori* is low. There is very little evidence that virulence factors initiate duodenal ulceration, but they may be related to failure of the ulcer to heal.

HELICOBACTER PYLORI VIRULENCE FACTORS AND DUODENAL ULCERATION

Following Warren and Marshall's historic paper in 1984[1], evidence for an association between duodenal ulceration and *Helicobacter pylori (H pylori)* infection has been strengthened. Earlier publications, however, were from developed countries, where the overall prevalence of *H pylori* infection was between 40% and 60%. The difficult problem that remained was why everyone with *H pylori* infection did not develop duodenal ulceration. This problem was increased by the reporting of the "African enigma" from the savannah regions of the West Coast of Africa[2,3], where the prevalence of *H pylori* infection was much higher (> 90%), but the prevalence of duodenal ulceration was relatively low. This was followed by an increasing number of reports from other countries, where a high prevalence of *H pylori* infection did not correlate with a high prevalence of duodenal ulceration (Africa[4-8], India[9-11], China[12,13], Japan[14,15], Korea[16], Peru[17], Iran[18], Vietnam[4]).

When it was later reported that some strains of *H pylori* were more virulent than others, this seemed a possible explanation of the paradox. In the more developed countries, the virulent factors, cagA (cytotoxin associated antigen) and vacA (vacuolating factor), were present in between 40% and 60% of *H pylori* strains, and it was suggested that these strains might prove to be the causal factors in duodenal ulceration and account for the discrepancies[19-24].

There is no doubt about the association of these factors with duodenal ulceration in countries, where the overall prevalence of *H pylori* infection and virulence factors is relatively low, compared with countries where it is high. However, an increasing number of reports from countries, where *H pylori* infection is almost ubiquitous (70-90+%) and 77%-88% of the strains carry the viru-

lence factors cagA and vacA, have shown no relationship between these factors and clinical outcome (South Africa[6], India[25,26], China[27-31], Japan[32-43], Korea[44-47], China Taiwan[48], Thailand[49], Sudan[50], Turkey[51-53], Nigeria[54,55], Sri Lanka[56], Bangladesh[57], Serbia Montenegro[58], Estonia[59], Brazil[60], Singapore[61,62], Mexico[63]). A few similar reports have also come from countries with a low prevalence (Germany[45,64], France[65,66], Finland[67], UK[68,69], USA[42]).

Most cagA positive strains also carry the vacA gene. When present, the cagA gene secretes the toxic CagA protein, but not all vacA strains secrete a toxigenic protein. There are different allelic types of vacA, the types vacAs1 and vacAs1m1 are toxic and strongly associated with duodenal ulceration, mostly in countries with a relatively low prevalence of *H pylori* infection. Once again, however, reports from countries with a high prevalence of these factors show no link between the presence of vacAs1 [6,26,29,38,44,47-49,56,57,60,70] or vacAs1m1[25,26,29,41,44,52,55,59,65,70] and clinical outcome.

Other virulence markers have been reported: ice-A1gene [induced by contact with gastric epithelium] and babA2 gene (blood group antigen binding adhesin), which binds to Lewis B present on gastric epithelial cells, show an association with duodenal ulceration in countries, where the prevalence of *H pylori* and these strains is low. However, reports from many countries with a high prevalence of *H pylori* and these virulence markers again show that in these areas they are not predictive of the clinical outcome (iceA1[6,26,31,41,45,48,52,55,57,61,62,71-73], babA2[6,32,39,41,42,47,61]).

Thus, there remains the anomaly that, although duodenal ulceration is strongly associated with *H pylori* infection and certain virulence factors in countries with a relatively low prevalence of both *H pylori* infection and virulence factors, this association disappears in many countries[74] where these prevalences are high, and where *H pylori* infection and virulence factors do not predict clinical outcome. This casts doubt upon whether *H pylori* initiates duodenal ulcer. This doubt is strongly supported by the finding that most patients with a short history[75] or all with less than 6 month`s history[76] of duodenal ulcer symptoms were uninfected with *H pylori*.

Nonetheless the importance of *H pylori* infection and virulence factors cannot be dismissed. There is no doubt that the eradication of *H pylori* infection leads to healing of duodenal ulceration and the risk of recurrence is greatly reduced. There is also no doubt about the strong association of *H pylori* and virulence factors with duodenal ulceration in countries where the overall prevalence of *H pylori* infection is relatively low.

The tendency for *H pylori* to be absent in the early case suggests that the organism is not the primary cause producing duodenal ulcer. The evidence that the chronic course of healing→recurrence→etc. of the typical chronic duodenal ulcer is converted in most cases into stable healing by eradicating *H pylori* suggests that the organism, when present, interferes with the healing process.

There remains the question why the virulence factors are related to the presence of duodenal ulceration in the countries with a low prevalence of *H pylori* infection. It is possible that colonization of nearby areas of antral epithelium or of gastric metaplasia in the duodenum by *H pylori* leads to the local release of toxins that produces the duodenal ulcer. However, this straightforward model has not been substantiated. The toxins concerned have been demonstrated and their toxic effects are determined mostly by their interaction with gastric epithelium[19] and there are only two papers reporting about the damage caused by toxins to duodenal mucosa. One paper[77] reports about the prevention of healing of mechanically abraded human duodenal epithelium *in vitro* by strains of wild *H pylori,* particularly those carrying the vacA gene, and also by supernatant fluid containing the vacA cytotoxin. The other paper[78] reports about increased duodenal mucosal permeability, when exposed to *H pylori* culture fluid in rats. It must be emphasized that neither paper reports about the *initiation* of ulceration.

As an alternative explanation, we advance the following more complicated model which we have partly suggested before[11,76]. *H pylori* is killed by excess acid[79,80]. In countries, where the overall prevalence of *H pylori* infection is low, duodenal ulcer patients initially may be free from *H pylori* infection because of their high acid output. In the early stages of ulceration, many subjects, prior to seeking definitive treatment, control their symptoms with antacids, some including H2 antagonists, which are available without prescription. This reduces the defense against infection with the organism in patients who have hitherto been resistant (since they start *H pylori* negative). This partial reduction in resistance can be overcome by virulent, but not by non - virulent strains, so there is an association between the virulent strains and the chronic ulcer patients, most of whom have become *H pylori* - positive for the organism by 6 months time[75,76]. The high baseline of infection with virulent strains, in the countries with a high prevalence, obscures this effect.

REFERENCES

1 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315

2 **Wolf G**. Function of the protein RPE65 in the visual cycle. *Nutr Rev* 2005; **63**: 97-100

3 **Holcombe C**, Omotara BA, Eldridge J, Jones DM. H. pylori, the most common bacterial infection in Africa: a random serological study. *Am J Gastroenterol* 1992; **87**: 28-30

4 **Mégraud F**, Brassens-Rabbé MP, Denis F, Belbouri A, Hoa DQ. Seroepidemiology of Campylobacter pylori infection in various populations. *J Clin Microbiol* 1989; **27**: 1870-1873

5 **Segal I**, Ally R, Sitas F, Walker AR. Co-screening for primary biliary cirrhosis and coeliac disease. Helicobacter pylori: the African enigma. *Gut* 1998; **43**: 300-301

6 **Segal I**, Ally R, Mitchell H. Helicobacter pylori--an African perspective. *QJM* 2001; **94**: 561-565

7 **Wyatt JI**, De Caestecker JS, Rathbone BJ, Heatley RV. Campylobacter pylori in Tropical Africa. *Gut* 1987; 28: A1409-A1410

8 **Tovey FI**, Hobsley M, Segal I, Jayaraj AP. Duodenal ulcer in South Africa: home-pounded versus milled maize. *J Gastroenterol Hepatol* 2005; **20**: 1008-1011

9 **Prasad S**, Mathan M, Chandy G, Rajan DP, Venkateswaran S, Ramakrishna BS, Mathan VI. Prevalence of Helicobacter pylori in southern Indian controls and patients with gastroduodenal disease. *J Gastroenterol Hepatol* 1994; **9**: 501-506

10 **Madanagopalan N**, Balakumar K, Gajaraj AJ. Epidemiology of peptic ulcer disease in India. *Indian J Gastroenterol* 1986; **5 Suppl**: 3-6

11 **Tovey FI**, Hobsley M, Kaushik SP, Pandey R, Kurian G, Singh K, Sood A, Jehangir E. Duodenal gastric metaplasia and Helicobacter pylori infection in high and low duodenal ulcer-prevalent areas in India. *J Gastroenterol Hepatol* 2004; **19**: 497-505

12 **Ching CK**, Lam SK. Helicobacter pylori epidemiology in relation to peptic ulcer and gastric cancer in south and north China. *J Gastroenterol Hepatol* 1994; **9 Suppl 1**: S4-S7

13 **Wong BC**, Ching CK, Lam SK, Li ZL, Chen BW, Li YN, Liu HJ, Liu JB, Wang BE, Yuan SZ, Xu CP, Hou XH, Zhang AT, Zheng ZT. Differential north to south gastric cancer-duodenal ulcer gradient in China. China Ulcer Study Group. *J Gastroenterol Hepatol* 1998; **13**: 1050-1057

14 **Schlemper RJ**, van der Werf SD, Biemond I, Lamers CB. Seroepidemiology of gastritis in Japanese and Dutch male employees with and without ulcer disease. *Eur J Gastroenterol Hepatol* 1996; **8**: 33-39

15 **Kawai K**, Shirakawa K, Misaki F, Hayashi K, Watanabe Y. Natural history and epidemiologic studies of peptic ulcer disease in Japan. *Gastroenterology* 1989; **96**: 581-585

16 **Malaty HM**, Kim JG, Kim SD, Graham DY. Prevalence of Helicobacter pylori infection in Korean children: inverse relation to socioeconomic status despite a uniformly high prevalence in adults. *Am J Epidemiol* 1996; **143**: 257-262

17 **Burstein M**, Monge E, León-Barúa R, Lozano R, Berendson R, Gilman RH, Legua H, Rodriguez C. Low peptic ulcer and high gastric cancer prevalence in a developing country with a high prevalence of infection by Helicobacter pylori. *J Clin Gastroenterol* 1991; **13**: 154-156

18 **Massarrat S**, Saberi-Firoozi M, Soleimani A, Himmelmann GW, Hitzges M, Keshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. *Eur J Gastroenterol Hepatol* 1995; **7**: 427-433

19 **Atherton JC**. H. pylori virulence factors. *Br Med Bull* 1998; **54**: 105-120

20 **Crabtree JE**, El - Omer E, Bugnoli M, Covacci A, Eyre D, Rappuoli R, McColl KEL. Serum cagA IgG antibodies in Helicobacter pylori positive healthy volunteers and patients with dyspeptic illness. *Gut* 1994; **35**: S35

21 **Hamlet A**, Thoreson AC, Nilsson O, Svennerholm AM, Olbe L. Duodenal Helicobacter pylori infection differs in cagA genotype between asymptomatic subjects and patients with duodenal ulcers. *Gastroenterology* 1999; **116**: 259-268

22 **van Doorn LJ**, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, Quint W. Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori. *Gastroenterology* 1998; **115**: 58-66

23 **Figueiredo C**, Van Doorn LJ, Nogueira C, Soares JM, Pinho C, Figueira P, Quint WG, Carneiro F. Helicobacter pylori genotypes are associated with clinical outcome in Portuguese patients and show a high prevalence of infections with multiple strains. *Scand J Gastroenterol* 2001; **36**: 128-135

24 **Mitchell HM**, Hazell SL, Li YY, Hu PJ. Serological response to specific Helicobacter pylori antigens: antibody against CagA antigen is not predictive of gastric cancer in a developing country. *Am J Gastroenterol* 1996; **91**: 1785-1788

25 **Chattopadhyay S**, Datta S, Chowdhury A, Chowdhury S, Mukhopadhyay AK, Rajendran K, Bhattacharya SK, Berg DE, Nair GB. Virulence genes in Helicobacter pylori strains from West Bengal residents with overt H. pylori-associated disease and healthy volunteers. *J Clin Microbiol* 2002; **40**: 2622-2625

26 **Mukhopadhyay AK**, Kersulyte D, Jeong JY, Datta S, Ito Y, Chowdhury A, Chowdhury S, Santra A, Bhattacharya SK, Azuma T, Nair GB, Berg DE. Distinctiveness of genotypes of Helicobacter pylori in Calcutta, India. *J Bacteriol* 2000; **182**: 3219-3227

27 **Pan ZJ**, van der Hulst RW, Feller M, Xiao SD, Tytgat GN, Dankert J, van der Ende A. Equally high prevalences of infection with cagA-positive Helicobacter pylori in Chinese patients with peptic ulcer disease and those with chronic gastritis-associated dyspepsia. *J Clin Microbiol* 1997; **35**: 1344-1347

28 **Wong BC**, Yin Y, Berg DE, Xia HH, Zhang JZ, Wang WH, Wong WM, Huang XR, Tang VS, Lam SK. Distribution of distinct vacA, cagA and iceA alleles in Helicobacter pylori in Hong Kong. *Helicobacter* 2001; **6**: 317-324

29 **Qiao W**, Hu JL, Xiao B, Wu KC, Peng DR, Atherton JC, Xue H. cagA and vacA genotype of Helicobacter pylori associated with gastric diseases in Xi'an area. *World J Gastroenterol* 2003; **9**: 1762-1766

30 **Xu C**, Li ZS, Tu ZX, Xu GM, Gong YF, Man XH. Distribution of cagG gene in Helicobacter pylori isolates from Chinese patients with different gastroduodenal diseases and its clinical and pathological significance. *World J Gastroenterol* 2003; **9**: 2258-2260

31 **Yin Y**, Zhang JZ, Wang ZY, Xia HX, Lin ZX. [Association between Helicobacter pylori virulence and duodenal ulcer disease in patients from Hong Kong in China]. *Zhonghua Liuxingbingxue Zazhi* 2003; **24**: 123-126

32 **Mizushima T**, Sugiyama T, Komatsu Y, Ishizuka J, Kato M, Asaka M. Clinical relevance of the babA2 genotype of Helicobacter pylori in Japanese clinical isolates. *J Clin Microbiol* 2001; **39**: 2463-2465

33 **Maeda S**, Kanai F, Ogura K, Yoshida H, Ikenoue T, Takahashi M, Kawabe T, Shiratori Y, Omata M. High seropositivity of anti-CagA antibody in Helicobacter pylori-infected patients irrelevant to peptic ulcers and normal mucosa in Japan. *Dig Dis Sci* 1997; **42**: 1841-1847

34 **Maeda S**, Ogura K, Yoshida H, Kanai F, Ikenoue T, Kato N, Shiratori Y, Omata M. Major virulence factors, VacA and CagA, are commonly positive in Helicobacter pylori isolates in Japan. *Gut* 1998; **42**: 338-343

35 **Maeda S**, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, Shiratori Y, Omata M. Structure of cag pathogenicity island in Japanese Helicobacter pylori isolates. *Gut* 1999; **44**: 336-341

36 **Ogura K**, Kanai F, Maeda S, Yoshida H, Ogura M, Lan KH, Hirota K, Kawabe T, Shiratori Y, Omata M. High prevalence of cytotoxin positive Helicobacter pylori in patients unrelated to the presence of peptic ulcers in Japan. *Gut* 1997; **41**: 463-468

37 **Takata T**, Fujimoto S, Anzai K, Shirotani T, Okada M, Sawae Y, Ono J. Analysis of the expression of CagA and VacA and the vacuolating activity in 167 isolates from patients with either peptic ulcers or non-ulcer dyspepsia. *Am J Gastroenterol* 1998; **93**: 30-34

38 **Tokumaru K**, Kimura K, Saifuku K, Kojima T, Satoh K, Kihira K, Ido K. CagA and cytotoxicity of Helicobacter pylori are not markers of peptic ulcer in Japanese patients. *Helicobacter* 1999; **4**: 1-6

39 **Höcker M**, Hohenberger P. Helicobacter pylori virulence factors--one part of a big picture. *Lancet* 2003; **362**: 1231-1233

40 **Kodama K**, Ito A, Nishizono A, Fujioka T, Nasu M, Yahiro K, Hirayama T, Uemura N. Divergence of virulence factors of Helicobacter pylori among clinical isolates does not correlate with disease specificity. *J Gastroenterol* 1999; **34 Suppl 11**: 6-9

41 **Yamaoka Y**, Kodama T, Gutierrez O, Kim JG, Kashima K, Graham DY. Relationship between Helicobacter pylori iceA, cagA, and vacA status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999; **37**: 2274-2279

42 **Yamaoka Y**, Souchek J, Odenbreit S, Haas R, Arnqvist A, Borén T, Kodama T, Osato MS, Gutierrez O, Kim JG, Graham DY. Discrimination between cases of duodenal ulcer and gastritis on the basis of putative virulence factors of Helicobacter pylori. *J Clin Microbiol* 2002; **40**: 2244-2246

43 **Zhou W**, Yamazaki S, Yamakawa A, Ohtani M, Ito Y, Keida Y, Higashi H, Hatakeyama M, Si J, Azuma T. The diversity of vacA and cagA genes of Helicobacter pylori in East Asia. *FEMS Immunol Med Microbiol* 2004; **40**: 81-87

44 **Park SM**, Park J, Kim JG, Yoo BC. Relevance of vacA genotypes of Helicobacter pylori to cagA status and its clinical outcome. *Korean J Intern Med* 2001; **16**: 8-13

45 **Li L**, Graham DY, Gutierrez O, Kim JG, Genta RM, El-Zimaity HM, Go MF. Genomic fingerprinting and genotyping of Helicobacter pylori strains from patients with duodenal ulcer or gastric cancer from different geographic regions. *Dig Dis Sci* 2002; **47**: 2512-2518

46 **Kim JW**, Kim JG, Chae SL, Cha YJ, Park SM. High prevalence of multiple strain colonization of Helicobacter pylori in

Korean patients: DNA diversity among clinical isolates from the gastric corpus, antrum and duodenum. *Korean J Intern Med* 2004; **19**: 1-9

47 **Park SM**, Park J, Kim JG, Cho HD, Cho JH, Lee DH, Cha YJ. Infection with Helicobacter pylori expressing the cagA gene is not associated with an increased risk of developing peptic ulcer diseases in Korean patients. *Scand J Gastroenterol* 1998; **33**: 923-927

48 **Perng CL**, Lin HJ, Sun IC, Tseng GY. Helicobacter pylori cagA, iceA and vacA status in Taiwanese patients with peptic ulcer and gastritis. *J Gastroenterol Hepatol* 2003; **18**: 1244-1249

49 **Mahachai V**, Tangkijvanich P, Wannachai N, Sumpathanukul P, Kullavanijaya P. CagA and VacA: virulence factors of Helicobacter pylori in Thai patients with gastroduodenal diseases. *Helicobacter* 1999; **4**: 143-147

50 **El-Mahdi AM**, Patchett SE, Char S, Domizio P, Fedail SS, Kumar PJ. Does CagA contribute to ulcer pathogenesis in a developing country, such as Sudan? *Eur J Gastroenterol Hepatol* 1998; **10**: 313-316

51 **Serin E**, Yilmaz U, Künefeci G, Ozer B, Gümürdülü Y, Güçlü M, Kayaselçuk F, Boyacioğlu S. Serum positive cagA in patients with non-ulcer dyspepsia and peptic ulcer disease from two centers in different regions of Turkey. *World J Gastroenterol* 2003; **9**: 833-835

52 **Bulent K**, Murat A, Esin A, Fatih K, MMMurat H, Hakan H, Melih K, Mehmet A, Bulent Y, Fatih H. Association of CagA and VacA presence with ulcer and non-ulcer dyspepsia in a Turkish population. *World J Gastroenterol* 2003; **9**: 1580-1583

53 **Abasiyanik MF**, Sander E, Salih BA. Helicobacter pylori anti-CagA antibodies: prevalence in symptomatic and asymptomatic subjects in Turkey. *Can J Gastroenterol* 2002; **16**: 527-532

54 **Rocha AM**, Rocha GA, de Magalhães Queiroz DM, Ani AE, Okeke EN, Bello CS, Malu AO. Anti-CagA antibodies in Helicobacter pylori-positive patients and blood donors from Nigeria. *Trop Doct* 2001; **31**: 147-149

55 **Smith SI**, Kirsch C, Oyedeji KS, Arigbabu AO, Coker AO, Bayerdöffer E, Miehlke S. Prevalence of Helicobacter pylori vacA, cagA and iceA genotypes in Nigerian patients with duodenal ulcer disease. *J Med Microbiol* 2002; **51**: 851-854

56 **Fernando N**, Holton J, Vaira D, DeSilva M, Fernando D. Prevalence of Helicobacter pylori in Sri Lanka as determined by PCR. *J Clin Microbiol* 2002; **40**: 2675-2676

57 **Rahman M**, Mukhopadhyay AK, Nahar S, Datta S, Ahmad MM, Sarker S, Masud IM, Engstrand L, Albert MJ, Nair GB, Berg DE. DNA-level characterization of Helicobacter pylori strains from patients with overt disease and with benign infections in Bangladesh. *J Clin Microbiol* 2003; **41**: 2008-2014

58 **Sokic-Milutinovic A**, Wex T, Todorovic V, Milosavljevic T, Malfertheiner P. Anti-CagA and anti-VacA antibodies in Helicobacter pylori-infected patients with and without peptic ulcer disease in Serbia and Montenegro. *Scand J Gastroenterol* 2004; **39**: 222-226

59 **Andreson H**, Lõivukene K, Sillakivi T, Maaroos HI, Ustav M, Peetsalu A, Mikelsaar M. Association of cagA and vacA genotypes of Helicobacter pylori with gastric diseases in Estonia. *J Clin Microbiol* 2002; **40**: 298-300

60 **Brito CA**, Silva LM, Jucá N, Leal NC, de Souza W, Queiroz D, Cordeiro F, Silva NL. Prevalence of cagA and vacA genes in isolates from patients with Helicobacter pylori-associated gastroduodenal diseases in Recife, Pernambuco, Brazil. *Mem Inst Oswaldo Cruz* 2003; **98**: 817-821

61 **Ho YW**, Ho KY, Ascencio F, Ho B. Neither gastric topological distribution nor principle virulence genes of Helicobacter pylori contributes to clinical outcomes. *World J Gastroenterol* 2004; **10**: 3274-3277

62 **Zheng PY**, Hua J, Yeoh KG, Ho B. Association of peptic ulcer with increased expression of Lewis antigens but not cagA, iceA, and vacA in Helicobacter pylori isolates in an Asian population. *Gut* 2000; **47**: 18-22

63 **Garza-Gonzalez E**, Bosques-Padilla FJ, Tijerina-Menchaca R, Perez-Perez GI. Characterisation of Helicobacter pylori isolates from the north-eastern region of Mexico. *Clin Microbiol Infect* 2004; **10**: 41-45

64 **Schilling D**, Ott MG, Nilius M, Köhler G, Zober A, Messerer P, Riemann JF. Specific Helicobacter pylori antigens unable to distinguish nonucler dyspepsia or peptic ulcer cases from asymptomatic seropositive controls: a nested case-control study in employees of a large company. *Dig Dis Sci* 2000; **45**: 2444-2450

65 **Miehlke S**, Yu J, Schuppler M, Frings C, Kirsch C, Negraszus N, Morgner A, Stolte M, Ehninger G, Bayerdörffer E. Helicobacter pylori vacA, iceA, and cagA status and pattern of gastritis in patients with malignant and benign gastroduodenal disease. *Am J Gastroenterol* 2001; **96**: 1008-1013

66 **Jenks PJ**, Mégraud F, Labigne A. Clinical outcome after infection with Helicobacter pylori does not appear to be reliably predicted by the presence of any of the genes of the cag pathogenicity island. *Gut* 1998; **43**: 752-758

67 **Heikkinen M**, Janatuinen E, Mayo K, Mégraud F, Julkunen R, Pikkarainen P. Usefulness of anti-Helicobacter pylori and anti-CagA antibodies in the selection of patients for gastroscopy. *Am J Gastroenterol* 1997; **92**: 2225-2229

68 **Graham DY**, Genta RM, Graham DP, Crabtree JE. Serum CagA antibodies in asymptomatic subjects and patients with peptic ulcer: lack of correlation of IgG antibody in patients with peptic ulcer or asymptomatic Helicobacter pylori gastritis. *J Clin Pathol* 1996; **49**: 829-832

69 **Stephens JC**, Stewart JA, Folwell AM, Rathbone BJ. Helicobacter pylori cagA status, vacA genotypes and ulcer disease. *Eur J Gastroenterol Hepatol* 1998; **10**: 381-384

70 **Wang J**, van Doorn LJ, Robinson PA, Ji X, Wang D, Wang Y, Ge L, Telford JL, Crabtree JE. Regional variation among vacA alleles of Helicobacter pylori in China. *J Clin Microbiol* 2003; **41**: 1942-1945

71 **Ribeiro ML**, Godoy AP, Benvengo YH, Mendonça S, Pedrazzoli J. Clinical relevance of the cagA, vacA and iceA genotypes of Helicobacter pylori in Brazilian clinical isolates. *FEMS Immunol Med Microbiol* 2003; **36**: 181-185

72 **Nogueira C**, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plaisier AP, Quint WG, Ruiz B, Correa P, van Doorn LJ. Helicobacter pylori genotypes may determine gastric histopathology. *Am J Pathol* 2001; **158**: 647-654

73 **Ito Y**, Azuma T, Ito S, Suto H, Miyaji H, Yamazaki Y, Kato T, Kohli Y, Keida Y, Kuriyama M. Sequence analysis and clinical significance of the iceA gene from Helicobacter pylori strains in Japan. *J Clin Microbiol* 2000; **38**: 483-488

74 **Mégraud F**. Impact of Helicobacter pylori virulence on the outcome of gastroduodenal diseases: lessons from the microbiologist. *Dig Dis* 2001; **19**: 99-103

75 **Pest P**, Zárate J, Varsky C, Man F, Schraier M. Helicobacter pylori in recently-diagnosed versus chronic duodenal ulcer. *Acta Gastroenterol Latinoam* 1996; **26**: 273-276

76 **Boulos PB**, Botha A, Hobsley M, Holton J, Oshowo AO, Tovey FI. Possible absence of Helicobacter pylori in the early stages of duodenal ulceration. *QJM* 2002; **95**: 749-752

77 **Tabel G**, Hoa NT, Tarnawski A, Chen J, Domek M, Ma TY. Helicobacter pylori infection inhibits healing of the wounded duodenal epithelium in vitro. *J Lab Clin Med* 2003; **142**: 421-430

78 **Watanabe K**, Joh T, Seno K, Takahashi N, Ohara H, Nomura T, Tochikubo K, Itoh M. Injurious effect of Helicobacter pylori culture fluid to gastroduodenal mucosa, and its detoxification by sucralfate in the rat. *Aliment Pharmacol Ther* 1999; **13**: 1363-1371

79 **Sjöström JE**, Larsson H. Factors affecting growth and antibiotic susceptibility of Helicobacter pylori: effect of pH and urea on the survival of a wild-type strain and a urease-deficient mutant. *J Med Microbiol* 1996; **44**: 425-433

80 **Dykhuizen RS**, Fraser A, McKenzie H, Golden M, Leifert C, Benjamin N. Helicobacter pylori is killed by nitrite under acidic conditions. *Gut* 1998; **42**: 334-337

S- Editor Guo SY **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 10-16
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Causal role of *Helicobacter pylori* infection and eradication therapy in gastric carcinogenesis

Masanori Ito, Shinji Tanaka, Tomoari Kamada, Ken Haruma, Kazuaki Chayama

Masanori Ito, Kazuaki Chayama, Department of Medicine and Molecular Science, Hiroshima University, Hiroshima, Japan
Shinji Tanaka, Department of Endoscopy, Hiroshima University Hospital, Hiroshima, Japan
Tomoari Kamada, Ken Haruma, Gastroenterology Unit, Department of Internal Medicine, Kawasaki Medical School, Kurashiki, Japan
Correspondence to: Shinji Tanaka, MD, PhD, Department of Endoscopy, Hiroshima University Hospital, Hiroshima, 734-8551, Japan. colon@hiroshima-u.ac.jp
Telephone: +81-82-257-5538 Fax: +81-82-257-5538
Received: 2005-06-09 Accepted: 2005-06-22

Abstract

Many epidemiological reports indicate that *Helicobacter pylori* (*H pylori*) infection plays an important role in gastric carcinogenesis. Several genetic and epigenetic alterations contribute to the initiation, promotion, and progression of the cancer cells in a multi-step manner. *H pylori* is known to induce chronic inflammation in the gastric mucosa. Its products, including superoxides, participate in the DNA damage followed by initiation, and the inflammation-derived cytokines and growth factors contribute to the promotion of gastric carcinogenesis. By eradicating *H pylori*, gastric inflammation can be cured; the therapy diminishes the levels not only of inflammatory cell infiltration, but also atrophy/intestinal metaplasia in part. A randomized controlled trial revealed that the eradication therapy diminished the gastric cancer prevalence in cases without precancerous conditions. In addition, recent epidemiological studies from Japanese groups demonstrated that the development of gastric cancer, especially of the intestinal type, was decreased by successful eradication therapy, although these were designed in a non-randomized manner. However, it should be mentioned that endoscopic detection is the only way to evaluate the degree of gastric carcinogenesis. We have reported that the endoscopic and histological morphologies could be modified by eradication therapy and it might contribute to the prevalence of gastric cancer development. Considering the biological nature of cancer cell proliferation, it is considered that a sufficiently long-term follow-up would be essential to discuss the anticancer effect of eradication therapy.

Key words: *H pylori*; Eradication; Gastritis; Gastric neoplasm; Endoscopy

Ito M, Tanaka S, Kamada T, Haruma K, Chayama K. Causal role of *Helicobacter pylori* infection and eradication therapy in gastric carcinogenesis. *World J Gastroenterol* 2006; 12(1): 10-16

http://www.wjgnet.com/1007-9327/12/10.asp

INTRODUCTION

Helicobacter pylori (H pylori), a Gram-negative bacillus discovered in 1983, is the most popular pathogenic bacteria in the world. Approximate-ly half of the population has *H pylori* infection worldwide, and the prevalence is thought to be 80-90% in developing countries and 30-50% in developed countries[1]. Many studies clarified the implication of the bacteria as the cause of many gastroduodenal diseases, including histological chronic gastritis, peptic ulcer, and mucosal-associated lymphoid tissue (MALT) lymphoma[2,3].

In particular, histological chronic atrophic gastritis is an important disease for gastric carcinogenesis. Two main causes for the promotion of atroph-ic gastrit-is are (1) *H pylori* and (2) autoimmune factors such as antiparietal cell antibody. Although these two factors work synergistically in the promotion of atrophic gastritis[4,5], autoimmune gastritis is a rare disease and most atrophic gastritis is thought to be induced by *H pylori* infection in East-Asian countries such as in Japan, China, and Korea[6]. Therefore, it is likely that *H pylori* is the most important factor for mucosal atrophy and we have confirmed this hypothesis in the Japanese population[7].

Gastric cancer is the second most common cancer (next to lung cancer) in the world and the number of newly diagnosed cases was calculated as 750000 persons per year[8]. As previously demonstrated by Correa *et al*., atrophic gastritis and the following intestinal metaplasia are regarded as an essential status for intestinal type cancer development[9]. It has been widely accepted that there is a strong association between *H pylori*-associated gastritis and gastric cancer. Nomura *et al*. and Parsonnet *et al*. first reported the relationship between *H pylori* infection and gastric cancer in 1991[10,11]. In 1994, the International Center for Cancer Research officially recogniz-ed that *H pylori* was a definite carcinogen for gastric cancer on the basis of several epidemiological reports[12]. Moreover, Huang *et al*. demonstrated the relationship between *H pylori* seropositivity and gastric cancer by meta-

analysis[13]. The odds ratios for gastric cancer were calculated as 1.92 (1.32-2.78; 95%CI), 2.24 (1.15-4.4), and 1.81 (1.16-2.84) for all studies, cohort and case-control studies, respectively. In addition, many investigations showed the tight relationship between *H pylori* and not only intestinal type cancer, but also diffuse type cancer[13,14]. Even in gastric cancer at a younger age, we were able to detect a tight relationship between gastric cancer and *H pylori*[15]. In 2001, Uemura *et al.*[16] clearly demonstrated that gastric cancer developed only in patients with *H pylori* infection with a prospective study. They prospectively followed 1 246 Japanese people for 7.8 years and found that gastric cancer had been detected in only 36 patients with *H pylori* infection. No gastric cancer was found in *H pylori*-negative patients in their study.

These findings strongly suggest the implication of *H pylori* in gastric carcinogenesis. Next, we should clarify the hypothesis as to whether we can control gastric carcinogenesis by the eradication of *H pylori*. In the present study, we have summarized the recent clinical evidence in this field and have attempted to answer this question.

Gastric inflammation induced by Helicobacter pylori infection and its carcinogenic effects

Persistent infection of *H pylori* induces the characteristic inflammation in the gastric mucosa with the histological finding of mononuclear cell/neutrophil infiltration. In particular, the superoxides, such as nitric oxide, in the gastric mucosa play an important role in the initiation of the carcinogenesis as a mediator of carcinogenic nitrosamine formation, DNA damage and tissue injury. Studies have also revealed that *H pylori* infection in human beings is associated with the enhanced expression of iNOS by tissue neutrophils and mononuclear cells[17,18]. Previously, we have reported that the expression of inducible nitric oxide synthase and nitrotyrosine in chronic gastritis is a predictive marker for a high risk of gastric cancer development[19]. The iNOS-producing gastritis, which is supposed to be strongly associated with gastric cancer, showed a characteristic cytokine profile and serum gastrin pattern[20].

Chronic inflammation results in the destruction of parietal cells of the oxyntic gland in the gastric corpus followed by the alteration of the pathophysiological status, including gastric acid secretion[21]. The pH of gastric juice is a major determinant of the nitrite and vitamin C concentrations in gastric juice. Previous studies reported a close linkage between low acid output and an increased concentration of nitrite and *N*-nitroso compounds in gastric juice[22]. Vitamin C was actively secreted into gastric juice and scavenges nitrite from the gastric juice and thereby acts to prevent the formation of nitrosamines. Vitamin C concentrations in gastric juice were found to be low in patients with hypochlorhydria status[23]. In addition, we have reported that gastric juice nitrite concentrations are higher and vitamin C concentrations are lower in patients with gastric cancer than those in atrophic gastritis, despite similar intra-gastric pH and *H pylori* status[24].

Furthermore, inflammation-derived cytokines and growth factors are known to act as promotion/proliferation factors for gastric cancer cells. Especially, hepatocyte growth factor has been reported to play a crucial role in epithelial/tumor cell proliferation, and its expression was promoted in *H pylori*-infected mucosa[25]. Churin *et al.* reported that *H pylori* activated c-Met (the receptor of HGF) in AGS cells, suggesting the important role of HGF in an autocrine manner[26]. In addition, the regulation of apoptotic signals is another important factor for the promotion/proliferation of cancer cells. *H pylori* was reported to be able to induce apoptosis of the gastric epithelial cells directly[27]. Nagasako *et al.*[28] have demonstrated that the Smad-5, which contributes to the apoptosis of gastric epithelial cells, is upregulated by *H pylori* infection.

Many recent advances have revealed the detailed molecular mechanisms of gastric inflammation induced by *H pylori*. The research on some virulent bacterial factors, VacA and CagA, were reported successively from Japanese groups. Fujikawa *et al.*[29] clarified that VacA binds to protein tyrosine phosphatase receptor type Z (Ptprz), which is a specific receptor on epithelial cells, and induces the intracellular pathway *via* G protein-coupled receptor kinase-interactor 1 (Git1) and pleiotrophin. On the other hand, the advantage of CagA is the most sensational in this field. Previously, Huang *et al.*[30]demonstrated the strong association between anti-CagA seropositivity and gastric carcinogenesis, suggesting the importance of CagA for gastric carcinogenesis. CagA protein produced in the bacterial cell is translocated into the host cell by a type IV secretory system[31], followed by translocation to the membrane[32], tyrosine phosphorylation of the EPIYA motif by *src*-family kinase and the activation of SHP-2 phosphatase, which is the important second messenger from CagA (Figure 1)[33]. Recent studies by Hatakeyama *et al.* clarified that the CagA protein showed diversity and was subclassified into two types, the Western type and East-Asian type, and the later type was reported to have a high affinity to SHP-2 and was regarded as a more harmful form[34]. It is possible to explain the international diversity of the prevalence of gastric cancer; in Western countries, the prevalence of gastric cancer is relatively low because Western type CagA (or Cag negative strain) is dominant. On the other hand in East-Asian countries, the more virulent strain (East-Asian type) is the major strain, and this is a reason for the higher prevalence of gastric cancer. However, it is still unclear why some Japanese/Chinese people do not show corpus atrophy, even if they carry the East-Asian type CagA. Mimuro *et al.*[35]suggested another signal pathway via growth factor receptor bound 2(Grb2), which is independent of CagA phosphorylation. Intragastric diversity or other bacterial factors must be examined to solve this question, as well as the host factors and environmental factors including a high intake of salt[36].

After successful *H pylori* eradication therapy, these harmful conditions are dramatically improved. There is no doubt that the eradication therapy can diminish the risk of the new development of gastric cancer or progression from the pre-malignant status. However, it should be emphasized that it takes a long time from when one cell transforms into a cancer cell to when we can detect the cancer tissue by endoscopic examination. Although the growth rate of gastric cancer cells differs

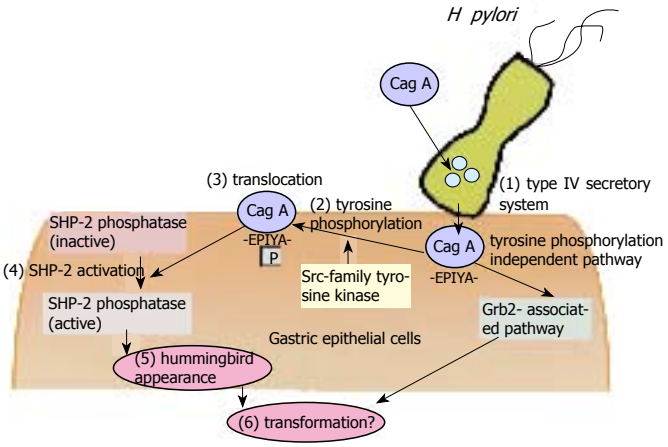


Figure 1 Intracellular molecular pathway starting from *H pylori* infection.

according to their biological or histological characteristics, Haruma *et al*.[37] reported that average doubling time of early gastric cancer is 16.6 mo in the polypoid type. From this fact, it is likely that when we detect the cancer lesion in the stomach by endoscopic examination, cancer cells must have already been present for many years, even if it is not detectable. Cancer cells have progressed through the multi-step process of genetic and epigenetic alteration of oncogenes[38]. These alterations must occur in the cancer cells in the initial stage, and it is unlikely that these could be always cancelled by eradication therapy alone.

Reversibility of pre-malignant status; atrophic gastritis and intestinal metaplasia

Many reports have mentioned the improvement of neutrophil/lymphocyte infiltration after *H pylori* eradication therapy. However, it is still controversial as to the improvement of glandular atrophy or intestinal metaplasia after the eradication therapy. Annibale *et al*. have concluded that eradication therapy does not improve mucosal atrophy[39]. Sung *et al*.[40] reported the results of a large-scale prospective randomized study and concluded that eradication therapy prevents the progression of atrophy, which was not reversible. However, some problems remain that the following period is relatively short in the former, and the basal grades of atrophy were mild in the latter study. On the other hand, Ohkusa *et al*.[41] and Haruma *et al*.[42] enrolled patients with atrophic gastritis and reported the reversibility of atrophy after *H pylori* eradication therapy. Furthermore, we previously followed up 22 patients in whom *H pylori* was eradicated for 5 years and confirmed that glandular atrophy is reversible both in the gastric corpus and in the antrum[43]. It should be noted that the reversibility of atrophy was found in the patients with moderate atrophy, and it took a long-time to confirm the reversibility. In cases with complete disappearance of the gland, such as cases with gastric adenoma, reconstruction of the gland may be unlikely. Recently, Sugiyama *et al*.[44] reported the regression of corpus atrophy after eradication. They emphasized the importance of the biopsy site, and demonstrated that the most suitable point is the lesser curvature of the gastric corpus.

Another major problem is the reversibility of the intestinal metaplasia. Although some reports have refuted the improvement of intestinal metaplasia[39,45], there has been a report suggesting the effect of eradication therapy in the improvement of intestinal metaplasia[41]. Correa *et al*.[46] reported that eradication therapy could regress not only the degree of atrophy, but also the intestinal metaplasia in a randomized controlled trial. Leung *et al*.[47] demonstrated that the eradication therapy could prevent the progression of intestinal metaplasia using a randomized controlled trial with a 5-year follow-up. However, to evaluate the status of intestinal metaplasia, biopsy specimens for point-diagnosis seem to be not suitable. Therefore, we performed dye-endoscopy using methylene blue solution, which allowed us to evaluate the degree of intestinal metaplasia as a "field". Our overall results suggested the reversibility of intestinal metaplasia by following up over long periods, even if all patients did not show a regression of the intestinal metaplasia[43]. At present, a large-scale prospective study (Japanese intervention trial of *H pylori*) has been ongoing. In this study, the reversibility of atrophic gastritis was examined by endoscopic and histological examinations, and its final result will be published in 2006. Although the improvement of atrophic gastritis or intestinal metaplasia links to the regression of gastric carcinogenesis at the extremely early stage, a long-term (more than 10 years) follow-up study will be necessary to confirm the effect of eradication therapy on gastric carcinogenesis.

Does eradication therapy truly diminish gastric cancer?

It is clinically important to clarify whether gastric carcinogenesis could be influenced by eradication therapy. Firstly, Uemura *et al*.[48] reported the reduced incidence of secondary developed gastric cancer by eradication therapy in patients with endoscopic resection of the primary gastric cancer. Because of the reason described above, their data did not suggest an anticarcinogenic effect of the eradication therapy, but instead an antiproliferative effect of cancer cell growth. Indeed, we have previously reported that the Ki-67 labeling index in cancer cells is lower in cancer lesions without *H pylori* infection than in those with *H pylori*[49]. Recently, Take *et al*.[50] reported that they followed up 1120 peptic ulcer patients with eradication therapy prospectively (mean 3.4 years) and found that gastric cancer is more frequently detected in patients with failed eradication than those with successful eradication. They have also demonstrated that gastric cancer was never detected in duodenal ulcer patients, in whom atrophic gastritis should be absent. On the other hand, a Chinese group has demonstrated, with a randomized controlled trial, that there is a relative decrease in cancer incidence in patients with eradication therapy in the overall population, but this difference did not reach a level of significance[51]. Only in a subgroup without pre-cancerous lesions (without atrophy or intestinal metaplasia) they demonstrated the statistically reduced incidence of gastric cancer by eradication therapy. These results may be partially conflicting to each other and the reason for this is uncertain. However, we should pay attention to the difference in the clinical stage of gastric cancer detected and in the diagnostic ability of endoscopic examination

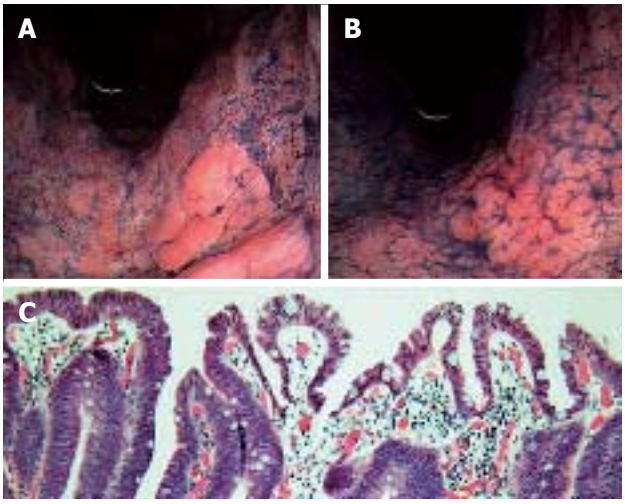


Figure 2 Dye-endoscopic features of gastric adenocarcinoma at pre- (**A**) and post-eradication therapy (**B**). Tumors became flattened and indistinct after eradication therapy. Histological features of gastric neoplasm at post-eradication are demonstrated in panel (**C**). Patient was a 67-year-old male.

between both trials. Whereas, in the Japanese study, most gastric cancer lesions were detected in the early stage as an intramucosal cancer, lesions found in Chinese study were those in a more advanced stage. We could not conclude that these two studies were designed with the same method because the endpoints may be different.

Recently, Kamada *et al*.[52] also prospectively followed up 1 787 patients with eradication therapy (median 4.5 years) and demonstrated that gastric cancer could be detected in 20 patients (1.1%). Most cancer lesions were detected in the early stage as intramucosal cancer and its histology was intestinal type dominant (75%). We also prospectively examined 101 patients with atrophic gastritis prospectively for more than 60 mo (mean 63.2 months) and found gastric cancer incidence in 8 patients[53]. Most gastric cancers were detected in the early stage and their histologies were of the intestinal type, and this is in complete agreement with previous studies. In addition, gastric cancer is more frequently found in elderly patients than in younger patients ($P < 0.05$). For the patients with atrophic gastritis, the age at the time of eradication therapy is an important factor for the occurrence of cancer after successful eradication. The long-term strict follow-ups after eradication seem to be necessary in elderly patients with atrophic gastritis.

Does alteration of the tumor appearance after eradication therapy modulate the incidence of cancer discovery?

As described above, the focus must be placed on the diagnostic ability of gastric cancer by endoscopic examination when we discuss the cancer discovery rate determined clinically. One of the difficulties of this field seems to be based on the methodology used to evaluate gastric carcinogenesis. Researchers can evaluate the degree of carcinogenesis only by the discovery rate of gastric cancer by endoscopic examination. Due emphasis must be placed on the differences in the diagnostic ability of each examination.

Moreover, endoscopic morphology might be influenced directly by eradication therapy, thus affecting the discovery rate of gastric cancer. Previously, Gotoda *et al*.[51] reported the endoscopic regression of gastric adenoma after successful eradication therapy. If the eradication itself influences the tumor morphology, this may affect the tumor discovery rate. Then, we investigated the morphological changes in the gastric neoplasm after *H pylori* eradication in Japanese patients. After a one-month follow-up, endoscopic re-evaluation revealed that one-third of the gastric tumor became indistinct and some tumors were difficult to discern with ordinary endoscopic observation[55]. All these altered lesions were of the superficial-elevated type, which is the characteristic appearance of intestinal type cancer, irrespective of the grade of histological appearance (adenoma or carcinoma) (Figure 2). The tumor appearance became flattened and the height of the lesions decreased, then the tumor became indistinct after eradication, even after a short time. In the depressed type cancer, that is typical for diffuse type gastric cancer, we could not find any morphological changes after eradication[55]. These results suggest that the morphology of the gastric neoplasm changes after eradication in the short-term, especially in the intestinal type gastric cancer, which were found characteristically in patients after eradication therapy. Even if the true incidence of cancer is not affected by eradication, the incidence of cancer discovery would be influenced by successful eradication therapy in cases of intestinal type cancer with elevated tumor features. Furthermore, we detected normal columnar epithelium over the neoplasm in some lesions (Figure 2). The appearance of normal foveolar epithelium must make it more difficult to detect gastric cancer by endoscopic observation. We could not explain the origin of this strange histological feature, but the most probable origin may be a regenerative change against the surface injury of the mucosal tumor tissue induced by the improved acid output after successful eradication. This must also contribute to the reduction in the rate of cancer discovery after successful eradication therapy and these overall alterations are quite rational for the explanation of the results reported by the Japanese researchers.

The mechanism of the antitumor promoting effect of *H pylori* eradication therapy is still unknown. In the *in vitro* studies, *H pylori* itself has been found to modify the expressions of several genes in gastric carcinoma cells[56]. Semino-Mora *et al*.[57] demonstrated the presence of *H pylori*-derived toxic proteins and mRNAs in gastric tumor cells *in vivo*. However, their theory is still controversial, and, until now, it has been believed that *H pylori* cannot exist in the gastric carcinoma cells. Thus, it is likely that *H pylori* indirectly influences tumor cell growth by regulating the inflammatory reaction around the tumor tissue. Several cytokines have been induced by *H pylori* infection and some of them may act as growth factors for tumor cells[25,58]. Suzuki *et al* [59] reported the decreased level of HGF in the gastric mucosa after eradication, which was linked to the decreased cell turnover. These indicate the importance of gastric inflammation in the gastric mucosa rather than *H pylori* itself on the luminal side. Gastrin is known to be an important gut-related hormone and a

growth factor for gastric cancer cells, and gastric tumor cells have been shown to contain its receptor[60]. However, it is unlikely that our new findings of the morphological changes were induced by a gastrin-related system[55].

There are two ways in which gastric tumors may grow; one is invasive downward growth and the other is expansive growth in the upward (luminal) direction. The latter may include the reactive (non-neoplastic) factor, which may be regulated by the gastric inflammation induced by *H pylori* infection. Kamada *et al*.[52] analyzed the macroscopic features of gastric cancers discovered after eradication therapy and demonstrated that most lesions are of the depressed type. If the eradication therapy mainly influences the expansive growth, the true biological behavior of the gastric malignancies may not be improved by eradication therapy.

Clinical manifestations of H pylori eradication therapy and what are the problems in the next step?

There is no doubt that eradication therapy diminishes the prevalence of cancer development. In the animal model with Mongolian gerbils, Shimizu *et al*.[61] demonstrated that *H pylori* eradication therapy diminishes the prevalence of gastric cancer incidence induced by *H pylori* infection and low-dose chemical carcinogen. Tatematsu *et al*.[62] also reported that *H pylori* eradication therapy regresses the heterotopic proliferative glands in the gastric mucosa of Mongolian gerbils, suggesting that the eradication reduces the promoting effect of the bacterium.

In human studies, we cannot discuss the anticarcinogenic effect of eradication therapy unless we follow up patients for a sufficiently long period (more than 10 years). As discussed above, it is very important that we distinguish the "development" of gastric cancer from the "discovery" of the tumor. In addition, it is clinically important to clarify whether eradication therapy can diminish the mortality rate for gastric cancer or not. It is very critical to know as to how we regress the biologically malignant gastric cancer, including diffuse-type cancer in younger patients. No reports have discussed this, and further study is necessary to answer this question.

Another problem to be solved is the "point of no return" of gastric carcinogenesis by eradication therapy. In the animal model, earlier eradication was demonstrated to be more effective for preventing the development of gastric cancer[63]. Since gastric cancer after eradication was frequently found in patients who received eradication in older age, we should consider that younger people should receive eradication therapy as early as possible. For the effective eradication for cancer prevention to be practical and economical, the selection of the population at higher risk should be clarified. It is especially necessary to identify the fundamental status of gastric inflammation in the development of diffuse type gastric cancer. Recent studies revealed that a high odds ratio for gastric cancer discovery was noticed in patients with nodular gastritis, which may be an important background for diffuse-type cancer[64,65]. This type of gastritis should be an important target for earlier eradication therapy to reduce cancer death. In addition, we should determine useful biomarkers for gastric inflammation, which would be beneficial for real clinical practice[66].

REFERENCES

1 **Goodman KJ**, Cockburn M. The role of epidemiology in understanding the health effects of Helicobacter pylori. *Epidemiology* 2001; **12**: 266-271
2 NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. *JAMA* 1994; **272**: 65-69
3 Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consensus Report. European Helicobacter Pylori Study Group. *Gut* 1997; **41**: 8-13
4 **Claeys D**, Faller G, Appelmelk BJ, Negrini R, Kirchner T. The gastric H+,K+-ATPase is a major autoantigen in chronic Helicobacter pylori gastritis with body mucosa atrophy. *Gastroenterology* 1998; **115**: 340-347
5 **Ito M**, Haruma K, Kaya S, Kamada T, Kim S, Sasaki A, Sumii M, Tanaka S, Yoshihara M, Chayama K. Role of anti-parietal cell antibody in Helicobacter pylori-associated atrophic gastritis: evaluation in a country of high prevalence of atrophic gastritis. *Scand J Gastroenterol* 2002; **37**: 287-293
6 **Haruma K**, Komoto K, Kawaguchi H, Okamoto S, Yoshihara M, Sumii K, Kajiyama G. Pernicious anemia and Helicobacter pylori infection in Japan: evaluation in a country with a high prevalence of infection. *Am J Gastroenterol* 1995; **90**: 1107-1110
7 **Kawaguchi H**, Haruma K, Komoto K, Yoshihara M, Sumii K, Kajiyama G. Helicobacter pylori infection is the major risk factor for atrophic gastritis. *Am J Gastroenterol* 1996; **91**: 959-962
8 **Correa P**. Helicobacter pylori infection and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 238s-241s
9 **Correa P**. Helicobacter pylori and gastric carcinogenesis. *Am J Surg Pathol* 1995; **19 Suppl 1**: S37-S43
10 **Nomura A**, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325**: 1132-1136
11 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131
12 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 1-241
13 **Huang JQ**, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between Helicobacter pylori seropositivity and gastric cancer. *Gastroenterology* 1998; **114**: 1169-1179
14 **Komoto K**, Haruma K, Kamada T, Tanaka S, Yoshihara M, Sumii K, Kajiyama G, Talley NJ. Helicobacter pylori infection and gastric neoplasia: correlations with histological gastritis and tumor histology. *Am J Gastroenterol* 1998; **93**: 1271-1276
15 **Haruma K**, Komoto K, Kamada T, Ito M, Kitadai Y, Yoshihara M, Sumii K, Kajiyama G. Helicobacter pylori infection is a major risk factor for gastric carcinoma in young patients. *Scand J Gastroenterol* 2000; **35**: 255-259
16 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789
17 **Sugiyama A**, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M. Helicobacter pylori infection enhances N-methyl-N-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. *Cancer Res* 1998; **58**: 2067-2069
18 **Rachmilewitz D**, Karmeli F, Eliakim R, Stalnikowicz R, Ackerman Z, Amir G, Stamler JS. Enhanced gastric nitric oxide synthase activity in duodenal ulcer patients. *Gut* 1994; **35**: 1394-1397
19 **Goto T**, Haruma K, Kitadai Y, Ito M, Yoshihara M, Sumii K, Hayakawa N, Kajiyama G. Enhanced expression of inducible nitric oxide synthase and nitrotyrosine in gastric mucosa of gastric cancer patients. *Clin Cancer Res* 1999; **5**: 1411-1415
20 **Kai H**, Ito M, Kitadai Y, Tanaka S, Haruma K, Chayama K.

Chronic gastritis with expression of inducible nitric oxide synthase is associated with high expression of interleukin-6 and hypergastrinaemia. *Aliment Pharmacol Ther* 2004; **19**: 1309-1314

21 **Yoshihara M**, Haruma K, Sumii K, Watanabe C, Kiyohira K, Kawaguchi H, Tanaka S, Kajiyama G. The relationship between gastric secretion and type of early gastric carcinoma. *Hiroshima J Med Sci* 1995; **44**: 79-82

22 **Reed PI**, Smith PL, Haines K, House FR, Walters CL. Gastric juice N-nitrosamines in health and gastroduodenal disease. *Lancet* 1981; **2**: 550-552

23 **O'Connor HJ**, Schorah CJ, Habibzedah N, Axon AT, Cockel R. Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease, and possible sources. *Gut* 1989; **30**: 436-442

24 **Kodama K**, Sumii K, Kawano M, Kido T, Nojima K, Sumii M, Haruma K, Yoshihara M, Chayama K. Gastric juice nitrite and vitamin C in patients with gastric cancer and atrophic gastritis: is low acidity solely responsible for cancer risk? *Eur J Gastroenterol Hepatol* 2003; **15**: 987-993

25 **Yasunaga Y**, Shinomura Y, Kanayama S, Higashimoto Y, Yabu M, Miyazaki Y, Kondo S, Murayama Y, Nishibayashi H, Kitamura S, Matsuzawa Y. Increased production of interleukin 1 beta and hepatocyte growth factor may contribute to foveolar hyperplasia in enlarged fold gastritis. *Gut* 1996; **39**: 787-794

26 **Churin Y**, Al-Ghoul L, Kepp O, Meyer TF, Birchmeier W, Naumann M. Helicobacter pylori CagA protein targets the c-Met receptor and enhances the motogenic response. *J Cell Biol* 2003; **161**: 249-255

27 **Martin JH**, Potthoff A, Ledig S, Cornberg M, Jandl O, Manns MP, Kubicka S, Flemming P, Athmann C, Beil W, Wagner S. Effect of H. pylori on the expression of TRAIL, FasL and their receptor subtypes in human gastric epithelial cells and their role in apoptosis. *Helicobacter* 2004; **9**: 371-386

28 **Nagasako T**, Sugiyama T, Mizushima T, Miura Y, Kato M, Asaka M. Up-regulated Smad5 mediates apoptosis of gastric epithelial cells induced by Helicobacter pylori infection. *J Biol Chem* 2003; **278**: 4821-4825

29 **Fujikawa A**, Shirasaka D, Yamamoto S, Ota H, Yahiro K, Fukada M, Shintani T, Wada A, Aoyama N, Hirayama T, Fukamachi H, Noda M. Mice deficient in protein tyrosine phosphatase receptor type Z are resistant to gastric ulcer induction by VacA of Helicobacter pylori. *Nat Genet* 2003; **33**: 375-381

30 **Huang JQ**, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003; **125**: 1636-1644

31 **Asahi M**, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C. Helicobacter pylori CagA protein can be tyrosine phosphorylated in gastric epithelial cells. *J Exp Med* 2000; **191**: 593-602

32 **Higashi H**, Yokoyama K, Fujii Y, Ren S, Yuasa H, Saadat I, Murata-Kamiya N, Azuma T, Hatakeyama M. EPIYA motif is a membrane-targeting signal of Helicobacter pylori virulence factor CagA in mammalian cells. *J Biol Chem* 2005; **280**: 23130-23137

33 **Higashi H**, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. *Science* 2002; **295**: 683-686

34 **Higashi H**, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci U S A* 2002; **99**: 14428-14433

35 **Mimuro H**, Suzuki T, Tanaka J, Asahi M, Haas R, Sasakawa C. Grb2 is a key mediator of helicobacter pylori CagA protein activities. *Mol Cell* 2002; **10**: 745-755

36 **Nozaki K**, Shimizu N, Inada K, Tsukamoto T, Inoue M, Kumagai T, Sugiyama A, Mizoshita T, Kaminishi M, Tatematsu M. Synergistic promoting effects of Helicobacter pylori infection and high-salt diet on gastric carcinogenesis in Mongolian gerbils. *Jpn J Cancer Res* 2002; **93**: 1083-1089

37 **Haruma K**, Suzuki T, Tsuda T, Yoshihara M, Sumii K, Kajiyama G. Evaluation of tumor growth rate in patients with early gastric carcinoma of the elevated type. *Gastrointest Radiol* 1991; **16**: 289-292

38 **Tahara E**. Genetic pathways of two types of gastric cancer. *IARC Sci Publ* 2004; : 327-349

39 **Annibale B**, Aprile MR, D'ambra G, Caruana P, Bordi C, Delle Fave G. Cure of Helicobacter pylori infection in atrophic body gastritis patients does not improve mucosal atrophy but reduces hypergastrinemia and its related effects on body ECL-cell hyperplasia. *Aliment Pharmacol Ther* 2000; **14**: 625-634

40 **Sung JJ**, Lin SR, Ching JY, Zhou LY, To KF, Wang RT, Leung WK, Ng EK, Lau JY, Lee YT, Yeung CK, Chao W, Chung SC. Atrophy and intestinal metaplasia one year after cure of H. pylori infection: a prospective, randomized study. *Gastroenterology* 2000; **119**: 7-14

41 **Ohkusa T**, Fujiki K, Takashimizu I, Kumagai J, Tanizawa T, Eishi Y, Yokoyama T, Watanabe M. Improvement in atrophic gastritis and intestinal metaplasia in patients in whom Helicobacter pylori was eradicated. *Ann Intern Med* 2001; **134**: 380-386

42 **Haruma K**, Mihara M, Okamoto E, Kusunoki H, Hananoki M, Tanaka S, Yoshihara M, Sumii K, Kajiyama G. Eradication of Helicobacter pylori increases gastric acidity in patients with atrophic gastritis of the corpus-evaluation of 24-h pH monitoring. *Aliment Pharmacol Ther* 1999; **13**: 155-162

43 **Ito M**, Haruma K, Kamada T, Mihara M, Kim S, Kitadai Y, Sumii M, Tanaka S, Yoshihara M, Chayama K. Helicobacter pylori eradication therapy improves atrophic gastritis and intestinal metaplasia: a 5-year prospective study of patients with atrophic gastritis. *Aliment Pharmacol Ther* 2002; **16**: 1449-1456

44 **Sugiyama T**, Sakaki N, Kozawa H, Sato R, Fujioka T, Satoh K, Sugano K, Sekine H, Takagi A, Ajioka Y, Takizawa T. Sensitivity of biopsy site in evaluating regression of gastric atrophy after Helicobacter pylori eradication treatment. *Aliment Pharmacol Ther* 2002; **16 Suppl 2**: 187-190

45 **Satoh K**, Kimura K, Takimoto T, Kihira K. A follow-up study of atrophic gastritis and intestinal metaplasia after eradication of Helicobacter pylori. *Helicobacter* 1998; **3**: 236-240

46 **Correa P**, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, Realpe JL, Malcom GT, Li D, Johnson WD, Mera R. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-helicobacter pylori therapy. *J Natl Cancer Inst* 2000; **92**: 1881-1888

47 **Leung WK**, Lin SR, Ching JY, To KF, Ng EK, Chan FK, Lau JY, Sung JJ. Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. *Gut* 2004; **53**: 1244-1249

48 **Uemura N**, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G. Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 639-642

49 **Sasaki A**, Kitadai Y, Ito M, Sumii M, Tanaka S, Yoshihara M, Haruma K, Chayama K. Helicobacter pylori infection influences tumor growth of human gastric carcinomas. *Scand J Gastroenterol* 2003; **38**: 153-158

50 **Take S**, Mizuno M, Ishiki K, Nagahara Y, Yoshida T, Yokota K, Oguma K, Okada H, Shiratori Y. The effect of eradicating helicobacter pylori on the development of gastric cancer in patients with peptic ulcer disease. *Am J Gastroenterol* 2005; **100**: 1037-1042

51 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194

52 **Kamada T**, Hata J, Sugiu K, Kusunoki H, Ito M, Tanaka S, Inoue K, Kawamura Y, Chayama K, Haruma K. Clinical features of gastric cancer discovered after successful

eradication of Helicobacter pylori: results from a 9-year prospective follow-up study in Japan. *Aliment Pharmacol Ther* 2005; **21**: 1121-1126

53 **Takata S**, Ito M, Tanaka S, Oka S, Ueda H, Egi Y, Kai H, Sasaki A, Masuda H, Kim S, Ueno Y, Kitadai Y, Kamada T, haruma K, Chayama K. Development of gastric cancer after successful eradication therapy of Helicobacter pylori: By long-term prospective study. *Gastroenterology* 2004; **126**: 182A

54 **Gotoda T**, Saito D, Kondo H, Ono H, Oda I, Fujishiro M, Yamaguchi H. Endoscopic and histological reversibility of gastric adenoma after eradication of Helicobacter pylori. *J Gastroenterol* 1999; **34 Suppl 11**: 91-96

55 **Ito M**, Tanaka S, Takata S, Oka S, Imagawa S, Ueda H, Egi Y, Kitadai Y, Yasui W, Yoshihara M, Haruma K, Chayama K. Morphological changes in human gastric tumours after eradication therapy of Helicobacter pylori in a short-term follow-up. *Aliment Pharmacol Ther* 2005; **21**: 559-566

56 **Kitadai Y**, Sasaki A, Ito M, Tanaka S, Oue N, Yasui W, Aihara M, Imagawa K, Haruma K, Chayama K. Helicobacter pylori infection influences expression of genes related to angiogenesis and invasion in human gastric carcinoma cells. *Biochem Biophys Res Commun* 2003; **311**: 809-814

57 **Semino-Mora C**, Doi SQ, Marty A, Simko V, Carlstedt I, Dubois A. Intracellular and interstitial expression of Helicobacter pylori virulence genes in gastric precancerous intestinal metaplasia and adenocarcinoma. *J Infect Dis* 2003; **187**: 1165-1177

58 **Yamaoka Y**, Kita M, Kodama T, Sawai N, Imanishi J. Helicobacter pylori cagA gene and expression of cytokine messenger RNA in gastric mucosa. *Gastroenterology* 1996; **110**: 1744-1752

59 **Suzuki M**, Suzuki H, Masaoka T, Tanaka S, Suzuki K, Ishii H. Helicobacter pylori eradication treatment modulates epithelial cell proliferation and tissue content of hepatocyte growth factor in the gastric mucosa. *Aliment Pharmacol Ther* 2004; **20 Suppl 1**: 158-164

60 **Ochiai A**, Yasui W, Tahara E. Growth-promoting effect of gastrin on human gastric carcinoma cell line TMK-1. *Jpn J Cancer Res* 1985; **76**: 1064-1071

61 **Shimizu N**, Ikehara Y, Inada K, Nakanishi H, Tsukamoto T, Nozaki K, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M. Eradication diminishes enhancing effects of Helicobacter pylori infection on glandular stomach carcinogenesis in Mongolian gerbils. *Cancer Res* 2000; **60**: 1512-1514

62 **Tatematsu M**, Tsukamoto T, Mizoshita T. Role of Helicobacter pylori in gastric carcinogenesis: the origin of gastric cancers and heterotopic proliferative glands in Mongolian gerbils. *Helicobacter* 2005; **10**: 97-106

63 **Nozaki K**, Shimizu N, Ikehara Y, Inoue M, Tsukamoto T, Inada K, Tanaka H, Kumagai T, Kaminishi M, Tatematsu M. Effect of early eradication on Helicobacter pylori-related gastric carcinogenesis in Mongolian gerbils. *Cancer Sci* 2003; **94**: 235-239

64 **Miyamoto M**, Haruma K, Yoshihara M, Sumioka M, Nishisaka T, Tanaka S, Inoue K, Chayama K. Five cases of nodular gastritis and gastric cancer: a possible association between nodular gastritis and gastric cancer. *Dig Liver Dis* 2002; **34**: 819-820

65 **Kamada T**, Miyamoto M, Ito M, Sugiu K, Kusunoki H, Hata J, Nagashima Y, KIDo S, Hamada H, Haruma K. Nodular gastritis is a risk factor for diffuse-type of gastric carcinoma in Japanese young patients. *Gastroenterology* 2004; **126**: A456

66 **Naito Y**, Ito M, Watanabe T, Suzuki H. Biomarkers in patients with gastric inflammation: a systematic review. *Digestion* 2005; **72**: 164-180

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 17-20
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Incidence and mortality of gastric cancer in China

Ling Yang

Ling Yang, National Office for Cancer Prevention and Control, Beijing 100021, China
Correspondence to: Dr. Ling Yang, National Office for Cancer Prevention and Control, 17 Panjiayuan Nanli, Chaoyang District, Beijing 100021, China. yangling71@yahoo.com
Telephone: +86-10-67767652 Fax: +86-10-67718227
Received: 2005-06-07 Accepted: 2005-07-22

Abstract

Gastric cancer is one of the most frequent cancers in the world; almost two-thirds of gastric cancer cases and deaths occur in less developed regions. In China, based on two national mortality surveys conducted in 1970s and 1990s, there is an obvious clustering of geographical distribution of gastric cancer in the country, with the high mortality being mostly located in rural areas, especially in Gansu, Henan, Hebei, Shanxi and Shaanxi Provinces in the middle-western part of China. Despite a slight increase from the 1970s to early 1990s, remarkable declines in gastric cancer mortality were noticed in almost the entire population during the last decade in China. These declines were largely due to the dramatic improvements in the social-economic environment, lifestyle, nutrition, education and health care system after economic reforms started two decades ago. Nevertheless, gastric cancer will remain a significant cancer burden currently and be one of the key issues in cancer prevention and control strategy in China. It was predicted that, in 2005, 0.3 million deaths and 0.4 million new cases from gastric cancer would rank the third most common cancer. The essential package of the prevention and control strategy for gastric cancer in China would focus on controlling *Helicobacter pylori* (*H pylori*) infection, improving educational levels, advocating healthy diet and anti-tobacco campaign, searching for cost-effective early detection, diagnosis and treatment programs including approaches for curable management and palliative care.

Key words: Gastric cancer; Incidence; Mortality

Yang L. Incidence and mortality of gastric cancer in China. *World J Gastroenterol*; 2006 12(1): 17-20

http://www.wjgnet.com/1007-9327/12/17.asp

GASTRIC CANCER IN THE WORLD

Gastric cancer is one of the most frequent cancers in the world, in both men and women. In the year 2002, the age standardized incidence rate was 22.0 per 100 000 in men, and 10.4 per 100 000 in women, and mortality rate was 16.3 per 100 000 in men, and 7.9 per 100 000 in women, according to the latest global estimation-GLOBOCAN 2002[1]. Despite a marked decrease, especially in mortality rate in many countries, the absolute number of gastric cancer cases and deaths is still a big burden of the local health program, since the world population and life expectancy are increasing. In 2002, it was estimated that there were 0.9 million new gastric cancer cases (0.60 million in men and 0.33 million in women) and 0.7 million deaths (0.45 million in men and 0.25 in women) from gastric cancer in the world. In men, gastric cancer ranks the third among the commonest cancers in incidence rate (after cancers of lung and prostate) and the second in mortality rate (after lung cancer), while in women, it is the fifth most common cancer in incidence and the fourth in mortality (after cancers of breast, cervix, lung and/or colon-rectum)[1].

In terms of geographic distribution, almost two-thirds of gastric cancer cases and deaths occur in less developed regions. High rates apply to Japan, China, Korea, Central and South America, Eastern Europe, and parts of the Middle East, and low rates to North America, Australia and New Zealand, Northern Europe, and India[1,2]. Five-year relative survivals of around 20% or less are frequently reported. The incidence ratios of men to women generally range between 1.5 and 2.5, with higher ratios for intestinal than diffuse cancers and in high-risk populations[2].

NATIONAL GASTRIC CANCER MORTALITY PATTERN IN THE 1970s AND 1990s

In China, two national mortality surveys were conducted in 1973-1975 and 1990-1992, respectively, both organized by the National Office for Cancer Prevention of Control, the Ministry of Public Health, China. These two surveys showed the detail pattern and distribution of mortality rates for cancers in China at that time[3-7]. The first mortality survey was a nation-wide study. An atlas was published based on the survey results[4], providing a visual geographic distribution of gastric cancer profile in early 1970s. A map of the gastric cancer mortality distribution for males is shown in (Figure 1) as an example. An obvious clustering of geographical distribution of gastric cancer appears in China, with the high mortality

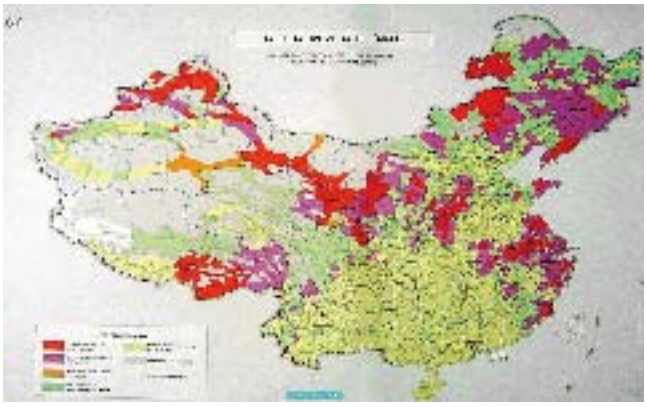


Figure 1 Mortality rates of gastric cancer in men in China, 1973-1975[4].

Table 1 The first 20 areas with high mortality rate for gastric cancer in China - according to the second national mortality survey in 1990-1992 (rate per 100 000)

Area	Rate	Area	Rate
Wuwei town, Gansu	117.78	Jiyuan town, Henan	66.59
Yangcheng county, Shanxi	104.01	Zhangye town, Gansu	64.43
Pingshun county, Shanxi	96.95	Jiaxian county, Shaanxi	64.36
Changle county, Fujian	93.38	Tianchang county, Anhui	63.04
Shexian county, Hebei	85.64	Tianzhu Tibet Autho county, Gansu	59.18
Lujiang county, Anhui	84.46	Putian county, Fujian	58.83
Zanghuang county, Hebei	77.67	Linxian county, Henan	58.56
Neixiang county, Henan	77.45	Wudu county, Gansu	58.54
Yuanqu county, Shanxi	74.22	Tianjian Dist., Fuzhou city, Fujian	57.52
Linze county, Gansu	66.69	Xianju county, Zhejiang	53.99

Table 2 Changes in the age-standardized mortality rates of gastric cancer between 1973 and 1975 and between 1990 and1992 in China (rate per 100 000)

Area	Year 1973-1975			Year 1990-1992			Changes (%)		
	Men	Women	Total	Men	Women	Total	Men	Women	Total
Whole country	27.1	13.0	19.8	30.1	13.8	21.8	11.0	6.3	10.0
Urban	27.3	13.3	20.1	21.2	9.8	15.3	-22.2	-26.7	-23.8
Rural	26.7	12.6	19.4	33.7	15.4	24.4	26.4	22.1	25.8

being mostly located in the north (Liaodong Peninsula, Shandong Peninsula, Yangtze River Delta and middle-western provinces along Taihang Mountain and 'Hexi Zoulang").

The second mortality survey covered 10% of the whole population that proved as a representative sample to reflect national cancer profile[5-7]. The crude mortality rate of gastric cancer in China was 25.2 per 100 000 (32.8 per 100 000 in men and 17.0 per 100 000 in women), which accounted for 23.2% of the total cancer deaths in 1990-1992, making gastric cancer the first leading cause of cancer death[8]. The geographic variety in the distribution was similar to that in the first survey in the 1970s. Table 1 shows the first 20 areas with high mortality rates for the gastric cancer (rates adjusted by the 1982 national census population). Most of these high-risk areas are located in rural areas, especially in Gansu, Henan, Hebei, Shanxi and Shaanxi Provinces in the middle-western part of China.

By comparing the above two national mortality surveys, an increasing trend for the age-standardized mortality rates of gastric cancer was shown in the entire population in China during the two decades. Nevertheless, the increase only appeared in rural areas, while a decreasing trend was noticed in urban population[9,10] (Table 2). In terms of age-specific mortality rates, a decreasing trend was noticed among almost all age groups (except age group 70-79 years) in urban residents, while an increasing trend was seen among most of age groups (except age group 30-44 years) in rural areas[10]. Nevertheless, with almost two-thirds of the population reside in rural areas, gastric cancer was still the first most common cancer in the entire population in China during 1970s and early 1990s, although it dropped to the third in urban areas.

In addition to the above two national mortality surveys, there were a few publications on gastric cancer mortality trends at local levels, either in big cities such as Beijing, Shanghai, or in high-risk areas such as in Henan and Hebei Provinces[11-14]. Similarly, the mortality rates of gastric cancer remain high although there was a slight decreasing trend during different time periods among those areas. Therefore, gastric cancer is still the major common cancer and a big burden for local health resources and facilities.

GASTRIC CANCER INCIDENCE AND MORTALITY PATTERNS AT THE NATIONAL LEVEL IN RECENT YEARS

Based on a routine mortality reporting system, covering 10% of the Chinese population, from the Ministry of Public Health which was submitted to the World Health Organization[15], the gastric cancer mortality trends in China were analyzed from 1987 to 1999, using the joinpoint model. The trends were further combined with the ratio of incidence to mortality from data in seven cancer registries in China that were published in the 8th edition of Cancer Incidence in Five Continents[16], to estimate and project the mortality and incidence for gastric cancer in 2000 and 2005 (by site, age, sex and area) at the national level, using the log-linear regression model with Poisson distribution assumption. These results have been serially published recently[17-19].

According to the data from the Center of Health Information Statistics (CHIS) under the Ministry of Public Health, gastric cancer mortality rates were higher in rural than urban areas, and in men than in women. During 1987-1999, slight but significant declines in mortality were noticed in almost the entire subpopulation (except in urban men before 1991) and for most age groups (except age group 15-34) in rural areas (Figures 2 and 3). In 2000, 0.3 million deaths and 0.4 million new cases were estimated for gastric cancer, similar to what was projected in 2005, while the latter had a slight decrease in men but increase in women (Table 3). In 2005, in terms of incidence rates, gastric cancer would rank the third among the most common cancers in China (after cancers of lung

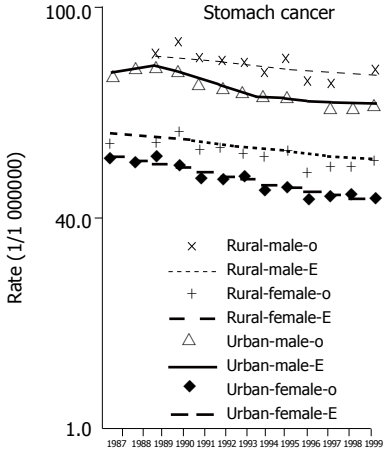


Figure 2 Time trends for age-standardized mortality rates (both observed and expected by model) of gastric cancer during 1987-1999 in China, by areas (rural and urban) and sex (per 100 000)

Table 3 Gastric cancer incidence and mortality in China in 2000 and 2005

		Men		Women	
		Year 2000	Year 2005	Year 2000	Year 2005
Mortality (per 100 000)					
rates	Age 45-54	45.5	43.3	20.5	19.0
	Age 55-64	109.4	89.4	46.6	40.1
	Age 65-74	229.4	198.3	104.6	91.3
	Age 75+	289.6	261.9	133.3	116.3
	ASR[1]	32.7	28.8	15.0	13.3
Number of deaths		200 518	197 222	95 942	97 843
Incidence (per 100 000)					
rates	Age 45-54	70	66.7	31.6	29.3
	Age 55-64	145.7	119	62.1	53.4
	Age 65-74	264.3	228.5	120.5	105.2
	Age 75+	288.8	261.2	133	116
	ASR[1]	41.9	37.1	19.5	17.4
Number of cases		256 256	253 110	121 485	123 883

[1]ASR: Age-standardized rate (adjusted by the world standard population).

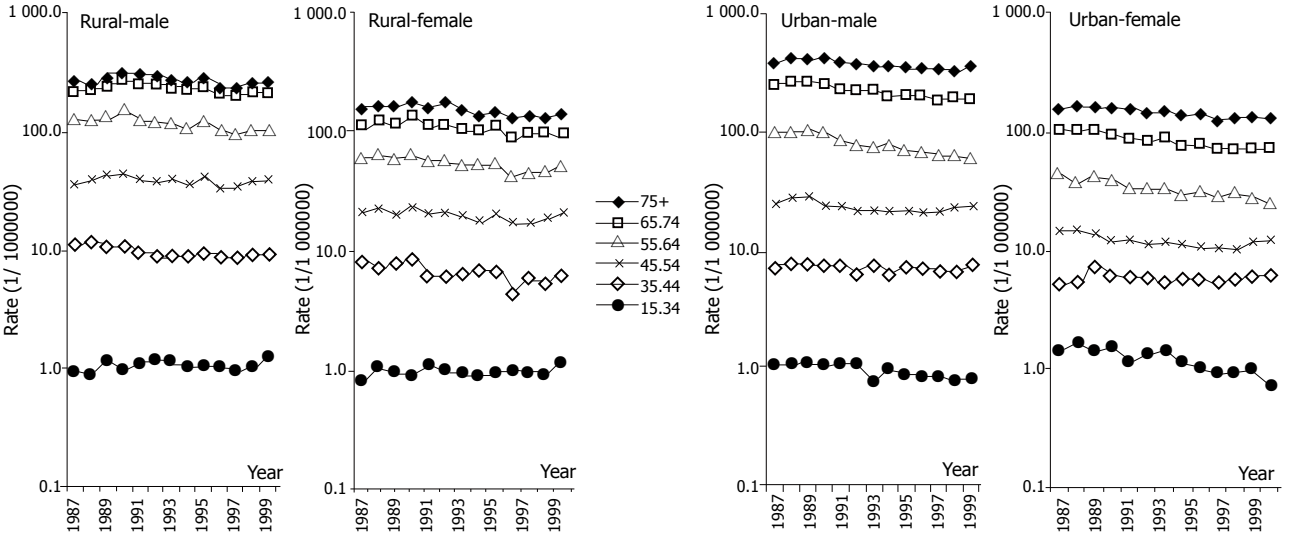


Figure 3 Time trends for age-specific mortality rates of gastric cancer during 1987-1999 in China, by areas (rural and urban) and sex, in CHIS data (rates per 100 000).

and liver in men and after cancers of breast and lung in women)[19].

FACTORS INFLUENCING THE GASTRIC CANCER PATTERN IN CHINA

Despite a slight increase from 1970s to early 1990s, remarkable declines in gastric cancer mortality were shown during the last decade in China. These declines were largely due to the dramatic improvements in the social-economic environment, lifestyle, nutrition, education and health care system after economic reforms started two decades ago. These include better socio-economic circumstances, higher educational levels, better refrigeration, reduced consumption of salted, smoked, and chemically preserved foods, eating more fruit and vegetables and remarkably improved sanitary conditions of the house and living standards, supplement intake nutrients such as vitamin C, vitamin E, beta-carotene, selenium and decreased intake of nitrosamine, which was strongly suspected as a major risk factor for gastric cancer[20-31]. *H pylori* infection, which is defined by the World Health Organization as a definite gastric carcinogen, is linked to crowded living conditions, family size, sharing a bedroom, low socio-economic status, low educational level and poor sanitation, and infrequent hand washing before meals. The prevalence of *H pylori* infection has reduced dramatically due to the improved socio-economic status and lifestyle changes during last decades, especially among urban areas. In addition, widespread prescription of antibiotics may be responsible for reducing *H pylori* infection. Finally, some locally popular customs, such as drinking green tea, consuming tofu, and ginger have been suggested to have a possible protective effect on gastric cancer[32-35].

However, huge demographic changes in China make the total number of incident cases and deaths from gastric cancer slightly declined in men between 2000 and 2005, while the number increased in women, despite the significant declining trends in rates among all age groups (Table 2)[17-19].

CONCLUSION

It is estimated that gastric cancer currently ranks the third among most common cancers, and will remain a significant cancer burden in China during the next decade. It will be, undoubtedly, one of the keys in cancer prevention and control strategy in China. The essential package would focus on controlling *H pylori* infection, improving educational levels, advocating healthy diet and anti-tobacco campaign, searching for cost-effective early detection, diagnosis and treatment programs including approaches for curable management and palliative care[36].

REFERENCES

1 **Ferlay J**, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide. Lyon: IARC Press, Cited 2005-04-15; Available from: URL: http: //www-dep.iarc.fr/

2 **Roder DM**. The epidemiology of gastric cancer. *Gastric Cancer* 2002; **5 Suppl 1**: 5-11

3 **National Office for Cancer Prevention and Control of the Chinese Ministry of Health (NOCPC)**. Investigate for malignant tumor mortality in China. Beijing: People's Health Publishing House, 1979: 175-201

4 **Editorial Committee for the Atlas of Cancer Mortality**. Atlas of cancer mortality in the People's Republic of China. Beijing: China Map Press, 1979: 23-30

5 **Li L**, Lu F, Zhang S. [Analysis of cancer modality and distribution in China from year 1990 through 1992--an epidemiologic study]. *Zhonghua Zhongliu Zazhi* 1996; **18**: 403-407

6 **Li L**, Zhang S, Lu F. [Research on characteristics of mortality spectrum and type composition of malignant tumors in China]. *Zhonghua Zhongliu Zazhi* 1997; **19**: 323-328

7 **Zhou YS,** Zhang SW, Li LD. Analysis for death for all and malignant tumors for Chinese population. *Bull Chin Cancer* 1997; **6**: 9-11

8 **Sun X**, Mu R, Zhou Y, Dai X, Qiao Y, Zhang S, Huangfu X, Sun J, Li L, Lu F. [1990-1992 mortality of stomach cancer in China]. *Zhonghua Zhongliu Zazhi* 2002; **24**: 4-8

9 **Li L**, Lu F, Zhang S. [Analyses of variation trend and short-term detection of Chinese malignant tumor mortality during twenty years]. *Zhonghua Zhongliu Zazhi* 1997; **19**: 3-9

10 **Sun XD**, Mu R, Zhou YS, Dai XD, Zhang SW, Huangfu XM, Sun J, Li LD, Lu FZ, Qiao YL. [Analysis of mortality rate of stomach cancer and its trend in twenty years in China]. *Zhonghua Zhongliu Zazhi* 2004; **26**: 4-9

11 **Wang QJ**, Zhu WX, Yuan GL. [Trend and prediction of cancer mortality in Beijing, China, during 1980-2001]. *Zhonghua Liuxingbingxue Zazhi* 1995; **16**: 195-198

12 **Gao YT**, Tu JT, Jin F, Gao RN. Cancer mortality in Shanghai during the period 1963-77. *Br J Cancer* 1981; **43**: 183-195

13 **Lu JB**, Sun XB, Dai DX, Zhu SK, Chang QL, Liu SZ, Duan WJ. Epidemiology of gastroenterologic cancer in Henan Province, China. *World J Gastroenterol* 2003; **9**: 2400-2403

14 **Li M**, Li ZS, Chen ZF, He YT, Zhao BS, Li ZY, Li YW, Hou J. Mortality report of malignant tumors in SheXian, Hebei Province, China, from the 1970's to the present. *Asian Pac J Cancer Prev* 2004; **5**: 414-418

15 **World Health Organization (WHO).** WHO Mortality Database as is WHO Statistical Information System (WHOSIS), cited 2005-04-15; Available from: URL: http: //www-depdb.iarc.fr/ and http: //www.who.int/whosis/

16 **Parkin DM,** Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer incidence in five continents Vol VIII. Lyon: IARC Press, 2002: 212-232

17 **Yang L**, Parkin DM, Li L, Chen Y. Time trends in cancer mortality in China: 1987-1999. *Int J Cancer* 2003; **106**: 771-783

18 **Yang L**, Parkin DM, Li LD, Chen YD, Bray F. Estimation and projection of the national profile of cancer mortality in China: 1991-2005. *Br J Cancer* 2004; **90**: 2157-2166

19 **Yang L**, Parkin DM, Ferlay J, Li L, Chen Y. Estimates of cancer incidence in China for 2000 and projections for 2005. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 243-250

20 **Kneller RW**, Guo WD, Hsing AW, Chen JS, Blot WJ, Li JY, Forman D, Fraumeni JF. Risk factors for stomach cancer in sixty-five Chinese counties. *Cancer Epidemiol Biomarkers Prev* 1992; **1**: 113-118

21 **Ma JL**, You WC, Gail MH, Zhang L, Blot WJ, Chang YS, Jiang J, Liu WD, Hu YR, Brown LM, Xu GW, Fraumeni JF. Helicobacter pylori infection and mode of transmission in a population at high risk of stomach cancer. *Int J Epidemiol* 1998; **27**: 570-573

22 **You WC**, Zhang L, Gail MH, Ma JL, Chang YS, Blot WJ, Li JY, Zhao CL, Liu WD, Li HQ, Hu YR, Bravo JC, Correa P, Xu GW, Fraumeni JF. Helicobacter pylori infection, garlic intake and precancerous lesions in a Chinese population at low risk of gastric cancer. *Int J Epidemiol* 1998; **27**: 941-944

23 **Yuan JM**, Yu MC, Xu WW, Cockburn M, Gao YT, Ross RK. Helicobacter pylori infection and risk of gastric cancer in Shanghai, China: updated results based upon a locally developed and validated assay and further follow-up of the cohort. *Cancer Epidemiol Biomarkers Prev* 1999; **8**: 621-624

24 **Muñoz N**. Is Helicobacter pylori a cause of gastric cancer? An appraisal of the seroepidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 445-451

25 **Limburg P**, Qiao Y, Mark S, Wang G, Perez-Perez G, Blaser M, Wu Y, Zou X, Dong Z, Taylor P, Dawsey S. Helicobacter pylori seropositivity and subsite-specific gastric cancer risks in Linxian, China. *J Natl Cancer Inst* 2001; **93**: 226-233

26 **Yang CS**. Vitamin nutrition and gastroesophageal cancer. *J Nutr* 2000; **130**: 338S-339S

27 **Guo W**, Blot WJ, Li JY, Taylor PR, Liu BQ, Wang W, Wu YP, Zheng W, Dawsey SM, Li B. A nested case-control study of oesophageal and stomach cancers in the Linxian nutrition intervention trial. *Int J Epidemiol* 1994; **23**: 444-450

28 **Ji BT**, Chow WH, Yang G, McLaughlin JK, Zheng W, Shu XO, Jin F, Gao RN, Gao YT, Fraumeni JF. Dietary habits and stomach cancer in Shanghai, China. *Int J Cancer* 1998; **76**: 659-664

29 **Blot WJ**, Li JY, Taylor PR, Guo W, Dawsey SM, Li B. The Linxian trials: mortality rates by vitamin-mineral intervention group. *Am J Clin Nutr* 1995; **62**: 1424S-1426S

30 **International Agency for Research on Cancer (IARC).** IARC Monograph Vol. 83. Tobacco smoke and involuntary smoking. Lyon: IARC Press, Cited 2005-04-15; Available from: URL: http: //www-cie.iarc.fr/htdocs/monographs/vol83/01-smoking.html

31 **Ji BT**, Chow WH, Yang G, McLaughlin JK, Gao RN, Zheng W, Shu XO, Jin F, Fraumeni JF, Gao YT. Body mass index and the risk of cancers of the gastric cardia and distal stomach in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 481-485

32 **Setiawan VW**, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC. Protective effect of green tea on the risks of chronic gastritis and stomach cancer. *Int J Cancer* 2001; **92**: 600-604

33 **Ji BT**, Chow WH, Yang G, McLaughlin JK, Gao RN, Zheng W, Shu XO, Jin F, Fraumeni JF, Gao YT. The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. *Cancer* 1996; **77**: 2449-2457

34 **Wu AH**, Yang D, Pike MC. A meta-analysis of soyfoods and risk of stomach cancer: the problem of potential confounders. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 1051-1058

35 **Mahady GB**, Pendland SL, Yun GS, Lu ZZ, Stoia A. Ginger (Zingiber officinale Roscoe) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. *Anticancer Res* 2003; **23**: 3699-3702

36 **World Health Organization (WHO).** National cancer control programmes: policies and managerial guidelines. Second version. Geneva: World Health Organization, 2002

S- Editor Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 21-28
World Journal of Gastroenterology ISSN 1007-9327

Berberine induces cell cycle arrest and apoptosis in human gastric carcinoma SNU-5 cell line

Jing-Pin Lin, Jai-Sing Yang, Jau-Hong Lee, Wen-Tsong Hsieh, Jing-Gung Chung

Jing-Pin Lin, School of Chinese Medicine, China Medical University, Taichung City 404, Taiwan, China
Jai-Sing Yang, Department of Medical Technology, Yuan-Pei University of Science and Technology, Hsinchu City, 300, Taiwan, China
Jau-Hong Lee, Department of Surgery, China Medical University Hospital, Taichung City, 404, Taiwan, China
Wen-Tsong Hsieh, Department of Pharmacology, China Medical University, Taichung City 404, Taiwan, China
Jing-Gung Chung, Department of Microbiology, School of Biological Science and Technology, China Medical University, Taichung City 404, Taiwan, China
Supported by The Grant CMU92-CM-02 from China Medical University
Co-first-authors: Jing-Pin Lin and Jai-Sing Yang
Co-correspondent: Jing-Pin Lin
Correspondence to: Dr Jing-Gung Chung, Department of Microbiology, School of Biological Science and Technology, China Medical University, 91 Hsueh-Shih Road, Taichung 404, Taiwan. China. jgchung@mail.cmu.edu.tw
Telephone: +886-4-2205-3366 Fax: +886-4-2205-3764
Received: 2005-07-05 Accepted: 2005-07-20

Abstract

AIM: To investigate the relationship between the inhibited growth (cytotoxic activity) of berberine and apoptotic pathway with its molecular mechanism of action.

METHODS: The *in vitro* cytotoxic techniques were complemented by cell cycle analysis and determination of sub-G_1 for apoptosis in human gastric carcinoma SNU-5 cells. Percentage of viable cells, cell cycle, and sub-G_1 group (apoptosis) were examined and determined by the flow cytometric methods. The associated proteins for cell cycle arrest and apoptosis were examined by Western blotting.

RESULTS: For SNU-5 cell line, the IC (50) was found to be 48 μmol/L of berberine. In SNU-5 cells treated with 25-200 μmol/L berberine, G_2/M cell cycle arrest was observed which was associated with a marked increment of the expression of p53, Wee1 and CDk1 proteins and decreased cyclin B. A concentration-dependent decrease of cells in G_0/G_1 phase and an increase in G_2/M phase were detected. In addition, apoptosis detected as sub-G_0 cell population in cell cycle measurement was proved in 25-200 μmol/L berberine-treated cells by monitoring the apoptotic pathway. Apoptosis was identified by sub-G_0 cell population, and upregulation of Bax, downregulation of Bcl-2, release of Ca^{2+}, decreased the mitochondrial membrane potential and then led to the release of mitochondrial cytochrome C into the cytoplasm and caused the activation of caspase-3, and finally led to the occurrence of apoptosis.

CONCLUSION: Berberine induces p53 expression and leads to the decrease of the mitochondrial membrane potential, Cytochrome C release and activation of caspase-3 for the induction of apoptosis.

Key words: Berberine; Cell cycle; Apoptosis; Caspase-3; ROS; MMP; SNU-5 cells

Lin JP, Yang JS, Lee JH, Hsieh WT, Chung JG. Berberine induces cell cycle arrest and apoptosis in human gastric carcinoma SNU-5 cell line. *World J Gastroenterol* 2006; 12(1): 21-28

http://www.wjgnet.com/1007-9327/12/21.asp

INTRODUCTION

The growth of tumor cells not like normal cells is uncontrolled. It is a strategy to change biological properties of cancer cells that lead to apoptosis of killing cancer cells in order to reach chemotherapeutic function for anticancer drugs. Apoptosis is a physiological mode of cell death, which can be selectively triggered by cells in response to the stimuli[1]. Therefore, the induction of apoptosis is a key factor for anticancer drugs.

Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxole[5,6-a]quinolizinium), a kind of alkaloid, was initially isolated from a Chinese herbal medicine and used as an antibiotic long ago; and it has effects against many bacterial species[2,3]. In the past years, berberine has subsequently been examined for anticancer activity following evidence of antineoplastic properties[3-5]. It has also been shown that berberine interacts with nucleic acids especially DNA[6] *in vitro*. Berberine exhibits the ability to induce apoptosis in human cancer cells[5,7] and promyelocytic leukemia HL-60 cells can form berberine complexes with DNA[8].

Cell cycle studies showed that berberine induces rapid apoptosis in a subpopulation (S phase) of the cells[8]. It is

also reported that berberine has dose-dependent effects of berberine on G_2/M phase and apoptosis in Balb/c 3T3 cells[9]. However, the effects of berberine on human gastric cells are still unclear. Therefore, the purpose of the present study was to find out the molecular mechanism of berberine underlying human gastric cancer cell line (SNU-5).

MATERIALS AND METHODS

Materials

Berberine, propidium iodide (PI), Tris-HCl, trypan blue, ribonuclease-A and Triton X-100 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Potassium phosphates, dimethyl sulfoxide (DMSO), and TE buffer were purchased from Merck Co. (Darmstadt, Germany). Iscove's modified Dulbecco's medium, glutamine, fetal bovine serum (FBS), and penicillin-streptomycin, trypsin-EDTA were obtained from Gibco BRL (Grand Island, NY, USA).

Human gastric carcinoma cell line (SNU-5)

SNU-5 cell line (human gastric carcinoma; 33 years, female) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cells were immediately placed into 75 cm×75 cm×75 cm tissue culture flasks and grown at 37 ℃ under a humidified 50 mL/L CO_2 and 950 mL/L air in 800 mL/L Iscove's modified Dulbecco's medium supplemented with 200 mL/L FBS, 10 g/L penicillin-streptomycin (1 MU/L penicillin and 10 g/L streptomycin) and 10 g/L glutamine as described previously[10].

Measurement of cell viability after cells were co-treated with berberine determined by trypan blue exclusion and flow cytometry

SNU-5 cells were plated in 12-well plates at a density of 5×10^5 cells/well and grown for 24 h. Various concentrations of berberine were added to the cells for final concentrations of 0, 25, 50, 100, and 200 μmol/L, while only DMSO (solvent) was added for the control regivnen and grown for a different period of time at 37 ℃, was added 50 mL/L CO_2 and 950 mL/L. The trypan blue exclusion and flow cytometry protocols were used as previously described for determining cell viability[7].

Flow cytometry analysis of DNA content for cell cycle and apoptosis analysis in SNU-5 cells co-treated with different concentrations of berberine

About 5×10^5 SNU-5 cells/well in 12-well plates were incubated with berberine (0, 25, 50, 100, and 200 μmol/L) for different time periods before the cells were harvested by centrifugation. The cells were fixed gently (drop by drop) in 700 mL/L ethanol (in PBS) in ice overnight at -20 ℃ and then re-suspended in PBS containing 40 g/L PI, 0.1 g/L RNase (Sigma) and 0/10 g/L Triton X-100. After 30 min at 37 ℃ in the dark, the cells were transferred to the tube, analyzed with flow cytometry (Becton-Dickinson, San Jose, CA, USA) equipped with an argon laser at 488 nm. Then cell cycle and apoptosis were determined and analyzed[7,10].

Inhibition of berberine-induced apoptosis by caspase inhibitor z-VAD-fmk in SNU-5 cells

In order to further examine whether caspase-3 activation was involved in apoptosis triggered by berberine, SNU-5 cells were pretreated with the cell permeable broad-spectrum caspase inhibitor z-VAD-fmk 3 h prior to the treatment with 100 μmol/L berberine. Apoptosis and caspase-3 activity were then determined as described above.

Detection of reactive oxygen species (ROS) in SNU-5 cells co-treated with berberine by flow cytometry

The level of ROS in the SNU-5 cells was examined and quantitated by flow cytometry (Becton Dickinson FACS Calibur), using 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, Sigma). SNU-5 cells were treated with or without berberine (100 mmol/L) for 0, 0.5, 1, 1.5, 2, 4, 6, and 12 h to detect the changes of ROS. The cells were harvested and washed twice, re-suspended in 500 mL of 2,7-dichlorodihydrofluorescein diacetate (10 μmol/L) and incubated at 37 ℃ for 30 min and analyzed by flow cytometry as described previously[11].

Detection of Ca^{2+} concentrations in SNU-5 cells co-treated with berberine by flow cytometry

The level of Ca^{2+} in the SNU-5 cells was determined and quantitated by flow cytometry (Becton Dickinson FACS Calibur), using the Indo 1/AM (Calbiochem; La Jolla, CA, USA). Cells were treated with or without berberine (100 mmol/L) for 0, 0.5, 1, 1.5, 2, 4, 6, and 12 h to detect the changes of Ca^{2+} concentrations. The cells were harvested and washed twice, and re-suspended in Indo 1/AM (3 mg/L) and incubated at 37 ℃ for 30 min and analyzed by flow cytometry as described previously[12].

Detection of mitochondrial membrane potential in SNU-5 cells co-treated with berberine by flow cytometry

The level of mitochondrial membrane potential in the SNU-5 cells was determined by flow cytometry (Becton Dickinson FACS Calibur), using $DiOC_6$ (4 mol/L) (Calbiochem, Inc., La Jolla, CA, USA). Cells were treated with or without various concentrations (0, 25, 50, 100, and 200 mmol/L) of berberine for 1, 2, 4, 6, 12, 24 h to detect the changes of mitochondrial membrane potential. The cells were harvested and washed twice, re-suspended in 500 mL of $DiOC_6$ (4 mol/L) and incubated at 37 ℃ for 30 min and analyzed by flow cytometry[11].

Detection of caspase-3 activity and apoptosis in SNU-5 cells co-treated with berberine by flow cytometry

The caspase-3 activity and apoptosis in the SNU-5 cells were determined by flow cytometry (Becton Dickinson FACS Calibur), using PhiPhiLux-G_2D_2 (4×10^{-4} mmol/L) (OncoImmunin, Inc., MD, USA). Cells were treated with or without various concentrations (0, 25, 50, 100, and 200 mmol/L) of berberine and co-treated with or without caspase-3 inhibitor (z-VAD-fmk) for 24 h to detect the changes of caspase-3 activity and apoptosis. The cells were harvested and washed twice, re-suspended in 50 mL PhiPhiLux-G_2D_2 of (4×10^{-4} mmol/L) and incubated at

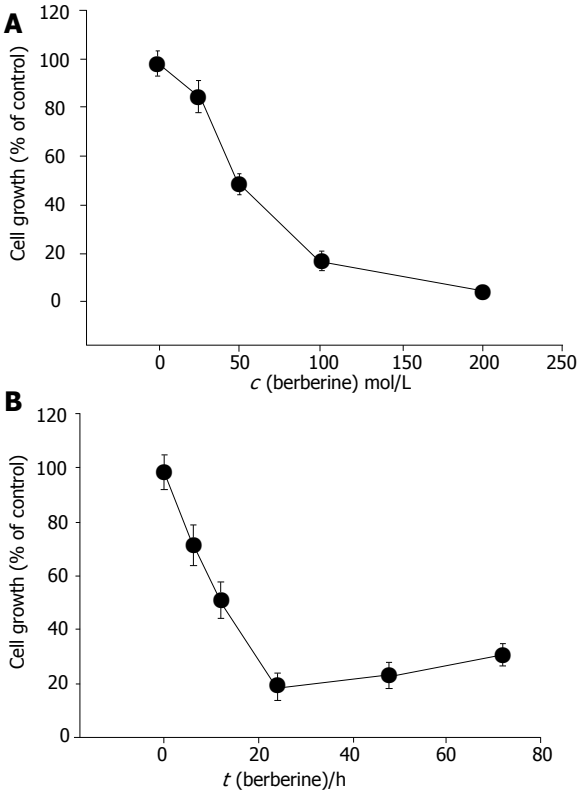


Figure 1 Percentage of viable SNU-5 cells treated with berberine with 24-h incubation. The SNU-5 cells (2×10^5 cells/well; 12-well plate) were plated in 80% Iscove's modified Dulbecco's medium+20% FBS with different concentrations of berberine for 24 h (Panel **A**) or 100 μmol/L berberine for 6, 12, 24, 48, and 72 h (Panel **B**). Then the cells were collected by centrifugation and the viable cells were determined by trypan blue exclusion and flow cytometry as described in Materials and Methods. Each point is mean ± SD of three experiments.

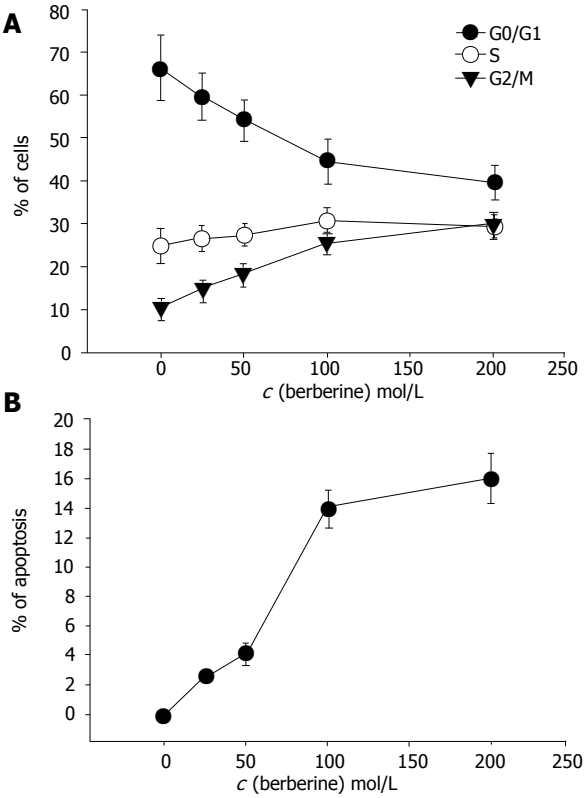


Figure 2 Flow cytometric analysis of the effects of berberine on SNU-5 cell cycle and sub-G_1 group. The SNU-5 cells were exposed to various concentrations of berberine for 48 h, and the cells were harvested and analyzed for cell cycle (Panel **A:** the percent of cells in phase) and sub-G_1 group (Panel **B:** the percent of cells in apoptosis) were analyzed by flow cytometry as described in Materials and methods. Data represents mean ± SD of three experiments.

37 ℃ for 30 min and analyzed by flow cytometry as described previously[11].

Effect of berberine on CDK1, Wee1, Cdc25, p53, JNK, Bcl-2, Bax, and cytochrome C of SNU-5 cells

The total protein was collected from SNU-5 cells treated with or without various concentrations of berberine for 48 h before CDK1, Wee1, Cdc25, p53, JNK, Bcl-2, Bax, and cytochrome C were measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot as described previously[12,13].

Statistical analysis

Student's *t*-test was used in statistical analysis between berberine-treated and control groups. $P<0.05$ was considered statisticahy significant

RESULTS

Effects of berberine on growth of SNU-5 cells

Percentage of cell growth was significantly different between the berberine-treated group and control groap. The effects of berberine on SNU-5 cells were dose-dependent (Figure 1A). Increasing the time of incubation led to the decrease of percentage of cell growth (Figure 1B). Apparently the effects of berberine on SNU-5 cells also were time dependent.

Cell cycle arrest and apoptosis induced by berberine in SNU-5 cells

First, we studied the cell cycle and occurrence of apoptosis induced by berberine. Cell cycle and apoptosis were detected by PI staining and annexin V method after 48 h of continuous exposure to berberine before analyzed by flow cytometry (Figures 2A and 2B). As shown in Figure 2, berberine induced G_2/M arrest and apoptosis in a concentration- and time-dependent manner.

Effect of berberine on cyclin B, CDK1, Wee1, and CDC25C of SNU-5 cells

Berberine increased the expression of Wee1 and CDC25C (Figures 3A and 3B) but decreased the expression of cyclin B and CDK1 (Figures 3C and 3D) as detected by western blotting.

Effects of berberine on the production of reactive oxygen species (ROS) in SNU-5 cell line

Percentage of ROS was significantly different between the berberine-treated group and control group. The effects of berberine on taking up of DCFH-DA dye by SNU-5 cells were dose-dependent (Table 1) and time dependent (Figure 4A).

Effects of berberine on the production of Ca^{2+} in SNU-5 cells

Percentage of Ca^{2+} concentrations was significantly

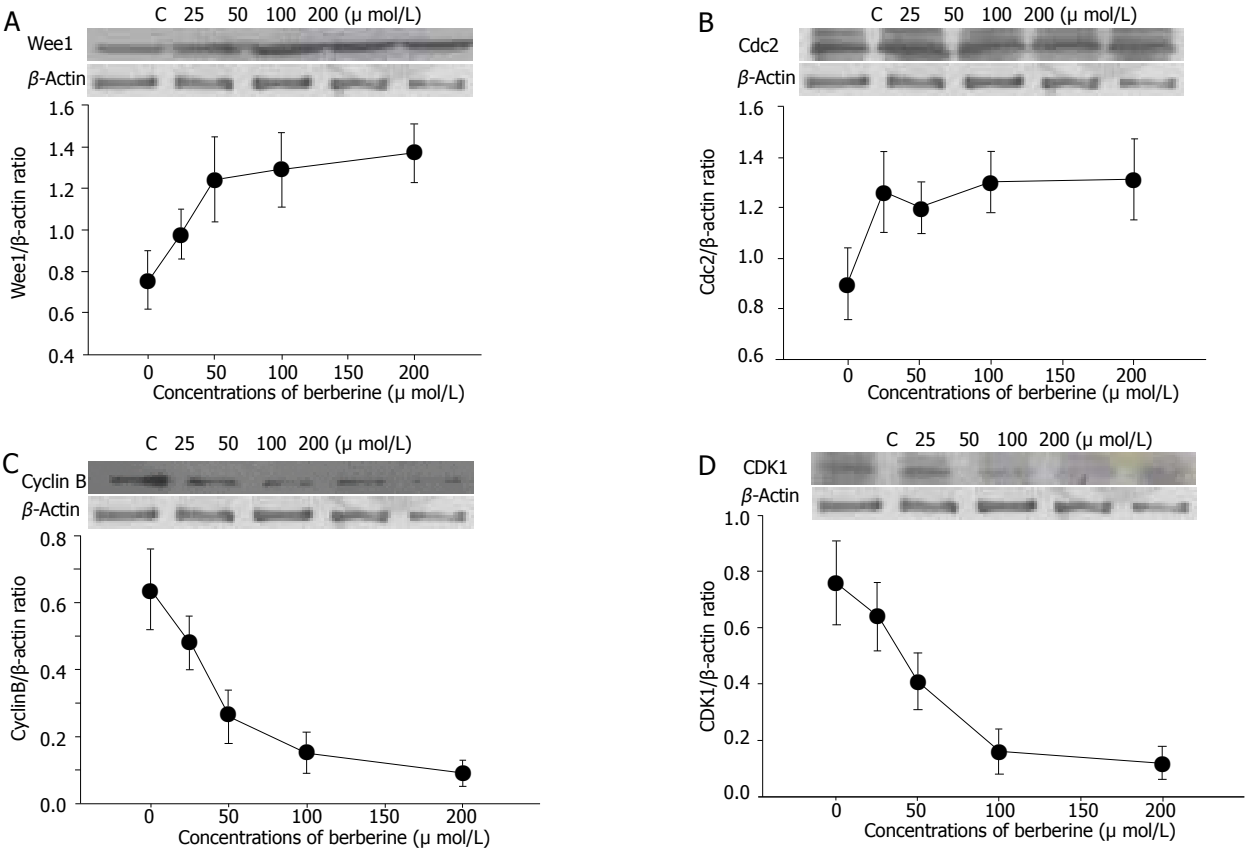


Figure 3 Changes of levels of CDK1 (A), cyclin B1 (B), Wee1 (C), and Cdc2 (D) in SNU-5 cells after exposure to berberine. SNU-5 cells (5×1 0 ^{9} / L) were treated with 0, 25, 50, 100, and, 200 μmol/L berberine for 24 h, then cytosolic fraction and total protein were determined as described in Materials and Methods.

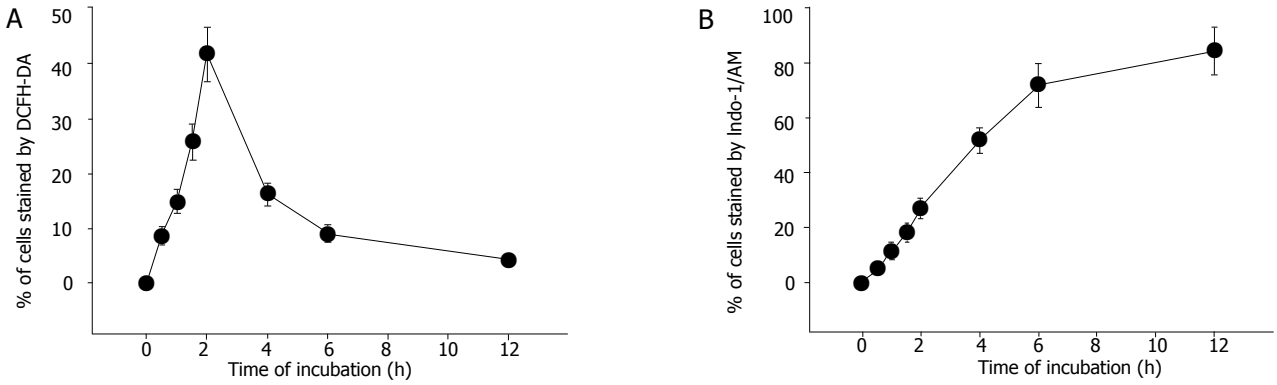


Figure 4 Flow cytometric analysis of reactive oxygen species (A) and Ca^{2+} concentration (B) in human gastric carcinoma SNU-5 cells with 100 μmol/L berberine for various time periods. The SNU-5 cells (5×10^{5} cells/mL) were treated with 100 μmol/L berberine for 0, 0.5, 1, 1.5, 2, 4, 6, and 12 h to detect the changes of ROS and Ca^{2+} cmcentration. The zero cancentration was defined as control. The percentage of cells stained with DCFH-DA dye was determined by flow cytometry as described in the Materials and Methods section.

different between the berberine-treated group and control groap. The effects of berberine on taking up of Indo-1/AM dye by SNU-5 cells were dose and time-dependent (Table 1, Figure 4B).

Effects of berberine on the mitochondrial membrane potential in SNU-5 cells

Percentage of mitochondrial membrane potential (MMP) was significantly different between the berberine-treated group and control group. Apparently the effects of berberine on the levels of MMP determined by the take up of the DiOL6 dye in SNU-5 cells were dose and time-dependent (Table 1, Figure 5).

Inhibition of berberine-induced caspase-3 activity and apoptosis by z-VAD-fmk in SNU-5 cells

The results indicate the caspase inhibitor that berberine increased caspase-3 activity in a dose- and time-dependent manner (Figure 6A). The SNU-5 cells were pretreated with the cell permeable broad-spectrum caspase inhibitor (z-VAD-fmk) 3 h prior to the treatment with berberine. The z-VAD-fmk decreased the caspase-3 activity. After

Table 1 Flow cytometric analysis of reactive oxygen species Ca^{2+} concentration and mitochondrial membrane potential in gastric carcinoma SNU-5 cells with treated various concentrations of berberine (mean ± SD)

Berberine (μ mol/L)	cells taking up DCFH-DA (%)	cells taking up Indo-1/AM (%)	cells taking up $DiOC_6$
0	0.24 ± 0.06	1.69 ± 0.22	94.20 ± 9.29
25	10.47 ± 2.02[a]	28.78 ± 2.49[a]	74.14 ± 8.28[a]
50	28.18 ± 3.14[a]	42.47 ± 3.96[a]	40.21 ± 5.08[a]
100	62.28 ± 5.46[a]	74.62 ± 6.48[a]	17.40 ± 1.87[a]
200	79.49 ± 7.08[a]	91.38 ± 8.41[a]	8.16 ± 1.04[a]

The SNU-5 cells (5×10^5 cells/mL) were treated with 0, 25, 50, 100, and 200 μmol/L of berberine. The zero concentration was defined as control. The percentage of cells taking up DCFH-DA dye, was determined by flow cytometry as described in the Materials and Methods. [a]$P < 0.05$. *vs* control.

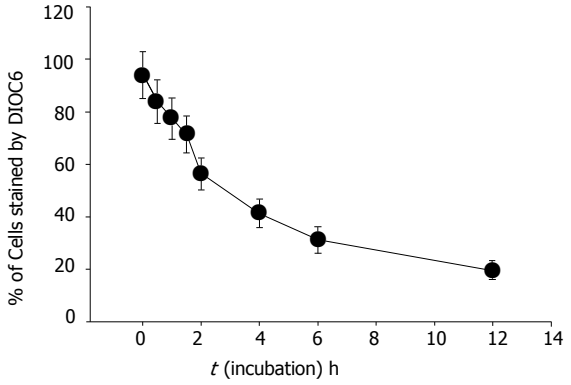


Figure 5 Flow cytometric analysis of mitochondrial membrane potential in human SNU-5 cells with 100 μmol/L berberine for various time periods. The SNU-5 cells (5×10^5 cells/L) were treated with various concentrations of berberine. The zero concentration was defined as control. The percentage of cells stained with $DiOL_6$ dye, was determined by flow cytometry as described in the Materials and Methods.

treated with berberine and z-VAD-fmk in SNU-5 cells, inhibition of berberine-mediated caspase-3 activation was accompanied with the marked attenuation of berberine-induced apoptotic cell death (Figure 6B).

Effect of berberine on p53, Bax, Bcl-2, and cytochrome C in SNU-5 cells

The results indicated that DADS increased the expression of p53 (Figure 3A), JNK phosphorylation (Figure 3B) and cytochrome C (Figure 3D) release but decreased the expression of Bcl-2 (Figure 3C) as detected by western blotting

DISCUSSION

Berberine (25-200 μmol/L) was cytotoxic to SNU-5 cells in a dose- and time-dependent manner. The IC_{50} for SNU-5 cells was 48 μmol/L (Figure 7). It is slightly different in HL-60 cells[8]. But the sensitivity of murine leukemia L1210 cells to the berberine is higher than that of HeLa cells[14]. Our results also showed that berberine induced ROS in a dose-dependent manner. It may be due to the cell death induced by ROS. Although it was demonstrated that berberine can decrease the intracellular ROS[5], such variable effects are not uncommon. However, cells after treated with berberine for 48 and 72 h slightly increased their viability. They therefore may lead to the resistance to berberine due to the expression of multidrug-resistant transporters (mdr) because berberine can modulate expression of mdr1 gene product (pgp-170) that leads to reduced response to paclitaxel in digestive track cancer cells[15].

Berberine arrests cells in S- and G_2/M-phase of the cell cycle, but the former effect is transient. However, G_2/M arrest is obvious. Apparently this effect is dose-dependent. Although it was reported that berberine could induce G_0/G_1 cell cycle arrest in murine L1210 cells[14], it was also reported that berberine can induce G_2/M cell cycle arrest in Balb/c 3T3 cells[4,16], suggesting that berberine induces cell cycle arrest depending on cell types. Therefore, the mechanism of berberine is not the same in all cell types. Western blot results from the present studies also demonstrated that berberine inhibits the levels of cyclin B and CDK1 but increases the levels of Wee1 and Cdc2, which may be the factors for G_2/M arrest in SNU-5 cells. It has been reported that increases of Cdc2 activity are in response to drug-induced G_2/M arrest[16,17]. Formation of Cdc2-cyclin B complex is necessary for G_2/M transition and cells to enter mitosis[16]. Our result demonstrated that berberine declined cyclin b levels in SNU-5 cells, and that G_2/M arrest could be controlled by cyclin B rather than by Cdc2 activation. Cyclin-dependent kinases (Cdks) are the central regulators of cell division cycle. Inhibitors of Cdks ensure proper coordination of cell cycle events and regulate cell proliferation in tissues and organs. Wee1 homologs phosphorylate a conserved tyrosine to inhibit the mitotic cyclin-dependent kinase Cdk1[18]. It was also reported that the induction of Cdc2 phosphorylation due to the increase of Wee1 and Myt1 as well as the reduction of Cdc2 and cyclin B1, is involved in 1,25 [OH] 2VD3-induced G_2/M arrest of keratinocytes[16,19]. It has been shown that multisite phosphorylation of either CDK, Cdc2, Wee1, or CDK-activating kinase is sufficient to generate dynamical behaviors including bistability and limited cycles[20,21]. Experimental depletion of Wee1 by a small interfering RNA directed to Wee1 mRNA could alleviate Vpr-induced G(2) arrest and allow normal progression from M into G phase[16].

Cell cycle analysis revealed the presence of apoptotic cell death (sub-G_1 group) following treatment with berberine. We also did morphological examination which showed cell shrinkage, loss of cell-to-cell contact, membrane blebbing and chromatin condensation elicited by increasing berberine concentration and length of exposure. These results were also confirmed by fluorescence microscopy and flow cytometry. So far, many signals and stimuli have been reported to join the induction of apoptosis, therefore the survival of specific cells is under the control of a wide complex of signals. Especially the caspase activities have been demonstrated to be the regulators of apoptosis[22,23]. Most apoptosis models are involved in caspase activation and two main pathways: caspases-8 and -3 or activation of caspases-9 and -3 activation[24]. The caspases-9 and -3 are involved in the

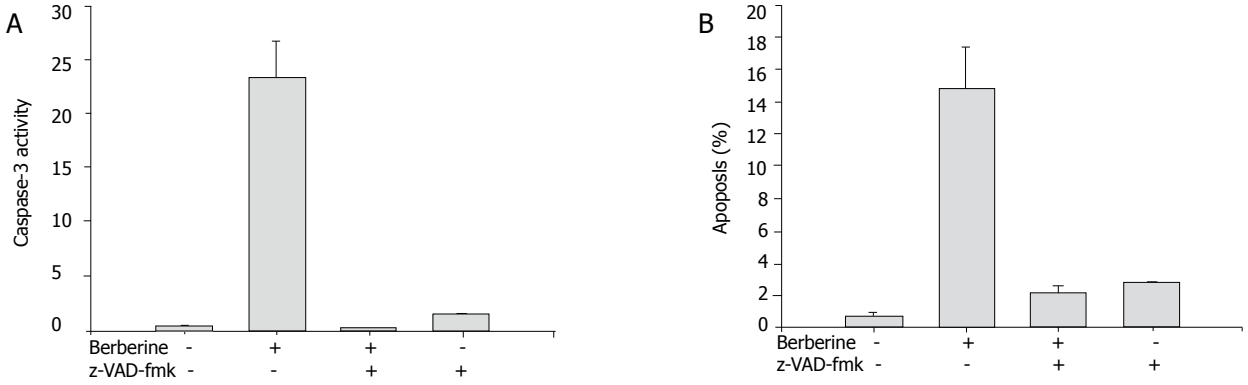


Figure 6 Flow cytometric analysis of the effects of berberine induced caspase-3 activity **(A)** and apoptosis **(B)**. The SNU-5 cells were incubated with 100 μmol/L berberine with or without z-VAD-fmk treatment for determination of caspase-3 activity and apoptosis.

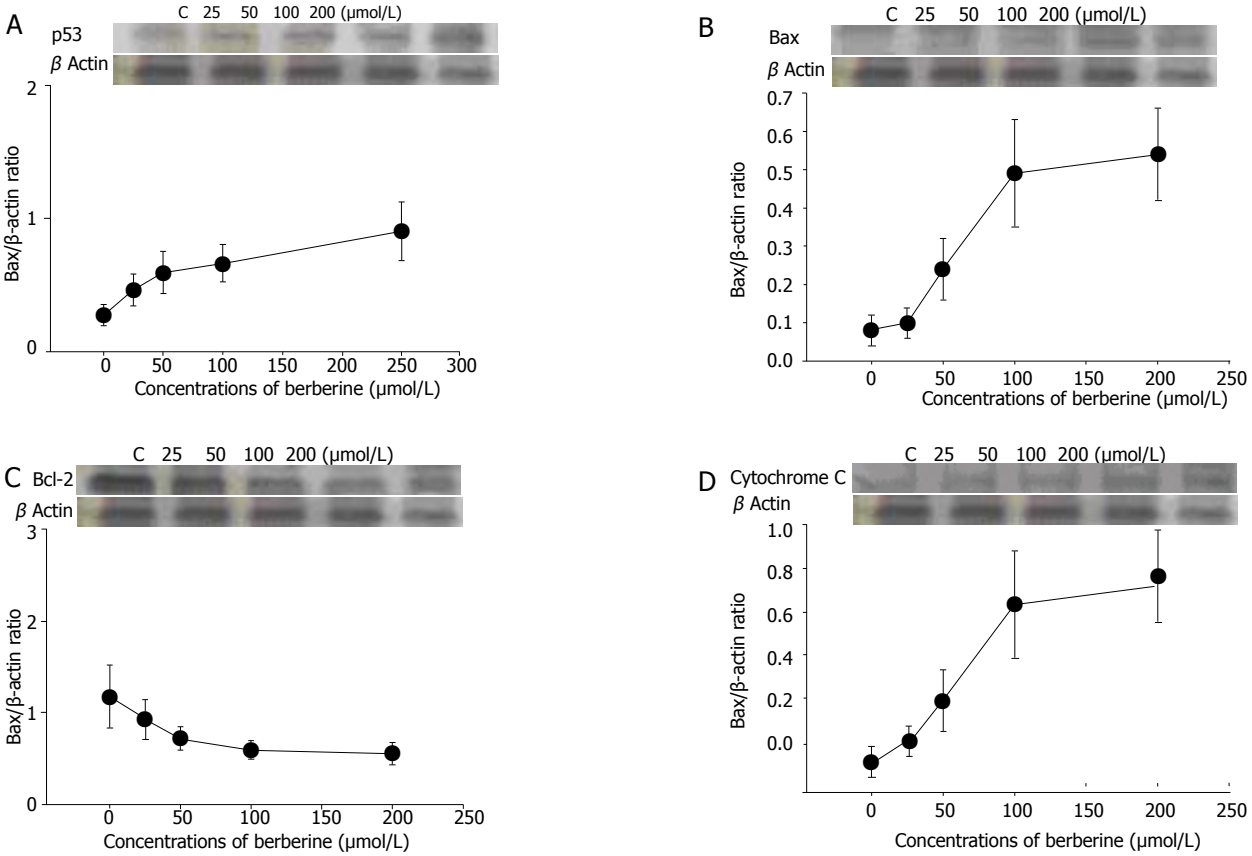


Figure 7 Changes of levels of p53**(A)**, Bcl-2**(B)**, Bax**(C)**, and cytochrome C **(D)** in SNU-5 cells after exposure to berberine. SNU-5 cells (5×10^6/mL) were treated with 0, 25, 50, 100, and 200 μmol/L berberine for 24 h, then cytosolic fraction and total protein were determined as described in Materials and Methods. The levels of p53, p21, Bcl-2, Bax, and cytochrome C were determind by Western blotting as described in Materials and Methods.

release of cytochrome C from mitochondria which causes the decrease of mitochondrial membrane potential. Our data demonstrated that berberine was able to induce the increase of Ca^{2+} and mitochondrial membrane potential loss and cytochrome C release in cytosolic fraction of SNU-5 cells, which correlates well with the activation of caspases-9 and -3. Our result also showed that berberine decreased the levels of Bcl-2 which control mitochondrial membrane potential and Bax was increased which promotes the cytochrome C release (Figure 8).

Berberine can decrease apoptosis induced by paclitaxel in human cancer cell lines including gastric cancer cells[25,26], whereas in the present study berberine induced cell cycle arrest and cell death (apoptosis) in SNU-5 cells in a dose- and time-dependent manner. Based on these findings, we suggest that berberine may contribute to the antineoplastic activity of gastric cancer cells. However, the molecular basis of such effects needs for further investigations. Although the exact binding sites or receptors of berbarine on the SNU-5 cells are still unknown, the antineoplastic activity of berberine may provide a basis for further studies.

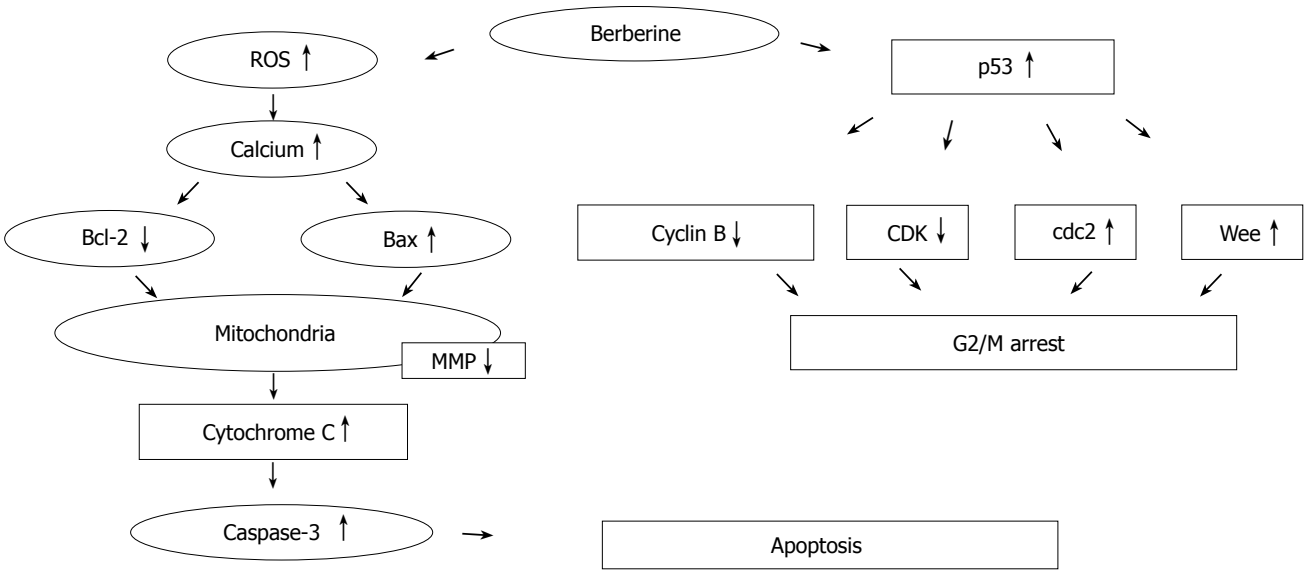


Figure 8 Proposed model of berberine mechanism of action on G2/M arrest and apoptosis in SNU-5 cells. Berberine induced p53 expression that led to the decrease of cyclin B and CDK1 but increase of the expression of cdc25c and Wee1 for G2/M arrest. Berberine induced ROS, Ca^{2+} production and decreased MMP levels led to cytochrome C release and caspase-3 activity, causing apoptosis in SNU-5 cells.

REFERENCES

1 **Kerr JF**, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972; **26**: 239-257
2 **Ghosh AK**, Bhattacharyya FK, Ghosh DK. Leishmania donovani: amastigote inhibition and mode of action of berberine. *Exp Parasitol* 1985; **60**: 404-413
3 **HANO K**, MIMURA F, OKU S, OKU K, KANI S, HAGIHARA A, OKAMURA T, NISHIYAMA T, MIYAZAKI S, HONDA F. [Pharmacological studies on metabolism of cancer tissues. XIII. pharmacological studies on carcinostatic effects of some plant components and their derivatives. I]. *Gan* 1957; **48**: 443-445
4 **Hoshi A**, Ikekawa T, Ikeda Y, Shirakawa S, Iigo M. Antitumor activity of berberrubine derivatives. *Gann* 1976; **67**: 321-325
5 **Zhang RX**, Dougherty DV, Rosenblum ML. Laboratory studies of berberine used alone and in combination with 1,3-bis(2-chloroethyl)-1-nitrosourea to treat malignant brain tumors. *Chin Med J (Engl)* 1990; **103**: 658-665
6 **YAMAGISHI H**. Interaction between nucleic acids and berberine sulfate. *J Cell Biol* 1962; **15**: 589-592
7 **Li YC**, Tyan YS, Kuo HM, Chang WC, Hsia TC, Chung JG. Baicalein induced in vitro apoptosis undergo caspases activity in human promyelocytic leukemia HL-60 cells. *Food Chem Toxicol* 2004; **42**: 37-43
8 **Kuo CL**, Chou CC, Yung BY. Berberine complexes with DNA in the berberine-induced apoptosis in human leukemic HL-60 cells. *Cancer Lett* 1995; **93**: 193-200
9 **Yang IW**, Chou CC, Yung BY. Dose-dependent effects of berberine on cell cycle pause and apoptosis in Balb/c 3T3 cells. *Naunyn Schmiedebergs Arch Pharmacol* 1996; **354**: 102-108
10 **Lin JP**, Lu HF, Lee JH, Lin JG, Hsia TC, Wu LT, Chung JG. (-)-Menthol inhibits DNA topoisomerases I, II alpha and beta and promotes NF-kappaB expression in human gastric cancer SNU-5 cells. *Anticancer Res* 2005; **25**: 2069-2074
11 **Lu HF**, Sue CC, Yu CS, Chen SC, Chen GW, Chung JG. Diallyl disulfide (DADS) induced apoptosis undergo caspase-3 activity in human bladder cancer T24 cells. *Food Chem Toxicol* 2004; **42**: 1543-1552
12 **Chung JG**, Yeh KT, Wu SL, Hsu NY, Chen GW, Yeh YW, Ho HC. Novel transmembrane GTPase of non-small cell lung cancer identified by mRNA differential display. *Cancer Res* 2001; **61**: 8873-8879
13 **Hahn FE,** Ciak, J Antibiotics. Vol.3. Editors: Gottliele D. P D, Shaw J.W. Cocoran. New York: Springer-Verlag, 1975: 577
14 **Jantová S**, Cipák L, Cernáková M, Kost'álová D. Effect of berberine on proliferation, cell cycle and apoptosis in HeLa and L1210 cells. *J Pharm Pharmacol* 2003; **55**: 1143-1149
15 **Lin HL**, Liu TY, Wu CW, Chi CW. Berberine modulates expression of mdr1 gene product and the responses of digestive track cancer cells to Paclitaxel. *Br J Cancer* 1999; **81**: 416-422
16 **Dai X**, Yamasaki K, Yang L, Sayama K, Shirakata Y, Tokumara S, Yahata Y, Tohyama M, Hashimoto K. Keratinocyte G2/M growth arrest by 1,25-dihydroxyvitamin D3 is caused by Cdc2 phosphorylation through Wee1 and Myt1 regulation. *J Invest Dermatol* 2004; **122**: 1356-1364
17 **Li XK**, Motwani M, Tong W, Bornmann W, Schwartz GK. Huanglian, A chinese herbal extract, inhibits cell growth by suppressing the expression of cyclin B1 and inhibiting CDC2 kinase activity in human cancer cells. *Mol Pharmacol* 2000; **58**: 1287-1293
18 **Yuan H**, Kamata M, Xie YM, Chen IS. Increased levels of Wee-1 kinase in G(2) are necessary for Vpr- and gamma irradiation-induced G(2) arrest. *J Virol* 2004; **78**: 8183-8190
19 **Stumpff J**, Duncan T, Homola E, Campbell SD, Su TT. Drosophila Wee1 kinase regulates Cdk1 and mitotic entry during embryogenesis. *Curr Biol* 2004; **14**: 2143-2148
20 **Martin SJ,** O'Brien GA, Nishikoa WK, Mcgahon AJ, Mahboubi A, Saido TC, Green DR. Protease activation during apoptosis: Death by a thousand cuts? *J Biol Chem* 1997; **270**: 6425-6431
21 **Yang L**, MacLellan WR, Han Z, Weiss JN, Qu Z. Multisite phosphorylation and network dynamics of cyclin-dependent kinase signaling in the eukaryotic cell cycle. *Biophys J* 2004; **86**: 3432-3443
22 **Krammer PH**. CD95(APO-1/Fas)-mediated apoptosis: live and let die. *Adv Immunol* 1999; **71**: 163-210
23 **Panaro NJ**, Popescu NC, Harris SR, Thorgeirsson UP. Flavone acetic acid induces a G2/M cell cycle arrest in mammary carcinoma cells. *Br J Cancer* 1999; **80**: 1905-1911
24 **Thornberry AT**, Rosen A, Nicholson DW. Control of apoptosis by proteases. In: Kaufmann SH, ed., Apoptosis. San Diego: Acad Press, 1997: 155-168
25 **Donaldson KL**, Goolsby GL, Kiener PA, Wahl AF. Activation of p34cdc2 coincident with taxol-induced apoptosis. *Cell Growth Differ* 1994; **5**: 1041-1050
26 **Wartenberg M**, Budde P, De Mareés M, Grünheck F, Tsang SY, Huang Y, Chen ZY, Hescheler J, Sauer H. Inhibition of

tumor-induced angiogenesis and matrix-metalloproteinase expression in confrontation cultures of embryoid bodies and tumor spheroids by plant ingredients used in traditional chinese medicine. *Lab Invest* 2003; **83**: 87-98

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 29-35
World Journal of Gastroenterology ISSN 1007-9327

Inhibitory effect of Polo-like kinase 1 depletion on mitosis and apoptosis of gastric cancer cells

Xue-Hua Chen, Bin Lan, Ying Qu, Xiao-Qing Zhang, Qu Cai, Bing-Ya Liu, Zheng-Gang Zhu

Xue-Hua Chen, Ying Qu, Xiao-Qing Zhang, Qu Cai, Bing-Ya Liu, Zheng-Gang Zhu, Department of Surgery, Shanghai Institute of Digestive Surgery, Ruijin Hospital, Medical School of Shanghai Jiao Tong University, Shanghai 200025, China
Bin Lan, Department of Surgical Oncology, the First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, Fujian Province, China
Supported by the Major State Basic Research Development Program of China, 973 program, No. 2002CB713700
Co-first-authors: Xue-Hua Chen and Bin Lan
Co-correspondents: Bing-Ya Liu and Zheng-Gang Zhu
Correspondence to: Dr Bing-Ya Liu Department of Surgery, Shanghai Institute of Digestive Surgery, Ruijin Hospital, Medical School of Shanghai Jiao Tong University, Shanghai 200025 China. liubysh@yahoo.com
Telephone: +86-21-64674654 Fax: +86-21-64373909
Received: 2005-05-13 Accepted: 2005-06-11

Abstract

AIM: Polo-like kinase 1 (PLK1) serine/threonine kinase plays a vital role in multiple phases of mitosis in gastric cancer cells. To investigate the effect of PLK1 depletion on mitosis and apoptosis of gastric cancer cells.

METHODS: PLK1 expression was blocked by small RNA interference(siRNA). The expression levels of PLK1, cdc2, cyclin B and caspase 3 were detected by Western blotting. Then, PLK1 depletion, cdc2 activity, cell proliferation, cell cycle phase distribution, mitotic spindle structure, and the rate of apoptosis of the PLK1 knockdown cells were observed.

RESULTS: PLK1 gene knockdown was associated with increased cyclin B expression, increased cdc2 activity (but not with the expression levels), accumulation of gastric cancer cells at G2/M, improper mitotic spindle formation, delayed chromosome separation and delayed or arrested cytokinesis. Moreover, PLK1 depletion in gastric cancer cells was associated with decreased proliferation, attenuated pro-caspase 3 levels and increased apoptosis.

CONCLUSION: Blockage of PLK1 expression may lead to decreased mitosis or even apoptosis in gastric cancer cells, indicating that PLK1 may be a valuable therapeutic target for gastric cancer.

Key words: Gastric cancer cells; PLK1 gene; Mitosis; Cell cycle; Apoptosis

Chen XH, Lan B, Qu Y, Zhang XQ, Cai Q, Liu BY, Zhu ZG. Inhibitory effect of Polo-like kinase 1 depletion on mitosis and apoptosis of gastric cancer cells. *World J Gastroenterol* 2006; 12(1): 29-35

http://www.wjgnet.com/1007-9327/12/29.asp

INTRODUCTION

The separation of chromosomes and the division of one cell into two daughter cells are the most dramatic events during the cell cycle. This process, known as mitosis, involves a series of structural changes in cells, including centrosome duplication, spindle formation and cytokinesis. The cdc2 - cyclinB protein complex, a mitosis promoting factor , is known to trigger and promote the completion of this complex process[1], but the mechanism responsible for activating the cdc2 - cyclinB complex remained unknown until the conserved Polo-like kinase 1 (PLK1) was cloned by Golsteyn *et al*.[2]. PLK1 accumulates markedly during the G_2/M phase in cells, where it phosphorylates and activates the cdc2 - cyclinB complex and other mitosis-involved substrates[3,4]. PLK1 is indispensable in some types of cell division including tumor cells[5]. Besides, PLK1 expression increases in multiple types of solid tumors such as non-small-cell lung cancer, head and neck cancer, esophageal cancer, colorectal cancer-associated glioma[6-10]. PLK1 is now considered as an oncogene, a potential target for cancer therapy. But to our knowledge, no previous work has examined the effect of PLK1 knockdown in gastric cancer cells. Here, we have used RNA interference technology to block the expression of PLK1 in gastric cancer cell line MKN45 and observed the changes in cell division phenotypes and cell viability.

MATERIALS AND METHODS

Cell culture

The gastric cancer cell line MKN45 was maintained in RPMI1640 medium containing 2 mmol/L glutamine and 100ml/L fetal bovine serum (Invitrogen, CA, USA) and incubated at 37 °C in an atmosphere containing 50 mL/L of CO_2.

PLK1 siRNA

The Ambion software was used to design RNAi sequences targeting human PLK1 (accession no. NM 005030) and

the siRNA sequence with the highest putative efficacy (5'CAACCAAAGTCGAATATGA 3') was synthesized by Shanghai GeneChem Co., Ltd. (PLK$^-$ group). Small RNA interference with randomized sequence (5' TTCTCCGAACGTGTCACGT3') against no gene (scrambled siRNA group) and only liposome (liposome group) were transfected as internal control.

For the experiments, cells transfected with the DOTAP liposomal transfection reagent (Roche, Germany) were seeded at 2×10^5 cells/well in six-well plates. After 24 h culture when cells were in the phase of log growth, 250 μL Opti-MEM I was mixed with 7.5 μL of 20 μmol/L siRNA duplex, while another 250 μL Opti-MEM I was separately incubated with 11.88 μL of DOTAP liposome. The two mixtures were gently mixed and incubated for about 30 min at room temperature. For transfection, the entire mixture was added to each well in 1.5 mL of fresh medium containing 100 ml/L FBS without antibiotics. The final transfected concentration of siRNA was 75 nmol/L. Cells were collected for further assay at 24, 48, and 72 h after transfection.

Western blotting

Cells were lysed in AM1 lysis buffer (Active Motif, USA) and protein concentrations were measured with the BCA protein assay kit (Pierce, USA). Total protein (50 μg) was resolved by 125 g/L SDS-PAGE and transferred onto PVDF membranes. After being blocked in TBST (20 mmol/L Tris, 137 mmol/L NaCl, 1 g/L Tween 20, pH 7.6) with 50 ml/L skim milk for 2 h at room temperature, membranes were incubated with PLK1, cyclinB, cdc2, pro-caspase 3, and β-actin primary antibodies (diluted 1:200; Santa Cruz Biotechnology, USA) for 2 h. Membranes were then washed thrice with TBST solution, followed by incubation for 1 h with HRP-linked secondary antibodies (1:1 000; Santa Cruz Biotechnology) at room temperature. Finally, membranes were visualized using the DAB reagent (Dako Corporation, Denmark).

Kinase analysis

Cdc2 kinase activity was measured using the cdc2-cyclinB kinase assay kit (MBL™ International, Japan) according to the manufacturer's instructions.

Fluorescence-activated cell sorting analysis

Cells were harvested by trypsin, washed with cold PBS and resuspended in 750 ml/L ethanol at 4°C for at least 8 h. The fixed cells were collected by brief centrifugation and resuspended in PBS containing 200 μg/mL RNase and 15 mg/L propidium iodide. After incubation at room temperature for 30 min, samples were subjected to flow cytometry (FCM) for Fluorescence-activated cell sorting assay and cell cycle phase analysis.

Immunofluorescence staining and confocal microscopy

Cells were grown on coverslips, fixed with 40g/L paraformaldehyde for 10 min and permeabilized with methanol for 2 min. After being washed thrice with 1 Triton X-100 in PBS, the coverslips were blocked with 20 ml/L FBS for 30 min, stained with 10 mg/L anti-α-tubulin primary antibody for 2 h at room temperature and incubated with FITC-conjugated secondary antibody (Santa Cruz Biotechnology) for 30 min. Finally, the DNA was stained with TO-PRO-3 iodide (Molecular Probes, USA) and confocal microscopy was performed to obtain detailed images of the subcellular structures.

MTT assay

The MTT method was used to measure cell proliferation. Briefly, 4×10^3 cells/well were cultured in 96-well plates. After 24 h, siRNA and liposome were added to transfect cells, and then at the designated time points (24, 48, 72, and 96 h after transfection), MTT (5 g/L) was added. Cells were incubated for 4 h and then the medium was removed and 100 μL DMSO solution was added for 5 min. The absorbance of the reaction solution was measured at 570 nm and the data from the various time points were used to generate cell proliferation curves.

Apoptosis detection

Apoptosis was assessed with an Annexin V kit (BD Corporation, USA). In brief, cells were harvested, washed in PBS and labeled with Annexin V antibody and PI according to the manufacturer's protocol. Labeled cells were then subjected to FCM for the determination of apoptosis.

Statistical analysis

All experiments were repeated thrice. Data were analyzed with SPSS software version 11.0. Student's *t*-test was used to evaluate the difference in cell phase distribution between PLK$^-$ and scrambled siRNA groups. One-way analysis of variance (ANOVA) was used to test the effects of the three treatments on cdc2 activity and apoptosis rate. Two-way analysis of variance was performed to detect the random effects of the three treatments on cell proliferation (MTT assay), and Student-Newman-Keuls test was used to detect the difference between any two groups. The χ^2 test was employed to examine the difference in cell mitosis phenotypes under confocal microscope. Cell morphology was observed under inverted microscope between experimental and control groups. $P<0.05$ was considered statistically significant.

RESULTS

Increase in cyclinB expression and cdc2 activity following PLK1 knockdown by siRNA

To determine the effect of siRNA on PLK1 depletion, we collected cell samples at 24, 48, and 72 h after transfection, extracted total cellular proteins and performed standard Western blotting of experimental cultures and scrambled siRNA-treated and liposome-only controls. PLK1 protein levels were reduced by 38.4% and 60.7% compared with the scrambled siRNA-treated control groups at 48 and 72 h, respectively, indicating that PLK1 gene expression was obviously blocked by the siRNAs (Figure 1A). We found that the cyclinB protein levels were 82.4% and 32.9% higher at 48 and 72 h, respectively in PLK1-depleted

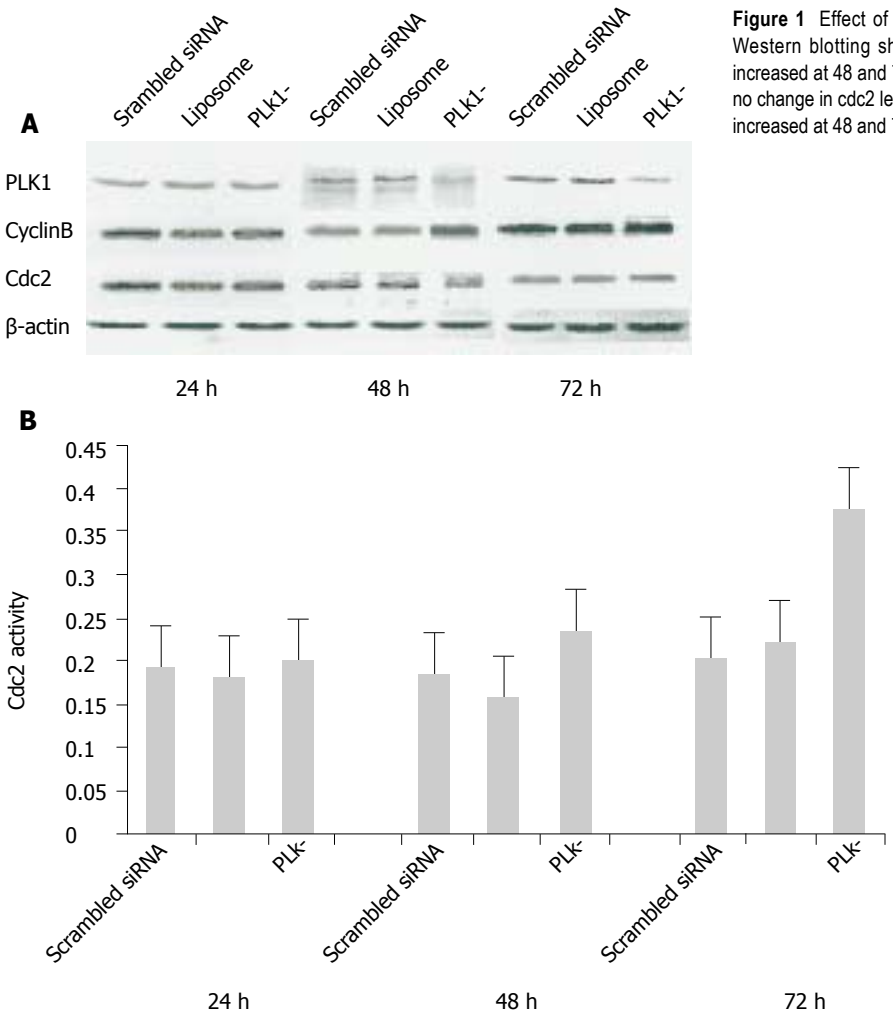


Figure 1 Effect of PLK1 depletion on members of the MPF complex. **A:** Western blotting showed that PLK1 levels decreased and cyclinB level increased at 48 and 72 h after RNAi targeting PLK, whereas there was almost no change in cdc2 level at 24, 48, and 72 h. **B:** Cdc2 activity was substantially increased at 48 and 72 h after PLK1 expression knockdown.

cells than in scrambled siRNA-treated cells (Figure 1A), while the mean activity of cdc2 kinase was increased in comparison to the controls (85.71% and 68% higher than scrambled siRNA-treated cells at 48 and 72 h, respectively, $P < 0.05$), but there was no apparent difference at the protein level (Figures 1A and 1B).

Tumor cell accumulation in G2/M phase caused by PLK1 siRNA

Cell cycle phase distribution was measured by FCM at 24, 48, and 72 h after siRNA transfection. The mean percentage of cells with G_2 DNA content in the PLK$^-$ group was 40.15%, 36.58%, and 59.88%, while that in the scrambled siRNA control group was 14.59%, 10.11%, and 17.69% at 24, 48, and 72 h, respectively ($P < 0.05$, Figure 2A). Inverted microscopy was used to examine the morphological changes of tumor cells under five randomly chosen inverted microscopic fields. The percentage of rounded cells was higher in the experimental group than in the scrambled siRNA control group (41% *vs* 13%, 38.55% *vs* 13.21%, and 44.55% *vs* 18.2% at 24, 48, and 72 h, respectively) ($P < 0.05$, Figure 2B).

Decreased tumor cell proliferation caused by PLK1 siRNA

MTT assays were used to determine whether PLK1 gene depletion affected tumor cell proliferation. The resultant proliferation curves indicated that the PLK1-depleted cells divided more slowly than cells in the two control groups ($P < 0.05$, Figure 3).

Spindle formation, chromosome separation and cytokinesis in gastric cancer cells delayed by PLK1 siRNA

Changes in the mitotic phenotypes of PLK1 knockdown and control cells were observed by immunofluorescence staining and confocal microscopy. The spindle structure lost its cohesiveness 48 h after siRNA transfection (Figure 4A). Images of the five different substages of mitosis could be acquired (Figure 4B). There were substantial differences in the amounts and percentages of cells between the experimental group and scrambled siRNA control group after 48 h ($P < 0.05$, Table 1) at the onset of mitosis. More PLK$^-$ cells (46% *vs* 20%) were at substage I (nuclear membrane breakdown and even chromosomal distribution in the cytoplasm) and fewer (1% *vs* 41%) were at substage II (chromosomal array along the equator plate) and III (2% *vs* 8%) (chromosomal segregation). Meanwhile, higher percentages of cells with dumbbell-shaped nuclei (33% *vs* 15%) and cytoplasmic bridges connecting two incompletely separated cells (8% *vs* 1%) were also shown in PLK$^-$ cells. These results collectively indicated that spindle formation, chromosome separation

Table 1 Effect of PLK1-specific siRNA on MKN45 mitosis phenotype[1] (%)

	I	II	III	IV	V	Other types	Total
Scrambled siRNA	20	41	8	15	1	15	100
PLK$^-$	46	1	2	33	8	10	100

[1]314 mitosis cells in control group and 233 mitosis cells in RNAi group were counted.

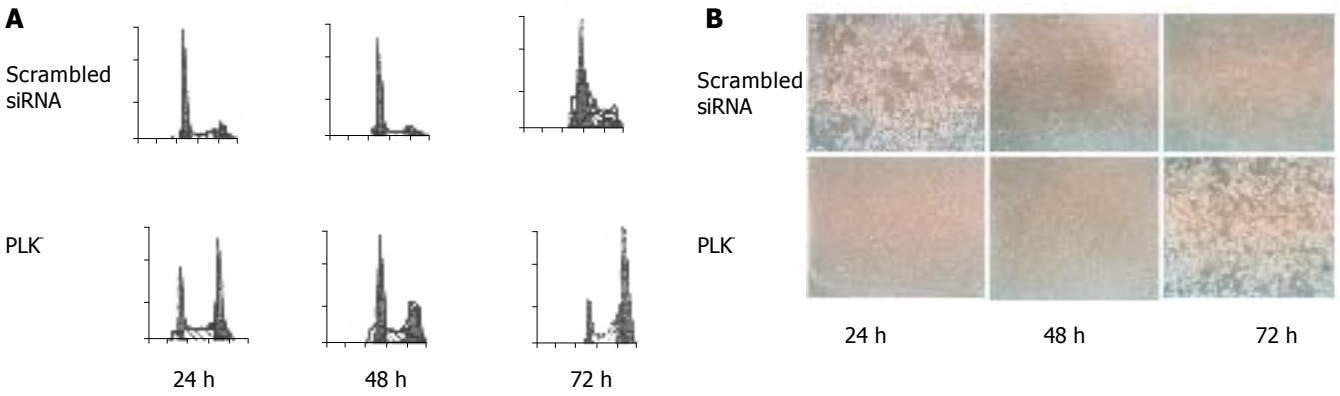


Figure 2 Changes in tumor cell cycle after PLK1 depletion. **A:** Fluorescence-activated cell sorting (FACS) analysis revealed that PLK$^-$ MKN45 cells had an increased G_2 DNA content and were accumulated at G_2/M phase. **B:** Cells treated with siRNA became rounder in shape.

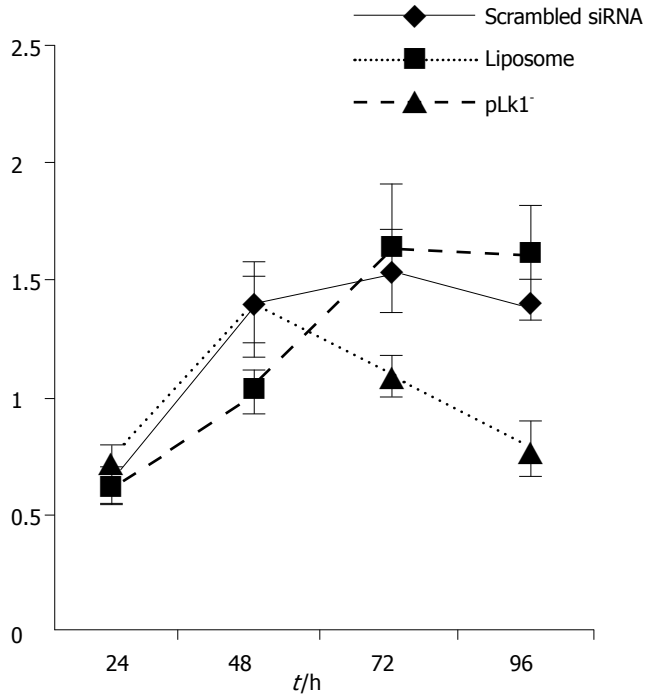


Figure 3 Effect of PLK1 depletion on cell proliferation.

and cytokinesis were delayed during mitosis in MKN45 cells transfected with PLK1-specific siRNA duplexes.

Increased apoptosis of MKN45 cells caused by PLK1 knockdown

To evaluate the role of PLK1 in tumor cell fate, PLK1-depleted cells were labeled with Annexin V antibody and subjected to FCM. The results showed that the mean apoptosis rate (including early and late apoptosis) at 48 and 72 h was higher in PLK1-depleted cells than in scrambled siRNA cells (42.4% *vs* 21.4%, 53.8% *vs* 32.9%, $P<0.05$, Figure 5A). Furthermore, Western blotting revealed that caspase 3 level was lower in PLK1-depleted cells (Figure 5B).

DISCUSSION

Gastric cancer is one of the most common malignancies in the world. Uncontrolled cellular proliferation is commonly associated with the poor prognosis of this disease. Human PLK1 plays a key role in some mitotic events[11,12]. Its expression is associated with tumor proliferation degree[13]. PLK1 depletion is associated with HeLa cell mitosis[5]. However, no previous work has examined the possible role of PLK1 in gastric cancer cell mitosis and cell fate. In this experiment, we have used siRNA technology to knockdown PLK1 gene expression and observed the changes in mitotic phenotypes of the gastric cancer cell line, MKN45.

The protein level of PLK1 was decreased by 38.4% and 60.7%, respectively at 48 and 72 h after the addition of the PLK1-specific siRNA, indicating that the interference is effective and this system is appropriate for the study of mitotic events under PLK1 knockdown conditions. A previous study has demonstrated that the direct motivation of driving cells from G_2 to M phase is caused by the cyclinB-cdc2 complex (also known as the mitosis promoting factor or MPF). PLK1 is responsible for phosphorylating and activating MPF at the G_2/M checkpoint[3] and mediates the degradation of cyclinB by activating the anaphase-promoting complex at the end of mitosis[14,15], indicating that PLK1 can initiate and maintain mitosis. In our PLK1 knockdown cells, the cyclinB level increased probably due to the failure of depleted PLK1

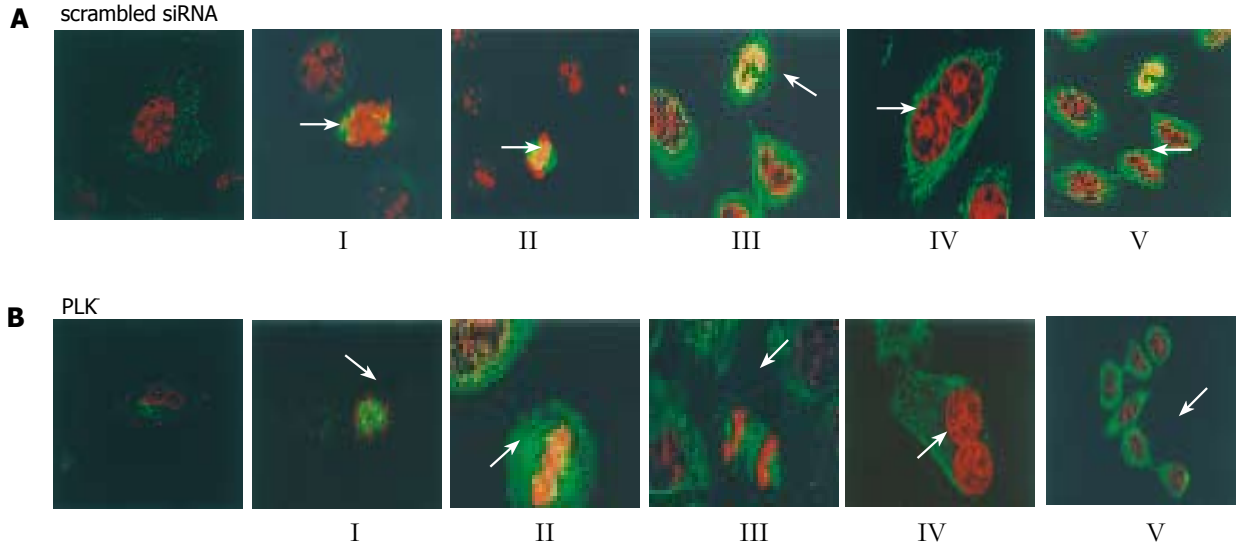


Figure 4 Tumor cell mitosis decreases following PLK1 depletion. **A:** Control cells showed clear mitotic frameworks, whereas the spindles of the PLK- depleted cells were unclear and broken when the sister chromosomes were separated. **B:** The five examined substages of mitosis (I-V) in both control and PLK- depleted cells.

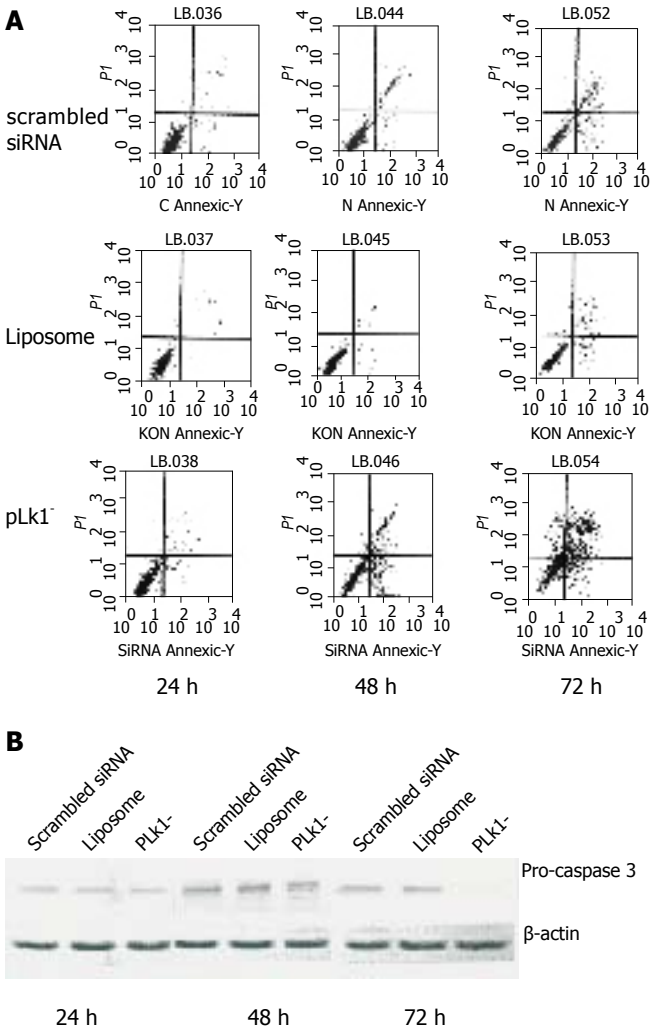


Figure 5 PLK1 siRNA induces MKN45 apoptosis. **A:** Annexin V staining and FCM revealed a higher percentage of apoptotic PLK1-depleted cells at 48 and 72 h; **B:** Western blotting showed reduced pro-caspase 3 levels in PLK- cells at 48 and 72 h.

to activate APC. In addition, the activity (but not the quantity) of cdc2 (a component of MPF) was increased by 85.7% and 68.0% at 48 and 72 h, respectively in PLK1 knockdown cells compared to scrambled siRNA controls. Previous reports indicate that PLK1 and its analogs seem to phosphorylate and activate cdc25[3,16], which then dephosphorylates and activates the cdc2/cyclinB complex in different organisms[17,18], suggesting that cdc2 may lose its activity under PLK1 knockdownconditions. Our observation of cdc2 activation seems to indicate that cdc2 is activated in a cdc25-independent manner.

Immunofluorescence and confocal microscopy revealed that the microtubule morphology was affected in PLK1-depleted cells, becoming broken and unclear. We were able to identify cells in both PLK1-depleted and control cultures corresponding to the five substages of mitosis: (I) nuclear membrane breakdown and even chromosomal distribution in cytoplasm; (II) chromosomal array along the equator plate; (III) chromosomal segregation; (IV) mitotic exit and nuclear membrane formation; (V) cytokinesis. These substages were observed at varying frequencies between the control and siRNA-treated cells. At the onset of mitosis, more siRNA-treated cells (46% *vs* 20%) were at substage I and fewer were at substage II (1% *vs* 41%) or III (2% *vs* 8%). Recent studies showed that PLK1 affects chromosomal separation by controlling the formation of mitotic spindle and phosphorylating cohesion to decrease the cohesion of sister chromosomes[19-21]. Our results revealed that in PLK1-depleted tumor cells, the mitotic spindle was disrupted (Figure 4A), which may be the reason why many PLK1-depleted cells are stalled in substage I and unable to progress to the subsequent stages requiring intact mitotic spindles.

In addition to its effect on chromosomal segregation, PLK1 is also closely associated with the exit from mitosis and subsequent cytokinesis. In the former, PLK1 appears to play a role in the activation of APC by destroying the APC inhibitor, Emi1[15,22]. In cytokinesis, PLK1 is associated with the phosphorylation and activation of motor-like protein (MKlp) 2[23] and nuclear distribution

gene C (NudC)[24,25]. In our study, 48 h after siRNA treatment, α-tubulin immunofluorescence and DNA staining revealed a higher percentage of dumbbell-shaped cell nucleoli (33% *vs* 15%) and increased the number of cytoplasmic bridges connecting two incompletely separated cells (8% *vs* 1%), indicating that PLK1-depleted cells cannot successfully exit mitosis and reform two new nuclear membranes at anaphase but arrest mitosis during cytokinesis. In our study, immunohistochemistry and FCM experiments showed that the expression of PLK1-specific RNAi affected mitotic processes, causing tumor cells to accumulate at the mitotic phase and blocking them from completing cell division. In addition, our MTT assays indicated that PLK1-depleted cells decreased their proliferation.

We examined whether PLK1 depletion affected the fate of MKN45 cells. We used Annexin V staining to identify early and late apoptosis. Our results revealed that apoptosis increased significantly 48 and 72 h after the addition of the PLK1-specific siRNA. There was no obvious change at 24 h, indicating a delay before the apoptotic mechanism is triggered. Further examination revealed a sharp decrease in pro-caspase 3 levels. PLK1 can bind to and suppress the pro-apoptotic molecule p53 in HeLa cells[26], while p53 is accumulated during PLK1-depletion-induced apoptosis[27]. Based on this, it may be interesting to clarify whether any other pathway is involved in PLK1 depletion-induced apoptosis.

In conclusion, PLK1 is vital to gastric cancer cell division. Gene or drug therapy aimed at depleting PLK1 level may have a potential value as a novel treatment for gastric cancer.

ACKNOWLEDGMENT

Bian Wei from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, for technical assistance with the confocal microscopy.

REFERENCES

1 **Ohi R**, Gould KL. Regulating the onset of mitosis. *Curr Opin Cell Biol* 1999; **11**: 267-273
2 **Golsteyn RM**, Schultz SJ, Bartek J, Ziemiecki A, Ried T, Nigg EA. Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases Drosophila polo and Saccharomyces cerevisiae Cdc5. *J Cell Sci* 1994; **107 (Pt 6)**: 1509-1517
3 **Toyoshima-Morimoto F**, Taniguchi E, Nishida E. Plk1 promotes nuclear translocation of human Cdc25C during prophase. *EMBO Rep* 2002; **3**: 341-348
4 **Lee M**, Daniels MJ, Venkitaraman AR. Phosphorylation of BRCA2 by the Polo-like kinase Plk1 is regulated by DNA damage and mitotic progression. *Oncogene* 2004; **23**: 865-872
5 **Liu X**, Erikson RL. Activation of Cdc2/cyclin B and inhibition of centrosome amplification in cells depleted of Plk1 by siRNA. *Proc Natl Acad Sci U S A* 2002; **99**: 8672-8676
6 **Wolf G**, Elez R, Doermer A, Holtrich U, Ackermann H, Stutte HJ, Altmannsberger HM, Rübsamen-Waigmann H, Strebhardt K. Prognostic significance of polo-like kinase (PLK) expression in non-small cell lung cancer. *Oncogene* 1997; **14**: 543-549
7 **Knecht R**, Elez R, Oechler M, Solbach C, von Ilberg C, Strebhardt K. Prognostic significance of polo-like kinase (PLK) expression in squamous cell carcinomas of the head and neck. *Cancer Res* 1999; **59**: 2794-2797
8 **Tokumitsu Y**, Mori M, Tanaka S, Akazawa K, Nakano S, Niho Y. Prognostic significance of polo-like kinase expression in esophageal carcinoma. *Int J Oncol* 1999; **15**: 687-692
9 **Takahashi T**, Sano B, Nagata T, Kato H, Sugiyama Y, Kunieda K, Kimura M, Okano Y, Saji S. Polo-like kinase 1 (PLK1) is overexpressed in primary colorectal cancers. *Cancer Sci* 2003; **94**: 148-152
10 **Dietzmann K**, Kirches E, von Bossanyi K, Mawrin C. Increased human polo-like kinase-1 expression in gliomas. *J Neurooncol* 2001; **53**: 1-11
11 **Golsteyn RM**, Lane HA, Mundt KE, Arnaud L, Nigg EA. The family of polo-like kinases. *Prog Cell Cycle Res* 1996; **2**: 107-114
12 **Mundt KE**, Golsteyn RM, Lane HA, Nigg EA. On the regulation and function of human polo-like kinase 1 (PLK1): effects of overexpression on cell cycle progression. *Biochem Biophys Res Commun* 1997; **239**: 377-385
13 **Ito Y**, Yoshida H, Matsuzuka F, Matsuura N, Nakamura Y, Nakamine H, Kakudo K, Kuma K, Miyauchi A. Polo-like kinase 1 (PLK1) expression is associated with cell proliferative activity and cdc2 expression in malignant lymphoma of the thyroid. *Anticancer Res* 2004; **24**: 259-263
14 **Kotani S**, Tugendreich S, Fujii M, Jorgensen PM, Watanabe N, Hoog C, Hieter P, Todokoro K. PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression. *Mol Cell* 1998; **1**: 371-380
15 **Moshe Y**, Boulaire J, Pagano M, Hershko A. Role of Polo-like kinase in the degradation of early mitotic inhibitor 1, a regulator of the anaphase promoting complex/cyclosome. *Proc Natl Acad Sci U S A* 2004; **101**: 7937-7942
16 **Karaïskou A**, Jessus C, Brassac T, Ozon R. Phosphatase 2A and polo kinase, two antagonistic regulators of cdc25 activation and MPF auto-amplification. *J Cell Sci* 1999; **112 (Pt 21)**: 3747-3756
17 **Karaïskou A**, Cayla X, Haccard O, Jessus C, Ozon R. MPF amplification in Xenopus oocyte extracts depends on a two-step activation of cdc25 phosphatase. *Exp Cell Res* 1998; **244**: 491-500
18 **Abrieu A**, Brassac T, Galas S, Fisher D, Labbé JC, Dorée M. The Polo-like kinase Plx1 is a component of the MPF amplification loop at the G2/M-phase transition of the cell cycle in Xenopus eggs. *J Cell Sci* 1998; **111 (Pt 12)**: 1751-1757
19 **Sumara I**, Vorlaufer E, Stukenberg PT, Kelm O, Redemann N, Nigg EA, Peters JM. The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. *Mol Cell* 2002; **9**: 515-525
20 **Peters JM**. Subunits and substrates of the anaphase-promoting complex. *Exp Cell Res* 1999; **248**: 339-349
21 **Sumara I**, Giménez-Abián JF, Gerlich D, Hirota T, Kraft C, de la Torre C, Ellenberg J, Peters JM. Roles of polo-like kinase 1 in the assembly of functional mitotic spindles. *Curr Biol* 2004; **14**: 1712-1722
22 **Hansen DV**, Loktev AV, Ban KH, Jackson PK. Plk1 regulates activation of the anaphase promoting complex by phosphorylating and triggering SCFbetaTrCP-dependent destruction of the APC Inhibitor Emi1. *Mol Biol Cell* 2004; **15**: 5623-5634
23 **Neef R**, Preisinger C, Sutcliffe J, Kopajtich R, Nigg EA, Mayer TU, Barr FA. Phosphorylation of mitotic kinesin-like protein 2 by polo-like kinase 1 is required for cytokinesis. *J Cell Biol* 2003; **162**: 863-875
24 **Zhou T**, Aumais JP, Liu X, Yu-Lee LY, Erikson RL. A role for Plk1 phosphorylation of NudC in cytokinesis. *Dev Cell* 2003; **5**: 127-138
25 **Aumais JP**, Williams SN, Luo W, Nishino M, Caldwell KA, Caldwell GA, Lin SH, Yu-Lee LY. Role for NudC, a dynein-associated nuclear movement protein, in mitosis and cytokinesis. *J Cell Sci* 2003; **116**: 1991-2003
26 **Ando K**, Ozaki T, Yamamoto H, Furuya K, Hosoda M, Hayashi S, Fukuzawa M, Nakagawara A. Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and

phosphorylation. *J Biol Chem* 2004; **279**: 25549-25561
27 **Liu X**, Erikson RL. Polo-like kinase (Plk)1 depletion induces apoptosis in cancer cells. *Proc Natl Acad Sci U S A* 2003; **100**: 5789-5794

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 36-42
World Journal of Gastroenterology ISSN 1007-9327

GASTRIC CANCER

CD44v6 in peripheral blood and bone marrow as micro-metastasis of patients with gastric cancer

Dao-Rong Wang, Guo-Yu Chen, Xun-Liang Liu, Yi Miao, Jian-Guo Xia, Lin-Hai Zhu, Dong Tang

Dao-Rong Wang, Lin-Hai Zhu, Dong Tang, Department of General Surgery, the First Affiliated Hospital of Yangzhou University, Yangzhou 225001, Jiangsu Province, China
Guo-Yu Chen, Xun-Liang Liu, Yi Miao, Jian-Guo Xia, Department of General Surgery, the First Affiliated Hospital of Nanjing Medical University, Nanjing 220029, Jiangsu Province, China
Supported by the grant from Science and Technology Committee of Jiangsu Province, No. 457-99064
Correspondence to: Dao-Rong Wang, PhD, Department of General Surgery, the First Affiliated Hospital of Yangzhou University, Yangzhou 225001, Jiangsu Province, China. daorong666@sina.com
Telephone: +86-514-3913499 Fax: +86-514-7937405
Received: 2005-05-12 Accepted: 2005-06-11

Abstract

AIM: To detect the expression of CD44 correlated with the ability of micro-metastasis in peripheral blood and bone marrow of patients with gastric cancer and to deduce its clinical significance.

METHODS: Preoperative peripheral blood and bone marrow specimens from 46 patients with gastric cancer and 6 controls were studied by semi-quantitative RT-PCR amplification of CD44v6mRNA. Preoperative and postoperative peripheral blood specimens from 40 patients with gastric cancer and 14 controls were studied by quantitative RT-PCR amplification of CD44v6mRNA in the corresponding period.

RESULTS: Semi-quantitative RT-PCR amplification showed that CD44v6mRNA expression of peripheral blood and bone marrow was positive in 39 (84.8%) and 40 (86.9%) of 46 patients with gastric cancer, respectively. In peripheral blood, CD44v6mRNA expression was positive for diffuse type in 30 (93.8%) of 32 patients and for intestinal type in 9 (64.3%) of 14 patients. On the other hand, in bone marrow, CD44v6mRNA expression was positive for diffuse type in 31 (96.9%) of 32 patients and for intestinal type in 10 (71.4%) of 14 patients. There was a significant difference between the diffuse type and intestinal type. Quantitative RT-PCR amplification demonstrated that CD44v6mRNA was not expressed in the peripheral blood of controls and CD44v6mRNA expression was positive for preoperative peripheral blood in 40 patients with gastric cancer, the expression levels being from 4.9×10^8 - 3.2×10^{11} copies/g RNA. The average expression level of CD44v6mRNA in peripheral blood was 3.9×10^{10} copies/g RNA. The expression levels of CD44v6mRNA in peripheral blood in gastric cancer patients after curative operation increased from 5.5×10^6 - 7.6×10^9 copies/g RNA and the average level was 2.4×10^8 copies/g RNA (Figure 3B) ($P = 0.00496$). After curative operation, the expression level decreased markedly.

CONCLUSION: Semi-quantitative and quantitative RT-PCR amplification for CD44v6mRNA is a sensitive and specific method for the detection of micro-metastasis in peripheral blood and bone marrow, which might be used as an indicator of tumor burden and therapeutic effect.

Key words: Gastric cancer; Micro-metastasis; Peripheral blood; Bone marrow; CD44v6

Wang DR, Chen GY, Liu XL, Miao Y, Xia JG, Zhu LH, Tang D. micro-metastasis of CD44v6 in peripheral blood and bone marrow of patients with gastric cancer. *World J Gastroenterol* 2006; 12(1):36-42

http://www.wjgnet.com/1007-9327/12/36.asp

INTRODUCTION

Micro-metastasis cannot be detected by a general clinical pathological method. Gastric cancer is one of the most frequent malignant tumors. Tumor invasion, metastasis, and relapse are correlated with the prognoses of gastric cancer. Tumors metastasize through lymph and blood circulation. Twenty percent of patients have micro-metastasis[1]. Tumor cell micro-metastasis in lymphocytes, peripheral blood, bone marrow, and abdominal cavity is the main reason of metastasis and relapse[2,3]. Discovering gastric cancer in time is not only important for predicting relapse and prognoses, but also important for making decisions concerning therapy. With the development of molecular biology technology, the method of inspecting micro-metastasis has become more reliable[4]. Semi-quantitative RT-PCR can reveal gastric cancer cells in peripheral blood and bone marrow[5,6]. CD44v6, a highly glycosylated cell surface protein, is involved in cell-cell and cell-matrix interactions and takes part in cell motility, tumor growth, and invasion[7].

CD44 is an integral membrane glycoprotein with an apparent molecular mass ranging 85-250 ku. It is

Table 1 Oligonucleotide primer and probe sequences used

Gene	Oligonucleotide	Location sequence PCR product	bp
CD44v	Upper primer	5′-TCCAGGCAACTCCTAGTAGT-3′	
	Lower primer	5′-CAGCTGTCCCTGTTGTCGAA-3′	740
β-Actin	Upper primer	5′-CTACAATGAGCTGCGTGTGGC-3′	
	Lower primer	5′-CAGGTCCAGACGCAGGATGGC-3′	206
Probe		5′-TGAGATTGGGTTGAAGAAATC-3′	

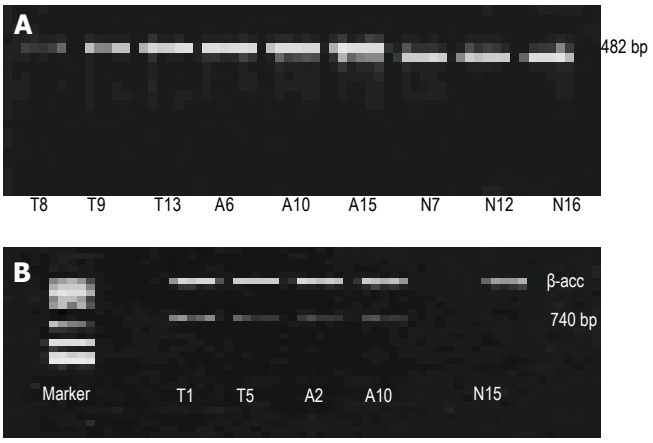


Figure 1 Expression of CD44s (**A**) and CD44v (**B**) in gastric mucosa.T1, T5, T8, T9, T13:bone marrow from patients with gastric cancer; A2, A6, A10, A15: peripheral blood from patients with gastric cancer; N7, N12, N15, N16:Controls.

originally described as a lymphocyte homing receptor on circulating lymphocytes[8]. At least 20 variants (v) of CD44 have been reported[9,10] due to the alternative splicing of 10 exons (v_1-v_{10}) that encode the membrane proximal portion of the extracellular domain. It has been reported that the expression of variant 6 of CD44 is correlated with invasion and metastasis of certain types of human cancer[11]. The expression of CD44v6 and CD44v5 is correlated with tumor progression, metastasis, and prognosis of colorectal cancer, breast cancer, and gastric cancer.

In our study, we have also determined the expression of CD44v6mRNA using semi-quantitative RT-PCR in peripheral blood and bone marrow from 46 patients with gastric cancer. We have also determined the quantitative expression of CD44v6mRNA in peripheral blood specimens from 40 patients with gastric cancer and 14 controls using quantitative RT-PCR to display the role of CD44v6mRNA in clinical stage and prognosis of gastric cancer.

MATERIALS AND METHODS

Patients and serum samples

Eighty-six patients were randomly divided into gastric cancer group (52 male and 34 female patients with an average age of 58.6 years, ranging 32-81 years) and control group (14 male and 8 female patients with an average age of 53.8 years, ranging 40-65 years).

Serum samples were obtained from 86 patients with primary gastric cancer prior to surgery at the Department of Gastroenterology, the First Affiliated Hospital of Yangzhou Medical University. The diagnosis was confirmed before surgery.

Extraction of total RNA

Before surgery, serum samples were obtained from peripheral blood and bone marrow 1 d before surgery and 9 d after surgery. Single nucleated cells were separated and stored at -20 °C. Total RNA was extracted. After being centrifuged at 2 500 r/min for 10 min, 5 mL S-ACR was added and bathed on ice for 15 min, then the process was repeated and the samples were stored at -70 °C.

cDNA synthesis, cDNA amplification, and semi-quantitative analysis

Primers sp1 and sp2 were from the cDNA sequence[12]. Primers p1, p2, and β-actin were from the cDNA sequence[13]. Primers were separately aimed at the standard and variant CD44s, CD44v6, and β-actin[14]. The three couples of primers were synthesized by Sagon Co., Canada and stored at -20 °C (sp1: 5' -GACACATATTGCTTCAATG CTTCAGC-3'; sp2: 5' -GATGCCAAGATGATCAGCCATTCTGGAAT-3'; P1: 5'-GACAGACACCTCAGTTTTTCTGGA-3'; P2: 5'-TTC CTTCGTGTGTGGGTAATGAGA-3'; forward β-actin: 5' -CTACAATGAGCTGCGTGTGGC-3'; backward β-actin: 5'-CAGGTCCAGACGCA GGATGGC-3'). After cDNA was synthesized and amplified, the product was analyzed and the density was scanned (semi-quantitative analysis). When the value of CD44v6mRNA/β-actin was less than 0.30 and more than 0.70, it was expressed as (-), (+), and (+).

Real-time RT-PCR

The point characterized the reactions during cycling, when the PCR product was first detected. The product was accumulated after a fixed number of cycles. The higher the starting quantity of the target molecule, the earlier the significant increase in fluorescence was observed. The parameter C+ (threshold cycle) was defined as the fractional cycle number at which the fluorescence was generated. CD44v6 target message in unknown samples was quantified by measuring C+ and using a calibration curve to determine the starting target message. The precise amount of total RNA added into each reaction mix (based on absorbance) and its quality were difficult to assess. For each experimental sample, the amount of targets and endogenous reference were determined by the calibration curve. The target amount was then divided by the endogenous reference amount to obtain a normalized target value. The relative gene target expression was also normalized to healthy control serum sample. Each of the normalized target values was divided by the calibrator-normalized target value to generate the final relative expression.

Primers, probes, and PCR consumables

Primers and probes for the CD44v6 gene were chosen with the assistance of the computer programs Oligo 4.0 (National Bioscience) and Primer Express (Perkin-Elmer Applied Bio-systems). We conducted BLAST searches against dbEST and nr to confirm the total gene specificity

Table 2 Relation between CD44v6mRNA expression in bone marrow and biologic behavior of gastric cancer

		Number of cases (*n*)	CD44v6mRNA/ β -actin				*P* value
			–	+	+	+	
Lymph node metastasis	(+) Positive	38	3	2	4	29	
	(-) Negative	8	4	1	1	3	0.0161
Clinicopathology	I-II	12	5	2	2	3	
	III-IV	34	1	2	3	28	0.0007
Tissue type	D	32	1	2	4	25	
	I	14	5	2	4	3	0.0003
Tumor size	>5 cm	24	3	2	6	13	
	≤5 cm	22	3	3	4	12	0.4547

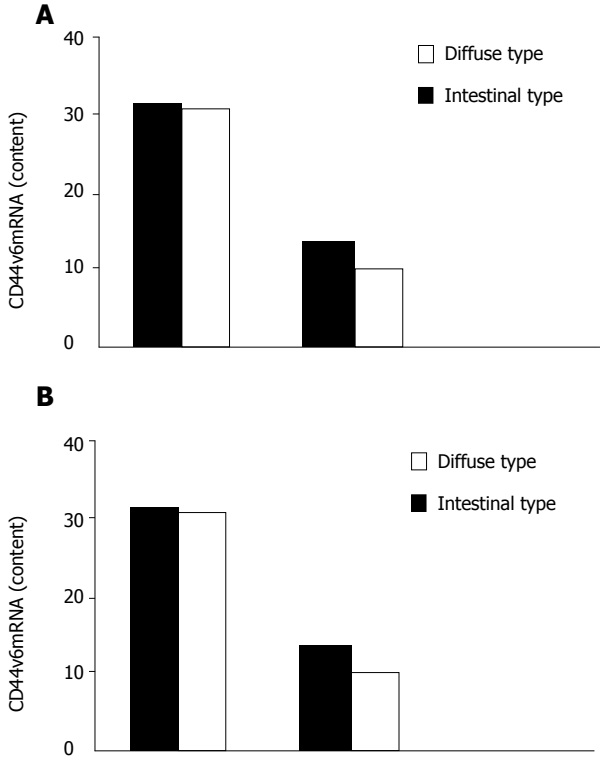


Figure 2 CD44v6mRNA expression in bone marrow (**A**) and peripheral blood (**B**) from patients with gastric cancer.

of the nucleotide sequences chosen for the primers and probes, and the absence of DNA polymorphisms. The primer for CD44v6 was selected and compared to the sequences of the closely related CD44v6 gene. The sequences of the oligonucleotide are shown in (Table 1). The primers and probes were designated by the nucleotide position corresponding to the 5'-position, followed by the letter U for upper (sense strand) or L for lower (antisense strand). To avoid amplification of contaminating genome DNA, one of the two primers or the probe was placed at the junction between two exons or in different exons.

RNA extraction

Total RNA was extracted from serum specimens of gastric cancer by the acid-phenol quantum method[15]. The quality of RNA samples was determined by electrophoresis through denaturation of agarose gels and staining with thallium bromide. The 18s and 28s RNA bands were visualized under ultraviolet light.

Calibration curve

Calibration curve was constructed with four fold serial dilutions of total RNA from healthy human serum. The diluted human total RNA was liquored and stored at -80 °C until use.

cDNA synthesis

Reverse transcription of RNA was performed in a final volume of 20 μL containing 1× RT-PCR buffer [500 mmol/L each dNTP, 3 mmol/L $MgCl_2$, 75 mmol/L KCl, 50 mmol/L Tris-HCl, pH 8.3, 10 μL of RNasin™ ribonuclease inhibitor (Promega), 10 mmol/L dithiothreitol, 50 μL of superscript RNase H^- reverse transcripts (Life Technologies), 1.5 mmol/L random hexanes and 1 μg of total RNA (calibration curve points and patient samples)]. The samples were incubated at 20 °C for 10 min and at 42 °C for 30 min. Reverse transcripts were inactivated by heating at 99 °C for 5 min and cooling at 5 °C for 5 min.

PCR amplification

All PCR reactions were performed on an ABI PRISM 7700 Sequence Detection System. For each PCR, a master mixture was prepared on ice with 1 × TaqMan buffer; 5 mmol/L $MgCl_2$; 200 mmol/L dATP, dCTP, and dGTP; 400 mmol/L dUTP; 300 mmol/L each primer; 150 mmol/L probe; 1.25 μL of Ampli Taq gold DNA. Polymers and 10 μL of each appropriately dilated reverse transcription sample were added to 40 μL of the PCR master mixture. The thermal cycling conditions were: an initial demodulation step at 95 °C for 10 min and 50 cycles at 95 °C for 15 s, at 65 °C for 1 min. Experiments were performed in duplicate for each data point. Each PCR run included five points of the calibration curve (fourfold serially diluted human normal gastric cDNA), a non-template control, cDNA calibrator. All patient samples with a cv of the number of CD44v6mRNA copies > 10% were retested.

Statistical analysis

The association of factors was evaluated by the χ^2 test. The significance of difference among the means was

Table 3 Relation between CD44v6mRNA expression in bone marrow and biologic behavior of gastric cancer

	Number of cases (*n*)		CD44v6m RNA/actin				*P* value
			–	+	+	+	
Lymph node metastasis	(+)	38	3	2	4	29	
	(-)	8	4	1	1	2	0.0131
Clinicopathology	I–II	12	5	2	2	3	
	III–IV	34	2	2	3	27	0.0005
Tissue type	D	32	2	2	4	25	
	I	14	5	2	4	3	0.0003
Tumor size	>5 cm	24	3	2	6	13	
	≤5 cm	22	4	3	4	11	0.4240

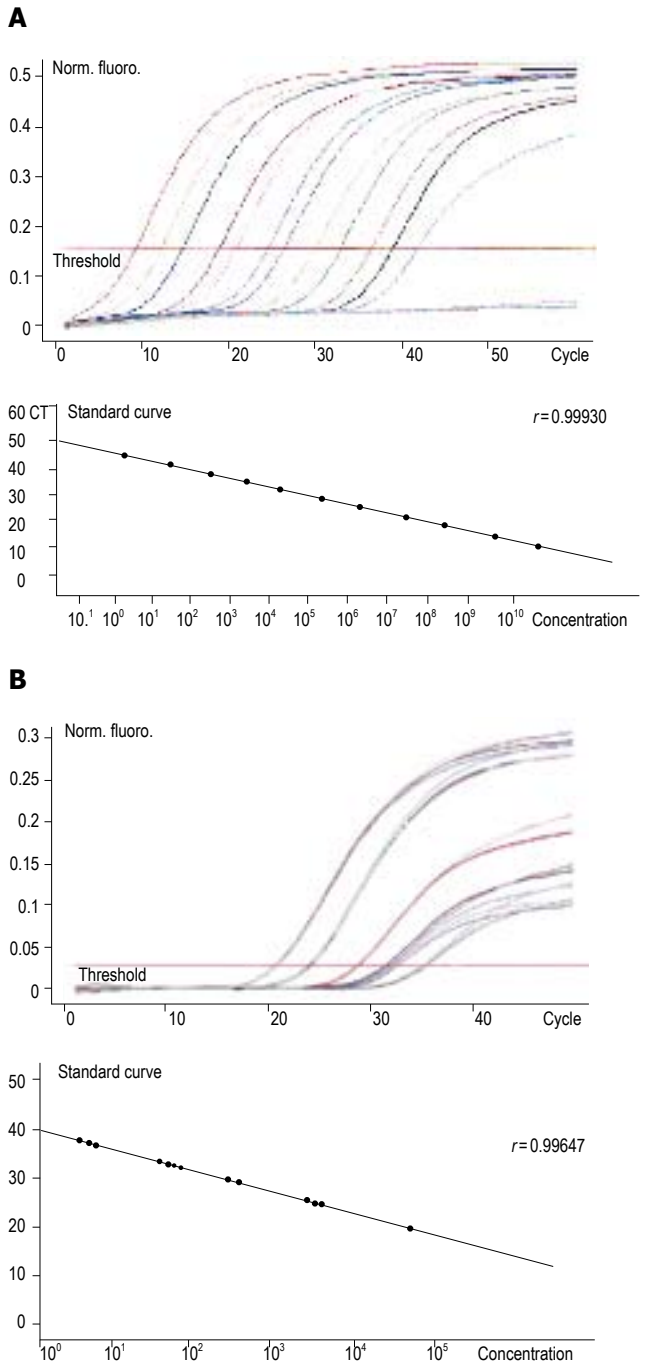


Figure 3 CD44v6mRNA expression in 20 peripheral blood samples from gastric cancer patients before (**A**) and after (**B**) surgery.

determined by the Student's t test and one-way analysis of variance. SPSS 11.0 for Windows 2000 was used. $P < 0.05$ was considered statistically significant.

RESULTS

Semi-quantitative analysis

Proliferation of CD44s in peripheral blood and bone marrow from patients with gastric cancer and controls is shown in Figure 1A.

CD44v6mRNA expression in bone marrow and peripheral blood was positive in 40 (86.9%) and 39 (84.8%) of 46 patients with gastric cancer. The positive CD44v expression was slightly higher in bone marrow than in peripheral blood ($P > 0.05$). The value of CD44v6mRNA expression was 64.6% ± 21.66% (Figure 1B).

In 46 patients with gastric cancer, diffuse type was found in 32 cases and intestinal type in 14 cases. Positive CD44v6mRNA expression in bone marrow was found in 31 (96.9%) of 32 diffuse type patients and 10 (71.4%) of 14 intestinal type patients (Figure 2A). On the other hand, positive CD44v6mRNA expression in peripheral blood was found in 30 (93.8%) of 32 diffuse type patients and 9 (64.3%) of 14 intestinal type patients (Figure 2B). There was a significant difference between the diffuse and intestinal types.

The relation between CD44v6mRNA expression and pathological type, lymph node metastasis, clinical pathology, and the size of tumor is presented in Tables 2 and 3.

Calibration curve and dynamic range of real-time RT-PCR

The calibration curve was constructed from the total RNA extracted from healthy human gastric serum diluted fourfold in mouse total RNA. The primer chosen to analyze the CD44v6 gene did not amplify human genomic DNA or mouse cDNA. The dynamic range was at least three orders of magnitude in samples containing 50 μg or 0.2 ng equivalent to total cDNA. A strong linear relation between Ct and log of the starting copy number was demonstrated ($r^2 \geq 0.99$). The efficiency of the reaction (E) was 90%-100% calculated by the formula: $E = 101/[m] - 1$, where m is the slope of calibration curve.

To determine the cut-off value for altered CD44v6 gene expression at the RNA level in gastric cancer serum, the CD44v6 value (ratio of CD44v6mRNA to β-actin) was determined for four normal gastric serum RNAs. Because this value fluctuated between 0.5 and 1.7, values of 3 or more were considered as overexpression of the CD44v6 gene in tumor RNA samples.

CD44v6mRNA status and clinical and pathological factors

We sought for links between CD44v6mRNA status and standard clinical, pathological and biological factors in gastric cancer. Significant association was found between the overexpression of CD44v6mRNA gene and standard histopathological grade ($P < 0.05$) and negative progesterone receptor status ($P < 0.001$). A trend toward a link between the overexpression of CD44v6 gene and

Table 4 Quantitative expression of CD44v6mRNA in peripheral blood of patients with gastric cancer before and after surgery

Id	Sex	Yr	Path	Metastasis	>5 cm	Histopathology	Real-time RT-PCR ($\times 10^2$ copies/μg RNA) before surgery	after surgery
1	M	56	D	+	T	III	3 100	300
2	M	52	I	–	F	II	4.9	2.0
3	W	60	D	+	T	IV	580	66
4	W	69	D	+	T	III	696	72
5	W	41	D	+	T	IV	3 200	460
6	M	39	D	+	T	IV	2 650	244
7	M	61	I	–	F	II	26	9.0
8	W	42	D	+	T	IV	360	48
9	M	71	I	–	F	II	590	72
10	M	61	I	–	F	II	430	58
11	M	41	D	+	T	II	1 230	142
12	W	47	D	+	T	IV	1 670	186
13	M	32	D	+	T	IV	1 340	166
14	M	49	I	+	T	III	280	36
15	W	61	D	+	T	II	1 080	128
16	M	76	I	+	F	I	480	68
17	M	81	I	–	F	II	36	6.9
18	W	60	I	+	F	III	290	42
19	M	37	D	+	T	III	980	112
20	W	40	D	+	T	IV	1 880	218
21	W	46	D	+	T	III	1 896	122
22	W	68	I	–	T	IV	2 208	342
23	M	72	I	–	T	III	1 960	140
24	M	64	I	+	F	II	868	22
25	W	51	D	+	T	III	1 020	100
26	M	32	D	+	T	IV	4 100	289
27	W	26	D	+	T	IV	3 908	432
28	M	72	I	–	F	II	680	107
29	W	74	I	–	T	III	1 024	210
30	M	61	D	+	T	IV	1 468	134
31	M	61	I		F	II	430	101
32	M	41	D	+	T	II	1 230	11
33	W	47	D	+	T	III	1 670	25
34	M	32	D	+	T	IV	1 340	40
35	M	49	I	+	T	I	280	11
36	W	61	D	+	T	II	1 080	120
37	M	76	I	+	F	I	480	91
38	M	81	I	–	F	I	36	137
39	W	60	I	+	F	II	290	43
40	M	37	D	+	T	III	980	69

estrogen receptor negativity was also observed ($P = 0.09$).

CD44v6mRNA amplification (patients for CD44v6mRNA expression)

As shown in Table 4, all the 20 peripheral blood samples of gastric cancer had the expression of CD44v6mRNA. The expression level ranged 4.9×10^8 - 3.2×10^{11} copies/g RNA and the average levels of peripheral blood was 3.9×10^{10} copies/g RNA (Figure 3A). The expression level of gastric cancer was 5.5×10^6 - 7.6×10^9 copies/g RNA and the average level was 2.4×10^8 copies/g RNA (Figure 3B) ($P = 0.00496$) after curative surgery.

DISCUSSION

Gastric cancer is one of the most frequent malignant tumors. The survival rate of patients after radical surgery of gastric cancer is 40% and patients always die because of metastasis and relapse[16]. Some patients have already existed micro-metastasis which is not detectable by general clinical pathology during the treatment. The detection of micro-metastasis is correlated with the prognosis of gastric cancer patients[17,18]. It was reported that micro-metastasis could be detected in lymph nodes of patients with intestinal cancer with negative pathology[19]. Micro-metastasis has been considered as an indicator of prognosis and the value of micro-metastasis is superior to the Duke's stage and tumor grade. Zhang *et al*.[20] demonstrated that tumor cells in metastatic lymph nodes of colorectal carcinoma possess cell proliferation activity and metastatic ability of tumor cells. Series slice examination has been used in inspecting tumor micro-metastasis since 1920s, but it is difficult to popularize[21]. RT-PCR is a sensitive and specific method which can find

a tumor cell from 1×10^6 peripheral blood monocytes[22]. Positive CD44v6mRNA expression was found in 39 of 46 patients with gastric cancer and negative CD44v6mRNA expression in patients with remote metastasis. The latter may be caused by sampling error[23].

In our research, the peripheral blood and bone marrow specimens from 46 patients with gastric cancer and 6 controls were compared. The positive CD44v6mRNA expression rate was 84.4% and 86.9%, respectively in patients with gastric cancer. The CD44v6mRNA expression rate of diffuse type cancers was higher than that of intestinal type cancers, suggesting that the expression of CD44v6mRNA is correlated with the malignant phenotype of gastric cancer and CD44v6mRNA can be used as an indicator of the degree of tumor invasion and lymph node metastasis[24]. The positive rate of CD44v6 is high in gastric cancer and may serve as a marker for diagnosing gastric cancer[25-27].

The CD44v6mRNA expression rate in patients with lymph node or remote metastasis was higher than that in those without lymph node metastasis. The positive micro-metastasis rate of grades III-IV gastric cancer was significantly higher than that of grades I-II gastric cancer, suggesting that the expression of CD44v6mRNA can be used as an indicator of relapse and metastasis. CD44v6 expression is a significant risk factor for lymph node metastasis in patients with advanced carcinoma. Expression of CD44v6 plays an important role in tumor progression and may be a useful predictor of lymph node metastasis[28-32].

The quantitative expression of CD44v6mRNA in 40 patients with gastric cancer before and after surgery showed that CD44v6mRNA was an indicator of tumor burden and therapeutic effect. The results showed that the expression level of CD44v6mRNA in peripheral blood of terminal gastric cancer patients (grades III-IV) was obviously higher than that in early gastric cancer patients (grades I-II). The expression level of CD44v6mRNA in gastric cancer patients with remote metastasis was obviously higher than that in those without metastasis. The expression level of CD44v6mRNA obviously decreased after curative surgery. The findings indicate that the expression level of CD44v6mRNA in peripheral blood is correlated with tumor burden.

Animal experiments indicate that 0.01% of tumor cells in circulation may lead to positive metastasis[31,33,34]. Gulmann *et al*[35] showed that CD44v6 expression is a late phenomenon in the transformation of intestinal metaplasia to dysplasia/cancer. Gene expression of severe gastric mucosal dysplasia displays an obviously latent malignant tendency and gastric carcinoma with the expression of CD44v6 protein has a stronger ability to infiltrate and to metastasize via lymph nodes[36]. Serum level of sCD44v6 could be taken as the criterion for evaluating the development and prognosis of gastric cancer, as well as the therapeutic target for anti-metastasis[37]. Therefore, micro-metastasis should be routinely detected to afford evidence for establishing individual therapy scheme[38]. Quantitative RT-PCR can improve the diagnostic sensitivity and specificity. Semi-quantitative RT-PCR is restricted because it cannot generate accurate gene quantification[15]. In our study, we have validated a RT-PCR method recently developed for the quantification of gene expression based on real-time analysis of PCR amplification and TaqMan methodology, which has several advantages over other RT-PCR-based quantitative assays such as competitive quantitative RT-PCR[39].

Xin *et al*[40,41] discovered that patients with positive CD44v6 have a lower 3- and 5-year survival rate ($P = 0.0002$). Immunohistochemical detection of CD44v6 could now be used as an indicator of tumor progression in patients with gastric carcinoma. Tumor cells in the bone marrow indicate that patients have blood micro-metastasis[42]. The probability of relapse and remote metastasis is great for patients who have postoperative bone marrow micro-metastasis and dynamic observation of micro-metastasis in these patients can predict the therapeutic effect and establish individual therapy scheme. Yamaguchi *et al*.[43] found that CD44v6-positive cancers are more frequently associated with hematogenous metastasis. Therefore, blood is another metastatic route of gastric cancer. Detecting peripheral blood micro-metastasis of early gastric cancer patients is of great importance in predicting the prognosis and deciding the rational therapy of gastric cancer patients.

REFERENCES

1 **Bustin SA**, Gyselman VG, Williams NS, Dorudi S. Detection of cytokeratins 19/20 and guanylyl cyclase C in peripheral blood of colorectal cancer patients. *Br J Cancer* 1999; **79**: 1813-1820

2 **Yuan HY**, Cheng FL, Wei ZZ, Yang GL, Chen JK. [Clinical significance of detecting lymph node micrometastasis of colorectal cancer by reverse transcriptase-polymerase chain reaction (RT-PCR)]. *Ai Zheng* 2004; **23**: 1069-1073

3 **Takayama O**, Yamamoto H, Ikeda K, Ishida H, Kato T, Okuyama M, Kanou T, Fukunaga M, Tominaga S, Morita S, Fujie Y, Fukunaga H, Ikenaga M, Ikeda M, Ohue M, Sekimoto M, Kikkawa N, Monden M. Application of RT-PCR to clinical diagnosis of micrometastasis of colorectal cancer: A translational research study. *Int J Oncol* 2004; **25**: 597-604

4 **Xia JZ**, Zhu ZG, Liu BY, Yan M, Yin HR. Significance of immunohistochemically demonstrated micrometastases to lymph nodes in gastric carcinomas. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 1113-1116

5 **Peck K**, Sher YP, Shih JY, Roffler SR, Wu CW, Yang PC. Detection and quantitation of circulating cancer cells in the peripheral blood of lung cancer patients. *Cancer Res* 1998; **58**: 2761-2765

6 **Noguchi S**, Hiratsuka M, Furukawa H, Aihara T, Kasugai T, Tamura S, Imaoka S, Koyama H, Iwanaga T. Detection of gastric cancer micrometastases in lymph nodes by amplification of keratin 19 mRNA with reverse transcriptase-polymerase chain reaction. *Jpn J Cancer Res* 1996; **87**: 650-654

7 **Jothy S**. CD44 and its partners in metastasis. *Clin Exp Metastasis* 2003; **20**: 195-201

8 **Picker LJ**, Nakache M, Butcher EC. Monoclonal antibodies to human lymphocyte homing receptors define a novel class of adhesion molecules on diverse cell types. *J Cell Biol* 1989; **109**: 927-937

9 **Screaton GR**, Bell MV, Jackson DG, Cornelis FB, Gerth U, Bell JI. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc Natl Acad Sci U S A* 1992; **89**: 12160-12164

10 **Harn HJ**, Isola N, Cooper DL. The multispecific cell adhesion molecule CD44 is represented in reticulocyte cDNA. *Biochem Biophys Res Commun* 1991; **178**: 1127-1134

11 **Mayer B**, Jauch KW, Günthert U, Figdor CG, Schildberg FW,

Funke I, Johnson JP. De-novo expression of CD44 and survival in gastric cancer. *Lancet* 1993; **342**: 1019-1022

12 **Li Y**, Zhang JH, Kuang G, Yang JQ, Zhao Q, Wang XL, Jiao ZK, Zhang ZD, Wang LL. Expresson of MUC,CD44v6,nm23 in gastric carcinomas and regional lymph node tissues and their association with invasion, metastasis, and prognosis the tumor. *Zhonghua Aizheng Zazhi* 2003; **22**: 985-989

13 **Jiang HG**, Tang WP, Liao XB, Cao J, Wu DM, Lin H. Significance and expression of CD44v6 and E-cadherin in gastric carcinomas and their lymph-node metastases. *Zhonghua Linchuang Weichangbingxue Zazhi* 2001; **13**: 60-63

14 **Wang DR**, Chen GY,Liu XL, Miao Y, Pan SY, Xia JG, Wu ZY. The serum concentration of soluble CD44v6 and the expression of CD44v6 in patients with gastric carcinoma. *Nanjing Daxue Xuebao* (Yixueban) 2002; **22**: 365-367

15 **Gibson UE**, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res* 1996; **6**: 995-1001

16 **Yang L**, Xia JG, Zheng SZ, Zhang XW, Chen GY, Xu ZQ, Yang HY, Zhou HB. The radioimmunoimaging of131I labelled CD44v6 monoclonal antibody in nude mice with metastatic focus of gastric cancer in vivo. *Nanjing Daxue Xuebao* (Yixueban) 2002; **22**: 282-283

17 **Liu ZH**, Li CM, Wang XJ, Long PB. Clinical application of gene detectionin micrometastases of gastric cancer. *Shijie Huaren Xiaohua Zazh*i 2004; **12**: 2033-2035

18 **Chen XM**, Chen GY, Wang ZR, Zhu FS, Wang XL, Zhang X. Detection of micrometastasis of gastric carcinoma in peripheral blood circulation. *World J Gastroenterol* 2004; **10**: 804-808

19 **Hayashi N**, Ito I, Yanagisawa A, Kato Y, Nakamori S, Imaoka S, Watanabe H, Ogawa M, Nakamura Y. Genetic diagnosis of lymph-node metastasis in colorectal cancer. *Lancet* 1995; **345**: 1257-1259

20 **Zhang JC**, Wang ZR, Cheng YJ, Yang DZ, Shi JS, Liang AL, Liu NN, Wang XM. Expression of proliferating cell nuclear antigen and CD44 variant exon 6 in primary tumors and corresponding lymph node metastases of colorectal carcinoma with Dukes' stage C or D. *World J Gastroenterol* 2003; **9**: 1482-1486

21 **Sun XW**, Shen BZ, Shi MS, Dai XD. Relationship between CD44v6 expression and risk factors in gastric carcinoma patients. *Shijie Huaren Xiaohua Zazhi* 2002; **10**: 1129-1132

22 **Zhou Y**, Zhu WQ, Shi YQ, Lu HF, Shi DR. Expression and prognostic significance of CD44v6 in primary gastric carcinoma. *Zhongguo Zhongliu* 2002; **12**: 391-394

23 **Chen Y**, Feng LM, Zhong BY, Xu Q, Xu GY, Wang L, Zhou DH. Detection of serum soluble CD44v6 and its clinical application in the patients with gastric carcinoma. *Zhongguo Shiyan Zhenduan* 2001; **5**: 169-170

24 **Lu PL**, JI F, Peng KR, Cui JH, Yu CH, Li YM. The expression of CD44v6 mRNA in human gastric cancer. *Zhejiang Yixue Zazhi* 2004; **26**: 184-186

25 **He Y**, Zhang L, Zhang PQ, Zhong ZJ, Jin XM. The expression and prognosis of tumor metastasis associated genes CD44v6 and p53 in the gastric carcinomas. *Beihua Daxue Xuebao* (Nat Sci) 2002; **3**: 399-401

26 **Zhou Y**, Xu H, Wang ZZ. Expression of Cd44v6 and PCNA in gastric cancer. *Zhengzhou Daxue Xuebao* (Med Sci) 2002; **37**: 308-310

27 **Peng AB**, Shi W, Hu SH, Zhao Q. [Expression of CD44v6 in gastric cancer and its correlation with Helicobacter pylori infection]. *Ai Zheng* 2003; **22**: 1184-1187

28 **Li Y**, Zhang JH, Kuang G, Yang JQ, Zhao Q, Wang XL, Jiao ZK, Zhang ZD, Wang LL. [Expression of MUC1, CD44v6, nm23 in gastric carcinomas and regional lymph node tissues and their association with invasion, metastasis, and prognosis of the tumor]. *Ai Zheng* 2003; **22**: 985-989

29 **Joo M**, Lee HK, Kang YK. Expression of E-cadherin, beta-catenin, CD44s and CD44v6 in gastric adenocarcinoma: relationship with lymph node metastasis. *Anticancer Res* 2003; **23**: 1581-1588

30 **Zhang S**, Li L, Lin H. [A multianalysis study on clinicopathologic factors related to lymph node metastasis in gastric cancer]. *Zhonghua Zhongliu Zazhi* 2001; **23**: 399-402

31 **Zhang JH**, Li Y, Wang XL, Cao YM, Kuang G, Wang LL. Relation between expressions of CD44v6,nm23-H1 in gastric carcinoma and lymph node tissues and clincal biological behavior. *Zhongguo Linchuang Zhongliu Zazhi* 2004; **31**: 256-258

32 **Wang DR**, Chen GY,Liu XL, Miao Y, Pan SY, Xia JG, Wu ZY. The serum content of soluble CD44v6 and the expression of CD44v6 in patients with gastric carcinoma. *Zhonghua Shiyan Waike Zazhi* 2002; **19**: 308-309

33 **Zhou Y**, Zong H, Xu H. Expression of CD44v6 in gastric cancer and its adjacent cancer tissues. *Zhengzhou Daxue Xuebao* (Med Sci) 2002; **37**: 593-595

34 **Fan KX**, Zhong WX, Sun LP, Li WM. Study on the expression of p16 and CD44v6 in gastric cancer. *Shiyong Zhongliuxue Zazhi* 2000; **15**: 391-393

35 **Gulmann C**, Grace A, Leader M, Butler D, Patchett S, Kay E. CD44v6: a potential marker of malignant transformation in intestinal metaplasia of the stomach? An immunohistochemical study using tissue microarrays. *Eur J Gastroenterol Hepatol* 2003; **15**: 981-986

36 **Mi JQ**, Zhang ZH, Shen MC. Significance of CD44v6 protein expression in gastric carcinoma and precancerous lesions. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 156-158

37 **Chen X**, Ouyang XN, Dai XH, Chen MH, Lin QC. [Relationship between sCD44v6 expression and TCM differentiation type of gastric carcinoma patients and influence of weitai capsule on the expression]. *Zhongguo Zhongxiyi Jiehe Zazhi* 2005; **25**: 12-15

38 **Qiu WC**, Chen G, Ding YT.The relationship between micrometastasis of gastric carcinoma and its prognosis. *Zhonghua Putong Waike Zazh*i 2003; **12**: 535-537

39 **Shi Y,** Zhao QZ. Advance in Adenovirus EIA Gene Research. *Zhongguo Zhongliu Shengwu Zhiliao Zazhi* 1999; **6**: 158-160

40 **Xin Y**, Grace A, Gallagher MM, Curran BT, Leader MB, Kay EW. CD44V6 in gastric carcinoma: a marker of tumor progression. *Appl Immunohistochem Mol Morphol* 2001; **9**: 138-142

41 **Xin Y**, Zhao FK, Zhang SM, Wu DY, Wang YP, Xu L. Relationship between Cd44v6 expression and prognosis in gastric carcinoma patients. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 210-214

42 **Shang XW**, Xu YF. The expression and chinical significance of CD44 splice variant V6 in human gastric cancer. *Zhongliu Yanjiu Yu Linchuang* 2001; **13**: 17-18

43 **Yamaguchi A**, Goi T, Yu J, Hirono Y, Ishida M, Iida A, Kimura T, Takeuchi K, Katayama K, Hirose K. Expression of CD44v6 in advanced gastric cancer and its relationship to hematogenous metastasis and long-term prognosis. *J Surg Oncol* 2002; **79**: 230-235

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 43-47
World Journal of Gastroenterology ISSN 1007-9327

Usefulness of endoscopic ultrasonography in preoperative TNM staging of gastric cancer

Tumur Tsendsuren, Sun-Ming Jun, Xu-Hui Mian

Tumur Tsendsuren, Department of Oncology, No. 1 Hospital, China Medical University, Shenyang 110001, Liaoning Province, China
Sun-Ming Jun, Department of Endoscopy, First Hospital, China Medical University, Shenyang 110001, Liaoning Province, China
Xu-Hui Mian, Department of Oncology, First Hospital, China Medical University, Shenyang 110001, Liaoning Province, China
Co-first-authors: Tumur Tsendsuren and Sun-Ming Jun
Co-correspondent: Tumur Tsendsuren
Correspondence to: Sun-Ming Jun, Department of Endoscopy, First Hospital, China Medical University, Shenyang 110001, Liaoning Province, China. smjmw@sina.com
Telephone: +86-24-81012947
Received: 2005-06-24 Accepted: 2005-07-15

Abstract

AIM: To evaluate the value of endoscopic ultrasonography (EUS) in the preoperative TNM staging of gastric cancer.

METHODS : Forty-one patients with gastric cancer (12 early stage and 29 advanced stage) proved by esophagogastroduodenoscopy and biopsies preoperatively evaluated with EUS according to TNM (1997) classification of International Union Contrele Cancer (UICC). Pentax EG-3630U/Hitachi EUB-525 echo endoscope with real-time ultrasound imaging linear scanning transducers (7.5 and 5.0 MHz) and Doppler information was used in the current study. EUS staging procedures for tumor depth of invasion (T stage) were performed according to the widely accepted five-layer structure of the gastric wall. All patients underwent surgery. Diagnostic accuracy of EUS for TNM staging of gastric cancer was determined by comparing preoperative EUS with subsequent postoperative histopathologic findings.

RESULTS: The overall diagnostic accuracy of EUS in preoperative determination of cancer depth of invasion was 68.3% (41/28) and 83.3% (12/10), 60% (20/12), 100% (5/5), 25% (4/1) for T1, T2, T3, and T4, respectively. The rates for overstaging and understaging were 24.4% (41/10), and 7.3% (41/3), respectively. EUS tended to overstage T criteria, and main reasons for overstaging were thickening of the gastric wall due to perifocal inflammatory change, and absence of serosal layer in certain areas of the stomach. The diagnostic accuracy of metastatic lymph node involvement or N staging of EUS was 100% (17/17) for N0 and 41.7% (24/10) for N+, respectively, and 66% (41/27) overall. Misdiagnosing of the metastatic lymph nodes was related to the difficulty of distinguishing inflammatory lymph nodes from malignant lymph nodes, which imitate similar echo features. Predominant location and distribution of tumors in the stomach were in the antrum (20 patients), and the lesser curvature (17 patients), respectively. Three cases were found as surgically unresectable (T4 N+), and included as being correctly diagnosed by EUS.

CONCLUSION: EUS is a useful diagnostic method for preoperative staging of gastric cancer for T and N criteria. However, EUS evaluation of malignant lymph nodes is still unsatisfactory.

Key words: Endoscopic ultrasonography; Preoperative staging; Gastric cancer

Tsendsuren T, Jun SM, Mian XH. Usefulness of endoscopic ultrasonography in preoperative TNM staging of gastric cancer. *World J Gastroenterol* 2006; 12(1):43-47

http://www.wjgnet.com/1007-9327/12/43.asp

INTRODUCTION

The incidence of gastric cancer is declining worldwide. However, it still remains the second most common cause of cancer-related death in the world[1,2]. Typically, gastric cancer is asymptomatic when cancer is at early stage of disease; therefore, majority of patients present in advanced stage, and the mortality rate of this disease is still very high. The diagnosis of gastric cancer is based on esophagogastroduodenoscopy with biopsy following double-contrast x-ray examination. Presently, endoscopic ultrasonography is the most reliable nonsurgical method obtainable for assessing the primary tumor with high diagnostic rate of staging gastric cancer and lymph node involvement. EUS is also becoming a promising diagnostic modality for the evaluation of gastrointestinal submucosal tumors and large gastric folds[3-7].

The complete treatment of gastric cancer is surgery, only tumor resection with involved lymph nodes associated with satisfactory prognosis. Survival after surgery is highly dependent on the stage of gastric cancer or anatomical extent of disease at the time of operation. Therefore, the accurate preoperative staging of gastric cancer is the most significant prognostic factor that predicts surgical outcome

Table 1 Relationship between EUS and anatomic layers of normal gastric wall

EUS	Histology
1st hypoechoic layer	Water interface and superficial mucosa
2nd hypoechoic layer	Deeper mucosa
3rd hyperechoic layer	Submucosa
4th hypoechoic layer	Muscularis propria
5th hyperechoic layer	Serosa and subserosal fat

Table 2 Correlation of UICC/AJCC classification for depth of primary esophageal or gastric cancer invasion (T) with EUS imaging for clinical staging[9]

Stage	EUS (abnormal)
T1-mucosa/submucosa	1st three layers
T2-muscularis propria	4th layer
T3-through adventitia/serosa	5th layer
T4-adjacent organ	Adjacent organ

Table 3 Accuracy of EUS in preoperative stage determination of 41 patients with gastric cancer

Histopathological T stage	*n*	EUS correct *n*/%	EUS over-staging *n*/%	EUS under-staging *n*/%
PT1	12	T1 10/83.3	T2 2/16.7	–
PT2	20	T2 12/60	T3 8/40	–
PT3	5	T3 5/100	–	–
PT4	4 (3)	T4 1/25	–	T2 2 (2)/50 T3 1 (1)/25
Total	41 (3)	28/68.3	10/24.4	3 (3)/7.3

Three cases of unresectable T4 N+ tumors were correctly diagnosed.

Table 4 Accuracy of EUS in preoperative determination of N stage in 41 patients with gastric cancer

Histopathological N stage	*n*	EUS correct *n*/%	EUS incorrect *n*/%
PN0	17	N 17/100	–
PN+	24 (3)	N+ 10 (3)/41.7	N0 14/58.3 false negative
All cases	41 (3)	27 (3)/66	14/34

and 5 years of survival and is essential for well-informed decisions on stage depending patient management to plan appropriate treatment. Such precise stage depending management will limit the occurrence of unnecessary exploratory surgical interventions[8].

The aim of the present study was to evaluate the usefulness of EUS in TNM staging of stomach cancer comparing with postoperative histopathological findings.

MATERIALS AND METHODS

Patients

Between April 2001 and April 2004, 41 patients (29 men and 12 women; age range, 28 - 80 years; mean age 57 years) with gastric cancer diagnosed by EGD and confirmed with biopsy specimen, underwent EUS examination prior to surgery for tumor depth of invasion and lymph node involvement at our Department of Endoscopy. Twelve of them were in early gastric cancer stage and 29 were in advanced stage. All patients underwent surgery.

Apparatus and EUS examination procedures

The Pentax EG - 3630U/Hitachi EUB - 525 echo endoscope with real-time ultrasound imaging linear scanning transducers (7.5 and 5.0 MHz) and Doppler information was used in the present study. This echo endoscope also provides the instrument channel for performing fine-needle aspiration biopsy. On the tip of the endoscope, a balloon is placed which is filled by deaerated water for improved coupling of the ultrasound waves to the gastrointestinal wall by producing a fluid interface and displacing intraluminal air. Prior to each EUS, examination was performed by EGD with biopsy to confirm gastric cancer. After oropharyngeal local anesthesia, patients were examined in a left lateral position. The echo endoscope was advanced into the stomach, and the lesions were first examined endoscopically. Next the stomach was insufflated with 200 - 500 mL deaerated water and observed from the pylorus to the cardia by moving the tip of the endoscope for revealing cancer abnormalities and lymph nodes involvement. The findings were recorded at the computer database of our department and interpreted following a standard protocol with regard to tumor invasion according to the widely accepted five-layer structure of the gastric wall (Table 1).

The assessment of tumor invasion depth or T stage was defined as a hypoechoic structure alternating five-layer ultrasonographic structure of gastric wall. Tumors were staged according to TNM (1997) classification criteria of International Union contrele Cancer (UICC). T1 lesion was seen as a disruption of the first three layers (tumor invades the mucosa or submucosa). T2 lesion was seen as an invasion of the fourth layer (tumor invades the muscularis propria). T3 lesion was seen as a penetration through the fifth layer (tumor invasion of the serosa). T4 lesion was seen as an invasion of the adjacent organs and structures (Table 2). T1 stage showed EUS images of early gastric cancer, T2-T4 stages showed EUS images of advanced gastric cancer. Lymph nodes had round border and hypoechoic structures were considered as malignant. Stage N0 referred to no sign of metastasis. N+ referred to metastases in perigastric lymph nodes.

RESULTS

Findings of the 41 patients at preoperative EUS were postoperatively compared with histopathologic findings for T and N staging.

Surgical findings

Tumors were located in the fundus and cardia region ($n = 4$) , in the body ($n = 11$), in the body and antrum ($n = 2$), in the antrum ($n = 20$) of the stomach, diffusely located ($n=2$) and residual stomach ($n = 2$). Distribution of tumors was in the anterior wall ($n = 5$), posterior wall ($n = 5$), greater curvature ($n = 5$), and the lesser curvature ($n = 17$) of the stomach and 9 were circumferential. Three cases

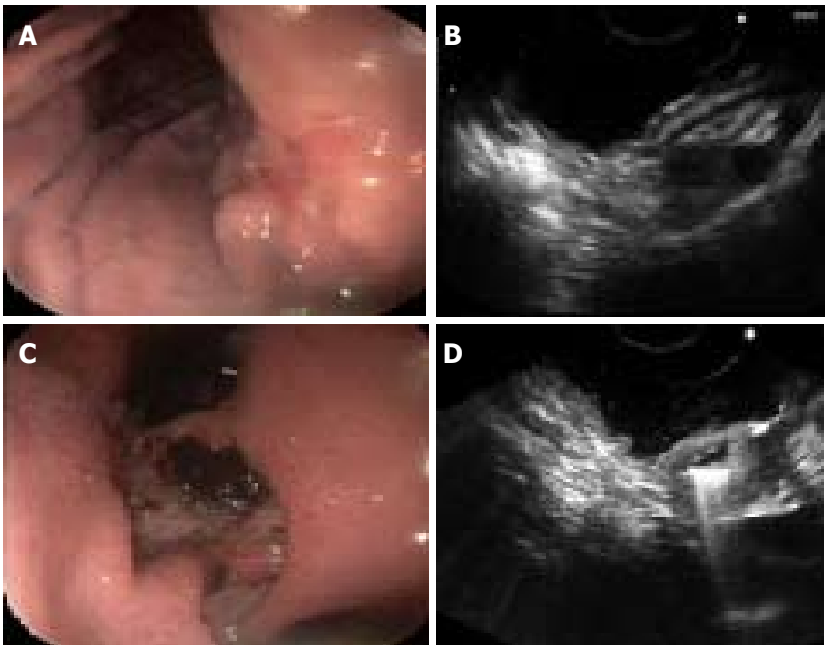


Figure 1 Early and advanced gastric cancer cases. **A:** Endoscopic view of superficial depressed type of early gastric cancer; **B:** EUS image shows cancer invasion of 1st and 2nd (mucosal) layers of gastric wall, while 3rd (submucosal) layer is clear (T1 category). Histopathological findings of the surgically resected specimen corresponded with the EUS findings; **C:** Endoscopic view of advanced Borrmann II type of gastric cancer; **D:** EUS images show disruption of 1-4 layers of the gastric wall with hypoechoic cancer tissue, but 5th (serosal) layer is not involved (T2 category).

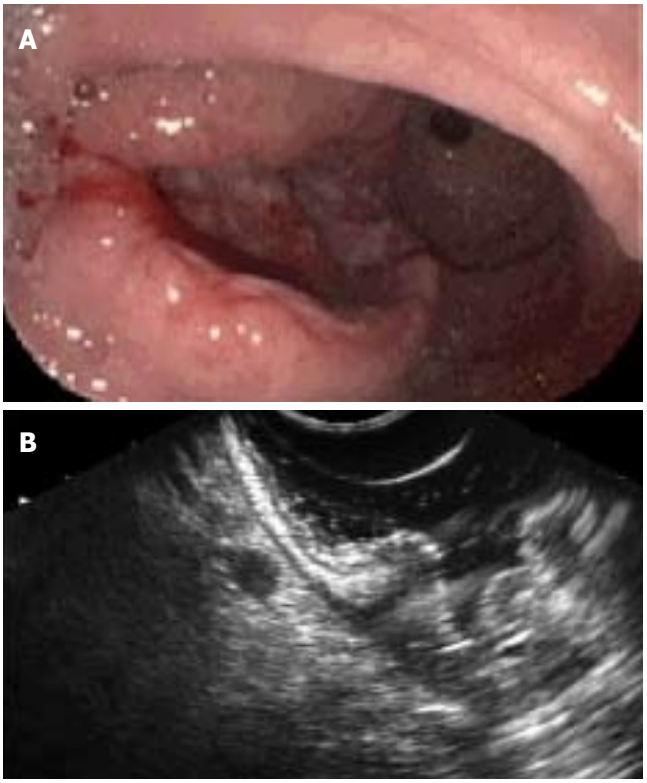


Figure 2 A case of advanced gastric cancer. **A:** Endoscopic view of Borrmann III type of gastric cancer; **B:** EUS image demonstrates T3 cancer with malignant lymph node. Note the hypoechoic structure and sharp margin of the lymph node (1.0 cm×0.6 cm).

were found as surgically unresectable (T4 N+).

Pathohistologic findings

T staging: The diagnostic accuracy of EUS was 83.3% in T1 staging, 60.0% in T2 staging, 100% in T3 staging, and 25% in T4 staging, respectively. Twenty-eight of forty-one cancers were staged correctly and the overall diagnostic accuracy of T stage was 68.3%. Ten cases were overstaged (24.4%) and 3 cases were understaged (7.3%) (Table 3). Echoendoscopic features of early and advanced gastric cancer are presented in (Figures 1A-1D).

N staging: EUS correctly determined 27 of 41 patients with the overall accuracy of 66.0%. The accuracy of EUS in N0 staging was high, all 17 patients without malignant lymph node metastasis were diagnosed correctly. However, EUS findings of preoperative positive metastatic lymph nodes in 10 patients were not confirmed histopathologically, and the accuracy of EUS in N+ staging was 41.7% (Table 4). The endosonographic features of advanced gastric cancer with malignant lymph nodes are shown in Figure 2.

DISCUSSION

The accurate staging of gastric cancer is the most important prognostic factor for patient management and EUS is the most reliable method in T and N staging of gastric cancer with high diagnostic rates. Such an accurate staging will apply the stage-depending correct management of the patients (radical surgery or palliative treatment) and will provide a great benefit avoiding unnecessary laparotomy on patients with unresectable disease. EUS is considered as the most accurate modality for T staging of gastric cancer in comparison with CT and intraoperative assessment[10,11].

The accuracy of EUS for gastric cancer from different authors ranges 64.8% - 92% in T staging and 50% - 90% in N staging (Table 5). These studies demonstrated that EUS is the most accurate staging method for gastric cancer with a few incidences of overstaging and understaging. The excellent results of accuracy of both T and N staging are shown in a study by Botet *et al.*[12] to be 92% and 78%, respectively. The high accuracy of EUS in preoperative staging of gastric cancer is proved by our results. In the current study, EUS had a diagnostic accuracy of 68.3% for tumor invasion. EUS had 24.4% overstaging in T staging, 2 of the 12 T1 tumors overstaged as T2, 3 of the 20 T2 tumors overstaged as T3. The main reason of

Table 5 Literalure summary of EUS studies on gastric cancer

	Author	Period	Number of patients	Accuracy (%)	
				T stage	N stage
1	Botet *et al*.[12] (USA)	1986–1988	50	92	78
2	Akahoshi *et al*.[13] (Japan)	1986–1990	74	81.1	50
3	Ziegler *et al*.[11] (Germany)	1986–1990	108	86	74
4	Lightdale[9] (USA)	1989–1991	525	81	76
5	Dittler *et al*[14] (Germany)	1989–1992	264	83	66
6	Francois *et al*.[15] (France)	1991–1993	35	79	79
7	Yanai *et al*.[16] (Japan)	1990–1995	104	64.8	Early stage
8	Meining *et al*.[17] (Germany)	1992–1996	33	66	Not reported
9	Yanai *et al*.[18] (Japan)	1996–1997	52	71	Early stage
10	Guo *et al*.[19] (China)	1996–1997	62	83.9	79
11	Hunerbein *et al*.[20] (Germany)	1997[1]	30	82	80
12	Habermann *et al*.[21] (Germany)	1998–2000	51	86	90
13	Hizawa *et al*.[22] (Japan)	1997–2002	234	78	Early stage
14	Xi *et al*.[23] (China)	2002[1]	32	80	68.6
15	Shimoyama *et al*.[24] (Japan)	1996–2003	45	71	80
16	Bhandari *et al*.[10] (Korea)	2003	63	87.5	79.1

[1]Year of article publication/duration of study not reported.

overstaging in T1 cancer is the thickening of gastric wall due to perifocal inflammatory reaction, which is difficult to distinguish from cancer tissue and imitates the presence of T2 cancer. Absence of serosal layer in certain regions of the stomach, the lesser curvature, the posterior wall of fundus and the anterior wall of antrum is the reason for overstaging T2 cancer. Cancers of these areas are classified histopathologically as T2 cancer, even carcinoma infiltrates through the whole gastric wall, because no serosal infiltration can be found.

EUS accuracy of metastatic lymph node involvement was 66% in the present study. Such slightly lower accuracy is related to the absence of standard differential echoendoscopic criteria for benign and malignant lymph nodes. Echoendoscopic features of metastatic lymph nodes from different authors include size > 10 mm, rounded structure, sharp demarcation of borders, and hypoechoic (dark) structure[25,26]. However, endoscopic ultrasonographic detection of metastatic lymph nodes is complicated, due to the difficulty of differentiation between malignant and inflammatory lymph nodes. Francois *et al*.[15] described that hypoechoic lymph nodes with well-defined margins and largest diameter/smallest diameter ratio less than 2 are considered to be malignant. Dittler and Siewert[14] noticed that, if EUS does not diagnose malignant lymph nodes in T1 or T2 stage, stage N0 can be assumed; if lymph nodes are visualized in stages T3 and T4, then they tend to be malignant. Results of certain studies[27] demonstrated that the EUS-guided fine-needle aspiration biopsy would be very useful to distinguish between benign and malignant lymph nodes.

Other reasons for inaccuracy of evaluation of tumor lymph nodes are related to the limited depth of transducer, and unsatisfactory visualization of distant lymph node by EUS. EUS cannot permit the assessment of tissue beyond the depth of about 5-6 cm.

The presence of ascites in gastric cancer patients is a poor prognostic sign and implies the presence of peritoneal metastasis. EUS-guided fine-needle aspiration biopsy also has been successfully used to detect malignant ascites[28-30].EUS detection of distant metastatic lymph nodes and distant metastasis or M staging of gastric cancer is insufficient due to limited penetration depth of this method as mentioned above. Therefore, combined use of EUS and CT, which is superior to EUS for gaining information about distant metastasis, should be effective for the management of gastric cancer patients for appropriate treatment options.

In conclusion, EUS is a useful diagnostic method for accurate preoperative staging for T and N criteria for gastric cancer. The accurate preoperative staging is extremely essential for proper stage-depending patient management, which improves the 5-year survival rate of this dismal prognostic disease. However, EUS evaluation of malignant lymph nodes is still unsatisfactory. Therefore, great effort should be taken to study differential criteria of malignant lymph nodes from benign lymph nodes.

REFERENCES

1 **Mehta VK**, Fisher G. Gastric cancer. Accessed 2004-11-03. Available from: URL: http: //www.emedicine.com/med/topic845.htm

2 **Neugut AI**, Hayek M, Howe G. Epidemiology of gastric cancer. *Semin Oncol* 1996; **23**: 281-291

3 **Chak A**. EUS in submucosal tumors. *Gastrointest Endosc* 2002; **56**: S43-S48

4 **Rösch T**, Kapfer B, Will U, Baronius W, Strobel M, Lorenz R, Ulm K. Accuracy of endoscopic ultrasonography in upper gastrointestinal submucosal lesions: a prospective multicenter study. *Scand J Gastroenterol* 2002; **37**: 856-862

5 **Chak A**, Canto MI, Rosch T, Dittler HJ, Hawes RH, Tio TL, Lightdale CJ, Boyce HW, Scheiman J, Carpenter SL, Van Dam J, Kochman ML, Sivak MV Jr. Endosonographic differentiation of benign and malignant stromal cell tumors. *Gastrointest Endosc* 1997; **45**: 468-473

6 **Zhang QL**, Nian WD. Endoscopic ultrasonography diagnosis in submucosal tumor of stomach. *Endoscopy* 1998; **30**(suppl 1): A69-A71

7 **Caletti G**, Fusaroli P, Bocus P. Endoscopic ultrasonography in large gastric folds. *Endoscopy* 1998; **30**(suppl 1): A72-A75

8 **Davies J**, Chalmers AG, Sue-Ling HM, May J, Miller GV, Martin IG, Johnston D. Spiral computed tomography and operative staging of gastric carcinoma: a comparison with histopathological staging. *Gut* 1997; **41**: 314-319

9 **Bhandari S**, Shim CS, Kim JH, Jung IS, Cho JY, Lee JS Lee MS, Kim BS. Usefulness of three-dimensional, multidetector row CT (virtual gastroscopy and multiplanar reconstruction) in the

evaluation of gastric cancer: a comparison with conventional endoscopy, EUS and histopathology. *Gastrointest Endosc* 2004; **59**: 619-626

10 **Ziegler K**, Sanft C, Zimmer T, Zeitz M, Felsenberg D, Stein H, Germer C, Deutschmann C, Riecken EO. Comparison of computed tomography, endosonography, and intraoperative assessment in TN staging of gastric carcinoma. *Gut* 1993; **34**: 604-610

11 **Botet JF**, Lightdale CJ, Zauber AG, Gerdes H, Winawer SJ, Urmacher C, Brennan MF. Preoperative staging of gastric cancer: comparison of endoscopic US and dynamic CT. *Radiology* 1991; **181**: 426-432

12 **Akahoshi K**, Misawa T, Fujishima H, Chijiiwa Y, Maruoka A, Ohkubo A, Nawata H. Preoperative evaluation of gastric cancer by endoscopic ultrasound. *Gut* 1991; **32**: 479-482

13 **Lightdale CJ**. Endoscopic ultrasonography in the diagnosis, staging and follow-up of esophageal and gastric cancer. *Endoscopy* 1992; **24 Suppl 1**: 297-303

14 **Dittler HJ**, Siewert JR. Role of endoscopic ultrasonography in gastric carcinoma. *Endoscopy* 1993; **25**: 162-166

15 **Francois E**, Peroux J, Mouroux J, Chazalle M, Hastier P, Ferrero J, Simon J, Bourry J. Preoperative endosonographic staging of cancer of the cardia. *Abdom Imaging* 1996; **21**: 483-487

16 **Yanai H**, Matsumoto Y, Harada T, Nishiaki M, Tokiyama H, Shigemitsu T, Tada M, Okita K. Endoscopic ultrasonography and endoscopy for staging depth of invasion in early gastric cancer: a pilot study. *Gastrointest Endosc* 1997; **46**: 212-216

17 **Meining A**, Dittler HJ, Wolf A, Lorenz R, Schusdziarra V, Siewert JR, Classen M, Höfler H, Rösch T. You get what you expect? A critical appraisal of imaging methodology in endosonographic cancer staging. *Gut* 2002; **50**: 599-603

18 **Yanai H**, Noguchi T, Mizumachi S, Tokiyama H, Nakamura H, Tada M, Okita K. A blind comparison of the effectiveness of endoscopic ultrasonography and endoscopy in staging early gastric cancer. *Gut* 1999; **44**: 361-365

19 **Guo W**, Zhang YL, Li GX, Zhou DY, Zhang WD. Comparison of preoperative staging of gastric carcinoma by endoscopic ultrasonography with CT examination. *China Natl J New Gastroenterol* 1997; **3**: 242-245

20 **Hünerbein M**, Ghadimi BM, Haensch W, Schlag PM. Transendoscopic ultrasound of esophageal and gastric cancer using miniaturized ultrasound catheter probes. *Gastrointest Endosc* 1998; **48**: 371-375

21 **Habermann CR**, Weiss F, Riecken R, Honarpisheh H, Bohnacker S, Staedtler C, Dieckmann C, Schoder V, Adam G. Preoperative staging of gastric adenocarcinoma: comparison of helical CT and endoscopic US. *Radiology* 2004; **230**: 465-471

22 **Hizawa K**, Iwai K, Esaki M, Matsumoto T, Suekane H, Iida M. Is endoscopic ultrasonography indispensable in assessing the appropriateness of endoscopic resection for gastric cancer? *Endoscopy* 2002; **34**: 973-978

23 **Xi WD**, Zhao C, Ren GS. Endoscopic ultrasonography in preoperative staging of gastric cancer: determination of tumor invasion depth, nodal involvement and surgical resectability. *World J Gastroenterol* 2003; **9**: 254-257

24 **Shimoyama S**, Yasuda H, Hashimoto M, Tatsutomi Y, Aoki F, Mafune K, Kaminishi M. Accuracy of linear-array EUS for preoperative staging of gastric cardia cancer. *Gastrointest Endosc* 2004; **60**: 50-55 **25 Grimm H**, Binmoeller KF, Hamper K, Koch J, Henne-Bruns D, Soehendra N. Endosonography for preoperative locoregional staging of esophageal and gastric cancer. *Endoscopy* 1993; **25**: 224-230

26 **Catalano MF**, Sivak MV, Rice T, Gragg LA, Van Dam J. Endosonographic features predictive of lymph node metastasis. *Gastrointest Endosc* 1994; **40**: 442-446

27 **Vilmann P**, Hancke S, Henriksen FW, Jacobsen GK. Endoscopic ultrasonography-guided fine-needle aspiration biopsy of lesions in the upper gastrointestinal tract. *Gastrointest Endosc* 1995; **41**: 230-235

28 **Chang KJ**, Albers CG, Nguyen P. Endoscopic ultrasound-guided fine needle aspiration of pleural and ascitic fluid. *Am J Gastroenterol* 1995; **90**: 148-150

29 **Chu KM**, Kwok KF, Law S, Wong KH. A prospective evaluation of catheter probe EUS for the detection of ascites in patients with gastric carcinoma. *Gastrointest Endosc* 2004; **59**: 471-474 [PMID: 15044880 DOI: 10.1016/S0016-5107(03)02873-6]

30 **Nguyen PT**, Chang KJ. EUS in the detection of ascites and EUS-guided paracentesis. *Gastrointest Endosc* 2001; **54**: 336-339

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 48-53
World Journal of Gastroenterology ISSN 1007-9327

LIVER CANCER

Hepatocellular carcinoma in extremely elderly patients: An analysis of clinical characteristics, prognosis and patient survival

Gengo Tsukioka, Satoru Kakizaki, Naondo Sohara, Ken Sato, Hitoshi Takagi, Hirotaka Arai, Takehiko Abe, Mitsuo Toyoda, Kenji Katakai, Akira Kojima, Yuichi Yamazaki, Toshiyuki Otsuka, Yutaka Matsuzaki, Fujio Makita, Daisuke Kanda, Katsuhiko Horiuchi, Tetsuya Hamada, Mieko Kaneko, Hideyuki Suzuki, Masatomo Mori

Gengo Tsukioka, Satoru Kakizaki, Naondo Sohara, Ken Sato, Hitoshi Takagi, Masatomo Mori, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan
Hirotaka Arai, Takehiko Abe, Department of Gastroenterology, Maebashi Red Cross Hospital, Maebashi, Gunma 371-0014, Japan
Mitsuo Toyoda, Kenji Katakai, Department of Internal Medicine, Isesaki Municipal Hospital, Isesaki, Gunma 372-0817, Japan
Akira Kojima, Department of Internal Medicine, Kiryu Kousei General Hospital, Kiryu, Gunma 376-0024, Japan
Yuichi Yamazaki, Toshiyuki Otsuka, Department of Internal Medicine, Tone Chuo Hospital, Numata, Gunma 378-0053, Japan
Yutaka Matsuzaki, Fujio Makita, Department of Gastroenterology, National Nishigunma Hospital, Shibukawa, Gunma 377-0027, Japan
Daisuke Kanda, Department of Gastroenterology, Saiseikai Maebashi Hospital, Maebashi, Gunma 371-0821, Japan
Katsuhiko Horiuchi, Department of Gastroenterology, Public Tomioka General Hospital, Tomioka, Gunma 370-2361, Japan
Tetsuya Hamada, Mieko Kaneko, Department of Internal Medicine, Fuji Heavy Industries Ltd., Health Insurance Society General Ota Hospital, Ota, Gunma 373-0056, Japan
Hideyuki Suzuki, Department of Gastroenterology, Shimada Memorial Hospital, Fujioka, Gunma 375-0024, Japan
Supported by the grant of the Center of Excellence, Biomedical Research using accelerator technology
Correspondence to: Satoru Kakizaki, MD, PhD, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan. kakizaki@showa.gunma-u.ac.jp
Telephone: +81-27-2208127 Fax: +81-27-2208136
Received: 2005-05-24 Accepted: 2005-07-05

Abstract

AIM: To identify the clinical and prognostic features of patients with hepatocellular carcinoma (HCC) aged 80 years or more.

METHODS: A total of 1310 patients with HCC were included in this study. Ninety-one patients aged 80 years or more at the time of diagnosis of HCC were defined as the extremely elderly group. Two hundred and thirty-four patients aged ⩾ 50 years but less than 60 years were regarded as the non-elderly group.

RESULTS: The sex ratio (male to female) was significantly lower in the extremely elderly group (0.90:1) than in the non-elderly group (3.9:1, $P < 0.001$). The positive rate for HBsAg was significantly lower in the extremely elderly group and the proportion of patients negative for HBsAg and HCVAb obviously increased in the extremely elderly group ($P < 0.001$). There were no significant differences in the following parameters: diameter and number of tumors, Child-Pugh grading, tumor staging, presence of portal thrombosis or ascites, and positive rate for HCVAb. Extremely elderly patients did not often receive surgical treatment ($P < 0.001$) and they were more likely to receive conservative treatment ($P < 0.01$). There were no significant differences in survival curves based on the Kaplan-Meier methods in comparison with the overall patients between the two groups. However, the survival curves were significantly worse in the extremely elderly patients with stage I/II, stage I/II and Child-Pugh grade A cirrhosis in comparison with the non-elderly group. The causes of death did not differ among the patients, and most cases died of liver-related diseases even in the extremely elderly patients.

CONCLUSION: In the patients with good liver functions and good performance status, aggressive treatment for HCC might improve the survival rate, even in extremely elderly patients.

Key words: Hepatocellular carcinoma; Extremely elderly patients; Survival analysis; Cause of death

Tsukioka G, Kakizaki S, Sohara N, Sato K, Takagi H, Arai H, Abe T, Toyoda M, Katakai K, Kojima A, Yamazaki Y, Otsuka T, Matsuzaki Y, Makita F, Kanda D, Horiuchi K, Hamada T, Kaneko M, Suzuki H, Mori M. Hepatocellular carcinoma in extremely elderly patients: An analysis of clinical characteristics, prognosis and patient survival. *World J Gastroenterol* 2006; 12(1): 48-53

http://www.wjgnet.com/1007-9327/12/48.asp

INTRODUCTION

With the arrival of the aging society, an increasing number of elderly patients with cancer is predicted in the future. Hepatocellular carcinoma (HCC) is one of the

Table 1 Age distribution of the 1 310 patients with hepatocellular carcinoma

Age (yr)	Number of HCC patients
<30	3
30-40	4
40-50	41
50-60	234
60-70	485
70-80	452
80<	91
Total	1 310

most common cancers and the age distribution of HCC patients has steadily increased because of the improved management of chronic liver diseases[1-5]. As a result, we sometimes encounter patients with HCC aged 80 years or more. However, the clinical characteristics and the long-term prognosis of these patients still remain obscure because they often have concomitant diseases and therefore long-term follow-up for such patients remains difficult. In recent studies, "the elderly" have usually been defined as to be at the ages of 60, 65, or 70 years and above[1-4]. The average life expectancy of Japanese males is 78.36 years, while that of females is 85.33 years[6]. With the increase in the average lifetime, the age at which a person is considered elderly is rising. Clarifying the optimal treatment strategy for extremely elderly patients with HCC has thus become an urgent necessity. However, to our knowledge, there have been so far few reports evaluating extremely elderly patients with HCC aged 80 years or more[5]. Hence, in this study, we aimed to clarify the age-specific clinical characteristics of HCC, and to evaluate the survival and characteristics of extremely elderly patients. We, therefore, undertook a retrospective study of 91 extremely elderly patients with HCC aged 80 years or more in comparison to the non-elderly patients from 1 310 consecutive HCC patients.

MATERIALS AND METHODS

A total of 1 310 consecutive patients were enrolled in this study. The patients were newly diagnosed with HCC and observed from January 1992 to December 2003 at the Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine and nine affiliated hospitals, namely, Maebashi Red Cross Hospital, Isesaki Municipal Hospital, Kiryu Kousei General Hospital, Tone Chuo Hospital, National Nishigunma Hospital, Saiseikai Maebashi Hospital, Public Tomioka General Hospital, Fuji Heavy Industries Ltd, Health Insurance Society General Ota Hospital, and Shimada Memorial Hospital. A diagnosis of HCC was confirmed histopathologically or clinicopathologically from biopsy specimens or combined examinations of ultrasonography, computed tomography, and selective angiography. For each patient, the following data were recorded: age, sex, hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCVAb), biochemical analysis (total bilirubin, albumin, prothrombin time, platelet count, and ICG R15), serum alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist II (PIVKA-II), diameter and number of tumors, Child-Pugh grading, tumor staging, presence of portal thrombosis or ascites, initial therapy, and survival. The AFP level was divided into two categories: 20 μg/L or less, and more than 20 μg/L. PIVKA-II level was also divided into two categories: < 40 and ≥ 40 AU/L. The diameter of the largest tumor was measured in its greatest dimension if the patient had two or more tumors. The number of HCCs was divided into two groups: solitary and non-solitary tumors. Portal thrombosis was defined as a protrusion of the tumor into the first and/or second branch, or into the main trunk of the portal vein. The presence of concomitant disease with a strong impact on the prognosis (e.g., malignant neoplasm, cardiovascular disease, and cerebrovascular disease) was recorded. The types of initial treatment for HCC were categorized into five categories: (1) transcatheter arterial embolization (TAE) or transcatheter arterial infusion (TAI); (2) percutaneous injection or ablation [percutaneous ethanol injection (PEI), microwave ablation (MA), or radiofrequency ablation (RFA)]; (3) surgical resection, including liver transplantation; (4) systemic or reservoir chemotherapy; and (5) supportive care. According to their age at the initial diagnosis, the patients were categorized into two groups: an extremely elderly group consisting of 91 HCC patients aged ≥ 80 years and a non-elderly group comprising 234 HCC patients aged ≥ 50 years but < 60 years.

Statistical analysis

Differences in the proportions were evaluated by Fisher's exact probability test. Differences in the means were evaluated by the Student's *t*-test. The survival curves according to the Kaplan-Meier method were compared using the log-rank test. Using Cox's proportional hazard model, a multivariate analysis was performed to evaluate the prognostic factors. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of extremely elderly and non-elderly groups

The age distribution of 1 310 patients with HCC is shown in Table 1. The mean age of the 1 310 patients with HCC was 66.7 ± 9.3 years (range, 20-94 years; median, 68 years). There were 91 patients who were older than 80 years, and 234 patients aged ≥ 50 years but less than 60 years. The characteristics of the extremely elderly group and the non-elderly group are summarized in Table 2. The sex ratio, with a comparable male to female ratio was 0.90:1 in the extremely elderly group and 3.9:1 in the non-elderly group, showing that women were more prevalent in the extremely elderly group ($P < 0.001$). The positive rate for HBsAg was significantly lower in the extremely elderly group ($P < 0.001$). The proportion of the patients negative for HBsAg and HCVAb markedly increased in the extremely elderly group ($P < 0.001$). The prothrombin time and platelet count were significantly higher in the extremely elderly group ($P < 0.001$ and $P < 0.01$, respectively), while ICGR15 was significantly lower in the extremely elderly group ($P < 0.001$) than in the non-elderly group.

Table 2 Profile of 91 extremely elderly patients and 34 non-erlderly patients with hepatocellular carcinoma

	Extremely elderly (80 yr or more)	Non-elderly (50-60 yr old)	*P*-value
Sex (M/F)	43/48	186/48	$P<0.001$
Mean age (range)	82.3 ± 2.7 (80-94)	55.3 ± 2.9 (50-59)	$P<0.001$
HBsAg (+/-)	3/88	36/198	$P<0.001$
HCV (+/-)	67/24	182/52	NS
non B, nonC/B or C	21/70	16/218	$P<0.001$
Diameter of HCC (mm)	39.5± 24.3	36.8± 27.0	NS
Number of HCC (1/2 or more)	47/44	110/124	NS
Child-Pugh grading (A/B/C/uk)	62/22/3/4	144/67/18/6	NS
Stage (I/II/III/IVA/IVB/uk)	11/37/25/11/3/4	44/85/53/36/10/5	NS
Portal thrombus (-/+)	77/14	189/45	NS
Ascites (-/+)	69/22	191/43	NS
T-Bil (μmol/L)	1.23 ± 1.29	1.5± 1.82	NS
Alb (g/dL)	3.55 ± 0.53	3.58 ± 0.6	NS
PT (%)	83.3 ± 15.9	73.6 ± 18.4	$P<0.001$
Plt (x 10^4/μL)	13.5 ± 7.7	10.7 ± 5.8	$P<0.01$
ICGR15 (%)	22.6 ± 11.8	31.5 ± 18.7	$P<0.001$
AFP (-/+/uk)	33/53/5	75/150/9	NS
PIVKA II (-/+/uk)	35/51/5	116/109/9	NS

uk; unknown

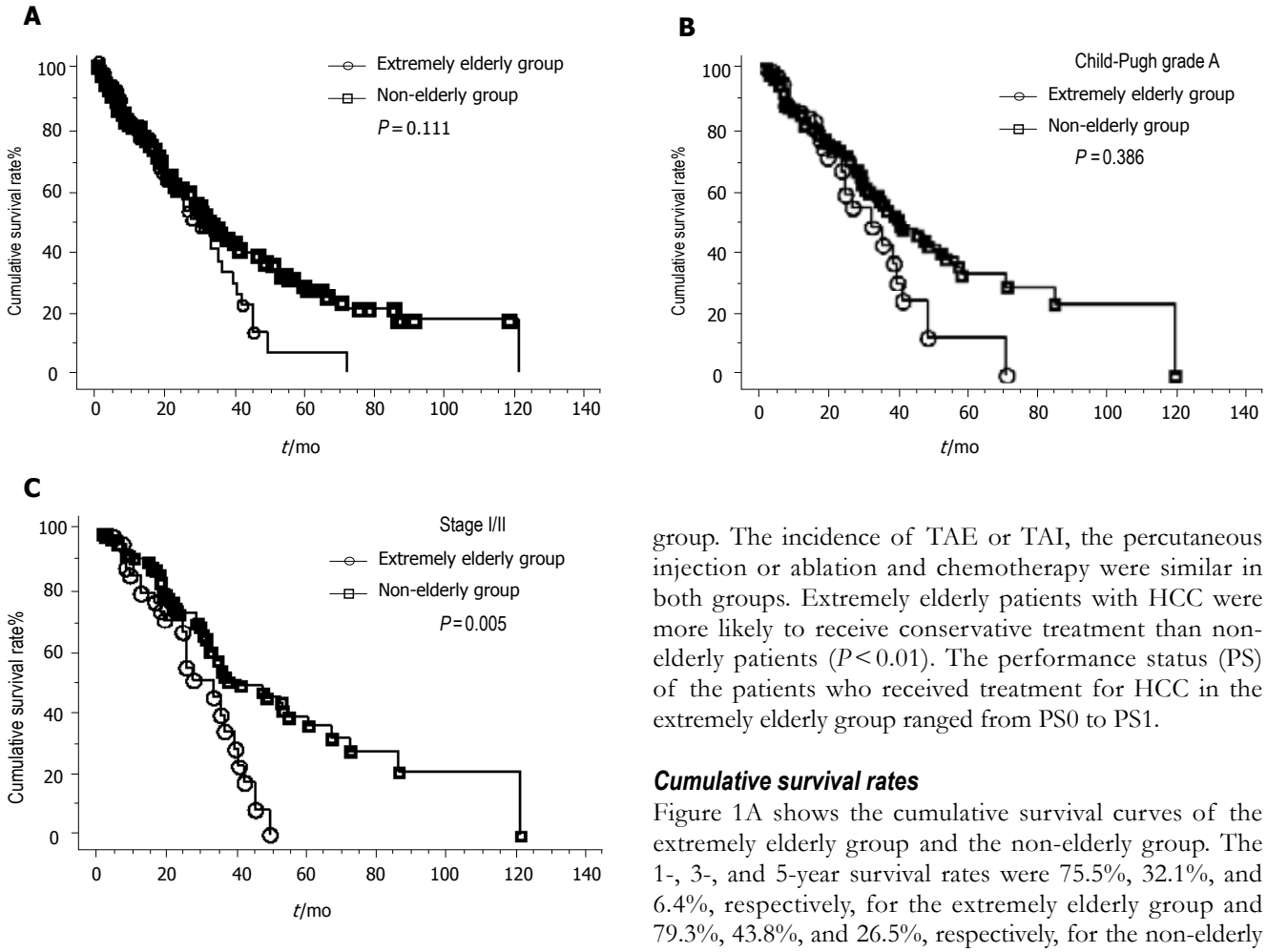


Figure 1 Survival curves of extremely elderly group and non-elderly group. **A:** All patients; **B:** Child–Pugh grade A patients; **C:** stage I/II patients.

There were no statistical differences in the prevalence of HCVAb, total bilirubin, albumin, AFP, PIVKAII, diameter and number of tumors, Child-Pugh grading, tumor stage, and presence of portal thrombosis or ascites. Table 3 shows the type of initial treatment. None of the patients underwent a surgical resection in the extremely elderly group. The incidence of TAE or TAI, the percutaneous injection or ablation and chemotherapy were similar in both groups. Extremely elderly patients with HCC were more likely to receive conservative treatment than non-elderly patients ($P<0.01$). The performance status (PS) of the patients who received treatment for HCC in the extremely elderly group ranged from PS0 to PS1.

Cumulative survival rates

Figure 1A shows the cumulative survival curves of the extremely elderly group and the non-elderly group. The 1-, 3-, and 5-year survival rates were 75.5%, 32.1%, and 6.4%, respectively, for the extremely elderly group and 79.3%, 43.8%, and 26.5%, respectively, for the non-elderly group. The difference in the survival rates between the two groups was not statistically significant ($P=0.114$). Figure 1B shows the cumulative survival curves of the patients with Child-Pugh grade A in the two groups. Although the survival tended to be worse in the extremely elderly patients, it did not reach statistical significance ($P=0.111$). There were also no significant differences in survival curves regarding the ChildPugh grade B/C patients ($P=0.386$). Due to the small number of patients with stage I tumors in the extremely elderly group, we compared

Table 3 Types of initial treatment for patients with hepatocellular carcinoma.

Treatment	Extremely elderly (80 yr or more)	Non-elderly (50-60 yr old)	*P*-value
TAE or TAI	59	128	NS
PEI or RFA or MA	18	62	NS
Surgery (liver transplantation)	0	28 (3)	$P < 0.001$
Chemotherapy	2	7	NS
Supportive care	12	9	$P < 0.01$

TAE, Transcatheter arterial embolization; TAI, Intranscatheter arterial infusion; PEI, percutaneous ethanol injection; MA, Microwave ablation; RFA, Radiofrequency ablation; NS, not significant.

Table 4 Causes of death in extremely elderly patients with HCC

Cause of death	Extremely elderly (80 yr or more)	Non-elderly (50-60 yr old)	*P*-value
HCC	27	77	NS
Hepatic failure	17	25	
Gastrointestinal-Bleeding	4	6	
Others	7	9	
Unknown	0	6	

the patients with stage I/II tumors between both groups. Figure 1C shows the cumulative survival curves of the stage I/II patients in both groups. The survival curves were significantly worse in the extremely elderly patients with stage I tumors in the extremely elderly group, we compared the patients with stage I/II tumors between both groups. Figure 1C shows the cumulative survival curves of the stage I/II patients for both groups. The survival curves were significantly worse in the extremely elderly patients with stage I/II tumors ($P = 0.005$). However, there were no significant differences in survival curves regarding stage III/IV ($P = 0.479$) or stage IV ($P = 0.794$) disease. In stage I/II and Child-Pugh grade A patients, the survival curves were significantly worse in the extremely elderly patients ($P = 0.005$). Survivals adjusted for the etiology of liver disease were also calculated. No significant differences were observed in the survival curves regarding HCV-related disease between the extremely elderly and non-elderly groups ($P = 0.142$). There were also no significant differences in the survival curves of the patients negative for HBsAg and HCVAb between the two groups ($P = 0.447$). Due to the small number of patients positive for HBsAg in the extremely elderly group, we could not compare those patients between both groups.

To evaluate the prognostic factors for the extremely elderly HCC patients and non-elderly patients, we performed a multivariate analysis using Cox's proportional hazard model, which showed that the Child-Pugh grading ($P < 0.05$), tumor staging ($P < 0.01$), albumin ($P < 0.01$), and platelet count ($P < 0.05$) were prognostic factors for extremely elderly patients with HCC. The Child-Pugh grading ($P < 0.05$), tumor staging ($P < 0.05$), and diameter of tumors ($P < 0.01$) were found to be prognostic factors for the non-elderly patients with HCC. The age, sex, HBsAg, HCVAb, total bilirubin, prothrombin time, number of tumors, and presence of portal thrombosis or ascites were not found to be prognostic factors, based on Cox's proportional hazard model. In the overall patients with HCC, the Child-Pugh grading ($P < 0.05$), tumor staging ($P < 0.05$) and diameter of tumors ($P < 0.01$) were found to be prognostic factors. The age was not found to be associated with the prognosis of the overall patients with HCC.

Cause of death of extremely elderly patients with HCC

Of the 91 patients aged ⩾ 80 years, 55 (60.4%) patients died during the observation period table 4. The causes of death among the 55 patients were HCC-related or hepatic failure in 44 patients (80.0%), gastrointestinal bleeding, including the rupture of esophageal varices in 4 patients (7.3%), and other diseases not related to HCC or liver cirrhosis in 7 patients (12.7%), such as pneumonia, renal failure and brain bleeding. In the non-elderly group, 123 (52.6%) patients died during the observation period. The causes of death among the 123 patients were HCC-related or hepatic failure in 102 patients (82.9%), gastrointestinal bleeding, including rupture of esophageal varices in six patients (5.0%), and other diseases not related to HCC or liver cirrhosis in nine (7.3%) and no records in six patients (5.0%).

Concomitant diseases of the patients with HCC

Of the 91 patients, 71 (78.0%) patients had concomitant diseases with liver disease in the extremely elderly group. As shown in Table 5, a variety of concomitant diseases were observed in the extremely elderly patients . "Others" in malignant neoplasms included: one ureteral carcinoma, one skin cancer, and one malignant lymphoma. "Other diseases" included: three benign prostatic hypertrophy, one idiopathic thrombocytopenia, one lichen planus, one ovarian cyst, one adrenal adenoma, and six others. On the other hand, 89 of 234 patients (38.0%) had concomitant diseases with liver disease in the non-elderly group. The ratio of patients having concomitant diseases was significantly higher in the extremely elderly group ($P < 0.001$).

DISCUSSION

Although the survival of the patients with an early stage and a good liver function was better in the non-elderly group, we could not find any significant difference in overall survival curves or causes of death between the extremely elderly and non-elderly groups with HCC. Even though the extremely elderly patients had various concomitant diseases, most of the patients demonstrating HCC died from HCC-related causes. As a result, HCC was a life-limiting factor even though the patients were extremely elder. In Japan, based on data from 2003[6], the average life expectancies at birth are 78.36 years for males and 85.33 years for females. In addition, an 80-year-old male has an average life expectancy of 8.26 years, while a female aged 80 years can expect to live another 11.04 years[6]. The 5-year survival rate in the extremely elderly group with HCC was only 6.4% in this study. We, therefore, have to treat HCC even though such patients are over 80 years of age and have concomitant diseases.

Table 5 Concomitant diseases in extremely elderly patients with hepatocellular carcinoma.

Concominant disease		Extremely elderly (80 yr or more)	Non-elderly (50-60 yr old)
Malignant neoplasm	Gastric cancer	2	2
	Colon cancer	3	4
	Prostatic cancer	2	0
	Others	3	1
Cardiovascular disease	Ischemic heart disease	9	3
	Hypertension	36	32
	Arrhythmia	7	2
	Congestive heart failure	2	0
	Valvular heart disease	2	0
	Aortic aneurysma	2	0
Respiratory disease	Chronic bronchitis	3	0
	Interstitial pneumonia	2	1
	Pneumonia	2	0
	Others	3	1
Gastrointestinal disease	Gall stone	4	3
	Chronic pancreatitis	1	4
	Peptic ulcer	2	5
	Reflux esophagitis	3	1
Endocrinal disease	Diabetes mellitus	23	47
	Gout	2	6
	Others	0	2
Neurological and cerebrovascular	Cerebral infarction or hemorrhage	7	2
	Dimentia	3	0
	Others	0	2
Renal disease	Chronic renal failure or nephritis	3	2
Other disease		13	5
No concominant disease		20	145

Dohmen *et al*.[5] analyzed 36 patients aged ≥ 80 years and concluded that the survival rates are not significantly different from the non-elderly group, and that the advanced stage of HCC, not an advanced age, has the greatest influence on the survival rate in extremely elderly patients[5]. Hoshida *et al*.[8] evaluated 135 patients with chronic liver disease aged ≥ 80 years and found that most patients (63.5%) would die of diseases other than liver diseases, such as pneumonia, especially in the non-cirrhosis group. It is reasonable that the extremely elderly patients without HCC die of diseases other than liver diseases. However, in their study, 18 patients with HCC at the start of observations had a poor prognosis.

Although there was no difference in the Child-Pugh grading, the extremely elderly group had good liver function as indicated by the prothrombin time or ICGR15 level. We speculated that patients with a poor liver function might die of liver failure and will not be able to live until 80 years of age when complicated with HCC. Otherwise, not only the liver function, but also other genetic or environmental factors may contribute to the development of HCC in extremely elderly patients. In this study, extremely elderly patients with HCC had a relatively good PS. The 10 hospitals included in this study are core hospitals for each area. The patients with a poor PS or poor liver function might have been excluded by the primary physician for aggressive treatment of HCC and thus might not have been introduced to these 10 hospitals.

Females were more prevalent in the extremely elderly group ($P < 0.001$). The proportion of females in the general population is known to gradually increase with increasing age[6]. Males are usually dominant in non-elderly patients with HCC and their prognosis of HCC is quite poor. We speculated that males with HCC might die of liver-related diseases before 80 years of age. However, we could not determine the reason why the female ratio significantly increased in the extremely elderly group of HCC. Previous studies reported that HBV-associated HCC is common in younger patients in Japan[11-13]. Our results were compatible with our expectation because only 3.3% of the HBV-associated HCC was identified in the extremely elderly group in contrast to 15.3% in the non-elderly group ($P < 0.001$). The ratio of non B and non C hepatitis patients significantly increased in the extremely elderly group. Other factors except for hepatitis virus infection such as alcohol or genetic disturbance may thus contribute to the development of HCC in the extremely elderly group.

Regarding the initial treatment, Collier *et al*.[10] reported in 1994 that elderly patients (≥ 65 years) with HCC are more likely to receive conservative treatment than younger patients (< 65 years), despite a similar disease stage. Conversely, Poon *et al*.[3] concluded that a hepatic resection and TAE for HCC in elderly patients (≥ 70 years) are well tolerated and show an improved survival rate. Percutaneous localized therapies or TAE were similarly performed in both the extremely elderly and non-elderly groups in this study and there were no obvious differences regarding adverse effects. However, no surgical resections were performed in the extremely elderly group. The oldest patient receiving a surgical resection as the initial treatment among these 1 310 patients was 77 years. The extremely elderly patients with HCC were more likely to receive conservative treatment than the non-elderly patients. Non-invasive treatments may have been selected due to the patient age or quality of life. Indeed, post-operative complications, such as pneumonia, show an incremental risk with age[14]. The elderly patients are more liable to succumb to post-operative organ failure and complications, especially infections[14]. The PS and physiological age are therefore considered to be more important than the chronological age in elderly patients with HCC.

In conclusion, there is no statistical difference in the overall survival rate between the extremely elderly and non-elderly groups after the diagnosis of HCC. HCC is considered to be a life-limiting factor even in extremely elderly patients. Therefore, in patients with good liver functions and good PS, aggressive treatment of HCC might improve the survival rate, even in extremely elderly patients.

REFERENCES

1 **Yanaga K**, Kanematsu T, Takenaka K, Matsumata T, Yoshida Y, Sugimachi K. Hepatic resection for hepatocellular carcinoma

in elderly patients. *Am J Surg* 1988; **155**: 238-241
2 **Nomura F**, Ohnishi K, Honda M, Satomura Y, Nakai T, Okuda K. Clinical features of hepatocellular carcinoma in the elderly: a study of 91 patients older than 70 years. *Br J Cancer* 1994; **70**: 690-693
3 **Poon RT**, Fan ST, Lo CM, Liu CL, Ngan H, Ng IO, Wong J. Hepatocellular carcinoma in the elderly: results of surgical and nonsurgical management. *Am J Gastroenterol* 1999; **94**: 2460-2466
4 **Hazama H**, Omagari K, Matsuo I, Masuda J, Ohba K, Sakimura K, Kinoshita H, Isomoto H, Murase K, Kohno S. Clinical features and treatment of hepatocellular carcinoma in eight patients older than eighty years of age. *Hepatogastroenterology* 2001; **48**: 1692-1696
5 **Dohmen K**, Shirahama M, Shigematsu H, Irie K, Ishibashi H. Optimal treatment strategy for elderly patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2004; **19**: 859-865
6 **Health and Welfare Statistics Association.** Life expectancy. *J Health Welfare Stat* 2004; 51: 67-68
7 **Dohmen K**, Shigematsu H, Irie K, Ishibashi H. Trends in clinical characteristics, treatment and prognosis of hepatocellular carcinoma. *Hepatogastroenterology* 2003; **50**: 1872-1877
8 **Arbaje YM**, Carbone PP. Hepatocellular carcinoma in the very elderly: to treat or not to treat? *Med Pediatr Oncol* 1994; **22**: 84-87
9 **Hoshida Y**, Ikeda K, Kobayashi M, Suzuki Y, Tsubota A, Saitoh S, Arase Y, Kobayashi M, Murashima N, Chayama K, Kumada H. Chronic liver disease in the extremely elderly of 80 years or more: clinical characteristics, prognosis and patient survival analysis. *J Hepatol* 1999; **31**: 860-866
10 **Namieno T**, Kawata A, Sato N, Kondo Y, Uchino J. Age-related, different clinicopathologic features of hepatocellular carcinoma patients. *Ann Surg* 1995; **221**: 308-314
11 **Kato Y**, Nakata K, Omagari K, Furukawa R, Kusumoto Y, Mori I, Tajima H, Tanioka H, Yano M, Nagataki S. Risk of hepatocellular carcinoma in patients with cirrhosis in Japan. Analysis of infectious hepatitis viruses. *Cancer* 1994; **74**: 2234-2238
12 **Dohmen K**, Shigematsu H, Irie K, Ishibashi H. Comparison of the clinical characteristics among hepatocellular carcinoma of hepatitis B, hepatitis C and non-B non-C patients. *Hepatogastroenterology* ; **50**: 2022-2027
13 **Collier JD**, Curless R, Bassendine MF, James OF. Clinical features and prognosis of hepatocellular carcinoma in Britain in relation to age. *Age Ageing* 1994; **23**: 22-27
14 **Koperna T**, Kisser M, Schulz F. Hepatic resection in the elderly. *World J Surg* 1998; **22**: 406-412

S- Editor Kumar M and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 54-59
World Journal of Gastroenterology ISSN 1007-9327

COLORECTAL CANCER

Clinical significance of subcellular localization of KL-6 mucin in primary colorectal adenocarcinoma and metastatic tissues

Qian Guo, Wei Tang, Yoshinori Inagaki, Yutaka Midorikawa, Norihiro Kokudo, Yasuhiko Sugawara, Munehiro Nakata, Toshiro Konishi, Hirokazu Nagawa, Masatoshi Makuuchi

Qian Guo, Wei Tang, Yutaka Midorikawa, Norihiro Kokudo, Yasuhiko Sugawara, Masatoshi Makuuchi, Hepatobiliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Yoshinori Inagaki, Munehiro Nakata, Department of Applied Biochemistry and Institute of Glycotechnology, Tokai University, Hiratsuka, Kanagawa 259-1292, Japan
Toshiro Konishi, Kanto Medical Center, NTT EC, 5-9-22 Higashigotanda, Shinagawa-ku, Tokyo 141-8625, Japan
Hirokazu Nagawa, Surgical Oncology Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Supported by Grants-in-aid from the Ministry of Education, Science, Sports and Culture of Japan and a grant for Hi-Tech Research from Tokai University
Correspondence to: Dr Wei Tang, Hepatobiliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyoku, Tokyo 113-8655, Japan. tang-sur@h.u-tokyo.ac.jp
Telephone: +81-3-5800-8654 Fax: +81-3-5684-3989
Received: 2005-05-21 Accepted: 2005-06-18

Abstract

AIM: To assess subcellular localization of KL-6 mucin and its clinicopathological significance in colorectal carcinoma as well as metastatic lymph node and liver tissues.

METHODS: Colorectal carcinoma tissues as well as metastatic lymph node and liver tissues were collected from 82 patients who underwent colorectomy or hepatectomy. Tissues were subjected to immunohistochemical analysis using KL-6 antibody.

RESULTS: Of the 82 colorectal carcinoma patients, 6 showed no staining, 29 showed positive staining only in the apical membrane, and 47 showed positive staining in the circumferential membrane and/or cytoplasm. Positive staining was not observed in non-cancerous colorectal epithelial cells surrounding the tumor tissues. The five-year survival rate was significantly lower in cases showing positive staining in the circumferential membrane and/or cytoplasm (63.0%) than those showing positive staining only in the apical membrane (85.7%) and those showing no staining (100%). Statistical analysis between clinicopathological factors and subcellular localization of KL-6 mucin showed that KL-6 localization in the circumferential membrane and/or cytoplasm was significantly associated with the presence of venous invasion (P = 0.0003), lymphatic invasion (P < 0.0001), lymph node metastasis (P < 0.0001), liver metastasis (P = 0.058), and advanced histological stage (P < 0.0001). Positive staining was observed in all metastatic lesions tested as well as in the primary colorectal carcinoma tissues.

CONCLUSION: The subcellular staining pattern of KL-6 in colorectal adenocarcinoma may be an important indicator for unfavorable behaviors such as lymph node and liver metastasis, as well as for the prognosis of patients.

Key words: KL-6 mucin; Colorectal carcinoma; Metastasis; Prognosis; Immunohistochemistry

Guo Q, Tang W, Inagaki Y, Midorikawa Y, Kokudo N, Sugawara Y, Nakata M, Konishi T, Nagawa H, Makuuchi M. Clinical significance of subcellular localization of KL-6mucin in primary colorectal adenocarcinoma and metastatic tissues. *World J Gastroenterol* 2006; 12(1): 54-59

http://www.wjgnet.com/1007-9327/12/54.asp

INTRODUCTION

MUC1, a transmembrane glycoprotein[1, 2], has been detected in various cancer cell lines and secretory epithelial cells lining the respiratory, reproductive, and gastrointestinal tracts[3-6]. It has been suggested that MUC1 may influence cell-to-cell adhesion, diminish the immune response, and be involved in intracellular signaling[7,8]. In carcinoma cells, it has been reported that high levels of MUC1 expression correlate with the invasive characteristic of tumors[9-13]. Our latest study has also shown that aberrant expression of MUC1, which was detected by KL-6 antibody, is associated with cancer progression in the carcinoma of the ampulla of Vater[14].

In normal epithelium, MUC1 is predominantly present on the apical surface of the epithelial cells[15,16]. Recently, it has been reported that, in breast carcinoma, MUC1 is expressed not only on the apical surface but also on

circumferential and basal membranes and in the cytoplasm of the carcinoma cells[15,16]. Furthermore, this aberrant localization of MUC1 has been reported to be associated with worse prognosis for the patient[17]. These findings suggest that subcellular observation of MUC1 expression in carcinoma cells is likely important for understanding the function of MUC1 and improving the prediction of prognosis. However, little is known about the clinical significance of subcellular localization of KL-6 mucin in other carcinomas.

In this study, we focused on subcellular localization of MUC1 in colorectal carcinoma as well as in the metastatic lymph nodes and liver tissue. MUC1 expression was immunohistochemically detected using KL-6 antibody, which recognizes the sialylated oligosaccharide moiety of MUC1 as a part of an epitope[18]. In colorectal carcinoma tissues, it has been suggested that the expression of KL-6 mucin is associated with tumor aggressiveness[10,19]. However, the subcellular localization and physiological function of KL-6 mucin in colorectal carcinoma have remained unknown. In this paper, we report that aberrant subcellular expression of KL-6 mucin in the circumferential membrane and/or cytoplasm is associated with lymph node and liver metastases and worse prognosis in colorectal carcinoma.

MATERIALS AND METHODS

Patients

Colorectal carcinoma tissues were collected from 82 consecutive patients (55 males and 27 females; 64.5 ± 11.4 years, mean ± SD) with a single primary colorectal adenocarcinoma who underwent surgical resection at the Department of Surgery, Graduate School of Medicine, the University of Tokyo, between January 1991 and December 1992. For all cases with lymph node and liver metastasis, whole specimens of resected lymph nodes and metastatic liver tissues were collected from 36 and 7 patients, respectively, in the study group. All specimens were classified according to Japanese Classification of Colorectal Carcinoma by the Japanese Society for Cancer of the Colon and Rectum[20], including the status of lymph node and liver metastasis at the time of surgical intervention and the depth of invasion (m, invasion of mucosa; sm, invasion of submucosa; mp, invasion of muscularis propria; ss, invasion of subserosa or subadventitia; se, invasion of serosa or adventitia; and si, invasion of adjacent structures).

Immunohistochemical staining

The immunohistochemical staining approach matched that of previous studies[14]. Briefly, 4 μm-thick sections were cut from archival formalin-fixed paraffin-embedded tissue blocks, deparaffinized, and dehydrated using a graded series of ethanol solutions. Endogenous peroxidase activity was halted through administration of 3 mL/L hydrogen peroxide/methanol for 30 min. The slides were rinsed with phosphate-buffered saline and then blocked with normal goat serum for 30 min at room temperature. The sections were then incubated with a KL-6 monoclonal antibody solution (1:200 dilution; Eisai, Tokyo, Japan) for 60 min at room temperature. After the sections were incubated with biotinylated secondary antibody for 60 min, bound biotinylated antibody was then tested by the biotin-streptavidin-peroxidase complex method following the manufacturer's instructions (Histofine SAB-PO kit; Nichirei, Tokyo, Japan). 3,3'-Diaminobenzidine was used as the chromogen, and hematoxylin was used as a counterstain. The negative control sections were treated by omitting the primary antibody to monitor background staining.

Evaluation of immunohistochemically stained carcinomas

Overall staining was evaluated in carcinoma cells observed in 10 random microscopic fields, or in the entire area if the tissue sample comprised less than 10 fields. Subcellular staining patterns were recorded by judging the apical membrane, circumferential membrane, and cytoplasm as described elsewhere[17]. Three investigators (Q.G., W.T., and N.K.) separately judged the staining characteristics, and the discrepancies were resolved through mutual observation and discussion of the microscopic fields.

Statistical analysis

The χ^2-test was used to evaluate the relationship between staining pattern and clinicopathological parameters. Survival curves were calculated using the Kaplan-Meier method and compared with the results of the log-rank test. Two patients (one in the no-staining group, another in the apical membrane staining group) were excluded from the data analysis for survival because the cause of death for these patients was not colorectal cancer. $P < 0.05$ was considered statistically significant. Statview 5.0J (Abacus Concepts, Berkeley, CA, USA) statistical software was used for data analyses.

RESULTS

Subcellular localization of KL-6 mucin

Among the 82 cases of colorectal carcinoma, 76 cases showed positive staining of KL-6 mucin. As shown in Figure 1, there was a considerable heterogeneity in the subcellular localization of KL-6 mucin. Staining was observed in either the apical or circumferential membrane (Figures 1A and 1B). Some cases showed positive staining in the cytoplasm in addition to the membranous region (Figures 1C and 1D). The number of cases showing the respective subcellular staining patterns are summarized in Table 1. It is notable that cytoplasmic staining tended to be accompanied by positive staining in the circumferential membrane (37/45, 82%) rather than in the apical membrane (8/45, 18%). Positive staining was not observed in non-cancerous colorectal epithelial cells in any case of this study (data not shown).

Relationship between survival and subcellular localization of KL-6 mucin

The five-year survival rate was 85.7% for cases showing positive staining only in the apical membrane (n = 28), 61.5% for cases showing positive staining in the circumferential membrane (n = 39), and 64.4% for cases showing positive staining in cytoplasm (n = 45) (data

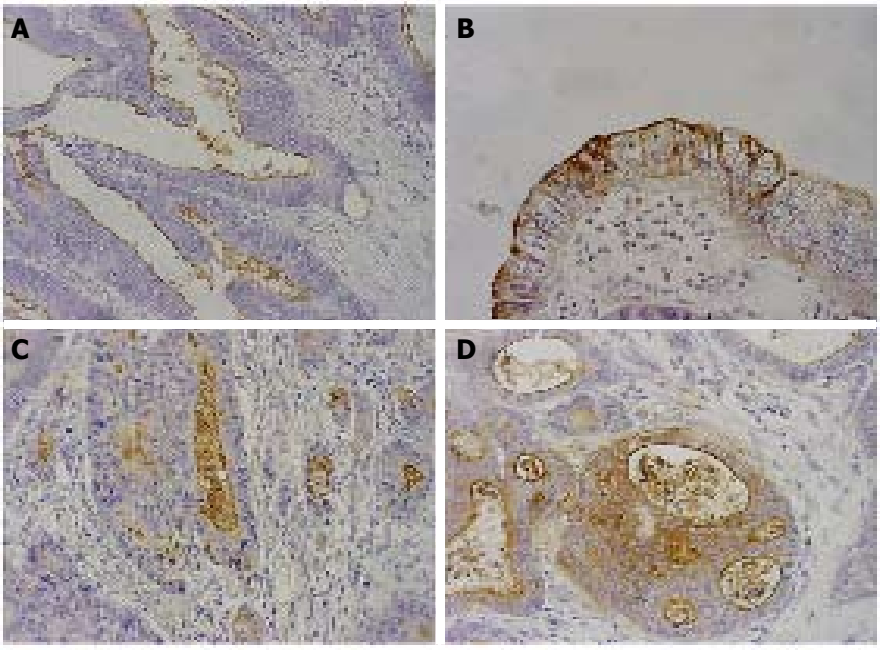


Figure 1 Immunohistochemical staining of colorectal adenocarcinoma tissues using KL-6 antibody (× 200). **A**: Positive staining in the apical membrane; **B**: positive staining in the circumferential membrane; **C:** positive staining in the apical membrane and cytoplasm; **D**: positive staining in the circumferential membrane and cytoplasm.

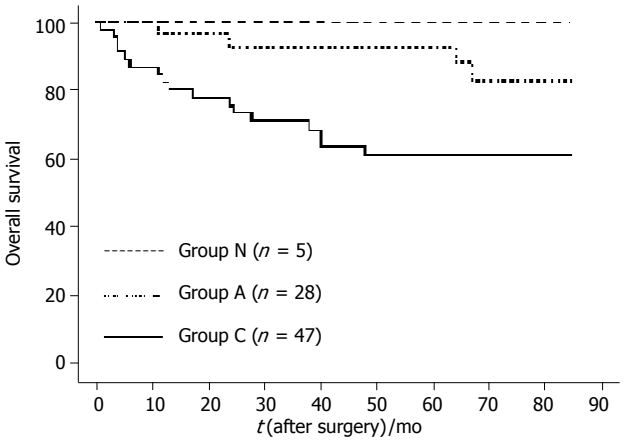


Figure 2 Kaplan-Meier curves for overall survival rates of patients with colorectal adenocarcinoma. Patients with KL-6 expression in the circumferential membrane and/or cytoplasm (solid line, group C, *n* = 46), in the apical membrane (dashed line, group A, *n* = 29) and without KL-6 staining (dotted line, group N, *n* = 5) were followed up for more than 70 mo. Two of 82 patients were excluded from the data analysis as described in Materials and Methods.

Table 1 Summary of subcellular staining of KL-6 mucin in colorectal adenocarcinoma

Group[1]	KL-6 mucin staining			*n*
	Apical membrane	Circumferential membrane	Cytoplasm	
N	Negative	Negative	Negative	6
A	Positive	Negative	Negative	29
C	Positive	Negative	Positive	8
C	Negative	Positive	Negative	2
C	Negative	Positive	Positive	37

[1]Patient groups N, A, and C were categorized according to the subcellular expression profile of KL-6 mucin (see text).

not shown). There were significant differences between the cases showing positive staining only in the apical membrane and the cases showing positive staining in the circumferential membrane ($P = 0.021$), and between the cases showing positive staining only in the apical membrane and the cases showing positive staining in cytoplasm ($P = 0.033$). On the other hand, the five-year survival rate was 100% for the cases showing no staining ($n = 5$). These results suggested that a subcellular KL-6 expression profile was associated with survival, and that cases showing positive staining in the circumferential membrane and/or cytoplasm showed worse prognosis.

As described above, cytoplasmic staining tended to be accompanied with positive staining of the circumferential membrane. Therefore, we classified the cases into the following three groups according to their subcellular staining profile: group N, negative ($n = 6$); group A, positive only in the apical membrane ($n = 29$); and group C, positive in the circumferential membrane and/or cytoplasm ($n = 47$) (Table 1). As shown in Figure 2, the five-year survival rate was significantly lower in group C (63.8%) than that in group A (85.7%; $P = 0.029$). On the other hand, group N showed the highest five-year survival rate (100%).

Relationship between clinicopathological factors and subcellular localization of KL-6 mucin

The relationship between clinicopathological factors and subcellular KL-6 mucin staining of the colorectal adenocarcinomas is summarized in Table 2. Positive staining in the circumferential membrane and/or cytoplasm was significantly associated with the presence of venous invasion, lymphatic invasion, and lymph node metastasis. This subcellular staining characteristic was also associated with the progression of the depth of invasion and histological stage (Table 2). Notably, all cases having lymph node ($n = 36$) or liver metastasis ($n = 7$) showed positive staining in the circumferential membrane and/or cytoplasm. This suggested that aberrant subcellular expression of KL-6 mucin in the circumferential

Table 2 Relationship between clinicopathological factors and sbcellular staining profile of KL-6 mucin in colorectal adenocarcinoma *n*(%)

Factors	*n*	Subcellular staining profile of KL-6 mucin			*P*
		No staining	Apical membrane	Circumferential membrane and/or cytoplasm	
Age (yr)					0.449
≤60	29	2 (6.9)	12 (41.4)	15 (51.7)	
>60	53	4 (7.5)	17 (32.1)	32 (60.4)	
Sex					0.469
Male	55	4 (7.3)	21 (38.2)	30 (54.5)	
Female	27	2 (7.4)	8 (29.6)	17 (63.0)	
Differentiation					0.055
Well	50	6 (12.0)	21 (42.0)	23 (46.0)	
Moderate	29	0 (0)	8 (27.6)	21 (72.4)	
Poor	3	0 (0)	0 (0)	3 (100)	
Venous invasion					0.0003
(+)	40	0 (0)	9 (22.5)	31 (77.5)	
(-)	42	6 (14.3)	20 (47.6)	16 (38.1)	
Lymphatic invasion					<0.0001
(+)	24	0 (0)	2 (8.3)	22 (91.7)	
(-)	58	6 (10.3)	27 (46.6)	25 (43.1)	
Depth of invasion					0.009
m	3	3 (100)	0 (0)	0 (0)	
sm, mp	14	2 (14.3)	8 (57.1)	4 (28.6)	
ss, se	59	1 (1.7)	20 (33.9)	38 (64.4)	
si	6	0 (0)	1 (16.7)	5 (83.3)	
Histological stage					<0.0001
0	3	3 (100)	0 (0)	0 (0)	
I	12	2 (16.7)	8 (66.6)	2 (16.7)	
II	29	1 (3.4)	20 (69.0)	8 (27.6)	
IIIa	29	0 (0)	1 (3.4)	28 (96.6)	
IIIb	6	0 (0)	0 (0)	6 (100)	
IV	3	0 (0)	0 (0)	3 (100)	
Lymph node metastasis					<0.0001
(+)	36	0 (0)	0 (0)	36 (100)	
(-)	46	6 (13.0)	29 (63.0)	11 (24.0)	
Liver metastasis					0.058
(+)	7	0 (0)	0 (0)	7 (100)	
(-)	75	6 (8.0)	29 (38.7)	40 (53.3)	

membrane and/or cytoplasm might participate in the metastasis of tumor.

Expression of KL-6 mucin in metastatic lesions

We next examined the expression of KL-6 mucin in lymph node and liver metastatic lesions (Figure 3). For all the 36 cases with lymph node metastasis and all seven cases with liver metastasis, positive staining was observed in metastatic lesions as well as in the primary colorectal carcinoma tissues.

DISCUSSION

MUC1 is abundantly expressed at the surface of epithelial cells in many tissues[3-6]. MUC1 expression is also observed in carcinomas that arise in several parts of the body, such as the colon, breast, lung, pancreas, papillary thyroid, and gallbladder[9-13]. The deduced amino acid sequence of MUC1 mucin reveals four distinct domains: the NH_2-terminal domain consisting of a hydrophobic signal sequence; a highly *O*-glycosylated tandem-repeat domain; a transmembrane domain; and a cytoplasmic domain[1,2,6]. Previous studies on MUC1 expression in colorectal carcinoma have primarily focused on the tandem-repeat domain, and suggested that tumor cells expressing high levels of MUC1 may have increased invasive and metastatic potential[10]. However, little is known about the detailed clinicopathological relationship among expression profile of MUC1, metastatic potentiality, and the prognosis for colorectal adenocarcinoma. On the other hand, although the processing of the full length MUC1 core proteins is similar in both normal and tumor cells, they have a remarkable diversity in oligosaccharide moieties[21,22]. Therefore, we targeted KL-6 mucin, a type of MUC1 bearing sialylated oligosaccharide recognized by KL-6 antibody[18]. Since sialylation of tumor cell glycoconjugates is thought to contribute to tumor progression and metastasis[23-26], targeting KL-6 mucin bearing sialylated oligosaccharide seems to be a reasonable strategy.

In our preliminary study, KL-6 mucin was observed in carcinoma cells but not in the surrounding normal cells. However, classification of KL-6 staining evaluated by overall expression level did not show significant relationships between the expression level of KL-6 mucin, metastasis, and patient's prognosis (data not shown). Recently, some reports on breast carcinoma have suggested a significant relationship between metastasis and subcellular location of MUC1 rather than its overall expression level, which led us to focus on the subcellular location of KL-6 mucin in colorectal carcinoma.

In the present study, the circumferential and/or cytoplasmic expression of KL-6 mucin was significantly correlated with lymph node metastasis in colorectal adenocarcinomas (Table 2). In addition, this aberrant localization of KL-6 mucin was likely to participate also in liver metastasis, since all cases having liver metastasis (n = 7) showed positive staining in the circumferential membrane and/or cytoplasm (Table 2). It is known that normal epithelial cells release a tailless, soluble form of MUC1 which targets exclusively the apical membrane in tissues[21]. However, in carcinoma cells with aberrant overexpression of MUC1, this apical polarization is lost, resulting in aberrant localization of MUC1 over the entire cell membrane and in the cytoplasm[15,16]. It has been proposed that MUC1 mediates anti-adhesion activity by interfering with cell-to-cell and/or cell-to-extracellular matrix interactions, thereby facilitating detachment of tumor cells from the primary growth[6, 27-29]. This is likely true of KL-6 mucin in colorectal adenocarcinoma, since a high frequency of metastasis was observed in cases showing any aberrant localization of KL-6 mucin (Table 2). This aberrant subcellular expression of KL-6 mucin might facilitate detachment of tumor cells from the primary growth, resulting in an increased ability of the tumor cells to metastasize.

It is notable that all the cases tested showed positive staining in metastatic lesions as well as the primary colorectal carcinoma tissues. Interestingly, in some cases presenting lymph node or liver metastasis, aberrant subcellular localization of KL-6 was observed in only

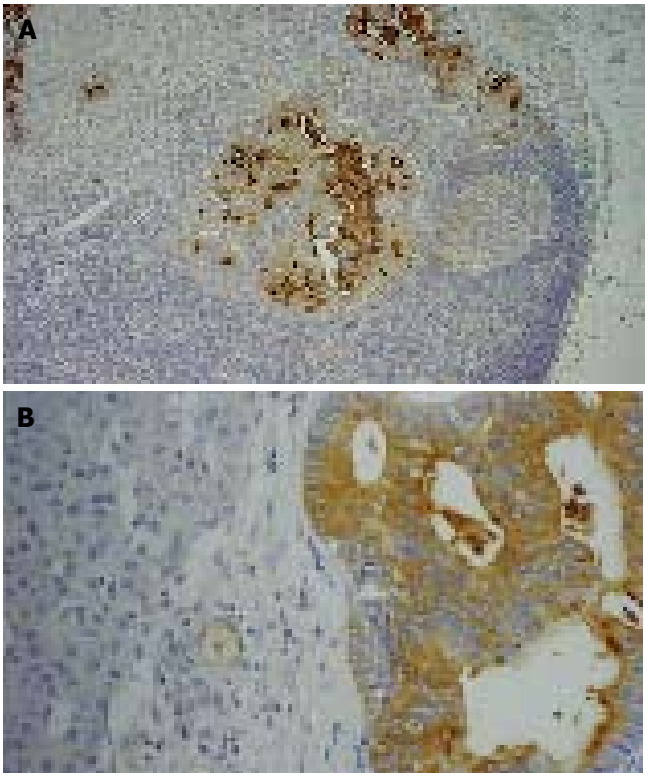


Figure 3 Immunohistochemical staining of metastatic lymph node and liver tissues using KL-6 antibody (×200). **A:**Positive KL-6 expression in a metastatic lymph node; **B:** positive KL-6 expression in metastatic liver tissues.

a few tumor cells (less than 5%) in the primary lesions (data not shown). This suggests that the significant factor is the subcellular location rather than the level of KL-6 expression, and is coincident with a report that breast carcinomas with the cytoplasmic staining pattern, even in a minor focus, are associated with a higher incidence of lymph node metastasis[17]. However, a considerable number of cases showing positive membranous and/or cytoplasmic staining did not present with metastases. Further investigation is needed to understand the role of KL-6 expression in metastatic events of colorectal adenocarcinoma.

Some studies have reported that, in breast carcinoma, patients expressing MUC1 in the non-apical membranes show worse prognosis than those expressing MUC1 in the apical membrane[17,30]. Our observation also showed that there was a significant relationship between subcellular location of KL-6 and prognosis in colorectal adenocarcinoma (Figure 2; $P = 0.029$). The five-year survival rates for the cases with apical membrane staining and those with circumferential and/or cytoplasmic staining of KL-6 mucin were 85.7% and 63.0%, respectively (Figure 2). Moreover, our data also showed that cases without KL-6 staining had a better prognosis than those with the apical staining of KL-6 mucin. These data suggest that the expression characteristics of KL-6 mucin would be useful for the prediction of a patient's prognosis in colorectal adenocarcinoma.

In conclusion, subcellular localization of KL-6 mucin plays a crucial role in determining disease outcome and expression of KL-6 mucin in the circumferential membrane and/or cytoplasm is an important indicator for lymph node and liver metastases as well as the prognosis of patients with colorectal adenocarcinoma.

REFERENCES

1 **Gendler SJ**, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, Pemberton L, Lalani EN, Wilson D. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem* 1990; **265**: 15286-15293
2 **Lan MS**, Batra SK, Qi WN, Metzgar RS, Hollingsworth MA. Cloning and sequencing of a human pancreatic tumor mucin cDNA. *J Biol Chem* 1990; **265**: 15294-15299
3 **Gendler SJ**, Spicer AP. Epithelial mucin genes. *Annu Rev Physiol* 1995; **57**: 607-634
4 **Hey NA**, Graham RA, Seif MW, Aplin JD. The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase. *J Clin Endocrinol Metab* 1994; **78**: 337-342
5 **Hilkens J**, Buijs F. Biosynthesis of MAM-6, an epithelial sialomucin. Evidence for involvement of a rare proteolytic cleavage step in the endoplasmic reticulum. *J Biol Chem* 1988; **263**: 4215-4222
6 **Hollingsworth MA**, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer* 2004; **4**: 45-60
7 **Meerzaman D**, Xing PX, Kim KC. Construction and characterization of a chimeric receptor containing the cytoplasmic domain of MUC1 mucin. *Am J Physiol Lung Cell Mol Physiol* 2000; **278**: L625-L629
8 **Agrawal B**, Krantz MJ, Reddish MA, Longenecker BM. Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. *Nat Med* 1998; **4**: 43-49
9 **Nakamori S**, Ota DM, Cleary KR, Shirotani K, Irimura T. MUC1 mucin expression as a marker of progression and metastasis of human colorectal carcinoma. *Gastroenterology* 1994; **106**: 353-361
10 **Hiraga Y**, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F, Kohno N. Immunoreactive MUC1 expression at the deepest invasive portion correlates with prognosis of colorectal cancer. *Oncology* 1998; **55**: 307-319
11 **Kashiwagi H**, Kijima H, Dowaki S, Ohtani Y, Tobita K, Tsukui M, Tanaka Y, Matsubayasi H, Tsuchida T, Yamazaki H, Nakamura M, Ueyama Y, Tanaka M, Tajima T, Makuuchi H. DF3 expression in human gallbladder carcinoma: significance for lymphatic invasion. *Int J Oncol* 2000; **16**: 455-459
12 **Zhang HK**, Zhang QM, Zhao TH, Li YY, Yi YF. Expression of mucins and E-cadherin in gastric carcinoma and their clinical significance. *World J Gastroenterol* 2004; **10**: 3044-3047
13 **Lüttges J**, Feyerabend B, Buchelt T, Pacena M, Klöppel G. The mucin profile of noninvasive and invasive mucinous cystic neoplasms of the pancreas. *Am J Surg Pathol* 2002; **26**: 466-471
14 **Tang W**, Inagaki Y, Kokudo N, Guo Q, Seyama Y, Nakata M, Imamura H, Sano K, Sugawara Y, Makuuchi M. KL-6 mucin expression in carcinoma of the ampulla of Vater: association with cancer progression. *World J Gastroenterol* 2005; **11**: 5450-5454
15 **Kufe D**, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma* 1984; **3**: 223-232
16 **Perey L**, Hayes DF, Maimonis P, Abe M, O'Hara C, Kufe DW. Tumor selective reactivity of a monoclonal antibody prepared against a recombinant peptide derived from the DF3 human breast carcinoma-associated antigen. *Cancer Res* 1992; **52**: 2563-2568
17 **Rahn JJ**, Dabbagh L, Pasdar M, Hugh JC. The importance of MUC1 cellular localization in patients with breast carcinoma: an immunohistologic study of 71 patients and review of the

literature. *Cancer* 2001; **91**: 1973-1982
18 **Kohno N**, Akiyama M, Kyoizumi S, Hakoda M, Kobuke K, Yamakido M. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. *Jpn J Clin Oncol* 1988; **18**: 203-216
19 **Aoki R**, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G, Shimamoto F, Kohno N. MUC-1 expression as a predictor of the curative endoscopic treatment of submucosally invasive colorectal carcinoma. *Dis Colon Rectum* 1998; **41**: 1262-1272
20 Japanese Society for Cancer of the Colon and Rectum. Japanese classification of colorectal carcinoma. 1st Eenglish ed. Tokyo: Kanehara, 1997: 4-82
21 **Julian J**, Carson DD. Formation of MUC1 metabolic complex is conserved in tumor-derived and normal epithelial cells. *Biochem Biophys Res Commun* 2002; **293**: 1183-1190
22 **Osako M**, Yonezawa S, Siddiki B, Huang J, Ho JJ, Kim YS, Sato E. Immunohistochemical study of mucin carbohydrates and core proteins in human pancreatic tumors. *Cancer* 1993; **71**: 2191-2199
23 **Ikeda Y**, Mori M, Kajiyama K, Haraguchi Y, Sasaki O, Sugimachi K. Immunohistochemical expression of sialyl Tn, sialyl Lewis a, sialyl Lewis a-b-, and sialyl Lewis x in primary tumor and metastatic lymph nodes in human gastric cancer. *J Surg Oncol* 1996; **62**: 171-176
24 **Li XW**, Ding YQ, Cai JJ, Yang SQ, An LB, Qiao DF. Studies on mechanism of Sialy Lewis-X antigen in liver metastases of human colorectal carcinoma. *World J Gastroenterol* 2001; **7**: 425-430
25 **Tang W**, Mafune K, Nakata M, Konishi T, Kojima N, Mizuochi T, Makuuchi M. Association of histochemical expression of Maackia amurensis leukoagglutinin-positive glycoconjugates with behaviour of human gastric cancer. *Histopathology* 2003; **42**: 239-245
26 **Tang W**, Guo Q, Usuda M, Kokudo N, Seyama Y, Minagawa M, Sugawara Y, Nakata M, Kojima N, Makuuchi M. Histochemical expression of sialoglycoconjugates in carcinoma of the papilla of Vater. *Hepatogastroenterology* 2005; **52**: 67-71
27 **Kohlgraf KG**, Gawron AJ, Higashi M, Meza JL, Burdick MD, Kitajima S, Kelly DL, Caffrey TC, Hollingsworth MA. Contribution of the MUC1 tandem repeat and cytoplasmic tail to invasive and metastatic properties of a pancreatic cancer cell line. *Cancer Res* 2003; **63**: 5011-5020
28 **Ligtenberg MJ**, Buijs F, Vos HL, Hilkens J. Suppression of cellular aggregation by high levels of episialin. *Cancer Res* 1992; **52**: 2318-2324
29 **Wesseling J**, van der Valk SW, Vos HL, Sonnenberg A, Hilkens J. Episialin (MUC1) overexpression inhibits integrin-mediated cell adhesion to extracellular matrix components. *J Cell Biol* 1995; **129**: 255-265
30 **Hayes DF**, Mesa-Tejada R, Papsidero LD, Croghan GA, Korzun AH, Norton L, Wood W, Strauchen JA, Grimes M, Weiss RB. Prediction of prognosis in primary breast cancer by detection of a high molecular weight mucin-like antigen using monoclonal antibodies DF3, F36/22, and CU18: a Cancer and Leukemia Group B study. *J Clin Oncol* 1991; **9**: 1113-1123

S- Editor Kumar M and Guo SY **L- Editor** Elsevier HK **E-Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 60-65
World Journal of Gastroenterology ISSN 1007-9327

COLORECTAL CANCER

Antithrombin reduces reperfusion-induced hepatic metastasis of colon cancer cells

Masanao Kurata, Kenji Okajima, Toru Kawamoto, Mitsuhiro Uchiba, Nobuhiro Ohkohchi

Masanao Kurata, Toru Kawamoto, Nobuhiro Ohkohchi, Department of Surgery, Functional and Regulatory Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan
Kenji Okajima, Department of Biodefense, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan
Mitsuhiro Uchiba, Department of Diagnostic Medicine, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan
Correspondence to: Nobuhiro Ohkohchi, Department of Surgery, Institute of Clinical Medicine,University of Tsukuba Tennoudai 1-1-1, Tsukuba,Japan. nokochi3@md.tsukuba.ac.jp
Telephone: +81-29-853-3221 Fax: +81-29-853-3222
Received: 2005-05-11 Accepted: 2005-06-09

Abstract

AIM: To examine whether antithrombin (AT) could prevent hepatic ischemia/reperfusion (I/R)-induced hepatic metastasis by inhibiting tumor necrosis factor (TNF)-α-induced expression of E-selectin in rats.

METHODS: Hepatic I/R was induced in rats and mice by clamping the left branches of the portal vein and the hepatic artery. Cancer cells were injected intrasplenically. The number of metastatic nodules was counted on day 7 after I/R. TNF-α and E-selectin mRNA in hepatic tissue, serum fibrinogen degradation products and hepatic tissue levels of 6-keto-$PGF_{1\alpha}$, a stable metabolite of PGI_2, were measured.

RESULTS: AT inhibited increases in hepatic metastasis of tumor cells and hepatic tissue mRNA levels of TNF-α and E-selectin in animals subjected to hepatic I/R. Argatroban, a thrombin inhibitor, did not suppress any of these changes. Both AT and argatroban inhibited I/R-induced coagulation abnormalities. I/R-induced increases of hepatic tissue levels of 6-keto-$PGF_{1\alpha}$ were significantly enhanced by AT. Pretreatment with indomethacin completely reversed the effects of AT. Administration of OP-2507, a stable PGI_2 analog, showed effects similar to those of AT in this model. Hepatic metastasis in congenital AT-deficient mice subjected to hepatic I/R was significantly increased compared to that observed in wild-type mice. Administration of AT significantly reduced the number of hepatic metastases in congenital AT-deficient mice.

CONCLUSION: AT might reduce I/R-induced hepatic metastasis of colon cancer cells by inhibiting TNF-α-induced expression of E-selectin through an increase in the endothelial production of PGI_2. These findings also raise the possibility that AT might prevent hepatic metastasis of tumor cells if administered during the resection of liver tumors.

Key words: Antithrombin; E-selectin; Hepatic ischemia/reperfusion; Metastasis; Prostacyclin; Tumor necrosis factor-α

Kurata M, Okajima K, Kawamoto T, Uchiba M, Ohkohchi N. Antithrombin reduces reperfusion-induced hepatic metastasis of colon cancer cells. *World J Gastroenterol* 2006; 12(1): 60-65

http://www.wjgnet.com/1007-9327/12/60.asp

INTRODUCTION

Hepatic ischemia/reperfusion (I/R) is sometimes induced by temporary clamping of the portal triad during resection of a liver tumor and a portal vein for advanced pancreatic head cancer[1, 2]. Hepatic I/R may promote hematogenous liver metastases of cancer cells that detach during the surgical procedure[3]. Endothelial leukocyte adhesion molecules such as E-selectin and intercellular adhesion molecule-1 have been demonstrated to be critically involved in the adhesion of tumor cells to endothelial cells, thereby promoting the metastasis of tumor cells[4-8]. Expression of these adhesion molecules in endothelial cells is markedly enhanced by pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, which plays a critical role in the development of I/R-induced liver injury[9, 10].

Antithrombin (AT), a physiological serine protease inhibitor, plays a critical role in the regulation of the coagulation cascade[11]. In addition to such regulatory role in the coagulation system, AT exerts an anti-inflammatory activity by increasing the endothelial production of prostacyclin (PGI_2), which is capable of inhibiting TNF-α production by monocytes[12-14]. According to this anti-inflammatory property, AT reduces I/R-induced liver injury by inhibiting TNF-α production in rats[15, 16]. These observations suggest that AT might reduce I/R-induced hepatic metastasis of cancer cells

by decreasing the expression of leukocyte adhesion molecules such as E-selectin through an increase in the endothelial production of PGI_2. In the present study, we have examined whether AT could reduce the metastasis of cancer cells injected intrasplenically in rats and congenital AT-deficient mice subjected to hepatic I/R.

MATERIALS AND METHODS

Reagents

AT and argatroban ((2R,4R)4-methyl-{N^2-[(3-methyl-1,2,3,4, tetrahydro-8-quinolyl) sulfonyl]-L-arginyl}-2-piperidinecarboxylic acid monohydrate) were obtained from the Mitsubishi-Welpharma Co., Ltd. (Osaka, Japan). OP-2507 (15 *cis*-(4-*n*-propylcyclohexyl)-16,17,18,19,20-pentanor-9-deoxy-6,9-α-nitrilo-prostaglandin F_1 methyl ester), a prostacyclin analog, was obtained from the Ono Pharmaceutical Co., Ltd. (Osaka, Japan). Indomethacin (IM), xylazine and RPMI 1640 were purchased from Sigma (St. Louis, MO, USA). Penicillin G, streptomycin, and fetal bovine serum (FBS) were obtained from Invitrogen (Gaithersburg, MD, USA). Ketamine hydrochloride was purchased from Parke-Davis (Morris Plains, NJ, USA). All other reagents were of analytical grade.

Animals

Pathogen-free male Fisher 344 (F344) rats, weighing 180-210 g, and C57BL/6 mice, weighing 18-22 g, were obtained from Clea Japan (Tsukuba, Japan). Congenital AT-deficient mice ($AT^{+/-}$) were kindly provided by Dr. Kojima, Nagoya University. Plasma levels of AT in $AT^{+/-}$ mice were about 50% of that in wild type[17]. Care and handling of the animals were in accordance with the National Institute of Health guidelines and the animals were fed standard animal chow and water. All the animal experiments were carried out in a humane manner after receiving permission from the Animal Experiment Committee of the university and in accordance with the Regulation of Animal Experiment and Japanese Governmental Law.

Cell lines

RCN-H4 cells, derived from hepatic metastasis of a F344 rat colon adenocarcinoma cell line, were provided by RIKEN Cell Bank (Tsukuba, Japan)[18, 19]. RCN-H4 cells were maintained in RPMI 1640 supplemented with 100 mL/L heat inactivated FBS, 100 kU/L penicillin G and 100 mg/L streptomycin in a humidified 950mL/L air-50 mL/L CO_2 at 37 ℃. B16-F10 melanoma cells, which are syngeneic to C57BL/6 mice, were gifted from Cell Resource Center of Tohoku University (Sendai, Japan). B16-F10 cells were maintained in the same medium that was used for RCN-H4 cell culture.

Rat hepatic metastasis model

Under anesthesia with diethyl ether, F344 rats underwent laparotomy. Warm ischemia of the median and left hepatic lobes was induced by clamping the left branches of the portal vein and the hepatic artery. The right lobe was perfused to prevent intestinal congestion. During the period of hepatic ischemia, the rat's abdomen was covered with a plastic wrap to prevent dehydration. AT (500 U/kg) and OP-2507 (3 μg/kg) were dissolved in double distilled water, and administered intravenously 20 min after ischemia. Argatroban (1 mg/kg) and IM (5 mg/kg) were administered subcutaneously 30 min prior to ischemia. After 30 min of ischemia, the clamp was removed and the right lobe and caudate lobe were resected to prevent shunting to them after reperfusion with 4-0 silk. After resection of the right and caudate lobes, RCN-H4 cells (2×10^6 cells/rat) were intrasplenically administrated using a 30-gauge needle and the wound was closed with 3-0 silk. Sham-operated animals underwent the same operation but without clamping. During the surgical operation, the rat' s body temperature was kept between 35.5 ℃ and 36.5 ℃ by using a heating pad. The rates of breathing and heart beating were stable during the operation. None of the animals died until the 7th day after the operation.

Mouse hepatic metastasis model

The same surgical procedure as for F344 rats was performed for every mouse except for the anesthesia method (ketamine, 75 mg/kg body weight plus xylazine, 25 mg/kg body weight) and ischemia time (60 min). After resection of the right and the caudate lobes, B16-F10 cells (2×10^5 cells/mouse) were intrasplenically administrated. AT (500 U/kg) was administered 10 min before reperfusion. None of the animals died until the 7th day after the operation.

Calculation of hepatic metastatic nodules

Animals were killed on the 7th day after the operation. The liver was removed, fixed with 10% buffered formalin for a few days, and cut into slices, each about 4 mm thick serially. These slices were stained with hematoxylin-eosin. The number of metastatic nodules was counted under a dissecting microscope (Olympus, Tokyo, Japan) at a magnification of ×100. The liver slice area was calculated with IP Lab software (Solution Systems Co., Ltd.) on a Macintosh computer. The number of metastatic nodules was normalized by the slice area (nodule number/cm^2).

Measurement of serum concentration of fibrin and fibrinogen degradation products

Ninety minutes after reperfusion, blood samples were collected in tubes containing 0.1 volume of 38g/L sodium citrate. Blood was centrifuged at 2 000 r/min for 10 min. Serum levels of fibrinogen degradation products (FDP (E)) were measured by a latex agglutination assay, as previously described[20].

Assay for 6-keto-$PGF_{1\alpha}$ in liver

Levels of 6-keto-$PGF_{1\alpha}$, a stable metabolite of PGI_2, in liver tissue were measured as described previously[15, 21]. In brief, tissue samples were weighed and homogenized in 0.1 mol/L phosphate buffer (pH 7.4) containing 0.5 g/L sodium azide. The homogenate was centrifuged (2 000 r/min, at 4 ℃ for 10 min) and the supernatant was stored at -80 ℃ until measurement. The concentration of 6-keto-$PGF_{1\alpha}$ was determined with a specific enzyme immunoassay kit (Amersham, Buckinghamshire, UK). The results were expressed as nanograms of 6-keto-$PGF_{1\alpha}$ per

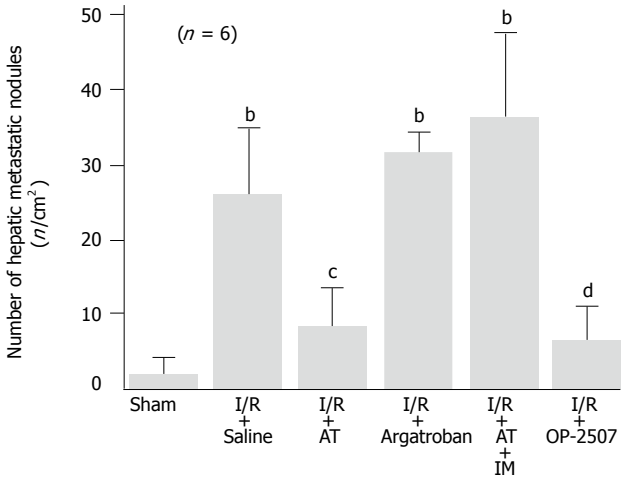


Figure 1 Effects of antithrombin (AT) and various agents on the number of hepatic metastatic nodules in rats after ischemia/reperfusion (I/R). [b]*P*<0.01 *vs* sham; [c]*P*<0.05, [d]*P*<0.01 *vs* I/R+saline.

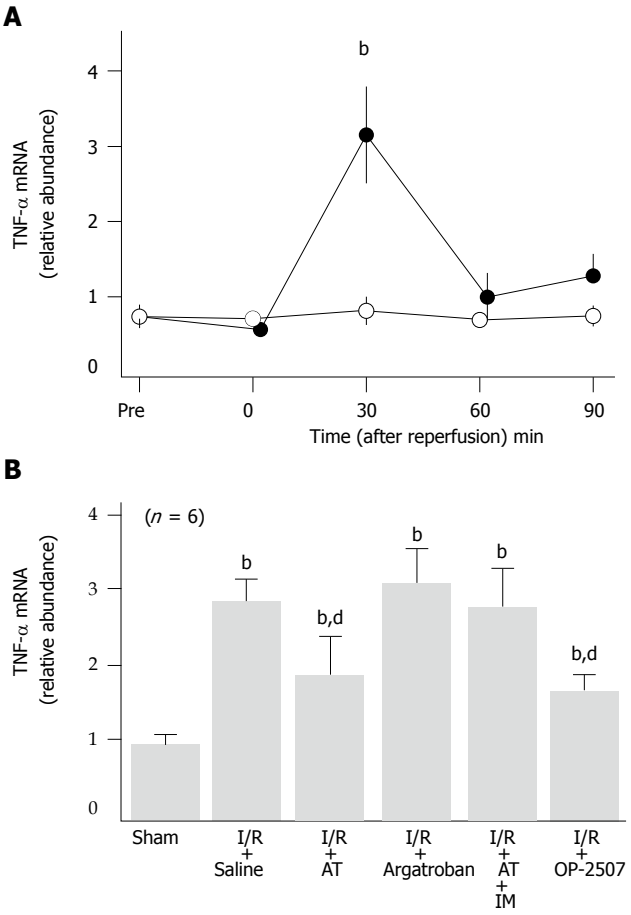


Figure 2 Changes in hepatic tumor necrosis factor (TNF)-α mRNA levels (**A**) and effects of antithrombin (AT) and various agents on TNF-α mRNA levels (**B**) in rats subjected to hepatic ischemia/reperfusion (I/R). [b]*P*<0.01 *vs* pre-ischemia. [d]*P*<0.01 *vs* sham and I/R+saline.

gram tissue.

RNA extraction and cDNA synthesis

Total RNA was isolated after homogenization of the tissue samples in TRIzol reagent according to the manufacturer's instructions. RNA was reverse transcribed into cDNA in a 20-μL reaction volume containing 2 μL RNA (1 μg) in RNase-free water, 2 μL of 10× RT buffer, 4.4 μL of 25 mmol/L $MgCl_2$, 4 μL deoxy NTPs mixture, 1 μL random hexamers, 0.4 μL RNase inhibitor, and 0.5 μL multiscribe reverse transcriptase (50 MU/L). Reverse transcription was performed at 25 ℃ for 10 min, at 37 ℃ for 60 min, and at 95 ℃ for 5 min, followed by quick chilling on ice and storage at 4 ℃ until subsequent amplification.

Real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Real-time quantitative RT-PCR analysis was performed in triplicate using the ABI PRISM 7700 Sequence Detection System instrument and software (Applied Biosystems, Foster City, CA, USA). The primers and probes for the TaqMan system were designed using Primer Express software (Applied Biosystems) and synthesized using PE ABI (Weiterstadt, Germany). The 5'-end nucleotide of the probe was labeled with a reporter dye (FAM). The sequences of the PCR primer sets and probes used for each gene were purchased from TaqMan Gene Expression Assays (Applied Biosystems). The reaction conditions and PCR cycles were set according to the manufacturer's directions.

Statistical analysis

All data were expressed as mean±SD. The results were compared using an analysis of variance (ANOVA) followed by Scheffe's test. An associated probability of $P<0.05$ was considered to be statistically significant.

RESULTS

Effects of AT and argatroban on I/R-induced increase of hepatic metastasis in rats

When intrasplenically injected, colon cancer cells formed a significant number of metastatic nodules in the liver of rats subjected to hepatic I/R on d 7 after the injection of cancer cells. The number of metastatic nodules in animals subjected to hepatic I/R was 13.6-fold than that observed in sham-operated animals (Figure 1). AT significantly inhibited the increase in the number of metastatic nodules in animals subjected to hepatic I/R (Figure 1). In contrast, argatroban, a synthetic selective inhibitor for thrombin, had no effect on this increase (Figure 1).

Effects of AT and argatroban on hepatic I/R-induced increase of TNF-α mRNA in hepatic tissue

Hepatic tissue levels of TNF-α mRNA did not significantly increase in sham-operated animals, while these significantly increased 30 min after hepatic I/R and decreased thereafter to pre-ischemia levels (Figure 2A). AT significantly inhibited the I/R-induced increases in the hepatic tissue TNF-α mRNA levels, whereas argatroban did not inhibit these increases (Figure 2B).

Effects of AT and argatroban on hepatic I/R-induced increase of E-selectin mRNA in hepatic tissue

Although hepatic tissue levels of E-selectin mRNA did not increase in sham-operated animals, these levels began

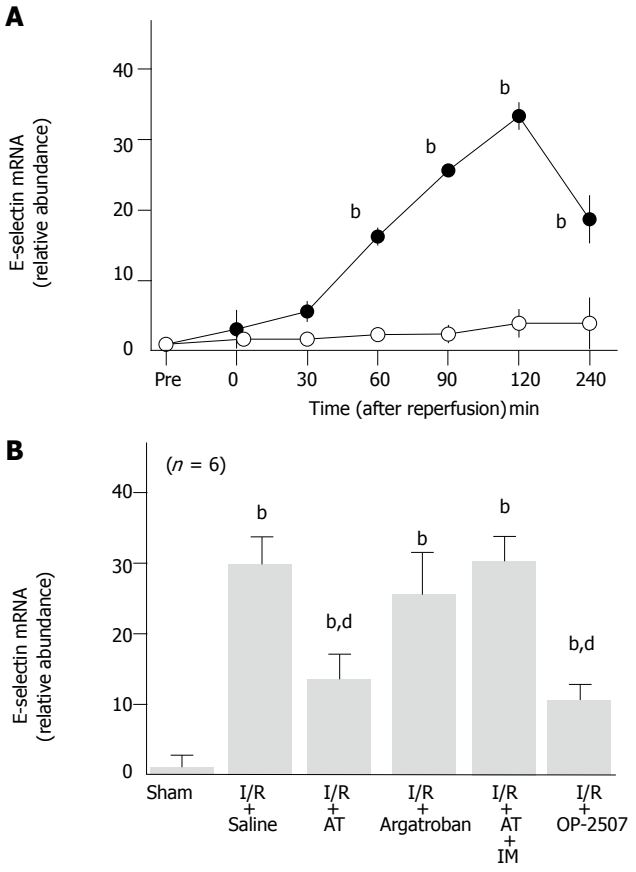


Figure 3 Changes in hepatic E-selectin mRNA levels (**A**) and effects of antithrombin (AT) and various agents on E-selectin mRNA levels (**B**) in rats subjected to hepatic ischemia/reperfusion (I/R). [b]*P*<0.01 *vs* pre-ischemia. [d]*P*<0.01 *vs* sham and I/R+saline.

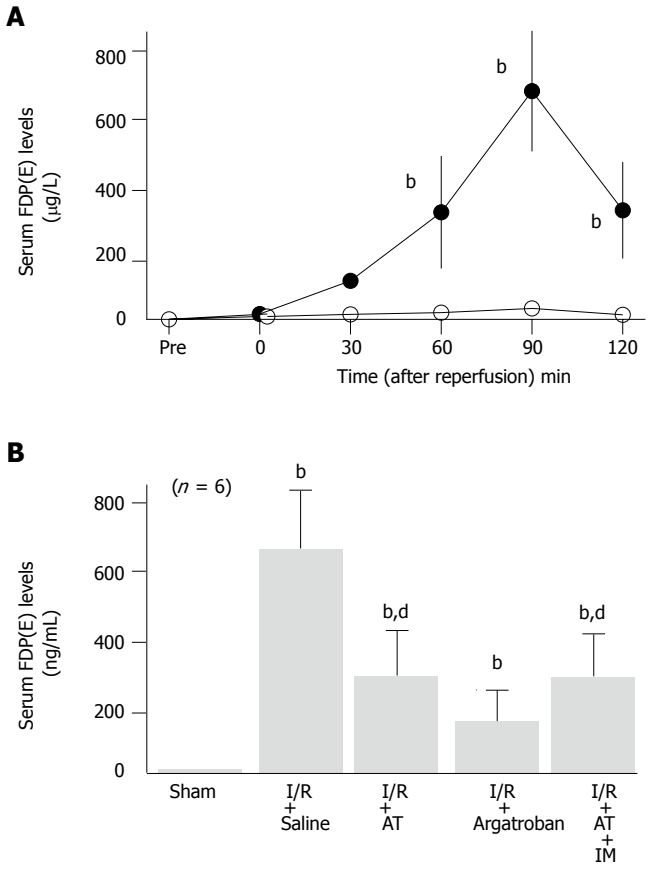


Figure 4 Changes in serum concentrations of fragment E of fibrin and fibrinogen degradation products (FDP(E)) (**A**) and effects of antithrombin (AT), argatroban and AT+indomethacin (IM) on serum concentrations of FDP(E) (**B**) in rats after hepatic ischemia/reperfusion (I/R). [b]*P*<0.01 *vs* pre-ischemia. [d]*P*<0.01 *vs* sham and I/R+saline.

to increase 60 min after reperfusion, peaking at 120 min after reperfusion in animals subjected to hepatic I/R (Figure 3A). AT significantly inhibited the I/R-induced increases of E-selectin mRNA levels observed in hepatic tissue 120 min after reperfusion, whereas argatroban did not inhibit these increases (Figure 3B).

Effects of AT and argatroban on I/R-induced increase of serum FDP(E)

Serum levels of FDP(E) significantly increased after reperfusion, peaking 90 min after reperfusion in animals subjected to hepatic I/R, while these levels did not increase in sham-operated animals (Figure 4A). Both AT and argatroban inhibited the hepatic I/R-induced increases of serum FDP(E) observed 90 min after reperfusion (Figure 4B).

Effect of AT on hepatic tissue levels of 6-keto-PGF$_{1\alpha}$

To examine whether AT reduced the hepatic I/R-induced increase in the number of metastatic nodules by promoting the endothelial release of PGI_2, we have analyzed the effect of AT on the hepatic tissue levels of 6-keto-$PGF_{1\alpha}$ after reperfusion. Hepatic tissue levels of 6-keto-$PGF_{1\alpha}$ increased after reperfusion, peaking 90 min after reperfusion (Figure 5A). These levels were significantly higher in the I/R group than in sham-operated animals (Figure 5B). Administration of AT significantly enhanced the I/R-induced increases in hepatic levels of 6-keto-$PGF_{1\alpha}$ 90 min after reperfusion, whereas argatroban did not (Figure 5B).

Effect of IM on anti-metastatic effect of AT observed in our experimental model

The anti-metastatic effect of AT was not observed in animals pretreated with IM which is known to inhibit cyclooxygenases (Figure 1). AT did not inhibit hepatic I/R-induced increases of TNF-α and E-selectin mRNA in hepatic tissue from animals pretreated with IM (Figures 2B and 3B). Hepatic I/R-induced increases in serum levels of FDP(E) were inhibited by AT in animals pretreated with IM (Figure 4B). Pretreatment of animals with IM significantly inhibited increases in hepatic tissue levels of 6-keto-$PGF_{1\alpha}$ in animals given AT 90 min after reperfusion (Figure 5B).

Effect of OP-2507 on hepatic I/R-induced increase in the number of metastatic nodules and hepatic tissue levels of TNF-α and E-selectin mRNA

To determine whether PGI_2 inhibited I/R-induced increase in the number of hepatic metastases, we have examined the effect of OP-2507 on the I/R-induced increase in the number of hepatic metastatic nodules. As shown in Figure 1 , the increase in the number of metastatic nodules in

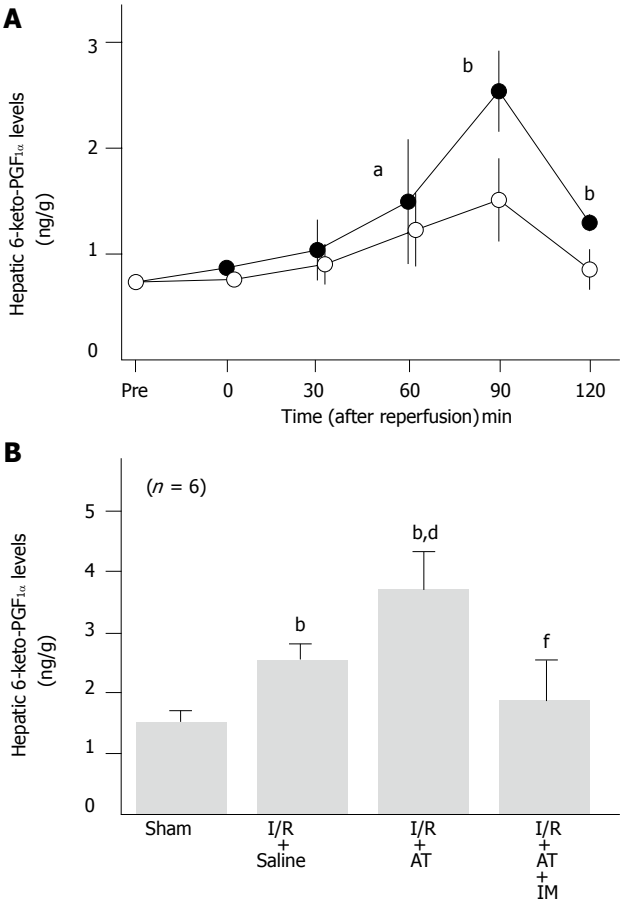


Figure 5 Changes in hepatic 6-keto-PGF$_{1\alpha}$ levels (**A**) and effects of antithrombin (AT) and AT plus indomethacin (IM) on hepatic 6-keto-PGF$_{1\alpha}$ levels (**B**) in rats subjected to hepatic ischemia/reperfusion (I/R). $^{a}P<0.05$, $^{b}P<0.01$ *vs* pre-ischemia. $^{d}P<0.01$ *vs* sham. $^{f}P<0.01$ *vs* I/R+saline and I/R+AT.

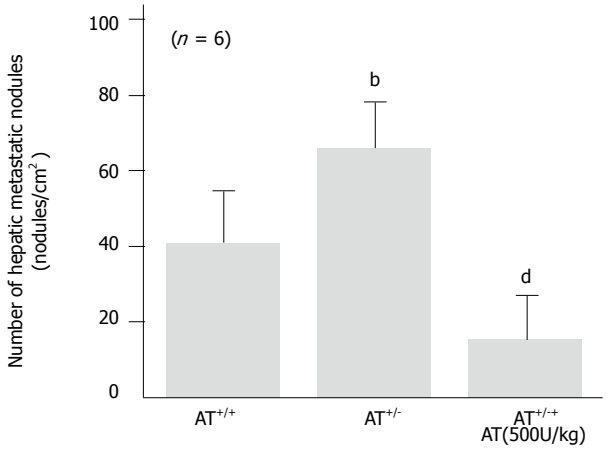


Figure 6 The number of hepatic metastatic nodules in congenital antithrombin (AT)-deficient mice. $^{b}P<0.01$ *vs* AT$^{+/+}$. $^{d}P<0.01$ *vs* AT$^{+/-}$

animals subjected to hepatic I/R was significantly inhibited by OP-2507. OP-2507 inhibited the I/R-induced increases in hepatic tissue levels of TNF-α and E-selectin mRNA observed 30 and 120 min after reperfusion, respectively (Figure 2B and 3B).

Hepatic I/R-induced metastasis of B16-F10 cells in congenital AT-deficient mice

To know whether endogenous AT also inhibited hepatic metastasis of B16-F10 cells injected intrasplenically, we have compared the number of hepatic metastatic nodules observed after I/R in congenital AT-deficient mice (AT$^{+/-}$) with that observed in wild-type C57BL/6J mice. The hepatic I/R-induced increase in the number of hepatic metastatic nodules observed 7 d after reperfusion was significantly higher in congenital AT$^{+/-}$ mice than in wild type mice (Figure 6). AT decreased the number of hepatic metastatic nodules in AT$^{+/}$ mice subjected to hepatic I/R (Figure 6).

DISCUSSION

In the present study, AT reduced the hepatic I/R-induced increase of hepatic metastasis in rats to which colon cancer cells were intrasplenically injected. TNF-α is a pro-inflammatory cytokine that increases the expression of leukocyte adhesion molecules such as E-selectin, thereby contributing to the lodgment of cancer cells on the sinusoidal surface, leading to metastatic nodule formation[22]. AT inhibited hepatic I/R-induced increases in hepatic tissue levels of TNF-α and E-selectin mRNA, suggesting that AT might inhibit the hepatic I/R-induced increase of colon cancer cells metastases in our rat model by inhibiting TNF-α production. TNF-α plays a critical role in microthrombus formation in hepatic I/R[23]. Since thrombin increases the expression of E-selectin by activating protease-activated receptor-1 on endothelial cells[24], AT might inhibit the hepatic metastasis of colon cancer cells by inhibiting thrombin activity. However, this possibility seemed less likely, since argatroban, a selective inhibitor of thrombin that inhibited hepatic I/R-induced increases of serum FDP (E) to the same extent as AT, did not inhibit the development of hepatic metastases in the present study. We have previously reported that AT inhibits hepatic I/R-induced increases in hepatic tissue levels of TNF-α by promoting the I/R-induced increase in endothelial production of PGI_2[16]. Consistent with this report is the present observation demonstrating that AT enhanced the hepatic I/R-induced increases in hepatic tissue levels of 6-keto-PGF$_{1\alpha}$. Pretreatment with IM reversed the effects of AT, including the inhibition of metastasis and increases in hepatic tissue levels of both TNF-α and E-selectin mRNA, suggesting that AT might inhibit hepatic I/R-induced increase in the metastasis of colon cancer cells by increasing endothelial production of PGI_2. Furthermore, OP-2507, a stable derivative of PGI_2, showed effects similar to those of AT, supporting the hypothesis described above.

Hepatic metastasis of intrasplenically injected cancer cells was significantly increased in heterozygous congenital AT-deficient mice whose plasma AT level is about half that of wild type mice[17] and replacement of AT in congenital AT-deficient mice reversed this increase in hepatic metastasis in the present study. These observations suggest that endogenous AT as well as AT given intravenously might be critically involved in the inhibition of hepatic I/R-induced increase of hepatic metastases in our experimental model.

Although the findings of the present study strongly

suggest that AT might inhibit the metastasis of colon cancer cells via the promotion of endothelial production of PGI_2, it is possible that a direct inhibition of tumor cell growth by AT also contribute to the inhibition of hepatic I/R-induced increase of colon cancer cell metastases. Our preliminary experiments using the monotetrazolium assay demonstrated that AT (5 kU/L) significantly inhibits the growth of RCN-H4 cells after incubation for 48 h[25]. Therefore, although IM pretreatment abrogated the anti-metastatic effect of AT in the present study, a direct effect of AT on cancer cell growth might contribute, at least in part, to the anti-metastatic effect of AT. This possibility should be further examined in future experiments.

AT concentrates are currently available for the treatment of disseminated intravascular coagulation and thrombosis in the clinical setting[26-28]. The findings of the present study raise the possibility that AT concentrates can effectively prevent hepatic metastasis of cancer cells during hepatectomy. These possibilities should be further examined in the clinical setting in the near future.

ACKNOWLEDGMENT

Prof T Kojima, Department of Medical Technology, Nagoya University School of Health Sciences for providing AT-deficient mice.

REFERENCES

1 **Arnoletti JP**, Brodsky J. Reduction of transfusion requirements during major hepatic resection for metastatic disease. *Surgery* 1999; **125**: 166-171
2 **Capussotti L**, Massucco P, Ribero D, Viganò L, Muratore A, Calgaro M. Extended lymphadenectomy and vein resection for pancreatic head cancer: outcomes and implications for therapy. *Arch Surg* 2003; **138**: 1316-1322
3 **Ku Y**, Kusunoki N, Shiotani M, Maeda I, Iwasaki T, Tominaga M, Kitagawa T, Fukumoto T, Suzuki Y, Kuroda Y. Stimulation of haematogenous liver metastases by ischaemia-reperfusion in rats. *Eur J Surg* 1999; **165**: 801-807
4 **Alexiou D**, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I, Tsigris C. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur J Cancer* 2001; **37**: 2392-2397
5 **Ye C**, Kiriyama K, Mistuoka C, Kannagi R, Ito K, Watanabe T, Kondo K, Akiyama S, Takagi H. Expression of E-selectin on endothelial cells of small veins in human colorectal cancer. *Int J Cancer* 1995; **61**: 455-460
6 **Uotani H**, Yamashita I, Nagata T, Kishimoto H, Kashii Y, Tsukada K. Induction of E-selectin after partial hepatectomy promotes metastases to liver in mice. *J Surg Res* 2001; **96**: 197-203
7 **Wittig BM**, Kaulen H, Thees R, Schmitt C, Knolle P, Stock J, Meyer zum Büschenfelde KH, Dippold W. Elevated serum E-selectin in patients with liver metastases of colorectal cancer. *Eur J Cancer* 1996; **32A**: 1215-1218
8 **Kitagawa T**, Matsumoto K, Iriyama K. Serum cell adhesion molecules in patients with colorectal cancer. *Surg Today* 1998; **28**: 262-267
9 **Burke J**, Zibari GB, Brown MF, Granger N, Kelly R, Singh I, McDonald JC. Hepatic ischemia-reperfusion injury causes E-selectin upregulation. *Transplant Proc* 1998; **30**: 2321-2323
10 **Colletti LM**, Cortis A, Lukacs N, Kunkel SL, Green M, Strieter RM. Tumor necrosis factor up-regulates intercellular adhesion molecule 1, which is important in the neutrophil-dependent lung and liver injury associated with hepatic ischemia and reperfusion in the rat. *Shock* 1998; **10**: 182-191
11 **Rosenberg RD**. Biochemistry of heparin antithrombin interactions, and the physiologic role of this natural anticoagulant mechanism. *Am J Med* 1989; **87**: 2S-9S
12 **Uchiba M**, Okajima K, Murakami K, Okabe H, Takatsuki K. Attenuation of endotoxin-induced pulmonary vascular injury by antithrombin III. *Am J Physiol* 1996; **270**: L921-L930
13 **Oh-ishi S**, Utsunomiya I, Yamamoto T, Komuro Y, Hara Y. Effects of prostaglandins and cyclic AMP on cytokine production in rat leukocytes. *Eur J Pharmacol* 1996; **300**: 255-259
14 **Ollivier V**, Parry GC, Cobb RR, de Prost D, Mackman N. Elevated cyclic AMP inhibits NF-kappaB-mediated transcription in human monocytic cells and endothelial cells. *J Biol Chem* 1996; **271**: 20828-20835
15 **Harada N**, Okajima K, Kushimoto S, Isobe H, Tanaka K. Antithrombin reduces ischemia/reperfusion injury of rat liver by increasing the hepatic level of prostacyclin. *Blood* 1999; **93**: 157-164
16 **Harada N**, Okajima K, Uchiba M, Kushimoto S, Isobe H. Antithrombin reduces ischemia/reperfusion-induced liver injury in rats by activation of cyclooxygenase-1. *Thromb Haemost* 2004; **92**: 550-558
17 **Ishiguro K**, Kojima T, Kadomatsu K, Nakayama Y, Takagi A, Suzuki M, Takeda N, Ito M, Yamamoto K, Matsushita T, Kusugami K, Muramatsu T, Saito H. Complete antithrombin deficiency in mice results in embryonic lethality. *J Clin Invest* 2000; **106**: 873-878
18 **Inoue Y**, Kashima Y, Aizawa K, Hatakeyama K. A new rat colon cancer cell line metastasizes spontaneously: biologic characteristics and chemotherapeutic response. *Jpn J Cancer Res* 1991; **82**: 90-97
19 **Okuno K**, Hirai N, Lee YS, Kawai I, Shigeoka H, Yasutomi M. Involvement of liver-associated immunity in hepatic metastasis formation. *J Surg Res* 1998; **75**: 148-152
20 **CLAUSS A**. [Rapid physiological coagulation method in determination of fibrinogen]. *Acta Haematol* 1957; **17**: 237-246
21 **Ramos E**, Closa D, Hotter G, Roselló-Catafau J, Gelpi E, Fernandez-Cruz L. The impact of arterialization on prostanoid generation after liver transplantation in the rat. *Transplantation* 1994; **58**: 140-144
22 **Brodt P**, Fallavollita L, Bresalier RS, Meterissian S, Norton CR, Wolitzky BA. Liver endothelial E-selectin mediates carcinoma cell adhesion and promotes liver metastasis. *Int J Cancer* 1997; **71**: 612-619
23 **Okajima K**, Harada N, Kushimoto S, Uchiba M. Role of microthrombus formation in the development of ischemia/reperfusion-induced liver injury in rats. *Thromb Haemost* 2002; **88**: 473-480
24 **Shankar R**, de la Motte CA, Poptic EJ, DiCorleto PE. Thrombin receptor-activating peptides differentially stimulate platelet-derived growth factor production, monocytic cell adhesion, and E-selectin expression in human umbilical vein endothelial cells. *J Biol Chem* 1994; **269**: 13936-13941
25 **Mosmann T**. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; **65**: 55-63
26 **Fourrier F**, Chopin C, Huart JJ, Runge I, Caron C, Goudemand J. Double-blind, placebo-controlled trial of antithrombin III concentrates in septic shock with disseminated intravascular coagulation. *Chest* 1993; **104**: 882-888
27 **Inthorn D**, Hoffmann JN, Hartl WH, Mühlbayer D, Jochum M. Antithrombin III supplementation in severe sepsis: beneficial effects on organ dysfunction. *Shock* 1997; **8**: 328-334
28 **Bucur SZ**, Levy JH, Despotis GJ, Spiess BD, Hillyer CD. Uses of antithrombin III concentrate in congenital and acquired deficiency states. *Transfusion* 1998; **38**: 481-498

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 66-69
World Journal of Gastroenterology ISSN 1007-9327

Helicobacter pylori

Discrepancies between primary physician practice and treatment guidelines for *Helicobacter pylori* infection in Korea

Byeong Gwan Kim, Ji Won Kim, Ji Bong Jeong, Young Jin Jung, Kook Lae Lee, Young Soo Park, Jin Huk Hwang, Jin Uk Kim, Na Young Kim, Dong Ho Lee, Hyun Chae Jung, In Sung Song

Byeong Gwan Kim, Ji Won Kim, Ji Bong Jeong, Young Jin Jung, Kook Lae Lee, Seoul National University Boramae Hospital; Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea
Young Soo Park, Jin Huk Hwang, Jin Uk Kim, Na Young Kim, Dong Ho Lee, Seoul National University Bundang Hospital; Seoul National University College of Medicine, Seoul, Korea
Hyun Chae Jung, In Sung Song, Seoul National University College of Medicine, Seoul, Korea
Correspondence to: Dr Dong Ho Lee, MD, Seoul National University Bundang Hospital; Department of Internal Medicine, Seoul National University College of Medicine, 300 Gumi-dong, Bundang-gu, 463-707 Seoul, Korea. dhljohn@snubh.org
Telephone: +82-31-787-7006 Fax: +82-31-787-4051
Received: 2005-06-08 Accepted: 2005-07-17

Kim BG, Kim JW, Jeong JB, Jung YJ, Lee KL, Park YS, Hwang JH, Kim JU, Kim NY, Lee DH, Jung HC, Song IS. Discrepancies between primary physician practice and treatment guidelines for *Helicobacter pylori* infection in Korea. *World J Gastroenterol* 2006; 12(1): 66-69

http://www.wjgnet.com/1007-9327/12/66.asp

Abstract

AIM: To evaluate the attitude of primary care physicians in the diagnosis and treatment of *Helicobacter pylori (H pylori)* infection.

METHODS: Primary care physicians in the Seoul metropolitan area answered self-administered questionnaire from January to March 2003.

RESULTS: One hundred and eight doctors responded to the questionnaire. The most frequent reasons for testing *H pylori* infection were gastric and duodenal ulcers (93.5% and 88.9%, respectively). For patients with *H pylori* positive dyspepsia, 28.7% of doctors always tried to eradicate the worm and 34.4% treated selectively. A large proportion (28.7%) of primary care physicians treated *H pylori* on a patient's request basis. Only 9.3% of primary care physicians always conducted follow-up testing after treating *H pylori* infection. When *H pylori* was not cleared by the first treatment, 40.7% of doctors reused the same regimen, 16.7% changed to another triple regimen and 25% to a quadruple regimen.

CONCLUSION: It has been well documented that the issuance of guidelines alone has little impact on practice. Communication between primary care physicians and gastroenterologists in the form of continuous medical education is required.

Key words: *Helicobacter pylori*; Guidelines; Primary care

INTRODUCTION

It is now accepted that infection of the gastric mucosa with *Helicobacter pylori* (*H pylori*) causes chronic gastritis. More than 90% of peptic ulcer patients are infected with *H pylori* and it has been shown that successful treatment prevents ulcer relapse[1-3]. National and international guidelines have been published on the management of *H pylori*, and it is tacitly assumed that these guidelines are adhered to in clinical practice[4-7]. But, there seems to be a discrepancy between current testing and treatment guidelines and clinical practice[8,9]. In particular, the approaches used in primary practice may differ markedly from those used in referral hospitals[10-13]. Patients commonly visit both primary care physicians and gastroenterologists because of upper gastrointestinal symptoms. Variable *H pylori* detection methods, including serologic assays and the urea breath test, are currently used. Although most physicians do not believe that *H pylori* causes non-ulcer dyspepsia, the majority often prescribe antibiotics for *H pylori* eradication. The extent to which research findings regarding *H pylori* have modified physicians' practice in general has not been well studied. We conducted the present study to determine whether current guidelines regarding *H pylori* infection have influenced diagnostic and therapeutic primary care practice.

MATERIALS AND METHODS

From January to March 2003, we conducted an observational, transverse study by using a self-administered questionnaire. Primary care physicians in the metropolitan area of Seoul were random-ly select-ed from the membership database of the Seoul Medical Association. Questionnaires were distributed by post, and non-respondents were sent reminders and then contacted by telephone. One hundred and thirty-five doctors participated in the study.

Table 1 Korean guidelines for *H pylori* treatment (Korean *H pylori* Study Group, 1998)

Indication for *H pylori* eradication	Peptic ulcer Regardless of the stage of ulcer Low-grade MALT associated lymphoma Stage IE1 After endoscopic mucosal resection (EMR) of early gastric cancer (EGC)
Recommended first line therapy	PPI-based triple therapy for 1-2 wk - PPI (omeprazole 20 mg or lansoprazole 30 mg or pantoprazole 40 mg) b.i.d. - Amoxicillin (not ampicillin) 1 000 mg b.i.d. - Clarithromycin (or metronidazole) 500 mg b.i.d.
Follow-up after eradication therapy	Urea breath test: test of choice, if available Or both biopsy urease test and histology At least 4 wk after completion of therapy Serology: not useful to confirm the eradication
Recommended second-line therapy	Quadruple therapy for 1 wk (PPI+conventional bismuth-based triple therapy) - PPI (omeprazole 20 mg or lansoprazole 30 mg or pantoprazole 40 mg) b.i.d. - Denol 120 mg b.i.d. - Metronidazole 400-500 mg t.i.d. - Tetracycline 500 mg q.i.d.

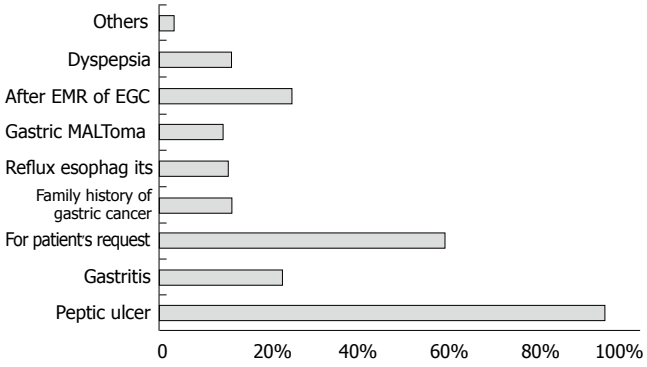


Figure 1 When do you test for *H pylori*?

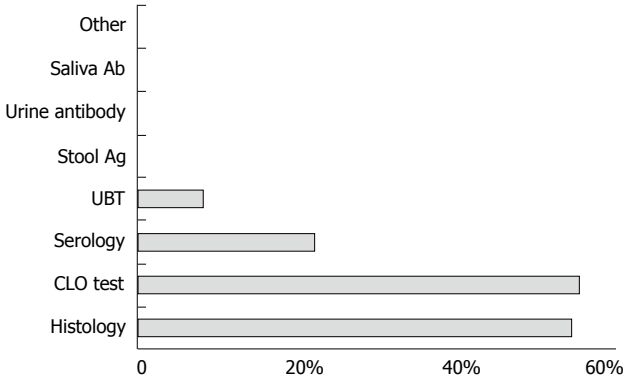


Figure 2 Which test do you prefer?

RESULTS

One hundred and eight doctors (80%) responded to the survey. We itemized below the physicians' responses to the questions given in the questionnaire.

When do you test for H pylori?

Primary care physicians widely used the *H pylori* test in cases of gastric ulcer and duodenal ulcer (88.9% and 93.5%, respectively, Figure 1). But they conducted tests for *H pylori* only in 26.9% and 13% of patients, after surgery for early gastric cancer or Maltoma (Figure 1). Frequently physicians tested for *H pylori* in cases of gastritis and due to a patient's request (25.0% and 58.3%, respectively, Figure 1).

Which test do you prefer?

More than half of the primary care physicians stated that they used the rapid urease test and biopsy, (55.6% and 54.6%, respectively, Figure 2), and a minority used the urea breath test and serology (8.3% and 22.2%, respectively, Figure 2).

How do you eradicate H pylori infection?

An 88% of physicians responded that they prescribed a proton pump inhibitor (PPI)-based triple regimen according to Korean guidelines (Table 1, Figure 3), and only small numbers were found to prescribed dual and quadruple regimens (2.8% and 2.8%, respectively, Figure 3).

How long do you treat H pylori infection?

The majority of physicians responded that they prescribe medication for 7,14 d (90.7%), according to Korean guidelines (Figure 4). A small number responded that they prescribed for less than 7, 21 or 28 d, accounting for 4.6%, 1.9%, 2.8%, respectively (Figure 4).

Do you perform follow-up testing after treating H pylori infection?

Only 9.3% replied that they always conducted follow-up testing after treating *H pylori* infection (Figure 5). The majority of primary care physicians stated that they selectively apply follow-up tests (Figure 5).

What kind of method do you prefer as follow-up test?

The majority percent of physicians stated that they used the rapid urease test or biopsy, accounting for 35.2% and 25.9%, respectively (Figure 6), while a minority favored urea breath testing or serology, accounting for 21.3% and 6.5%, respectively (Figure 6).

Treatment plan after failure to eradicate H pylori

Surprisingly, a large proportion (40.7%) of primary care physicians prescribed the original regimen after failure to eradicate *H pylori* (Figure 7). Only 25.0% physicians

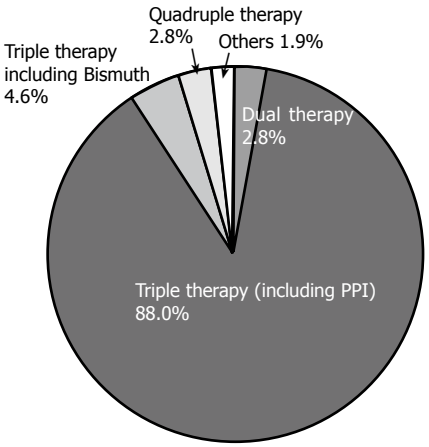


Figure 3 How do you eradicate *H pylori* infection?

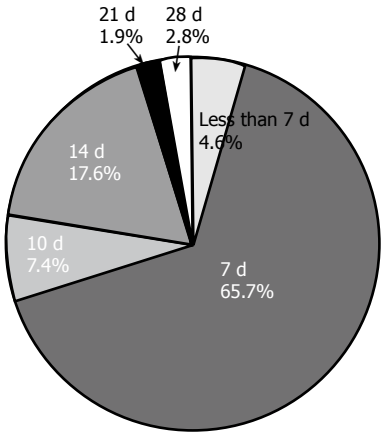


Figure 4 How long do you treat *H pylori* infection?

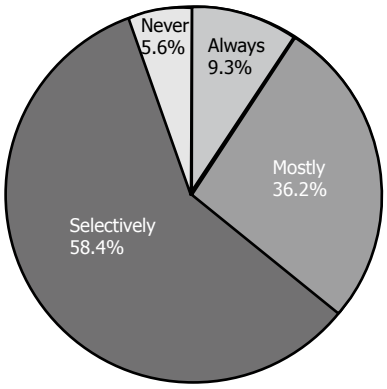


Figure 5 Do you perform follow-up testing after treating *H pylori* infection?

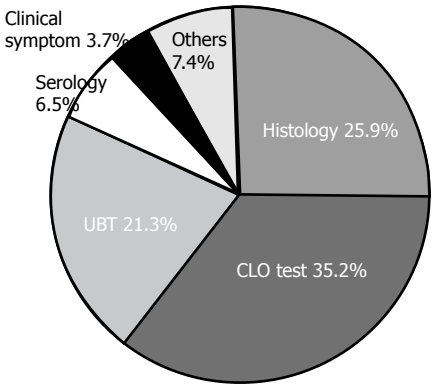


Figure 6 What kind of method do you prefer as follow-up test?

prescribed regimens complying with Korean guidelines (Figure 7).

Do you treat H pylori infection in dyspepsia patients without peptic ulcer?

Physicians (31.5%) responded that they always eradicate H pylori (Figure 8), while 10.2% treated *H pylori* on patient's request without testing (Figure 8).

Do you medicate for H pylori eradication by patient's request?

A large number (28.7%) of physicians responded positively (Figure 9).

DISCUSSION

The prevalence of *H pylori* among Korean adults is 60%-80%[14], and gastric cancer remains the most common malignancy in Korea[15]. Most Korean primary care physicians are interested in *H pylori*, and more frequently prescribe medication for the treatment of *H pylori* than gastroenterologist. The indications for *H pylori* eradication in Korea (Korean *H pylori* Study Group, 1998) are peptic ulcer, low-grade MALT-associated lymphoma, and post-endoscopic mucosal resection of early gastric cancer (Table 1). The recommended first-line therapy in Korea is the PPI-based triple therapy for 1 to 2 weeks. PPI would be the omeprazole, lansoprazole, or pantoprazole (Table 1). The treatment of choice in terms of antibiotics is the amoxicillin+clarithromycin (Table 1). The recommended second-line therapy according to the Korean guidelines is quadruple therapy for a week (Table 1), based on PPI, Denol, metronidazole, and tetracycline (Table 1). The issued Korean guidelines are similar to those issued by the Asia Pacific Consensus Conference on the management of *H pylori* infection[6]. The survey conducted for the present study involved mailing a questionnaire to primary physicians in Seoul, Korea. The questions primarily addressed physician decisions concerning the evaluation and treatment of *H pylori* infection in patients with gastroenterologic disease. Alternative treatment regimens were also examined. The survey results indicate that primary care physicians widely adopt *H pylori* testing in cases of gastric ulcer and duodenal ulcer according to Korean guidelines. However, physicians only conduct *H pylori* testing in 26.9% and 13.0% of postoperative early gastric cancer and Maltoma patients. In addition, they frequently test for *H pylori* in cases of gastritis and due to a patient's request, accounting for 25.0% and 58.3%, respectively, but many do not perform follow-up testing after *H pylori* treatment. Only 9.3% of primary care physicians always conduct follow-up testing after *H pylori* treatment. The majority of primary care physicians prefer the rapid urease test

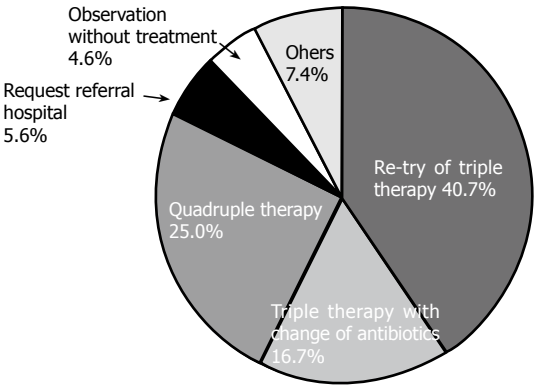


Figure 7 Treatment plan after failure to eradicate *H pylori*.

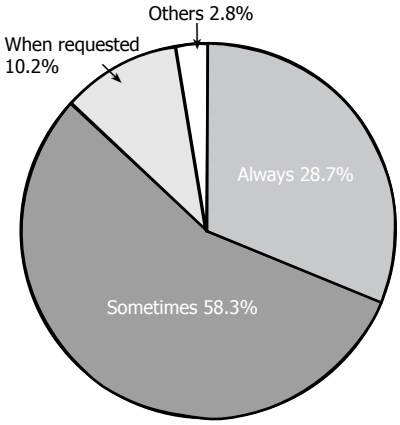


Figure 8 Do you treat *H pylori* infection in dyspepsia patients without peptic ulcer?

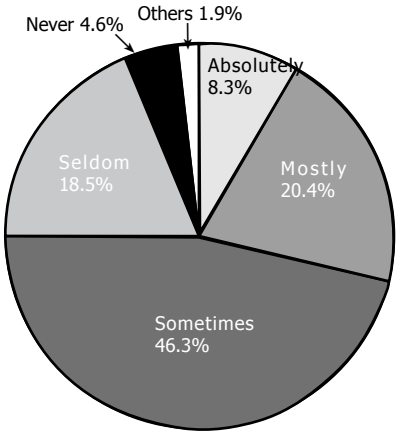


Figure 9 Do you medicate for *H pylori* eradication by patient's request?

or biopsy, accounting for 35.2%, 25.9%, respectively, as follow-up tests, because generally in Korea primary care physicians have an endoscopic unit, but not urea breath test equipment; 6.5% physicians use a serology-based follow-up test. Only 25.0% prescribe a quadruple regimen as second line therapy, contrary to the Korean guidelines and a large number (40.7%) of physicians prescribe the same regimen after failing to eradicate *H pylori*. In addition, they frequently treat *H pylori* in cases of non-ulcer dyspepsia and patient's request. This finding is at odds with the current guideline and primary care practice for the diagnosis and treatment of *H pylori* infetion in Korea. Moreover, the finding of the present study compare well with data published in other countries[10-13]. Thus, the issuance of guidelines has little impact on practice. Our findings suggest that communication programs, such as continuous medical education, between primary care physicians and gastroenterologists are needed. Moreover, schemes designed to ensure guideline implementation should be preceded by a detailed analysis of likely primary care physician response.

REFERENCES

1 **Marshall BJ**. Helicobacter pylori. *Am J Gastroenterol* 1994; **89**: S116-S128
2 **Rauws EA**, Tytgat GN. Cure of duodenal ulcer associated with eradication of Helicobacter pylori. *Lancet* 1990; **335**: 1233-1235
3 **Graham DY**, Lew GM, Klein PD, Evans DG, Evans DJ, Saeed ZA, Malaty HM. Effect of treatment of Helicobacter pylori infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. *Ann Intern Med* 1992; **116**: 705-708
4 Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consensus Report. European Helicobacter Pylori Study Group. *Gut* 1997; **41**: 8-13
5 American Gastroenterological Association medical position statement: evaluation of dyspepsia. *Gastroenterology* 1998; **114**: 579-581
6 **Lam SK**, Talley NJ. Report of the 1997 Asia Pacific Consensus Conference on the management of Helicobacter pylori infection. *J Gastroenterol Hepatol* 1998; **13**: 1-12
7 **Peterson WL**, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. Helicobacter pylori-related disease: guidelines for testing and treatment. *Arch Intern Med* 2000; **160**: 1285-1291
8 **Sharma VK**, Howden CW. A national survey of primary care physicians' perceptions and practices related to Helicobacter pylori infection. *J Clin Gastroenterol* 2004; **38**: 326-331
9 **Huang J**, Lam SK, Malfertheiner P, Hunt RH. Has education about Helicobacter pylori infection been effective? Worldwide survey of primary care physicians. *J Gastroenterol Hepatol* 2003; **18**: 512-520
10 **Provenzale D**, Ofman J, Gralnek I, Rabeneck L, Koff R, McCrory D. Gastroenterologist specialist care and care provided by generalists--an evaluation of effectiveness and efficiency. *Am J Gastroenterol* 2003; **98**: 21-28
11 **Cardin F**, Zorzi M, Furlanetto A, Guerra C, Bandini F, Polito D, Bano F, Grion AM, Toffanin R. Are dyspepsia management guidelines coherent with primary care practice? *Scand J Gastroenterol* 2002; **37**: 1269-1275
12 **Martínez-Sánchez G**, Saperas E, Benavent J, Mearin F, Piñol JL, Barenys M, Mascort JJ, Forné M, Bordas JM, Azagra R, Piqué JM. [The attitude of primary health care physicians in the metropolitan area of Barcelona about the diagnosis and treatment of Helicobacter pylori infection in gastroduodenal diseases]. *Gastroenterol Hepatol* 1998; **21**: 473-478
13 **Breuer T**, Goodman KJ, Malaty HM, Sudhop T, Graham DY. How do clinicians practicing in the U.S. manage Helicobacter pylori-related gastrointestinal diseases? A comparison of primary care and specialist physicians. *Am J Gastroenterol* 1998; **93**: 553-561
14 **Kim JH**, Kim HY, Kim NY, Kim SW, Kim JG, Kim JJ, Roe IH, Seo JK, Sim JG, Ahn H, Yoon BC, Lee SW, Lee YC, Chung IS, Jung HY, Hong WS, Choi KW. Seroepidemiological study of Helicobacter pylori infection in asymptomatic people in South Korea. *J Gastroenterol Hepatol* 2001; **16**: 969-975
15 **Bae JM**, Jung KW, Won YJ. Estimation of cancer deaths in Korea for the upcoming years. *J Korean Med Sci* 2002; **17**: 611-615

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M and Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 70-74
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Gallbladder bile composition in patients with Crohn's disease

Annika Lapidus, Jan-Erik Åkerlund, Curt Einarsson

Annika Lapidus, Jan-Erik Åkerlund, Curt Einarsson, Centre of Gastrointestinal Disease, Ersta Hospital, Department of Surgery, Danderyd Hospital and Department of Gastroenterology and Hepatology, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden
Supported by grants from the Swedish Research Council and Karolinska Institutet
Correspondence to: Dr Curt Einarsson, Department of Medicine, Division of Gastroenterology and Hepatology, K63, Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden. curt.einarsson@medhs.ki.se
Telephone: +46-8-5858-0000 Fax: +46-8-5858-2335
Received: 2005-05-12 Accepted: 2005-06-02

Abstract

AIM: To further elucidate the pathogenesis and mechanisms of the high risk of gallstone formation in Crohn's disease.

METHODS: Gallbladder bile was obtained from patients with Crohn's disease who were admitted for elective surgery (17 with ileal/ileocolonic disease and 7 with Crohn's colitis). Fourteen gallstone patients served as controls. Duodenal bile was obtained from ten healthy subjects before and after the treatment with ursodeoxycholic acid. Bile was analyzed for biliary lipids, bile acids, bilirubin, crystals, and crystal detection time (CDT). Cholesterol saturation index was calculated.

RESULTS: The biliary concentration of bilirubin was about 50% higher in patients with Crohn's disease than in patients with cholesterol gallstones. Ten of the patients with Crohn's disease involving ileum and three of those with Crohn's colitis had cholesterol saturated bile. Four patients with ileal disease and one of those with colonic disease displayed cholesterol crystals in their bile. About 1/3 of the patients with Crohn's disease had a short CDT. Treatment of healthy subjects with ursodeoxycholic acid did not increase the concentration of bilirubin in duodenal bile. Several patients with Crohn's disease, with or without ileal resection/disease had gallbladder bile supersaturated with cholesterol and short CDT and contained cholesterol crystals. The biliary concentration of bilirubin was also increased in patients with Crohn's colitis probably not due to bile acid malabsorption.

CONCLUSION: Several factors may be of importance for the high risk of developing gallstones of both cholesterol and pigment types in patients with Crohn's disease.

Key words: Bile acid; Biliary lipid composition; Bilirubin; Crohn's disease; Gallstone disease

Lapidus A, Åkerlund JE, Einarsson C. Gallbladder bile composition in patients with Crohn's disease. *World J Gastroenterol* 2006; 12(1): 70-74

http://www.wjgnet.com/1007-9327/12/70.asp

INTRODUCTION

The prevalence of gallstone disease in patients with Crohn's disease is about two-fold higher than that in general population[1-8]. This is true not only for patients with ileal disease/resection but also for patients with Crohn's colitis. No large studies of gallstone composition are available in patients with Crohn's disease but in two small series, while both pigment stones and cholesterol-rich stones have been reported[9,10]. The pathogenesis of gallstones in patients with Crohn's disease still remains to be elucidated.

One hypothesis for the increased prevalence of gallstone disease in patients with Crohn's disease is that the bile acid malabsorption in patients with diseased or resected ileum may lead to cholesterol supersaturated bile. In fact, supersaturated bile has been reported in some[11-14] but not all studies of patients with ileal disease or resection[8-10,15-19].

Another hypothesis for gallstone formation in patients with Crohn's disease is that patients with ileal disease or resection develop pigment stones as a consequence of increased spillage of malabsorbed bile acids into the colon where they solubilize unconjugated bilirubin and promote its absorption and thereby increase the rate of bilirubin secretion into the bile. In support of this hypothesis, an increased concentration of bilirubin in gallbladder bile or duodenal bile of patients with chronic ileitis or previous ileectomy has been reported[10,14,17,18]. Brink *et al.* [20] demonstrated that bile acid malabsorption after ileectomy of rats induces enterohepatic circulation of bilirubin and doubles the secretion rate of bilirubin into the bile. The same research group has also shown that adding ursodeoxycholic acid to the diet of mice and rats can increase the cecal bile acid levels and bilirubin secretion rates into the bile probably by inducing enterohepatic cycling of bilirubin[21].

The aims of the present study were to determine the biliary lipid composition, occurrence of cholesterol crystals, crystallization time, and bilirubin concentration in gallbladder bile of patients with ileal Crohn's disease

Table 1 Characteristics of patients with Crohn's disease and controls with gallstone disease

Patient group	*n*	Sex (M/F)	Mean age (range) (yr)	Previous surgery	Present surgery	Medical treatment
Crohn's disease						
Ileitis or ileocolitis	17	8/9	38 (23-70)	Ileal or ileocolonic Ileal, resection (*n* = 12)	Colonic or ileocolonic resection (*n* = 15) Cholecystectomy (*n* = 2)	Steroids (*n* = 11) Azathioprine (*n* = 4) Nitromidazole (*n* = 6) 5-ASA (*n* = 2)
Colitis	7	5/2	42 (28-57)	Partial colonic resection (*n* = 3)	Partial colonic resection or colectomy (*n* = 7)	Steroids (*n* = 5) Azathioprine (*n* = 1) Nitromidazole (*n* = 3)
Gallstone disease	14	1/13	47 (27-57)		Cholecystectomy (*n* = 14)	

with or without the involvement of colon and especially patients with Crohn's colitis in comparison to patients with cholesterol gallstone disease undergoing cholecystectomy served as control group and to study the influence of treatment with ursodeoxycholic acid on the duodenal concentration of bilirubin in human subjects.

MATERIALS AND METHODS

Patients and healthy subjects

Twenty-four patients with Crohn's disease admitted for elective surgery were included in the study. Clinical details are given in (Table 1). One patient had slightly elevated serum alkaline phosphatase level and three had slightly elevated transaminase and/or gamma glutamyl transpeptidase (GGT)-level. All other patients had normal laboratory tests of liver function including serum bilirubin. Seventeen patients had ileal or ileocolonic disease and 7 patients had Crohn's disease confined solely to the colon. Two patients with gallstones were cholecystectomized, one of them simultaneously underwent colectomy. All other patients were admitted for ileal, ileocolonic or colonic resection due to the failure of pharmacological treatment.

Fourteen consecutive patients with cholesterol gallstones admitted for cholecystectomy served as controls (Table 1).

In another experiment, 10 healthy subjects (6 men and 4 women, mean age 44 years) were studied before and after the treatment with ursodeoxycholic acid.

Informed consent was obtained from all the participants. The ethical aspect of the study was approved by the Ethical Committee of Karolinska Hospital Huddinge.

Experimental procedures

After the abdomen was opened, bile from the gallbladder was obtained by needle aspiration. The bile was collected in sterile tubes surrounded by foil and sent to the laboratory for analysis.

The healthy subjects were treated with ursodeoxycholic acid (Ursofalk® in 250 mg capsules, obtained from Dr Falk Pharma, Freiburg, Germany) at a daily dose of 15 mg/kg for 3 wk. Before and after the treatment, the bile was collected with an oroduodenal tube in the morning after an overnight fast. Gallbladder contraction was stimulated by an intravenous injection of cholecystokinin and 5-10 mL of the concentrated bile was obtained through the tube. The bile was collected in a test tube surrounded by a foil and was sent to the laboratory for analysis. Serum samples were also collected for analysis of bilirubin.

Biliary bilirubin concentration

An aliquot of the bile was immediately diluted with saline and the bilirubin concentration was determined by a similar procedure as for serum bilirubin as described previously[18]. The biliary concentration was expressed as mmol/L in gallbladder bile and as micromoles of bilirubin per millimole bile acid in duodenal bile.

Biliary lipids and bile acid composition

A portion of the gallbladder bile was immediately extracted with 20 volumes of chloroform–methanol 2:1 (vol/vol) and analyzed for cholesterol and phospholipids. Cholesterol was determined by an enzymatic method[22] and phospholipids by the method of Rouser *et al*.[23] The total bile acid concentration in one aliquot of the bile sample was determined using 3-alpha-hydroxy steroid dehydrogenase assay[24]. The relative concentration of cholesterol bile acids and phospholipids was expressed as molar percentage of the total biliary lipids. The cholesterol saturation was calculated according to Carey[25]. Bile acid composition was determined using gas-liquid chromatography[26].

Analysis of cholesterol crystals and crystallization time (CDT)

Gallbladder bile samples were examined for typical rhomboid monohydrate cholesterol crystals by polarizing light microscopy on pre-heated slides. CDT was determined by the method of Holan *et al*.[27] with minor modifications[28]. After centrifugation of about 6 mL bile at 100 000 *g* for 2 h, 3 mL from the middle phase was transferred into a sterile glass vial and sealed with a cap equipped with permeable silicon membrane. The vial was stored in darkness in an incubator at 37 ℃. About 3 μL from the top, middle and bottom portions was aspirated each day, mixed and placed on a pre-heated slide and viewed thoroughly by polarizing light microscopy. CDT was defined as the number of days until the appearance of typical rhomboid monohydrate cholesterol crystals.

Statistical analysis

Data were given as mean ± SE. Comparisons of the data between patients and healthy subjects were calculated using Mann-Whitney's rank sum test and Wilcoxon's sum of rank test. $P < 0.05$ was considered statistically significant.

Table 2 Biliary lipid composition and cholesterol saturation (mean ± SE)

	Crohn's disease ileitis or ileocolitis (n = 17)	Crohn's disease colitis (n = 7)	Crohn's disease all patients (n = 24)	Gallstone disease (n = 14)
Cholesterol (molar%)	7.4± 0.7	6.4 ± 0.9	7.1 ± 0.6	9.8 ± 1.2
Phospholipids (molar%)	22.3 ± 1.2	25.0 ± 1.8	23.1 ± 1.0	25.1 ± 1.8
Bile acids (molar%)	70.3 ± 1.6	68.6 ± 2.4	69.8 ± 1.3	65.1 ± 3.0
Cholesterol saturation (%)	103 ± 9	83 ± 9[a]	97 ± 7	138 ± 18

[a]$P < 0.05$ *vs* patients with gallstone disease.

Table 3 Biliary bile acid composition (mean ± SE)

	Crohn's disease ileitis or ileocolitis (n = 17)	Crohn's disease colitis (n = 7)	Crohn's disease all patients (n = 24)	Gallstone disease (n = 14)
Cholic acid (%)	44.5 ± 3.3	43.2 ± 2.2	44.1 ± 2.4[a]	35.4 ± 2.7
Chenodeoxycholic acid (%)	43.0 ± 2.9[b]	45.3 ± 3.8[b]	43.7 ± 2.3[b]	31.2 ± 2.4
Deoxycholic acid (%)	8.4 ± 2.3[c]	10.7 ± 3.2[c]	9.1 ± 1.8[c]	30.7 ± 4.3
Lithocholic acid (%)	0.10 ± 0.09[c]	0.01 ± 0.01[c]	0.07 ± 0.07[c]	1.5 ± 0.2
Ursodeoxycholic acid (%)	3.6 ± 1.8	0.7 ± 0.5	2.7 ± 1.3	1.2 ± 0.3

$P < 0.05$, [2]$P < 0.01$, [3]$P < 0.001$ *vs* patients with gallstone disease.

Table 4 Bilirubin concentrations in bile (mean ± SE)

	Crohn's disease ileitis or ileocolitis (n = 15)	Crohn's disease colitis (n = 6)	Crohn's diseaseall patients (n = 21)	Gallstone disease (n = 14)
Bilirubin (mmol/L)	4.6 ± 0.7[a]	5.9 ± 1.6	5.0 ± 0.7	2.6 ± 0.2

* Significantly different from corresponding value of patients with gallstone disease. [a]$P < 0.05$ *vs* patients with gallstone disease.

RESULTS

Gall bladder bile composition

Data on biliary lipid composition are given in (Table 2). The cholesterol saturation of bile was significantly lower in patients with Crohn's disease confined to the colon than in patients with the gallstone. In contrast, 10 out of 17 patients with Crohn's disease involving the ileum had cholesterol-saturated bile. Nevertheless, all patients with CD as a group tended to have lower cholesterol saturation compared to patients with gallstone ($P = 0.055$).

Bile acid composition is shown in (Table 3). Cholic acid, chenodeoxycholic acid and deoxycholic acid were the dominant bile acids both in patients with Crohn's disease and in patients with gallstone. The patients with Crohn's disease had significantly lower proportions of deoxycholic acid and lithocholic acid than the patients with gallstone. The proportions of cholic acid and chenodeoxycholic acid were concomitantly increased.

The biliary bilirubin concentration was about 50% higher in patients with Crohn's disease than in patients with gallstone (Table 4). No difference was obtained between patients with Crohn's disease confined to the colon and those with ileal involvement. The bilirubin concentration tended to be higher in patients with Crohn's disease confined to the colon in patients with ileal involvement but the difference did not reach statistical significance.

Cholesterol crystals and CDT

Cholesterol crystals were present in four gallbladder samples of the 17 patients with Crohn's disease involving the ileum. One of the patients with cholesterol crystals also had gallstones and was cholecystectomized. The gallbladder bile was saturated with cholesterol. Only one of the patients with Crohn's colitis displayed cholesterol crystals. Also this patient had gallstones in saturated bile and was cholecystectomized. Most of the patients (9 out of 14) with cholesterol gallstones displayed cholesterol crystals.

CDT was measured only in patients with Crohn's disease. Six out of sixteen patients with ileal involvement had a short CDT (mean 4 d, range 1-7 d). Two of six patients with Crohn's colitis also had a short CDT (4 and 5 d, respectively).

Treatment with ursodeoxycholic acid

The results are summarized in (Table 5). Ursodeoxycholic acid accounted for (0.5 ± 0.5)% of the total biliary bile acids before the treatment. Treatment with ursodeoxycholic acid increased the bile acid to (54.8 ± 3.8)%. Cholic acid, chenodeoxycholic acid, and deoxycholic acid were concomitantly decreased. Treatment with ursodeoxycholic acid decreased the cholesterol saturation from (101 ± 10)% to (52 ± 5)%. The biliary bilirubin concentration expressed as mmol/mol bile acid did not change after the treatment with ursodeoxycholic acid. The serum concentration of bilirubin also did not change after the treatment with ursodeoxycholic acid.

DISCUSSION

Several studies have shown that patients with ileal Crohn'

Table 5 Data on healthy subjects treated with ursodeoxycholic acid (UDCA)

Patients	Sex	Age (yr)	BMI	Cholesterol saturation (%)		Cholesterol (molar %)		Bile acids (molar %)		UDCA i (%)		Bile acids (mmol/mL)		Bilirubin (mmol/L)		Bilirubin/bile acids (mmol/mol)	
				A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	F	39	22.2	103	31	6.6	1.7	74.5	82.8	0.0	49.9	57.1	100.9	490	1 058	8.6	10.5
2	F	40	24.4	74	38	4.8	2.4	75.8	79.2	0.0	65.3	92.8	95.6	1 466	571	15.8	6.0
3	F	30	26.8	116	46	9.1	3.4	65.8	72.7	0.0	–	75.4	65.8	1 031	798	13.7	12.1
4	F	58	26.3	32	42	2.4	2.8	71.7	76.1	0.0	55.8	117.8	73.6	1 265	667	10.7	9.1
5	M	33	29.4	125	51	7.4	3.8	76.2	71.4	5.2	55.1	52.1	117.4	1 045	1 111	20.0	9.5
6	M	30	24.0	104	60	6.1	3.7	77.6	78.2	0.0	66.5	97.9	32.1	1 301	418	13.3	13.0
7	M	34	24.9	128	38	8.9	2.5	70.5	76.7	0.0	54.3	52.3	26.3	993	375	19.0	14.3
8	M	73	26.2	118	55	8.9	4.3	67.5	68.6	0.0	35.7	31.0	61.9	1 168	1 497	37.7	24.2
9	M	49	23.2	77	79	5.6	4.9	71.3	77.7	0.0	41.3	33.5	12.8	662	365	19.8	28.5
10	M	55	24.3	135	77	9.8	4.9	68.6	76.7	0.0	69.7	16.4	8.5	568	197	34.5	23.3
Mean		44	25.2	101	52[b]	7.0	3.4[b]	72.0	76.0[a]	0.5	54.8[b]	62.6	59.5	999	706	19.3	15.1
SE		4.5	0.7	10	5	0.7	0.3	1.26	1.3	0.5	3.8	10.3	12.1	104	130	3.1	2.4

A = before UDCA feeding; B = after UDCA feeding; [a]$P < 0.05$, [b]$P < 0.01$ *vs* before UDCA feeding.

s disease and/or previous ileal resection have elevated bilirubin levels in the bile[10,14,17,18]. Animal experiments showed that ileectomy-induced bile acid malabsorption increases bilirubin secretion into the bile[20], suggesting that the increased bilirubin levels in patients with ileal disease and/or resection may be due to induced enterohepatic cycling of bilirubin because of bile acid malabsorption. Orally given ursodeoxycholic acid can compete with ileal absorption of endogenous bile acids and cause bile acid malabsorption in rodents as well as in human subjects[29-31]. Meéndez-Saánchez *et al* [21] also showed that oral administration of ursodeoxycholic acid to rodents induces biliary secretion of bilirubin and increases cecal bile acid levels as well as bilirubin concentrations. In the present study however, oral administration of ursodeoxycholic to healthy subjects did not increase the bilirubin concentration in bile, which makes it unlikely that bile acid malabsorption increases bilirubin secretion into the bile in human beings. This is further confirmed by our finding in the present study that patients with Crohn's colitis but without the involvement of the distal ileum and apparent bile acid malabsorption also had elevated bilirubin levels in the bile. In fact, bilirubin concentrations tended to be higher in patients with Crohn's disease confined to the colon than in those with ileal involvement. In contrast to our results, Brink *et al* [10] and Pereira *et al* [14] have shown that bilirubin level is normal in the gallbladder bile of patients with Crohn's colitis.

If the increased bilirubin concentration in the bile of patients with Crohn's disease is not due to an enhanced enterohepatic circulation of bilirubin because of bile acid malabsorption, what could then be the explanation? Theoretically increased bilirubin content can be explained by an increased formation and excretion into the bile and/or a decreased metabolism of bilirubin in the intestine with subsequent absorption and enterohepatic circulation of bilirubin. An increased formation of bilirubin may originate from hemolysis. However, none of the patients in the present study had hemolysis or hyperbilirubinemia. Therefore, the most likely explanation for the increased biliary content of bilirubin in the patients is an increased intestinal absorption. Normally, bilirubin is deconjugated and degraded to urobilinogen and other products in the colon[32]. In Crohn's disease, an altered colonic bacterial flora may enhance the deconjugation with a subsequently increased absorption of unconjugated bilirubin from the intestine and an increased excretion of bilirubin into the bile[33].

Two patients with Crohn's disease, one with the disease involving the ileum and the other one with Crohn's colitis were cholecystectomized. The gallbladder bile in both of them was supersaturated with cholesterol and contained cholesterol crystals, indicating that the stones are cholesterol type. Another three patients with ileal involvement but with out Crohn's colitis displayed cholesterol crystals in the gallbladder bile. About 1/3 of the patients with Crohn's disease with but without the ileal disease had a short CDT. Half of these patients had unsaturated gallbladder bile. This finding is in agreement with a recent report by Keulemans *et al*[19] who showed that patients with Crohn's disease have an increased tendency to form cholesterol crystals. They have also found that the crystallization behavior is the same in patients with ileal disease as in those with the disease confined to the colon and is caused by increased cholesterol crystallization promoting activity.

Several of the patients with ileal disease but without gallstones displayed cholesterol saturated gallbladder bile (Table 5). However, the mean value of the cholesterol saturation in this group of patients was the same as that obtained in the healthy subjects, which is in agreement with our previous finding that patients with ileal resection due to Crohn's disease have a normal saturation of the bile[16,18].

In conclusion, patients with Crohn's disease involving the ileum and those with Crohn's colitis have elevated concentration of bilirubin in the gallbladder bile. Oral administration of ursodeoxycholic acid to healthy subjects does not increase the biliary concentration of bilirubin. These results speak against the previously described hypothesis that the increased concentration of bilirubin in the bile samples from patients with Crohn's ileitis

or previous ileal resection is due to malabsorption of bile acids that spill into the colon where they solubilize unconjugated bilirubin and increase its absorption and enterohepatic circulation. In some patients with ileal disease/resection, the gallbladder bile is supersaturated with cholesterol and contains cholesterol crystals. About 1/3 of the patients with Crohn's disease with but without the ileal involvement have a short CDT probably because of increased cholesterol crystallization promoting activity in the gallbladder bile. Thus, several factors including cholesterol supersaturated bile, short CDT and increased bilirubin concentration, may be of importance for the high risk of developing gallstones of both cholesterol and pigment types in Crohn's disease.

ACKNOWLEDGMENT

The skillful technical assistance of Ms Lisbet Benthin and Ms Ingela Arvidsson is acknowledged.

REFERENCES

1 **Cohen S**, Kpplan M, Gottlieb L, Patterson J. Liver disease and gallstones in regional enteritis. *Gastroenterology* 1971; **60**: 237-245
2 **Harrower AD**, Davidson NM, Yap PL, Nairn IM, Fyffe JA, Horn DB, Strong JA. Hypothalmic-pituitary adrenal responsiveness to dexamethasone-insulin tolerance test in acromegalic patients before and during treatment with bromocriptine. *Acta Endocrinol* (Copenh) 1978; **88**: 18-22
3 **Hill GL**, Mair WS, Goligher JC. Gallstones after ileostomy and ileal resection. *Gut* 1975; **16**: 932-936
4 **Whorwell PJ**, Hawkins R, Dewbury K, Wright R. Ultrasound survey of gallstones and other hepatobiliary disorders in patients with Crohn's disease. *Dig Dis Sci* 1984; **29**: 930-933
5 **Lorusso D**, Leo S, Mossa A, Misciagna G, Guerra V. Cholelithiasis in inflammatory bowel disease. A case-control study. *Dis Colon Rectum* 1990; **33**: 791-794
6 **Hutchinson R**, Tyrrell PN, Kumar D, Dunn JA, Li JK, Allan RN. Pathogenesis of gall stones in Crohn's disease: an alternative explanation. *Gut* 1994; **35**: 94-97
7 **Lapidus A**, Bångstad M, Aström M, Muhrbeck O. The prevalence of gallstone disease in a defined cohort of patients with Crohn's disease. *Am J Gastroenterol* 1999; **94**: 1261-1266
8 **Fraquelli M**, Losco A, Visentin S, Cesana BM, Pometta R, Colli A, Conte D. Gallstone disease and related risk factors in patients with Crohn disease: analysis of 330 consecutive cases. *Arch Intern Med* 2001; **161**: 2201-2204
9 **Magnuson TH**, Lillemoe KD, Pitt HA. How many Americans will be eligible for biliary lithotripsy? *Arch Surg* 1989; **124**: 1195-119; discussion 1195-119
10 **Brink MA**, Slors JF, Keulemans YC, Mok KS, De Waart DR, Carey MC, Groen AK, Tytgat GN. Enterohepatic cycling of bilirubin: a putative mechanism for pigment gallstone formation in ileal Crohn's disease. *Gastroenterology* 1999; **116**: 1420-1427
11 **Dowling RH**, Bell GD, White J. Lithogenic bile in patients with ileal dysfunction. *Gut* 1972; **13**: 415-420
12 **Marks JW**, Conley DR, Capretta TL, Bonorris GG, Chung A, Coyne MJ, Schoenfield LJ. Gallstone prevalence and biliary lipid composition in inflammatory bowel disease. *Am J Dig Dis* 1977; **22**: 1097-1100
13 **Rutgeerts P**, Ghoos Y, Vantrappen G. Effects of partial ileocolectomy and Crohn's disease on biliary lipid secretion. *Dig Dis Sci* 1987; **32**: 1231-1238
14 **Pereira SP**, Bain IM, Kumar D, Dowling RH. Bile composition in inflammatory bowel disease: ileal disease and colectomy, but not colitis, induce lithogenic bile. *Aliment Pharmacol Ther* 2003; **17**: 923-933
15 **Färkkilä MA**. Biliary cholesterol and lithogeneity of bile in patients after ileal resection. *Surgery* 1988; **104**: 18-25
16 **Lapidus A**, Einarsson K. Effects of ileal resection on biliary lipids and bile acid composition in patients with Crohn's disease. *Gut* 1991; **32**: 1488-1491
17 **Dawes L**, Stryker S, Rege R, Nahrwold D. Gallbladder bile composition in Crohn's disease. *Surg Forum* 1991; **42**: 188-189
18 **Lapidus A**, Einarsson C. Bile composition in patients with ileal resection due to Crohn's disease. *Inflamm Bowel Dis* 1998; **4**: 89-94
19 **Keulemans YC**, Mok KS, Slors JF, Brink MA, Gouma DJ, Tytgat GN, Groen AK. Concanavalin A-binding cholesterol crystallization inhibiting and promoting activity in bile from patients with Crohn's disease compared to patients with ulcerative colitis. *J Hepatol* 1999; **31**: 685-691
20 **Brink MA**, Méndez-Sánchez N, Carey MC. Bilirubin cycles enterohepatically after ileal resection in the rat. *Gastroenterology* 1996; **110**: 1945-1957
21 **Méndez-Sánchez N**, Brink MA, Paigen B, Carey MC. Ursodeoxycholic acid and cholesterol induce enterohepatic cycling of bilirubin in rodents. *Gastroenterology* 1998; **115**: 722-732
22 **Roda A**, Festi D, Sama C, Mazzella G, Alini R, Roda E, Barbara L. Enzymatic determination of cholesterol in bile. *Clin Chim Acta* 1975; **64**: 337-341
23 **Rouser G**, Fkeischer S, Yamamoto A. Two dimensional then layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* 1970; **5**: 494-496
24 **Fausa O**, Skålhegg BA. Quantitative determination of bile acids and their conjugates using thin-layer chromatography and a purified 3alpha-hydroxysteroid dehydrogenase. *Scand J Gastroenterol* 1974; **9**: 249-254
25 **Carey MC**. Critical tables for calculating the cholesterol saturation of native bile. *J Lipid Res* 1978; **19**: 945-955
26 **Angelin B**, Einarsson K, Leijd B. Biliary lipid composition during treatment with different hypolipidaemic drugs. *Eur J Clin Invest* 1979; **9**: 185-190
27 **Holan KR**, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology* 1979; **77**: 611-617
28 **Sahlin S**, Ahlberg J, Angelin B, Reihnér E, Einarsson K. Nucleation time of gall bladder bile in gall stone patients: influence of bile acid treatment. *Gut* 1991; **32**: 1554-1557
29 **Keelan M**, Thomson AB. Feeding diets containing 2% cheno- or urso-deoxycholic acid or cholestyramine to rats for two weeks alters intestinal morphology and bile acid absorption. *Can J Physiol Pharmacol* 1991; **69**: 592-598
30 **Marteau P**, Chazouilléres O, Myara A, Jian R, Rambaud JC, Poupon R. Effect of chronic administration of ursodeoxycholic acid on the ileal absorption of endogenous bile acids in man. *Hepatology* 1990; **12**: 1206-1208
31 **Owen RW**, Dodo M, Thompson MH, Hill MJ. Faecal steroid loss in healthy subjects during short-term treatment with ursodeoxycholic acid. *J Steroid Biochem* 1987; **26**: 503-507
32 **LESTER R**, SCHMID R. Intestinal absorption of bile pigments. II. Bilirubin absorption in man. *N Engl J Med* 1963; **269**: 178-182
33 **Neut C**, Bulois P, Desreumaux P, Membré JM, Lederman E, Gambiez L, Cortot A, Quandalle P, van Kruiningen H, Colombel JF. Changes in the bacterial flora of the neoterminal ileum after ileocolonic resection for Crohn's disease. *Am J Gastroenterol* 2002; **97**: 939-946

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 75-81
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Crohn's disease in Stockholm County during 1990-2001: An epidemiological update

Annika Lapidus

Annika Lapidus, Medical Department, Centre of Gastrointestinal Disease, Ersta Hospital, Karolinska Institute at Karolinska University Hospital, Huddinge, Stockholm, Sweden
Supported by the grants from Knut and Alice Wallenbergs foundation
Correspondence to: Annika Lapidus MD, Centre of Gastroenterology, Ersta Hospital, Box 4622, S-11691 Stockholm, Sweden. annika.lapidus@ersta.se
Telephone: +46-8-7146100
Received: 2005-05-13 Accepted: 2005-06-18

Key words: Crohn's disease; Inflammatory bowel disease; Epidemiology; Incidence; Prevalence

Lapidus A. Crohn's disease in Stockholm County during 1990-2001: An epidemiological update. *World J Gastroenterol* 2006; 12(1): 75-81

http://www.wjgnet.com/1007-9327/12/75.asp

Abstract

AIM: To further assess of the incidence and localization of Crohn's disease (CD) in a well-defined population during the 1990s and to evaluate the prevalence of CD on the 1st of January 2002.

METHODS: In a retrospective population based study, all 16-90 years old citizens of Stockholm County diagnosed as having CD according to Lennard Jones' criteria between 1990 and 2001 were included. Case identification was made by using computerized inpatient and outpatient registers. Moreover private gastroenterologists were asked for possible cases. The extent of the disease and the frequency of anorectal fistulae were determined as were the ages at diagnosis. Further, the prevalence of CD on the 1st of January 2002 was assessed.

RESULTS: All the 1 389 patients, 689 men and 700 women, fulfilled the criteria for CD. The mean incidence rate for the whole period was 8.3 per 10^5 (95%CI 7.9 -8.8). There was no difference between the genders. The mean annual incidence of the whole study period for colorectal disease and ileocecal disease, was 4.4 (95%CI 4.0-4.7) and 2.4 (95%CI 2.1-2.6) per 10^5, respectively. Perianal disease occurred in 13.7% (95%CI 11.9-15.7 %) of the patients. The prevalence of CD was 213 per 100 000 inhabitants.

CONCLUSION: The incidence of CD has markedly increased during the last decade in Stockholm County and 0.2% of the population suffers from CD. The increase is attributed to a further increase of colorectal disease, while the incidence of ileocecal disease has remained stable.

INTRODUCTION

Crohn's disease (CD) is one of the major inflammatory bowel diseases (IBD) that challenges many gastroenterologists in their everyday work. Although CD has been recognized for over 70 years[1], its etiology still remains unclear. An explanatory factor for the unsolved etiology may be the heterogeneous appearance of the disease.

Although descriptive epidemiological studies can hardly prove the etiology of the disease, they are very important in the pursuit of risk factors that can be further assessed in etiological epidemiological studies. Furthermore, they are useful for planning the health care system, development of new pharmaceuticals and therapeutic endeavors.

The former increasing incidence of CD in countries located in the north[2-5] seems to have leveled off during the eighties. Nevertheless, the incidence continued to increase in Scotland and Iceland during the eighties and early nineties[6,7]. An increasing incidence of CD has also been assessed in former low-incidence countries in southern Europe, Asia, and Japan[8-10]. Although numerous epidemiological studies of CD have been performed since the 1960s, there are only few population-based studies that have explored the incidence of CD during the last decade[11-14].

When Burrill B Crohn and his colleagues first described CD in 1932[1], the most common site of the disease was the ileocecal area. It was not until 1960 that CD localized to the colon and/or rectum was regarded as an entity of its own[15], unrelated to ulcerative colitis (UC). Pure colorectal disease has become more common over the years. In a previous survey of patients diagnosed with CD in Stockholm between 1955 and 1989, the proportion of colorectal disease doubled from 14% to 32% during the study period[16]. This phenomenon has been reported from other areas as well[6,7,17].

As the mortality due to CD is low[18], an increasing

number of patients will suffer from CD. There are scarce current prevalence rates available[13,19,20].

The primary aim of this study was to further assess the incidence and localization of CD in a well-defined population during the 1990s and to evaluate the prevalence of CD on the 1st of January 2002.

MATERIALS AND METHODS

Study area and population

Stockholm County covers an area of 6 519 km^2 with both urban and rural parts. The population increased from 1.64 to 1.84 million inhabitants between 1990 and 2001[21]. The number of individuals aged 16-90 years increased from 1.33 to 1.47 million during the same period and attributed to approximately 80% of the total population. The distribution between the genders was constant during the study period with a share of 49% men. The proportion of aliens was 9.4% in 2001.

There were 9 major hospitals and further 13 established gastroenterologists in private practice taking care of patients with IBD during the study period. The number of lower GI endoscopies (i.e, sigmoidoscopies and colonoscopies) performed in Stockholm County increased from 4 787 to 19 778 per year between 1993 and 2001[22].

Case identification

In Stockholm County, there has been a computerized central registration of all diagnoses for inpatients since 1969. Since 1993, diagnoses have also been successively registered for outpatients and all patients attending the outpatient clinic at the hospitals. The general practitioners have got their diagnoses registered since 1996. Diagnoses were registered according to the International Statistical Classification of Disease (ICD-9 1987-1996, ICD-10 1997 and onwards).

A survey of possible cases was made from the records registered as CD (ICD code 555 and K.50) in the inpatient register between 1990 and 2002 and the outpatient register between 1993 and 2002.

Gastroenterologists in private practice were asked about possible cases and their colonoscopy and histology reports were assessed. All records (paper, microfilmed, and electronic) were retrospectively scrutinized. Patients with an established probable or definite diagnosis of CD according to Lennard Jones' criteria[23] were included in the study. Included patients should be citizens of Stockholm County and were diagnosed as having CD between 1st January 1990 and 31st December 2001. Patients younger than 16 years at diagnosis were not included in the study.

Definitions

The diagnosis of CD and extent of the disease at diagnosis were based on radiological and/or endoscopical reports including macroscopic and microscopic findings as previously described in detail[16].

Date of diagnosis was defined as the first examination revealing signs of CD. If the diagnosis was changed from UC to CD, the first diagnosis date of IBD was considered. Date of presentation of symptoms which are consistent with IBD was approximated to months with a certainty expressed as either the first or the 15th day of the month. In cases, where only the year could be estimated, the date of onset was set as the 1st of July.

The type of diagnostic examination(s) performed at the time for diagnosis was noted. The localization of CD was determined based on these diagnostic examinations and classified into five groups: oro-jejunal disease, small bowel disease (inflammation of the small bowel excluding the distal 30 cm of the terminal ileum), ileocecal disease (inflammation including the distal 30 cm of the ileum with or without isolated involvement of the cecum), ileocolonic disease (continuous or discontinuous inflammation of the ileum and colon) and colorectal disease (inflammation in the colon only and/or rectum). The group of oro-jejunal disease consisted of patients with solely oro-jejunal disease and patients with mainly oro-jejunal disease but also inflammation in a more distal part of the intestine.

The extent of colorectal disease was further classified into three groups. For assessment of the disease extent, the colon was schematically divided into four areas consisting of the ascending, transverse, descending, and sigmoid respectively. Colonic disease could either be segmental disease with the involvement of one, two or three of the four schematic areas, left-sided disease extending proximally from the rectum but not beyond the splenic flexure, or total colonic disease with inflammation in all the four areas. Patients with segmental or total colonic disease could have either rectal involvement or rectal sparing. Anorectal fistulae included fistulas and abscesses in the rectum, anal canal or perianal area that appeared before the diagnosis of CD, at the time of diagnosis or at any time during the follow-up period.

If data concerning the parameters mentioned above were uncertain to assess, the data were labeled as unreliable and excluded from actual analysis.

Data management and statistical methods

The cases of CD as well as the population were grouped into 15-year age specific groups: 16-30 years, 31-45 years, 46-60, 61-75, and 75-90. Information about the population in Stockholm County was obtained from the National Central Statistical Register[21].

Incidence rates were calculated for 2-year periods and expressed as annual incidence rates. For each 2-year period, the age specific incidence was calculated by dividing the number of cases by the number of person years in each age group. The population was not adjusted (i.e, subtracted) for previous incidence cases. Calculations were made separately for men and women. The annual incidence was adjusted for sex and age by using the population in Stockholm County in 1995 as a standard population. Incidence rates were expressed as cases per 10^5 inhabitants with a 95% confidence interval (CI) based on the assumption that the number of cases follows a Poisson distribution. A Poisson model (Breslow) was used to study the relationship between sex, calendar year, age and the risk of CD. The hazard function of individuals aged 16-88 years was assumed to be exp $[\beta_0+\beta_1 \cdot \text{minimum (age, 45)}+\beta_2 \cdot \text{maximum (0, minimum (age-45, 70-45)}+\beta_3 \cdot \text{maximum (0, age-70)}+\beta_4 \cdot \text{minimum (calendar year-1990, 6)}+\beta_5 \cdot \text{maximum (0, calendar year-}$

Table 1 New cases of Crohn's disease in Stockholm County during 1990–2001 grouped by 2-year intervals, in relation to gender, age and localization of disease at diagnosis

	1990-1991	1992-1993	1994-1995	1996-1997	1998-1999	2000-2001	1990-2001
Total	174	219	234	286	235	241	1 389
Gender							
Male	74	114	112	143	127	119	689
Female	100	105	122	143	108	122	700
Age at diagnosis							
16-30	60	86	89	121	87	88	531
31-45	49	52	62	66	57	56	342
46-60	27	44	40	47	51	61	270
61-75	29	27	33	40	32	26	187
76-90	9	10	10	12	8	10	59
Localization of disease at diagnosis							
Oro-jejunal	2	4	5	7	7	5	30
Small bowel	8	14	9	9	5	4	49
Ileocecal	58	55	61	85	61	73	393
Ileocolonic	15	28	38	35	32	41	189
Colorectal	91	118	121	150	130	118	728

1996)+β_6 · sex]. The coefficients β_1, β_2, β_3, β_4, β_5, and β_6 reflected the trends of the risk, $\beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta_5 = \beta_6 = 0$ corresponded to no change of risk[24].

Time between the onset of symptoms and diagnosis was calculated as the median.

The extent of disease at diagnosis was assessed as incidence rates as well as proportions, i.e. percentages of the total number of cases. Comparison between men and women with respect to the localization of disease was performed by χ^2 test. The Kruskal-Wallis test was applied for comparison of age at onset and calendar time at onset between localization of disease. If a significant importance of localization was obtained, each localization was compared to all the others by use of Fisher's permutation test.

The probability of anorectal fistulae and rectal involvement, depending on age, sex, and localization of the disease, was estimated by the use of logistic regression. The two-tailed tests were used.

The Ethics Committee at Huddinge University Hospital approved the study.

RESULTS

Basic data are shown in Table 1.

Altogether 1 389 patients fulfilled the inclusion criteria. A total of 569 patients (41%) had a definite diagnosis of CD and 820 patients (59%) had a possible diagnosis of CD according to Lennard Jones' criteria.

Information regarding the onset of symptoms, methods of diagnosis, occurrence of anorectal fistulae and rectal involvement was not available in 26, 9, 96, and 31 patients respectively. Twenty-eight percent of the patients were prescribed azathioprine or equivalent immunosuppressive medicine at any time during the follow-up.

Time between onset of symptoms and diagnosis

The median time between the onset of symptoms and diagnosis was 6.5 mo (range 0-376 mo) for the whole study period. Patients with small bowel disease and ileocecal disease had a longer median time to diagnosis (12.8 and 10.9 mo, respectively) than patients with ileocolonic and colorectal disease (6.1 and 5.9 mo, respectively) ($P < 0.005$).

Table 2 Diagnostic procedures performed at the time of diagnosis (n = 1 389)

	Main examination, n (%)	Total number, n (%)
Colonoscopy	690 (49.7)	690 (49.7)
Ileocolonoscopy	285 (20.5)	285 (20.5)
Large bowel barium enema	138 (9.9)	250 (18.0)
Enteroclysis	62(4.5)	602 (43.3)
Surgery	82 (5.9)	141 (10.2)
Rigid sigmoidoscopy	20 (1.4)	NA
Sigmoidoscopy	12 (0.9)	46 (3.3)
Miscellaneous	45 (3.2)	
Unknown	55 (4.0)	

NA, not assessed.

Diagnostic procedures

The diagnostic procedures performed between the first-time visit and the diagnosis of CD are shown in Table 2.

If a colonoscopy/ileocolonoscopy was performed, the examination was considered as the "main examination". Otherwise, the examination that provided most information for diagnosing and evaluation of the extent of disease was labeled as "main examination". The total number of procedures reflected how many patients underwent each specific examination.

Altogether 70.2% of the patients underwent an endoscopic examination of the colon and the terminal ileum was inspected in 29.2% of these patients. An enteroclysis was performed in 43% of all patients. Six percent of the patients were diagnosed by surgery. The remaining patients underwent only flexible/rigid sigmoidoscopy or miscellaneous examinations.

Incidence

The mean annual incidence rate for the entire study period was 8.3% (95%CI 7.9%-8.8%) per 100 000 inhabitants. The mean annual incidence rate was 7.7% (95%CI 7.1-8.3%) and 8.9 % (95%CI 8.3-9.6%), respectively, during the first and second half of the study period (Figure 1).

The mean increase in incidence per year was estimated to be 7.6% (95%CI 4.3-11.1%) between 1990 and 1996 with the highest increase (21.0%) between 1990 and 1992 (95%CI 5.4-38.8%) and (14.1%) between 1994 and 1996 (95%CI 1.8-27.8%). The maximum incidence rate was found in 1996-1997 with an annual mean of 10.3% (95%CI 9.1-11.5%) per 100 000 inhabitants. After 1996, the annual incidence decreased with a mean of 4.6% per year (95%CI 1.0-8.0%).

No statistical difference in incidence rates was found between men and women [8.6% (95%CI 7.9-9.2%) *vs* 8.1% (95%CI 7.5-8.7%)] for the entire study period.

Age at diagnosis

The highest age specific incidence was found among those aged 16-30 years at diagnosis with a mean incidence rate

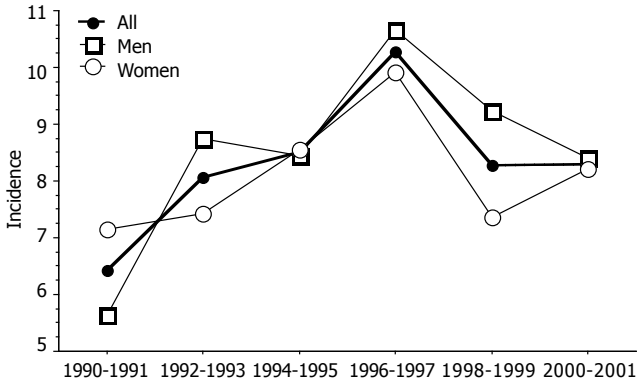


Figure 1 Annual incidence rate (mean per two-year periods x 10^{-5}) of Crohn´s disease in Stockholm County (1990-2001).

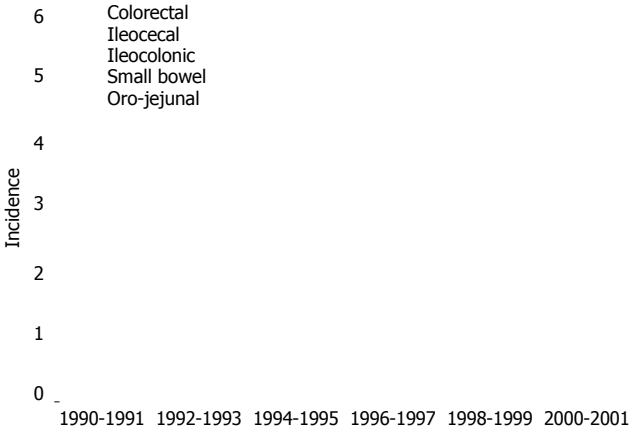


Figure 3 Incidence of Crohn´s disease in Stockholm County by the extent of disease at diagnosis per 10^5 inhabitants (1990-2000).

of 12.3 per 10^5 (95%CI 10.1-14.6) for the entire study period. A maximum incidence rate was observed during 1996-1997 with 17.1 per 10^5.

The relationship between age and the risk of CD by using a Poisson model, with the calendar year fixed to 1995, allowed the curve to bend at the arbitrary ages 30, 45, 60, and 75 years (Figure 2). With increasing age, the incidence decreased on an average of 1.7% per year between 16 and 30 years ($P < 0.05$) and 3.9% per year between 30 and 45 years ($P < 0.001$). Thereafter, the incidence increased again on a yearly average of 2.2% ($P < 0.05$) resulting in a second peak at the age of 60 years. In the elderly, the incidence rate decreased with an average of 1.2% and 3.9% per year between 60 and 75 years and over 75 years, respectively. Although the crude number of cases was lower in the older age groups, the bimodal peak in incidence was attributed to fewer person-years of follow-up in the older age groups compared with the younger age groups.

The proportion of patients older than 60 years at diagnosis was 17.7% and the proportion of all patients older than 75 years at diagnosis was 4.2%.

Extent of disease at diagnosis

The incidence of CD by the extent of disease at diagnosis is shown in Figure 3.

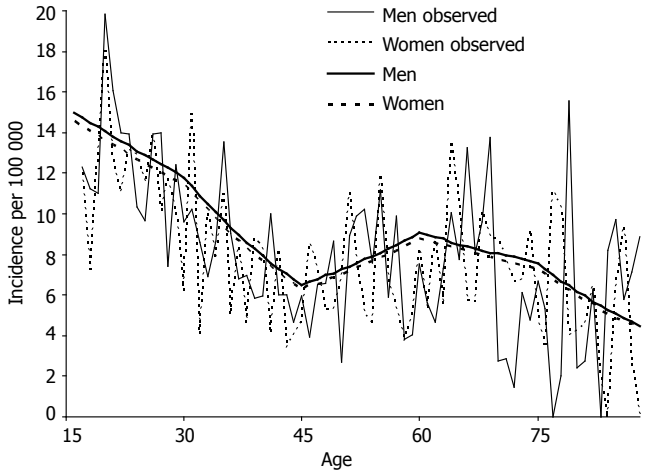


Figure 2 Relationship between age and the incidence of Crohn´s disease in Stockholm County (1990-2001), determined by using a poisson model, with the calender year fixed to 1995, allowing the curve to bend at the arbitrary ages 30, 45, 60, and 75 yr.

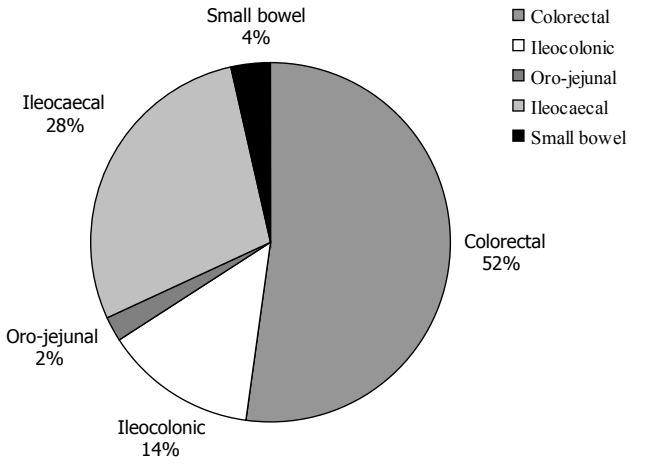


Figure 4 Localiation of Crohn´s disease in Stockholm County (1990-2001).

The mean annual incidence in the whole study period for colorectal and ileocecal disease was 4.4% (95%CI 4.0-4.7%) and 2.4% (95%CI 2.1-2.6%), respectively. Corresponding figures for ileocolonic disease, small bowel disease and oro-jejunal disease were 1.1% (95%CI 1.0-13%), 0.3% (95%CI 0.2-0.4%) and 0.2% (95%CI 0.1-0.2%), respectively. The only significant change over time was a decreasing incidence of small bowel disease throughout the study period ($P < 0.05$).

The overall proportion of colorectal and ileocecal disease was 52.3% (95%CI 49.7-55.0%) and 28.3% (95%CI 26.0-30.7%), respectively (Figure 4).

Age influenced the localization of CD at diagnosis. By comparing each disease localization to the others, patients with colorectal ($P < 0.005$) and small bowel disease ($P < 0.005$) were found to be older at diagnosis. In contrary, patients with ileocolonic ($P < 0.001$) and proximal disease ($P = 0.055$) were younger.

In pure colorectal disease, 36.3% of the patients had total colonic involvement, 40.2% had segmental disease and 23.5% had distal colorectal disease. Rectal

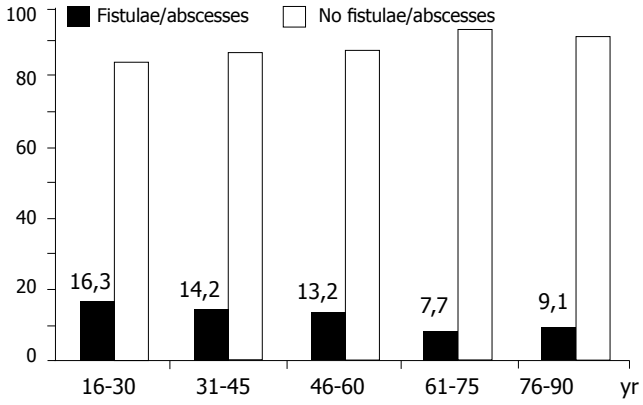


Figure 5 Percentage of patients with Crohn´s disease (n = 1293) having perianal fistulae/abscesses in Stockholm County during 1990-2001 by age specific groups ($P < 0.05$).

inflammation at diagnosis was found in 67.8% of the patients with colorectal disease.

Distal colorectal disease was associated with a higher age at diagnosis ($P < 0.005$) and inversely total colonic disease was associated with a lower age at diagnosis compared to the rest of the patients ($P < 0.001$). Age did not influence the risk of segmental colorectal disease.

Gender had no influence on either disease localization or extent of colorectal disease at diagnosis.

Anorectal fistulae

Anorectal fistulae occurred before or after the diagnosis of CD in 13.7% (95%CI 11.9-15.7%) of the patients whose data were assessable (n = 1 293). Gender as well as age had a significant impact on the occurrence of fistulae. Men were more likely to develop this kind of perianal disease than women (15.8% *vs* 11.6%, $P < 0.05$). The impact of age on anorectal fistulae is shown in Figure 5. The disease localization did not influence the frequency of fistulae.

Prevalence

Calculations were based on all adult patients (> 16 years) with CD living in Stockholm County on the 1st of January 2002. Of the 1 389 patients diagnosed between 1990 and 2001, 71 patients were dead and 76 patients had moved out from the area. From the earlier cohort of 1 936 patients diagnosed during 1955-1989[16], 368 patients were dead and 239 patients had moved out. Twelve patients diagnosed in Stockholm before 1955 were still living in the area.

The present case identification survey revealed 327 patients diagnosed outside the Stockholm County, 128 patients diagnosed as UC before 1990 and later got the diagnosis changed from UC to CD and 97 patients diagnosed as CD during 1955-1989 but not included in the previous study[16] for unknown reasons. The prevalence was calculated by dividing the total number of patients with CD (3 135) by the adult population (> 16 years) in Stockholm County (1 469 048). Accordingly, the prevalence of CD among the adults in Stockholm County on the 1st of January 2002 was estimated to be 213 per 100 000 inhabitants.

DISCUSSION

Including a former published study[16], the development of CD has now been documented for 45 years in Stockholm County. Thereby, one of the largest population based cohort of patients with CD in the world has been collected.

There are pros and cons regarding study designs. A prospective study may be considered more faultless but is unfeasible in a large study area with many hospitals and gastroenterologists. Shortcoming of a retrospective study can be lack of uniform diagnostic criteria or incomplete data collection. Patients in Stockholm are handled by a limited number of gastroenterologists and the diagnosis is therefore uniform. However, endoscopy has been escalating during the last years. A potential aim to retrospectively assess smoking habits in this study was abandoned due to the lack of reliable data, else sufficient data regarding different parameters were available. The pro of the present retrospective study is a long follow-up period when case ascertainment can be accomplished in the meantime.

The number of patients having a definite diagnosis of CD according to Lennard Jones' criteria[23] in this study was lower (41%) than that in our previous study of CD in Stockholm (73%)[16]. One explanation could be that fewer patients underwent surgery. Specimens for histopathological examination were less available and thereby granulomas and submucosal signs of CD might be undetected. Some uncertainty may arise about the "probable" cases of CD according to Lennard Jones' criteria. Case ascertainment was assured by scrutinizing the records retrospectively. By following the clinical course of CD, the diagnosis was further ascertained and all included cases were bonafide cases of CD.

Long-term incidence data are scarce in the literature from the late 1990s and onwards[12-14,25-27]. The incidence of CD among the adults in the present study was 70% higher than that in Stockholm during 1985-1989, with the highest incidence rate in 1996-1997. The registry of outpatients begun in 1993 and theoretically, the lower incidence found in 1990-1993 may represent an incomplete case ascertainment. Similarly, the drop-off in incidence during the last 4 years of the study could represent decreased ascertainment. However, all patients with CD in Stockholm are regularly followed up and should be identified sooner or later. Diagnoses are registered continuously and a marked delay of identification is unlikely, thus the case ascertainment is reliable.

A 55% increase of the incidence rate was also seen in Denmark where the incidence rate reached a maximum with approximately 10 per 10^5 in 1998-2002[12]. In Northern France, the incidence of CD increased 23% during a similar time period[14]. Still, the overall incidence rate is lower in France than in Stockholm. The highest incidence rates have previously been reported from North Eastern Scotland[7] and Manitoba in Canada[19] and now also from Sweden. This may illustrate a north-south gradient of CD that still exists although less pronounced than believed earlier[10]. The proportion of immigrants in Stockholm has not changed during the last decade and

should therefore not affect the results[21].

CD is in general believed to affect women more frequently than men[14,17,28] although several studies have not found any difference[4,10]. This study confirmed earlier observations from Stockholm that gender does not influence the risk for CD[16].

CD mostly occurs in young adults, but can present itself at any time in life[29]. The age specific distribution in this study is consistent with some former studies with the highest incidence found among young adults and thereafter decreasing incidence rates with increasing age and a second peak in the elderly[7,16,17,30]. Nevertheless, the overall incidence among the elderly was substantially higher in this study compared with other studies[31,32].

Pure colorectal disease and small bowel disease are associated with a high age in contrast to our previous study where only small bowel disease was related to a high age at diagnosis. It has been suggested that smoking CD patients are more likely to develop small bowel disease than non-smoking patients[33]. Smoking habits were not evaluated in this retrospective study. However, one can speculate whether smoking was more tolerable in the society previously with an ensuing larger group of smokers among the older population. This assumption is strengthened by the fact that this entity of CD was the only one that decreased throughout the study period. Colorectal CD may be misclassified as ischemic colitis as well as diverticulosis. Both conditions are common in this age group. CD patients with concomitant diverticular disease are more likely to have granulomas but the misclassification should be limited by finding granulomas in other parts of the intestine[31]. Focal inflammation is one of the criteria of CD and distinguishes colorectal CD from UC.

This study focused on CD in adults since a study of children with IBD in northern Stockholm has been recently performed[27]. That study showed an increasing incidence of pediatric CD during the latter 1990s with an incidence of 8.4 per 10^5 in 1999-2001, thus identical rates were found in adults in the present study.

Anorectal fistulae, strongly associated with CD, have been reported in almost 40% of the patients, preferably in those with colorectal involvement[34]. Though patients with colorectal disease accounted for a huge proportion, the frequency of anorectal fistulae was low in the present study and on the whole, no relation was found between the frequency of fistulae and the disease localization. Inclusion of patients with mild colorectal disease might cause a dilution effect and a false low frequency of fistulae. A short follow-up period may be an alternative explanation and moreover, a general increased use of azathioprine may theoretically have prevented the development of fistulae.

The increasing incidence of CD is found in patients with solely colorectal disease. This entity of CD was first described in 1960 but an increasing proportion of colorectal CD was not reported before the late eighties[15].

The increase of colorectal disease can be attributed to a factual increase, a switch from UC to CD or more accurate diagnostic procedures. Unfortunately, there are no contemporary data regarding the development of UC in Stockholm County. However, Jacobsen *et al*[12] have shown a concomitant substantial increase of both UC and CD in North Jutland in Denmark between 1988 and 2002, which supports a factual increase of CD and contradicts a diagnostic shift from UC to CD. In contrast, the incidence of UC decreased by 17% in Northern France during a comparable time period[14]. If hypothetically, the decreasing incidence of UC should represent a corresponding increase of CD, still there would be a 50% increase of CD not due to a reclassification from UC to CD in Stockholm during the 1990s. The diagnosis of CD is partly based on histopathology and may vary between different pathologists[35]. In this study one single pathologist did most of the histopathological examinations.

The number of lower GI endoscopies performed in the area has increased fourfold[22]. This escalating access to medical service may have led to an examination of more patients with only mild symptoms. Patients with minor colonic inflammation that might have been overlooked with previously used barium enemas could therefore have attributed to the increased incidence of CD.

The median time between presentation of symptoms and diagnosis is rather similar to that found in 1955-1989[16]. The shortest time is found in patients with disease in the colon, while patients with small bowel disease have a longer period of symptoms before the diagnosis. This fact may illustrate the advantage of endoscopic technique in the diagnosis of IBD. Recently, the capsule endoscopy has been introduced in Sweden and is used to a limited extent[36]. With a more widespread use of this new technique, disease in the ileum may be more frequently detected[37]. Consequently an increased number of patients with small bowel CD will be diagnosed and thereby the incidence of CD will probably further increase in the future.

In conclusion, this follow-up study of CD in Stockholm County shows that the incidence increases by 80% from the 1980s to the 1990s. As much as 0.2% of the adult population in Stockholm County suffers from CD. The disease appears at any age and there is no predominance regarding gender. Colorectal CD is the most common localization and the frequency of anorectal fistulae is less evident than that in the past.

ACKNOWLEDGMENT

Mrs Monica Johansson is gratefully acknowledged for help with secretarial assistance. Mrs Helena Johansson is gratefully acknowledged for statistical support.

REFERENCES

1 **Crohn BB**, Ginzburg L, Oppenheimer GD. Regional ileitis. A pathological and clinical entity. *JAMA* 1932; **99**: 1923-1929
2 **Ekbom A**, Helmick C, Zack M, Adami HO. The epidemiology of inflammatory bowel disease: a large, population-based study in Sweden. *Gastroenterology* 1991; **100**: 350-358
3 **Lee FI,** Nguyen-Van-Tam J. Prospective study of incidence of Crohn's disease in northwest England: no increase since late 1970's. *Eur J Gastroenterol Hepatol* 1994; 6: 27-31
4 **Lindberg E**, Jörnerot G. The incidence of Crohn's disease is not decreasing in Sweden. *Scand J Gastroenterol* 1991; **26**: 495-500
5 **Loftus EV**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and

survival. *Gastroenterology* 1998; **114**: 1161-1168
6 **Björnsson S**, Jóhannsson JH. Inflammatory bowel disease in Iceland, 1990-1994: a prospective, nationwide, epidemiological study. *Eur J Gastroenterol Hepatol* 2000; **12**: 31-38
7 **Kyle J**. Crohn's disease in the northeastern and northern Isles of Scotland: an epidemiological review. *Gastroenterology* 1992; **103**: 392-399
8 **Iida M**, Yao T, Okada M. Long-term follow-up study of Crohn' s disease in Japan. The Research Committee of Inflammatory Bowel Disease in Japan. *J Gastroenterol* 1995; **30 Suppl 8**: 17-19
9 **Leong RW**, Lau JY, Sung JJ. The epidemiology and phenotype of Crohn's disease in the Chinese population. *Inflamm Bowel Dis* 2004; **10**: 646-651
10 **Shivananda S**, Lennard-Jones J, Logan R, Fear N, Price A, Carpenter L, van Blankenstein M. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut* 1996; **39**: 690-697
11 **Blanchard JF**, Bernstein CN, Wajda A, Rawsthorne P. Small-area variations and sociodemographic correlates for the incidence of Crohn's disease and ulcerative colitis. *Am J Epidemiol* 2001; **154**: 328-335
12 **Jacobsen B**, Fallingborg J, Rasmussen H. The incidence of IBD in North Jutland County, Denmark 1978-2002. *Gut* 2003; **52**: A79
13 **Loftus C G**, Loftus EV, Jr., Sandborn WJ, Tremaine WJ, Harmsen AR, Zinsmeister AR, Melson LJ. Update on incidence and prevalence of Crohn's disesae (CD) and ulcerative colitis (UC) in Olmsted County Minnesota 2003. *DDW* 2003; A278
14 **Molinié F**, Gower-Rousseau C, Yzet T, Merle V, Grandbastien B, Marti R, Lerebours E, Dupas JL, Colombel JF, Salomez JL, Cortot A. Opposite evolution in incidence of Crohn's disease and ulcerative colitis in Northern France (1988-1999). *Gut* 2004; **53**: 843-848
15 **LOCKHART-MUMMERY HE**, MORSON BC. Crohn's disease (regional enteritis) of the large intestine and its distinction from ulcerative colitis. *Gut* 1960; **1**: 87-105
16 **Lapidus A**, Bernell O, Hellers G, Persson PG, Löfberg R. Incidence of Crohn's disease in Stockholm County 1955-1989. *Gut* 1997; **41**: 480-486
17 **Yapp TR**, Stenson R, Thomas GA, Lawrie BW, Williams GT, Hawthorne AB. Crohn's disease incidence in Cardiff from 1930: an update for 1991-1995. *Eur J Gastroenterol Hepatol* 2000; **12**: 907-911
18 **Wolters FL**, Russel MG, Stockbrügger RW. Systematic review: has disease outcome in Crohn's disease changed during the last four decades? *Aliment Pharmacol Ther* 2004; **20**: 483-496
19 **Bernstein CN**, Blanchard JF, Rawsthorne P, Wajda A. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am J Epidemiol* 1999; **149**: 916-924
20 **Rubin GP**, Hungin AP, Kelly PJ, Ling J. Inflammatory bowel disease: epidemiology and management in an English general practice population. *Aliment Pharmacol Ther* 2000; **14**: 1553-1559
21 **Sweden Statistics**. Population statistics for the years 1990-2002. Stockholm: Statistics Sweden, 2004
22 **Wéden M.** Statistics on endoscopy in Stockholm County 1993-2003. Stockholm: 2004
23 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-9
24 **Breslow N**, Day N. Statistical methods in cancer research volume II. *Scientific Publications* 1987; **32**: 131-135
25 **Lakatos L**, Mester G, Erdelyi Z, Balogh M, Szipocs I, Kamaras G, Lakatos PL. Striking elevation in incidence and prevalence of inflammatory bowel disease in a province of western Hungary between 1977-2001. *World J Gastroenterol* 2004; **10**: 404-409
26 **Armitage E**, Drummond HE, Wilson DC, Ghosh S. Increasing incidence of both juvenile-onset Crohn's disease and ulcerative colitis in Scotland. *Eur J Gastroenterol Hepatol* 2001; **13**: 1439-1447
27 **Hildebrand H**, Finkel Y, Grahnquist L, Lindholm J, Ekbom A, Askling J. Changing pattern of paediatric inflammatory bowel disease in northern Stockholm 1990-2001. *Gut* 2003; **52**: 1432-1434
28 **Munkholm P**, Langholz E, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of Crohn's disease in the county of Copenhagen, 1962-87: a sixfold increase in incidence. *Scand J Gastroenterol* 1992; **27**: 609-614
29 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517
30 **Thomas GA**, Millar-Jones D, Rhodes J, Roberts GM, Williams GT, Mayberry JF. Incidence of Crohn's disease in Cardiff over 60 years: 1986-1990 an update. *Eur J Gastroenterol Hepatol* 1995; **7**: 401-405
31 **Heresbach D**, Alexandre JL, Bretagne JF, Cruchant E, Dabadie A, Dartois-Hoguin M, Girardot PM, Jouanolle H, Kerneis J, Le Verger JC, Louvain V, Pennognon L, Richecoeur M, Politis J, Robaszkiewicz M, Seyrig JA, Tron I. Crohn's disease in the over-60 age group: a population based study. *Eur J Gastroenterol Hepatol* 2004; **16**: 657-664
32 **Pironт P**, Louis E, Latour P, Plomteux O, Belaiche J. Epidemiology of inflammatory bowel diseases in the elderly in the province of Liège. *Gastroenterol Clin Biol* 2002; **26**: 157-161
33 **Lindberg E**, Järnerot G, Huitfeldt B. Smoking in Crohn's disease: effect on localisation and clinical course. *Gut* 1992; **33**: 779-782
34 **Sandborn WJ**, Fazio VW, Feagan BG, Hanauer SB. AGA technical review on perianal Crohn's disease. *Gastroenterology* 2003; **125**: 1508-1530
35 **Farmer M**, Petras RE, Hunt LE, Janosky JE, Galandiuk S. The importance of diagnostic accuracy in colonic inflammatory bowel disease. *Am J Gastroenterol* 2000; **95**: 3184-3188
36 **Höög C**, Antfolk A, Wirlöf C, Sjöqvist U. [Capsule endoscopy is better than other methods. 66 examinations performed at Sodersjukhuset prove a high diagnostic yield]. *Lakartidningen* 2004; **101**: 4102-4106
37 **Swain P**. Wireless capsule endoscopy and Crohn's disease. *Gut* 2005; **54**: 323-326

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 82-88
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Proton pump inhibitor treatment of patients with gastroesophageal reflux-related chronic cough: A comparison between two different daily doses of lansoprazole

Fabio Baldi, Roberta Cappiello, Carlotta Cavoli, Stefania Ghersi, Francesco Torresan, Enrico Roda

Fabio Baldi, Roberta Cappiello, Carlotta Cavoli, Stefania Ghersi, Francesco Torresan, Enrico Roda, Department of Gastroenterology, Metabolic and Infectious Diseases, S.Orsola-Malpighi Hospital, Bologna, Italy
Correspondence to: F Baldi, Department of Gastroenterology, Metabolic and Infectious Diseases, S.Orsola-Malpighi Hospital, Via Massarenti 9, 40138 Bologna, Italy. baldi@med.unibo.it
Telephone: +39-51341435 Fax: +39-51341435
Received: 2005-06-06 Accepted: 2005-07-20

Baldi F, Cappiello R, Cavoli C, Ghersi S, Torresan F, Roda E. Proton pump inhibitor treatment of patients with gastroesophageal reflux-related chronic cough: A comparison between two different daily doses of lansoprazole. *World J Gastroenterol* 2006; 12(1): 82-88

http://www.wjgnet.com/1007-9327/12/82.asp

Abstract

AIM: To compare two different daily doses of lansoprazole given for 12 weeks and to assess the role of gastrointestinal (GI) investigations as criteria for selecting patients.

METHODS: Out of 45 patients referred for unexplained chronic persistent cough, 36 had at least one of the GI investigations (endoscopy, 24-h esophageal pH-metry and a 4-week trial of proton pump inhibitor (PPI) therapy) positive and were randomly assigned to receive either 30 mg lansoprazole o.d. or 30 mg lansoprazole b.i.d. for 12 weeks. Symptoms were evaluated at baseline (visit 1) after the PPI test (visit 2) and after the 12-week lansoprazole treatment period (visit 3).

RESULTS: Thirty-five patients completed the study protocol. Twenty-one patients (60.0%) reported complete relief from their cough with no difference between the two treatment groups (58.8% and 61.1% had no cough in 30 mg lansoprazole and 60 mg lansoprazole groups, respectively). More than 80% of the patients who had complete relief from their cough at the end of the treatment showed a positive response to the PPI test.

CONCLUSION: Twelve weeks of lansoprazole treatment even at a standard daily dose, is effective in patients with chronic persistent cough. A positive response to an initial PPI test seems to be the best criterion for selecting patients who respond to therapy.

Key words: Gastroesophageal reflux; Cough; Proton pump inhibitors; Lansoprazole

INTRODUCTION

There is increasing evidence that many otolaryngologic or pulmonary conditions, ranging from very mild symptoms such as hoarseness to very severe diseases such as cancer, may be caused by gastroesophageal reflux (GER)[1]. In particular, GER seems to account for a relevant proportion of patients with asthma, cough, and laryngitis. Chronic persistent cough is a very common and disabling complaint for which patients seek medical care. It has been shown that in non-smoking patients with a normal chest X-ray and not taking angiotensin-converting enzyme (ACE) inhibitors the four most common causes of cough are post-nasal drip syndrome (PNDS), asthma, GER and chronic bronchitis[2]. These four conditions may account for as many as 90% of the cases of chronic cough. Moreover, GER by itself has been found to be responsible for 10%-40% of them even in the absence of reflux symptoms[3]. For these reasons, antisecretory drugs have been proposed for the treatment of patients with chronic cough possibly due to GER. Most studies on this topic have been performed with proton pump inhibitors (PPIs) at higher daily doses and for generally longer periods than those usually employed for typical GER disease. These studies have shown better results in terms of symptom resolution and improvement of laryngeal signs for PPIs *vs* placebo, but the success rate has been relatively low and quite variable, ranging from 35% to 60%[4-6]. This variability in response may be due to the differences either in selection criteria or in treatment regimens. Since the poorest outcomes[4] have been obtained in the study in which PPIs were given at the highest daily dose and for the longest period, patient selection may be the critical factor.

Thus, published studies suggest that antireflux treatment of patients with GER-related respiratory symptoms, particularly chronic cough, must be carried

out with profound and prolonged acid inhibition in order to achieve satisfactory results in about two-thirds of the cases. However, the optimal treatment regimen and the criteria for patient selection need to be better defined.

The aim of our study was to evaluate the relative efficacy of two different daily doses of lansoprazole given for 12 weeks to patients with chronic persistent cough that could be reasonably ascribed to GER, and to assess the role of gastrointestinal (GI) investigations (endoscopy, 24-h esophageal pH-metry and a 4-week trial of empiric PPI treatment) as criteria for selecting patients with chronic cough who could benefit from antireflux treatment.

MATERIALS AND METHODS

Patients

The study protocol was approved by the Ethics Committee of the S. Orsola-Malpighi Hospital, Bologna, Italy and all participants gave their written informed consent. A pretrial analysis determined that 15 patients were required in each treatment to demonstrate equivalence between 30 and 60 mg/d lansoprazole treatment groups in terms of cough severity and frequency with 80% power at an alfa level of 0.05.

Patients aged 18-70 years with unexplained chronic persistent cough (i.e. for at least 3 days per week for a minimum of 3 months) were enrolled in the study over a period of 1 year (from June 2002 to June 2003). All patients were consecutively referred by otolaryngologists and pulmonologists after the exclusion of oropharyngeal or respiratory diseases potentially responsible for the cough (particularly asthma and PNDS) by means of a diagnostic evaluation which included medical history, physical examination, methacholine challenge test, chest X-ray and fiber-optic laryngoscopy. Patients were excluded if they were pregnant or breastfeeding; had systemic diseases, cardiac and pulmonary disorders, viral and bacterial or fungal infections, neoplasia or Zollinger –Ellison syndrome; or received previous treatment with drugs that interfered with their gastric acid secretion (H2-antagonists, PPIs) and chronic treatment with NSAIDs, phenytoin, warfarin, tricyclic antidepressants, reserpine, beta-agonists, anticholinergics, antihistamines, inhaled steroids, or ACE inhibitors. Patients with chronic alcohol or drug abuse were also excluded, as were smokers.

After an initial clinical evaluation aimed at assessing the presence of associated typical reflux symptoms (heartburn and regurgitation) and the severity of cough, all patients underwent a diagnostic work-up which included upper GI endoscopy, 24 h esophageal pH-metry and a trial of empiric PPI therapy (PPI test, 30 mg lansoprazole b.i.d. for 4 weeks). The investigations were always performed in the same sequence, i.e. in the order of endoscopy, pH-metry and the PPI test.

A patient was considered eligible for 12 wk of lansoprazole treatment if at least one of the GI investigations was positive.

Symptom assessment

The severity of cough was evaluated according to a visual analog scale (VAS) graded from 0 to 10 and to a four-level scoring system, regarding the previous week, calculated as follows:

- *Overall frequency*: 0 = absent, 1 = occasional (<3 d/wk), 2 = often (3-6 d/wk), 3 = every day
- *Daily frequency*: 0 = absent, 1 = 1 episode, 2 = 2-3 episodes, 3 = >3 episodes
- *Severity*: 0 = absent, 1 = mild (not interfering with daily activities), 2 = moderate (sometimes interfering with daily activities), 3 = severe (regularly interfering with daily activities and/or sleep).

Symptoms were evaluated at baseline (visit 1), after a 4-wk PPI trial (visit 2) and a 12-week lansoprazole treatment period (visit 3).

Upper GI endoscopy

All patients underwent upper GI endoscopy performed by the same gastroenterologist. The presence of esophagitis was noted and graded according to the Savary-Miller classification: grade I = single erosive or exudative lesion, oval or linear, on only one longitudinal fold; grade II = non-circular multiple erosions or exudative lesions on more than one longitudinal fold with or without confluence; grade III = circular erosive or exudative lesions; grade IV = chronic lesions, ulcers, strictures, or short esophagus, isolated or associated with grade I-III lesions; grade V = Barrett's epithelium, isolated or associated with grade I-III lesions[7]. Grades I-IV were considered diagnostic of erosive esophagitis and endoscopy was considered positive for the purpose of this study.

Twenty-four h esophageal pH-metry

After an overnight fast, two glass electrodes previously calibrated with buffer solutions at pH 7.0 and 1.0, were assembled with the sensors 15 cm apart and introduced into the stomach by the nasopharyngeal route. The distal electrode was positioned 5 cm above the lower esophageal sphincter (LES) so that the proximal one was located distal to the upper esophageal sphincter (UES). The location of the LES was determined by a combination of pH step-up technique, manometry (always performed in patients with hiatus hernia; 8 cases) and visualization under fluoroscopy if needed. The probe was connected to a digital portable recording unit (pH-day, Menfis, Italy) with a sampling frequency of one signal every second. Patients were asked to maintain their usual lifestyle and diet. They were provided with a diary card to record timing of meals, duration of nocturnal rest, time of cough occurrence and type of symptoms. Moreover, they were instructed to push an event marker button on the recording unit at the time of occurrence of their major complaint (cough). The pH-metry was stopped after 23-24 h. After being extracted, the electrodes were calibrated again with buffer solutions at pH 7.0 and 1.0. GER was evaluated as acid exposure fraction time (percent of time with pH <4). For the purpose of this study, pH-metry was considered positive if the percentage of total time with pH <4 was >4.73%[8] at the distal recording site or >1.00% at the proximal site (these values exceeded the 95th percentile of those previously obtained in normal subjects in our laboratory). In patients with normal values at both

Table 1 Demographics, clinical details and outcome of investigations performed in the 45 patients with chronic cough

	All patients	GERD symptoms	
		Present	Absent
Subjects	45	25	20
Age (mean±SD)	54.5 ± 11.1	54.6 ± 12.8	54.4 ± 8.6
Gender, male (%)	7 (15.5)	3 (12.0)	4 (20.0)
Upper endoscopy positive (%)	7 (15.5)	3 (12.0)	4 (20.0)
pH-metry positive (%)	26 (57.8)	13 (52.0)	13 (65.0)
PPI test positive (%)	23 (51.1)	13 (52.0)	10 (50.0)

No statistical differences between the groups in any of the parameters.

Table 2 Analysis of concordance between 24 h pH-metry and PPI test

	24 h pH-metry		Total
	Positive	Negative	
PPI test positive	12	11	23
PPI test negative	14	8	22
Total	26	19	

Youden's index = -0.1174 K (measure of reliability) = -0.115 (no reliability).

recording sites, we calculated the symptom index (SI), i.e., the number of cough episodes associated with acid reflux (that was simultaneous with or occurring within 5 min before or after the reflux episode) divided by the total number of cough episodes multiplied by 100, together with the symptom sensitivity index (SSI), i.e., the number of cough episodes associated with acid reflux, divided by the total number of reflux episodes multiplied by 100. We considered the investigation positive, if the SI was >50% with a SSI >20%[9].

PPI test

After endoscopy and 24-h pH -metry, all patients received a 4-week open-label course of 30 mg lansoprazole administered before breakfast and dinner. Before PPI trial (visit 1) was started and at the end of the treatment course (visit 2) the patients were interviewed regarding their main symptom, i.e., cough, and asked to rate symptom severity during the previous week on a VAS graded 0-10[10]. The test was considered positive, if the post-treatment value was 0 or if the difference between the pre- and post-treatment values was $\geqslant$5.

Intervention

Patients with a positive finding in at least one of the three assessments (endoscopy, pH-metry and symptom relief during a 4-week PPI trial) were randomized to either 30 mg lansoprazole oral capsules in the morning and identical placebo capsules in the evening or 30 mg lansoprazole in the morning and 30 mg lansoprazole in the evening before meals for 12 wk. Active drug and placebo were both supplied by Takeda Italia Pharmaceuticals (Rome, Italy). Both patients and investigators were blinded to the treatment status. Patients were interviewed and VAS was assessed at the end of treatment, and pill counting was performed to check for compliance with treatment.

Symptomatic response was defined as complete (VAS and score values = 0), partial (VAS value $\geqslant$ 1 but $\leqslant$ 50% of the baseline value and score value <5) or absent.

Statistical analysis

Data were analyzed by the Student's *t* test for independent data of continuous variables, by the Mann-Whitney's non-parametric test for discrete variables and by the χ^2 test for nominal variables reported as contingency tables[11]. The Wilcoxon's rank-sum test or the Friedman test was used for the comparison of symptom scores. A two-sided significance level of 0.05 was chosen.

Equivalence between the two treatment regimens was determined using the Schuirman one-sided test procedure. Mean VAS values recorded at visit 3 (after 12 wk of double-blind therapy) in the reference group (60 mg/day lansoprazole) were transformed to 100, and then the same transformation equation was applied to the mean VAS values at visit 3 in the 30 mg/d lansoprazole group. Equivalence was demonstrated if the 90% confidence intervals (CIs) for the difference between the transformed mean VAS values in each group were within the standard deviation (± 20).

RESULTS

Patient selection

Forty-five patients (7 men, mean age 54.5 ± 11.1 years, age range 29-70 years) referred for unexplained chronic persistent cough underwent the GI investigations aimed at the detection of GER disease (Table 1). Most of the patients were middle-aged females and 25 (55.5%) also reported typical reflux symptoms (heartburn and/or regurgitation occurring at least once per week). Thirty-six (80%) patients were positive for at least one test. Only a few patients (n = 7, 15.5%) had endoscopy-proven esophagitis, while about 50% had pathological GER and a positive PPI test. Twenty-four patients (53.3%) were found to have pathological reflux, 12 at both recording sites, 10 at the distal site only and just 2 at the proximal site only. Two out of 21 patients with negative pH-metry were found to have a positive SI and considered as positive for pH-metry. Twenty-three patients had a positive PPI test, five of them reported complete disappearance of cough, while the remaining 18 had a reduction in cough symptoms of 76.8% ± 10.1%. There was no association between an abnormal result of any of the aforementioned tests and the presence or absence of associated GER symptoms. There was a lack of concordance between the results of pH-metry and those of the PPI test (Table 2). In fact, 12 patients were positive for both tests and 8 were negative for both tests (44.4%), while in the other 25 cases the tests provided opposite results.

Of the 36 patients who satisfied the criteria for admission to the 12-week lansoprazole treatment, 12 had positive PPI test and pH-metry, 11 had a positive PPI test only and 13 had positive pH-metry only. Esophagitis was not the only pathological finding in any of the patients. One of the patients did not continue the study due to

Table 3 Characteristics of the patients entering the randomized double-blind phase of the study

	Lansoprazole (30 mg/d) (*n* = 17)	Lansoprazole (60 mg/d) (*n* = 18)
Age (mean±SD)	57.5 ± 11.9	52.4 ± 10.0
Gender, male (%)	3 (17.6)	2 (11.1)
Upper endoscopy positive (%)	4 (23.5)	3 (16.7)
pH-metry positive (%)	13 (76.5)	10 (55.5)
PPI test positive (%)	11 (64.7)	11 (61.1)

No statistical differences between the groups in any of the parameters.

Table 4 Patient symptoms evaluated at baseline (visit 1), after the PPI test (visit 2) and at the end of the 12-week lansoprazole treatment period (visit 3)

	Visit 1		Visit 2		Visit 3	
	VAS	Score	VAS	Score	VAS	Score
Lansoprazole (30 mg/d) (*n* = 17)	8 (7.5-9.5)	9 (8-9)	2 (1.5-5.5)[b]	4 (3-7)[a]	1 (0-4.5)[b]	3 (0-6.5)[b]
Lansoprazole (60 mg/d) (*n* = 18)	9 (8-9)	8 (7-9)	2 (1-6.5)[b]	3.5 (3-7)[a]	1 (0-5)[b]	3 (0-6.25)[b]

Data expressed as median (25%-75% quartiles). [b]$P < 0.001$ *vs* visit 1; [a]$P < 0.005$ *vs* visit 1.

unwillingness, while the remaining 35 completed the study protocol (17 on 30 mg/d lansoprazole and 18 on 60 mg/d lansoprazole) and were considered for our efficacy analysis. There was no statistically significant difference between the two treatment groups with regard to age, gender, presence of esophagitis, or 24-h pH-metry and PPI trial outcome (Table 3).

Effect of treatment

Patients' symptoms as assessed with both the subjective (VAS) and the objective (score) system, improved significantly at the end of the 12-wk treatment period *vs* baseline in both treatment groups, with no statistically significant difference either between the two groups or between the values at visits 2 and 3 (Table 4). The 90%CIs of the difference in the transformed mean VAS scores at 12 wk were within the standard deviation, thereby demonstrating equivalence of the two regimens.

At the end of the 12-wk treatment period 21 patients (60.0%) reported complete relief of their cough with no difference between the two treatment groups (10/17 and 11/18 had no cough in the 30 mg/d lansoprazole and 60 mg/d lansoprazole groups, respectively, Figure 1). In patients selected for the 12-wk course of lansoprazole a PPI was actually taken for 16 wk (including the 4-wk PPI test). At the end of the PPI test, 10 (28.6%) of the 35 patients who continued to complete 12 wk of therapy showed complete relief of their cough. During the following course of PPI the percentage of symptom-free patients increased up to about 60% at the end of the 3-mo period, independently of the daily dose of the drug.

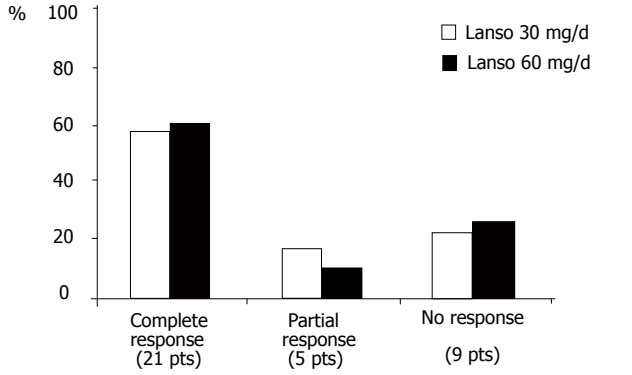


Figure 1 Percentage of patients showing symptomatic response at the end of 12-wk treatment with 30 mg/d (*n* = 17) or 60 mg/d (*n* = 18) lansoprazole.

Patient characteristics and outcome of treatment

In order to determine the factors that may have influenced the treatment outcome, we compared the characteristics of patients who achieved complete relief of their symptoms with those who showed only partial relief or no symptomatic response (Table 5). As shown in the table, the positivity of the PPI test was significantly different between the two groups. In fact, more than 80% of patients who had complete relief of their cough at the end of treatment had a positive response to the PPI trial in comparison to only 28.6% of those who did not satisfactorily respond. None of the other factors, including the daily lansoprazole dose, was significantly correlated with the positive outcome of the treatment. Moreover, the rate of complete symptom relief at the end of the 12-wk course in patients classified as PPI test-positive was 81.8% in comparison to only 23.1% in those with a negative test (Figure 2).

DISCUSSION

The correlation between respiratory or otolaryngologic symptoms and GER is usually ascertained in patients with unexplained chronic cough by the exclusion of other common causes such as asthma and PNDS, and the finding of pathognomonic signs such as posterior laryngitis, also called "reflux laryngitis", as well as the demonstration of pathological reflux[4-6].

In our study, the pathogenetic role of reflux was suspected after the exclusion of common otolaryngologic or pneumological causes and after a careful work-up aimed at the detection of GER disease. The presence of posterior laryngitis was not included in our criteria because the sensitivity of this finding is generally thought to be low, while its specificity is ill-defined[12]. About 50% of our patients complained of typical reflux symptoms, such as heartburn and regurgitation, but this finding seemed to be of little relevance, since it was not correlated with the presence of esophagitis, pathological reflux or specifically, a positive response to the PPI trial (Table 1).

As expected, only a few patients had endoscopically proven esophagitis. This is in agreement with the previous observations in patients with so-called extra-esophageal manifestations of GER disease, showing that

Table 5 Comparison between patients with symptomatic response and non-responders

	Responders [*n* = 21 (%)]	Non-responders [*n* = 14 (%)]
GER symptoms	14 (66.7)	5 (35.7)
Treatment with 60 mg/d lansoprazole	11 (52.4)	7 (50.0)
Erosive esophagitis	5 (23.8)	2 (14.3)
pH-metry positive	10 (47.6)	13 (92.8)
PPI test positive	18 (85.7)	4 (28.6)[b]

Responders = patients with VAS and score values of 0; non-responders = patients with VAS and score values ≥1; [b]$P < 0.01$ *vs* responders.

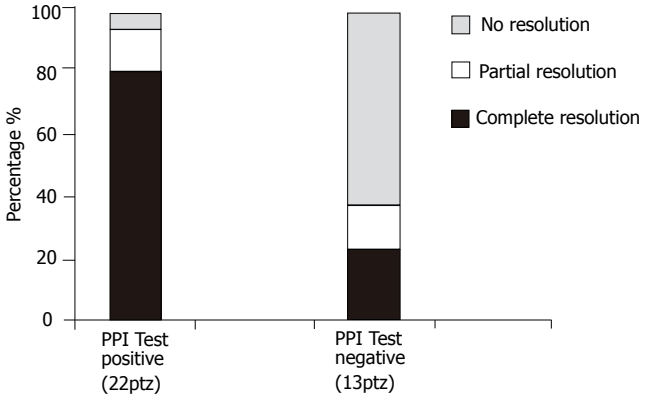


Figure 2 Percentage of patients showing symptomatic response at the end of 12-wk treatment with 30 or 60 mg/d lansoprazole (n = 35), subdivided according to the outcome of PPI test. $P<0.05$ *vs* negative PPI test.

endoscopic evidence of esophagitis is found in 25%-40% of patients with asthma[13-14] and in only 10%-25% of patients with otolaryngologic disorders[6,15]. Therefore, upper GI endoscopy has a very low sensitivity in patients with atypical GER symptoms and, in the absence of other clinical indications, should not be included in the diagnostic work-up of these patients.

Ambulatory 24-h esophageal pH-metry is considered the gold standard for GER detection, with a high sensitivity in patients with typical symptoms[16]. Studies performed in patients with extra-esophageal symptoms showed that diagnostic sensitivity ranges 50%-80% in patients with asthma[17,18] and otolaryngologic manifestations, such as laryngitis or cough[4,6,19]. Moreover, the use of a double esophageal probe has been proposed in the latter patient group[19]. However, the usefulness of proximal pH-metry has been questioned since its sensitivity and reproducibility are too low[14,20]. Our results have confirmed the findings of others, showing that the diagnostic gain provided by dual-channel pH-metry is very low[4].

Since the presence of "physiological" reflux does not necessarily imply the absence of a casual link between reflux and symptoms, the diagnostic sensitivity of 24-h pH-metry may be improved by including the evaluation of a symptom/reflux correlation (SI), especially in patients with atypical symptoms, such as non-cardiac chest pain[21]. On the other hand, the SI has two major potential limitations: (1) it is feasible only in patients with symptoms during the recording period and this is not always the case (in our patient group 77.7% reported cough episodes during pH-metry), and (2) its value may be questioned if patients have only few symptomatic episodes during the recording period. For this reason, as suggested by previous studies[22], we considered a correlation between a given symptom and a reflux event to be present for SI values > 50% together with a SSI > 20%. We performed this evaluation as part of the analysis of our 24 h pH-metry tracings and found that 17 of the 21 patients with negative pH-metry reported cough during the recording period, with 2 of them showing a positive SI, thus increasing the diagnostic value of this investigation.

An empirical trial with PPIs (the so called "omeprazole or PPI test") has been recently proposed for the diagnosis of GER disease in patients with various clinical presentations, especially those with atypical or extra-esophageal symptoms. The diagnostic sensitivity of this test is generally validated *vs* 24-h pH-metry as a gold standard. The variability of results reported thus far depends on the differences in treatment schedules (daily doses, duration) and the outcomes chosen (cut-off point for symptom improvement, VAS, questionnaires, *etc.*). Doses used in clinical studies range 40-80 mg of omeprazole or 60 mg of lansoprazole daily from 1 to 14 d in patients with symptoms suggestive of GER disease or non-cardiac chest pain[23–29] and from 7 to 90 d in patients with extra-esophageal manifestations[4,30,31]. In patients with laryngeal symptoms, in which a longer duration and a high daily dosage are considered necessary for obtaining a significant symptom improvement, the test is in general fairly sensitive, with values ranging 62.5%-81%[4,30,31]. Thus, our PPI test consisted of 4-week administration of 60 mg/d lansoprazole. According to a similar study[28] we used a VAS graded 0-10 to assess as objectively as possible the severity of symptoms perceived by our patients and we considered the PPI test positive if symptoms disappeared (grade 0) or were reduced by at least 50% in comparison to the pre-administration value. In our study the test was positive in 63.9% of patients with a sensitivity similar to that previously reported.

In our study there was a lack of concordance between the results of the 24 h esophageal pH-metry and those of the PPI test (Table 2). In fact there was agreement in only 20 patients (44.4%). It was reported that there is a significant direct correlation between pH-metry and response to the omeprazole test in patients with typical reflux symptoms (65.8% of patients with concordant results, $P = 0.04$)[23]. In contrast, a study[4] performed in patients with chronic cough showed concordance between the two tests in only half the cases (52.2%). This lack of concordance is not surprising and may depend on the intrinsic limitations of both pH-metry (i.e. absence of symptoms during the recording period in patients with "physiological" reflux) and the PPI test (inadequacy of treatment dose or duration). It may also depend on the simultaneous and independent existence of two conditions, GER and cough, not causally linked.

For all these reasons in our study, we proposed that at least one of the GI investigations (endoscopy, pH-metry,

PPI test) had to be positive in order to select the cough patients in whom a 12-wk lansoprazole course could be reasonably proposed as a treatment choice.

The efficacy of PPI treatment in patients with extra-esophageal manifestations has been previously established[4-6]. Our study showed that both lansoprazole regimens significantly reduced symptom scores in comparison to baseline values (Table 4). There was a further reduction of the values recorded at visit 3 in comparison to those at visit 2, but the difference was not statistically significant. Moreover, it is interesting to note that there was a good concordance between the subjective (VAS) and objective (score) analysis, suggesting that they can be used interchangeably for symptom assessment. The success rate was also evaluated on an individual basis in terms of symptom resolution (Figure 1) which was graded on three levels according to the complete, partial or poor improvement of the symptoms. At the end of the 12-week treatment course about 60% of the patients reported complete relief of their cough (58.8% and 61.1% had no cough in the 30 mg/d lansoprazole and 60 mg/d lansoprazole groups, respectively). We did not find significant differences between the two treatment regimens but our study was only powered to show equivalence rather than efficacy. Studies performed in patients with chronic cough treated with PPIs have reported a complete relief of the symptom in a percentage of patients ranging 26%-43%[4,5] but the comparison among the studies is difficult because of different patient selection criteria and treatment regimens. A study that used the same drug and treatment duration as the present study[6] has reported a complete resolution of symptoms in 50% of patients with idiopathic laryngitis, which is very similar to our results.

In our study, the patients selected for the 12-wk course of PPI after the test period actually took lansoprazole for 16 weeks, and some of these patients experienced symptom relief at the end of the first 4 weeks, 10 of them showing complete resolution of their cough. This suggests that 1-month PPI administration at a double daily dose may be an effective therapy in about one-third of these cases. The percentage of symptom-free patients increased up to about 60% at the end of the 12-wk period, independently of the daily dose of the drug.

The analysis of factors that could have influenced the outcome (Table 5) clearly showed that the positivity of the PPI test was significantly different between complete and incomplete responders to PPI treatment. None of the other factors, particularly the presence of GER symptoms ,seemed to be associated with the positive outcome of treatment. This is in agreement with previous studies in patients with cough[4-6] and laryngitis[6]. Indeed, the response to an initial treatment with a PPI has been suggested as the best method for identifying patients with GER-related chronic cough[4]. Our results show that PPI test may predict response to a longer course of PPI treatment in patients with atypical reflux symptoms. The rate of complete symptom relief at the end of the 12-week course in our patients classified as PPI test-positive was 81.8% (the highest, to our knowledge, among those reported in similar studies) in comparison to only 23.1% of those with a negative test (Figure 2).

In conclusion, a 12-wk course of lansoprazole is effective in relieving symptoms of patients with unexplained chronic persistent cough. A positive response to an initial 4-wk administration of PPI at a double daily dose seems to be an effective criterion for selecting patients who obtain the best results from the PPI treatment.

REFERENCES

1 **Richter JE.** Extraesophageal presentations manifestations of gastroesophageal reflux disease. *Semin Gastrointest Gastroenterol Dis* 1997; **8**: 75-89
2 **Irwin RS**, Richter JE. Gastroesophageal reflux and chronic cough. *Am J Gastroenterol* 2000; **95**: S9-14
3 **Irwin RS**, Curley FJ, French CL. Chronic cough. The spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of specific therapy. *Am Rev Respir Dis* 1990; **141**: 640-647
4 **Ours TM**, Kavuru MS, Schilz RJ, Richter JE. A prospective evaluation of esophageal testing and a double-blind, randomized study of omeprazole in a diagnostic and therapeutic algorithm for chronic cough. *Am J Gastroenterol* 1999; **94**: 3131-3138
5 **Kiljander TO**, Salomaa ER, Hietanen EK, Terho EO. Chronic cough and gastro-oesophageal reflux: a double-blind placebo-controlled study with omeprazole. *Eur Respir J* 2000; **16**: 633-638
6 **El-Serag HB**, Lee P, Buchner A, Inadomi JM, Gavin M, McCarthy DM. Lansoprazole treatment of patients with chronic idiopathic laryngitis: a placebo-controlled trial. *Am J Gastroenterol* 2001; **96**: 979-983
7 **Armstrong D**, Blum AL, Savary M. Reflux disease and Barrett' s oesophagus. *Endoscopy* 1992; **24**: 9-17
8 **Baldi F**, Ferrarini F, Longanesi A, Bersani G. Ambulatory 24-hour oesophageal pH monitoring in normal subjects: a multicentre study in Italy. G.I.S.M.A.D. GOR Study Group. *Ital J Gastroenterol* 1991; **23**: 477-480
9 **Singh S**, Richter JE, Bradley LA, Haile JM. The symptom index. Differential usefulness in suspected acid-related complaints of heartburn and chest pain. *Dig Dis Sci* 1993; **38**: 1402-1408
10 **Nyrén O**, Adami HO, Bates S, Bergström R, Gustavsson S, Lööf L, Sjödén PO. Self-rating of pain in nonulcer dyspepsia. A methodological study comparing a new fixed-point scale and the visual analogue scale. *J Clin Gastroenterol* 1987; **9**: 408-414
11 **Armitage P,** Berry G, Matthews JNS. Statistical Methods in Medical Research. 4rd Edition. Oxford: Blackwell Scientific Publications, 2002
12 **Young JL**, Shaw GY, Searl JP, Miner PB. Laryngeal manifestations of gastroesophageal reflux disease: endoscopic appearance and management. *Gastrointest Endosc* 1996; **43**: 225-230
13 **Larrain A**, Carrasco E, Galleguillos F, Sepulveda R, Pope CE. Medical and surgical treatment of nonallergic asthma associated with gastroesophageal reflux. *Chest* 1991; **99**: 1330-1335
14 **Sontag SJ**, Schnell TG, Miller TQ, Khandelwal S, O'Connell S, Chejfec G, Greenlee H, Seidel UJ, Brand L. Prevalence of oesophagitis in asthmatics. *Gut* 1992; **33**: 872-876
15 **Richter JE**, Hicks DM. Unresolved issues in gastroesophageal reflux-related ear, nose, and throat problems. *Am J Gastroenterol* 1997; **92**: 2143-2144
16 **Jamieson JR**, Stein HJ, DeMeester TR, Bonavina L, Schwizer W, Hinder RA, Albertucci M. Ambulatory 24-h esophageal pH monitoring: normal values, optimal thresholds, specificity, sensitivity, and reproducibility. *Am J Gastroenterol* 1992; **87**: 1102-1111
17 **Sontag SJ**, O'Connell S, Khandelwal S, Miller T, Nemchausky B, Schnell TG, Serlovsky R. Most asthmatics have gastroesophageal reflux with or without bronchodilator therapy. *Gastroenterology* 1990; **99**: 613-620

18 **Schnatz PF**, Castell JA, Castell DO. Pulmonary symptoms associated with gastroesophageal reflux: use of ambulatory pH monitoring to diagnose and to direct therapy. *Am J Gastroenterol* 1996; **91**: 1715-1718
19 **Koufman JA**. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope* 1991; **101**: 1-78
20 **Vaezi MF**, Richter JE. Twenty-four-hour ambulatory esophageal pH monitoring in the diagnosis of acid reflux-related chronic cough. *South Med J* 1997; **90**: 305-311
21 **Hewson EG**, Sinclair JW, Dalton CB, Richter JE. Twenty-four-hour esophageal pH monitoring: the most useful test for evaluating noncardiac chest pain. *Am J Med* 1991; **90**: 576-583
22 **Breumelhof R**, Smout AJ. The symptom sensitivity index: a valuable additional parameter in 24-hour esophageal pH recording. *Am J Gastroenterol* 1991; **86**: 160-164
23 **Schenk BE**, Kuipers EJ, Klinkenberg-Knol EC, Festen HP, Jansen EH, Tuynman HA, Schrijver M, Dieleman LA, Meuwissen SG. Omeprazole as a diagnostic tool in gastroesophageal reflux disease. *Am J Gastroenterol* 1997; **92**: 1997-2000
24 **Fass R**, Fennerty MB, Ofman JJ, Gralnek IM, Johnson C, Camargo E, Sampliner RE. The clinical and economic value of a short course of omeprazole in patients with noncardiac chest pain. *Gastroenterology* 1998; **115**: 42-49
25 **Johnsson F**, Weywadt L, Solhaug JH, Hernqvist H, Bengtsson L. One-week omeprazole treatment in the diagnosis of gastro-oesophageal reflux disease. *Scand J Gastroenterol* 1998; **33**: 15-20
26 **Fass R**, Ofman JJ, Sampliner RE, Camargo L, Wendel C, Fennerty MB. The omeprazole test is as sensitive as 24-h oesophageal pH monitoring in diagnosing gastro-oesophageal reflux disease in symptomatic patients with erosive oesophagitis. *Aliment Pharmacol Ther* 2000; **14**: 389-396
27 **Cremonini F**, Wise J, Moayyedi P, Talley NJ. Diagnostic and therapeutic use of proton pump inhibitors in non-cardiac chest pain: a metaanalysis. *Am J Gastroenterol* 2005; **100**: 1226-1232
28 **Juul-Hansen P**, Rydning A, Jacobsen CD, Hansen T. High-dose proton-pump inhibitors as a diagnostic test of gastro-oesophageal reflux disease in endoscopic-negative patients. *Scand J Gastroenterol* 2001; **36**: 806-810
29 **Pandak WM**, Arezo S, Everett S, Jesse R, DeCosta G, Crofts T, Gennings C, Siuta M, Zfass A. Short course of omeprazole: a better first diagnostic approach to noncardiac chest pain than endoscopy, manometry, or 24-hour esophageal pH monitoring. *J Clin Gastroenterol* 2002; **35**: 307-314
30 **Metz DC**, Childs ML, Ruiz C, Weinstein GS. Pilot study of the oral omeprazole test for reflux laryngitis. *Otolaryngol Head Neck Surg* 1997; **116**: 41-46
31 **Jaspersen D**, Diehl KL, Geyer P, Martens E. [Diagnostic omeprazole test in suspected reflux-associated chronic cough]. *Pneumologie* 1999; **53**: 438-441

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 89-93
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Benefits of early postoperative jejunal feeding in patients undergoing duodenohemipancreatectomy

Takehiro Okabayashi, Michiya Kobayashi, Isao Nishimori, Tekeki Sugimoto, Toyokazu Akimori, Tsutomu Namikawa, Ken Okamoto, Saburo Onishi, Keijiro Araki

Takehiro Okabayashi, Michiya Kobayashi, Tekeki Sugimoto, Toyokazu Akimori, Tsutomu Namikawa, Ken Okamoto, Keijiro Araki, Department of Tumor Surgery, Kochi Medical School, Nankoku, Kochi 783-8505, Japan
Isao Nishimori, Saburo Onishi, Department of Gastroenterology and Hepatology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan
Co-correspondence to: Michiya Kobatashi
Correspondence to: Dr Michiya Kobayashi, Department of Tumor Surgery, Kochi Medical School, Nankoku, Kochi 783-8505, Japan
Telephone: +81-88-880-2370 Fax: +81-88-880-2371
Received: 2005-06-16 Accepted: 2005-07-08

Abstract

AIM: To study whether early postoperative enteral nutrition reduces the incidence of complications and/or improves nutritional status following duodenohemipancre atectomy (DHP).

METHODS: We studied 39 patients who underwent DHP for a peri-ampullary mass. Twenty-three patients received total parental nutrition and then started to have an oral intake of nutrition between postoperative day (POD) 7 and 14 [late postoperative enteral nutrition (LPEN) group]. Sixteen patients started to have enteral feeding through a jejunostomy catheter the day after the operation [early postoperative enteral nutrition (EPEN) group]. The incidence of complications and laboratory data at the early postoperative stage were studied in comparison between LPEN and EPEN groups.

RESULTS: Serum levels of albumin and total protein in the EPEN group were significantly higher than those in the LPEN group. The loss of body mass index was significantly suppressed in the EPEN group as compared to the LPEN group. The lymphocyte count decreased immediately after the operation was restored significantly faster in the EPEN group than in the LPEN group. The EPEN group showed significantly fewer incidences of postoperative pancreatic fistulas, as well as a significantly shorter length of hospitalization than the LPEN group. There were no significant differences in the incidences of other postoperative complications between the two groups, such as delayed gastric emptying, surgical site infection, cholangitis, and small bowel obstruction.

CONCLUSION: EPEN is a safe and beneficial opportunity for patients who have undergone DHP for a peri-ampullary mass.

Key words: Nutrition; Postoperative jejunal feeding; Pancreaticoduodenectomy; Enteral nutrition

Okabayashi T, Kobayashi M, Nishimori I, Sugimoto T, Akimori T, Namikawa T, Okamoto K, Onishi S, Araki K. Benefits of early postoperative jejunal feeding in patients undergoing duodenohemipancreatectomy. *World J Gastroenterol* 2006; 12(1): 89-93

http://www.wjgnet.com/1007-9327/12/89.asp

INTRODUCTION

Postoperative nutritional support was shown to reduce the incidence of complications and/or to shorten the hospitalization period[1,2]. Recently, early postoperative enteral nutrition (EPEN) has been proposed as the novel method for nutritional support after surgery, especially after gastric and colorectal resection[3-9]. Several studies suggested that EPEN possibly improves also the postoperative outcome of patients after duodenohemi pancreatectomy (DHP)[10-13], which is one of the most invasive operations in the upper abdominal surgery with a high incidence of postoperative complications[14-19]. Whereas, EPEN has been introduced with complications such as troubles of jejunal feeding tube and delayed gastric emptying[10-13,20,21]. Taken together, the overall benefit of EPEN after DHP remains controversial.

The purpose of the present study was to evaluate the influence of EPEN on the incidence of postoperative complications. Moreover, by analyzing a variety of clinical parameters including laboratory data, body mass index (BMI), and the duration of hospitalization, we attempted to determine which method of postoperative nutritional support, enteral or non-enteral, was more advantageous in the total management of patients who had undergone DHP.

MATERIALS AND METHODS

Materials

We investigated a total of 39 patients who had undergone

Table 1 Patient profiles

	LPEN group (n = 23)	EPEN group (n = 16)	P value
Demographics			
Age (range)	67.2 yrs (42-82)	68.0 yrs (54-81)	0.8137
Gender: male	65.2%	56.3%	0.8168
Underlying disease (%)			0.6206
Cholangiocarcinoma	9 (39.1)	2 (12.5)	
Pancreatic carcinoma	6 (26.1)	8 (50.0)	
IPMN	4 (17.4)	3 (18.8)	
CPV	3 (13.1)	1 (6.2)	
Others	1 (4.3)	2 (12.5)	

IPMN: Intraductal papillary mucinous neoplasm; CPV: Carcinoma of the papilla of Vater; Other diseases: Gastrointestinal stromal tumor, primary sclerosing cholangitis, and pancreatic metastasis of renal cell carcinpma

Table 2 Operative characteristics

GroupCharacteristics	LPEN group (n = 23)	EPEN (n = 16)	P value
Operative procedure (%)			0.0371
PpPD	12 (52.2)	14 (87.5)	
PD	11 (47.8)	2 (12.5)	
Demographics (range)			
Operative time (min)	516 (360-765)	509 (370-605)	0.5417
Blood loss volume (mL)	1 014 (400-1 650)	908 (400-1 600)	0.0593
Transfused patients (%)	17 (73.9)	8 (53.3)	0.3384
RC-MAP (U)	1.9 (0-10)	1.1 (0-6)	0.0599
FFP (U)	9.2 (0-40)	5.3 (0-10)	0.1220
IOR (%)	2 (8.7)	7 (43.8)	0.0190

PD: Pancreaticoduodenectomy; PpPD: Pylorus-preserving pancreaticoduodenectomy; RC-MAP: Red cells in mannitol-adenine-phosphate solution; FFP: Fresh frozen plasma; IOR: intra-operative radiotherapy.

DHP for a peri-ampullary mass from 2000 to 2005 at Kochi Medical School, including 24 men and 15 women (mean age of 67.5 years; 43-82 years). Among these 39 patients, there were 14 cases of pancreatic invasive ductal carcinoma, 11 of cholangiocarcinoma, 7 of intraductal papillary mucinous neoplasms, 4 of carcinoma of the papilla of Vater, 1 of gastrointestinal stromal tumor, 1 of primary sclerosing cholangitis, and 1 of metastatic renal cell carcinoma (Table 1). All patients underwent a complete perioperative physical examination and laboratory investigations. Moreover, a variety of relevant parameters regarding the operative procedure and anesthesia were recorded in all cases.

Operative procedure

In all patients, the reconstructive technique was used to anastomose the pancreas first, followed by the hepatic duct and the duodenum with a Braun anastomosis. The pancreatic-enteric anastomosis was performed as a pancreaticojejunostomy in an end-to-side fashion. In patients of the EPEN group, a feeding jejunostomy catheter was placed at the end of surgery and before closing the wound through the anterior wall of the stomach using a modified Witzel technique. Furthermore, in all cases of pylorus-preserving pancreaticoduodenectomy (PpPD), the gastrostomy tube was inserted from the afferent loop of the jejunum into the stomach for the purpose to prevent delayed gastric emptying.

Postoperative nutrition

We determined the amount of calories required for postoperative nutrition according to the Harris-Benedict equation[22]. Some patients received total parenteral nutrition and then started to have oral intake of nutrition usually between POD 7 and 14, as determined late postoperative enteral nutrition (LPEN) group. The second group of patients started to have EPEN through a catheter-feeding jejunostomy on POD1 (within 24 h after surgery), as determined EPEN group. Enteral feeding was started at a rate of 20 mL/h and gradually increased by 10 mL/h a day up to the final rate (70 mL/h).

Laboratory and clinical investigations

The operation time, blood loss volume, and amount of blood transfusion during and after the surgery were carefully recorded. Samples for laboratory investigations were taken on POD 1, 4, 6, and 14. The laboratory parameters included serum levels of total protein, albumin, total bilirubin, cholinesterase, alanine transaminase, aspartate transaminase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, amylase, urea nitrogen, and creatinine. The BMI was measured before surgery and on POD 6 and 14. Postoperative complications, including surgical site infection, leakage from anastomosis, pancreatic fistula, cholangitis, small bowel obstruction and delayed gastric emptying, were carefully monitored every day. The duration of hospitalization was defined as the time from the day of the surgery to the day of discharge. The progress of all patients, following their discharge from hospital, was monitored by our hospital.

Pancreatic fistula

We determined the occurrence of pancreatic fistula based on the following criteria: the concentrations of amylase and lipase in the drainage fluid being three times higher than that in the serum on consecutive PODs, and the drainage volume being more than 10 mL/d. Amylase and/or lipase concentrations in the serum and drainage fluid were checked on POD 1, 3, 4, 5, and 7, and twice a week thereafter[23-25].

Statistical analysis

We tested for statistical significance using the χ^2 test, the Fisher's exact test and the t test. $P < 0.05$ was considered statistically significant. Where appropriate, values were expressed as mean ± SD.

RESULTS

We retrospectively reviewed 39 patients who had undergone DHP between 2000 and 2004 at Kochi Medical School, and subdivided them into two groups, 23 patients in the LPEN group and 16 patients in the EPEN group (Table 1). Hospital mortality was 2.6%. There were no significant differences between the two groups in age,

Table 3 Postoperative outcome of patients with pancreatic surgery

Characteristics	LPEN group (n = 23)	EPEN group (n = 16)	P value
BMI			
Baseline	21.75 ± 3.16	22.58 ± 2.60	0.8044
POD 6	20.43 ± 2.67	2.40 ± 2.59	0.0305
POD 14	20.14 ± 2.50	22.38 ± 2.55	0.0111
Postoperative complications (%)			
Anastomotic leakage	0 (0.0)	0 (0.0)	NS
Surgical site infection	2 (8.7)	2 (12.5)	0.8797
Pancreatic fistula	9 (39.1)	1 (6.3)	0.0279
Ventral hernia	0 (0.0)	1 (6.3)	0.4103
Cholangitis	6 (26.1)	5 (31.3)	0.9926
Small bowel obstruction	1 (4.3)	1 (6.3)	0.6362
Delayed gastric emptying	1 (4.3)	2 (12.5)	0.5571
Length of hospitalization (days)	44.3 ± 19.0	31.7 ± 8.8	0.0011

NS: not significant

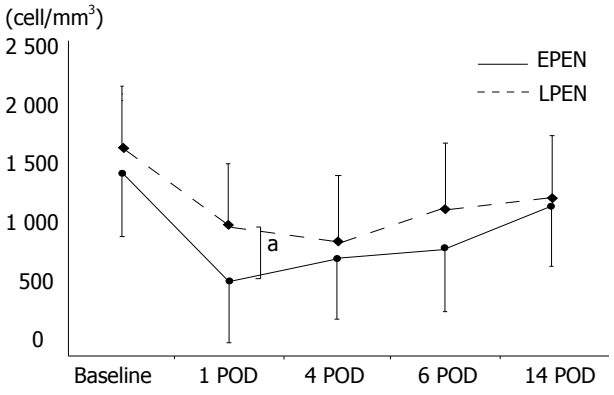


Figure 1 Peripheral lymphocyte coun. [a]P<0.05 *vs* LPEN.

gender, and the incidence of underlying diseases.

PpPD was carried out in 26 cases and PD in 13 cases. The frequencies of PpPD and intra-operative radiotherapy (IOR) were significantly higher in the EPEN group than in the LPEN group, because we started IOR for all patients with pancreatic carcinoma in 2002 ($P < 0.05$). There were no significant differences between the two groups in other parameters (Table 2) including operation time, blood loss volume, and the proportion of patients who received blood transfusion including red blood cells in mannitol-adenine-phosphate solution (RC-MAP) and fresh frozen plasma (FFP).

The baseline preoperative values of all laboratory parameters were comparable between the two groups. In the postoperative course, however, several parameters showed a significant difference. The lymphocyte count decreased immediately after the operation (at POD 1) in both groups (Figure 1), which was more obviously seen in the EPEN group compared to the LPEN group ($P < 0.05$), probably due to the higher incidence of IOR (Table 2). However, during POD 1 and 4, lymphocyte count in the LPEN group continuously decreased, but increased in the EPEN group. There was no significant difference between the two groups in lymphocyte count at POD 4 and

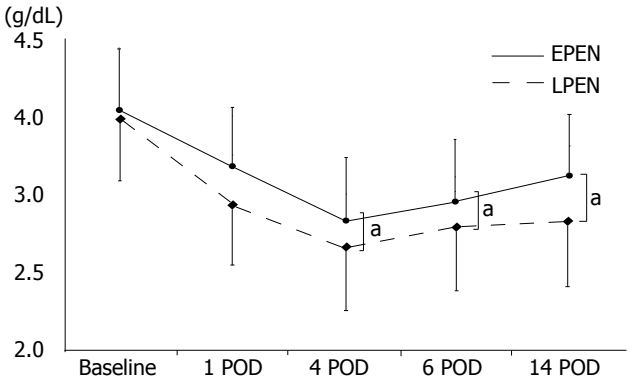


Figure 2 Serum albumin level. [a]P<0.05 *vs* LPEN.

thereafter. The serum levels of albumin (Figure 2) and the total protein (data not shown) decreased immediately after the operation in both groups. However, albumin levels at POD 4, 6, and 14 (Figure 2) and total protein levels at POD 6 and 14 (data not shown) in the EPEN group were significantly higher than those in the LPEN group. These findings in the restoration of nutritional parameters were consistent with the alteration in BMI. Loss of BMI was significantly suppressed in the EPEN group as compared to the LPEN group (Table 3).

None of the cases had a complication of anastomotic leakage. The EPEN group had a significantly lower incidence of pancreatic fistula than the LPEN group ($P < 0.05$). There was no significant difference between the two groups in the incidence of other postoperative complications, including surgical site infection, ventral hernia, cholangitis, small bowel obstruction, and delayed gastric emptying (Table 3). Finally, the EPEN group also required a significantly shorter length of hospital stay than the LPEN group ($P < 0.01$).

DISCUSSION

In the present study, we employed 39 patients who underwent DHP and subdivided them into two groups according to the procedures of the postoperative nutritional support: the EPEN and LEPN groups. There was no significant difference in baseline profiles between the two groups except for the following two parameters, which should be considered in the analysis of the results. First, the number of patients with PD was greater in the LPEN group than in the EPEN group. EPEN after DHP was introduced in our department in 2002, in order to assess its utility. Until then, almost all patients who underwent PD for a peri-ampullary mass were postoperatively administered with total parenteral nutrition. However, we believed that there were no significant differences in the surgical procedure because two surgeons performed the pancreatic surgery in this study period. Second, the number of patients with IOR was much greater in the EPEN group than the LPEN group. The difference was caused by the fact that we started IOR for all patients with pancreatic carcinoma in 2002[26-28].

Although the serum levels of albumin and total protein

dropped remarkably in all the patients after the operation, they recovered quickly in the EPEN group, and were significantly higher than those in the LPEN group at the early postoperative stage. Consistent with these findings, loss of BMI was significantly suppressed in the EPEN group as compared to the LPEN group. These findings indicate that EPEN modulates a metabolic response, favoring the synthesis of proteins. We believe that nutritional improvement observed in the EPEN group was not influenced by a large proportion of patients with PpPD (87.5%), since PpPD has been reported to provide a long-term nutritional support for operated patients but has not any benefit for nutritional status in early postoperative stage[29].

Furthermore, the lymphocyte count fell immediately after surgery in the EPEN group probably due to the IOR, resulting in the significantly lower level as compared to the LPEN group ($P < 0.05$). However, the lymphocyte count in the EPEN group increased thereafter, and reached at the similar level observed in the LPEN group at POD 14. These findings suggest that the administration of EPEN not only improves the nutritional status but also improves whole-body protein kinetics.

There was no significant difference between two groups in the incidence of infectious complications, such as surgical site infection and cholangitis, and also noninfectious complications, such as ventral hernia and small bowel obstruction. Although the EPEN group contained a significantly greater number of patients with PpPD than the LPEN group, there were no significant differences in the occurrence of delayed gastric emptying between the two groups. The incidence of delayed gastric emptying has been reported in 7-36% of patients with DHP[18,30-34]. Usually, the delayed gastric emptying is more frequently seen in patients with PpPD than in patients with PD, and is typically associated with prolonged hospitalization. In our study, tube gastrostomy was created in all patients who underwent PpPD, and thus patients with delayed gastric emptying had no vomiting.

Pancreatic fistula is considered as a major postoperative complication of DHP and has been reported in 5-24% of patients with DHP[23,35-38]. In our study, surprisingly, the incidence of pancreatic fistula was significantly lower in the EPEN group (6.3%) than in the LPEN group (39.1%). There have been some concerns that EPEN could increase the possibility of pancreatic fistulae because of its stimulatory effect on exocrine pancreatic secretion. Our data show that EPEN has no bad influence upon the occurrence of pancreatic fistula but rather works to prevent it.

In conclusion, EPEN is a safe and beneficial procedure for patients who have undergone DHP. EPEN improves early postoperative outcomes, including nutritional status and whole-body protein kinetics. Furthermore, EPEN contributes to a significantly lower incidence of pancreatic fistula, resulting in a shorter duration of hospitalization compared to the LPEN group. Based on these findings, EPEN can provide regular postoperative nutritional support following DHP.

REFERENCES

1 **Kudsk KA**, Croce MA, Fabian TC, Minard G, Tolley EA, Poret HA, Kuhl MR, Brown RO. Enteral versus parenteral feeding. Effects on septic morbidity after blunt and penetrating abdominal trauma. *Ann Surg* 1992; **215**: 503-11; discussion 511-3

2 **Moore FA**, Feliciano DV, Andrassy RJ, McArdle AH, Booth FV, Morgenstein-Wagner TB, Kellum JM, Welling RE, Moore EE. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. The results of a meta-analysis. *Ann Surg* 1992; **216**: 172-183

3 ASPEN Board of Directors: Guidelines for the use of parenteral and enteral nutrition in adult and pediatrric patients. *JPEN J Parenter Enteral Nutr* 1993; **17**: 1SA-26SA

4 Round table conference on metabolic support of the critically ill patients--March 20-22, 1993. *Intensive Care Med* 1994; **20**: 298-299

5 **Jolliet P**, Pichard C, Biolo G, Chioléro R, Grimble G, Leverve X, Nitenberg G, Novak I, Planas M, Preiser JC, Roth E, Schols AM, Wernerman J. Enteral nutrition in intensive care patients: a practical approach. Working Group on Nutrition and Metabolism, ESICM. European Society of Intensive Care Medicine. *Intensive Care Med* 1998; **24**: 848-859

6 **Farreras N**, Artigas V, Cardona D, Rius X, Trias M, González JA. Effect of early postoperative enteral immunonutrition on wound healing in patients undergoing surgery for gastric cancer. *Clin Nutr* 2005; **24**: 55-65

7 **Feo CV**, Romanini B, Sortini D, Ragazzi R, Zamboni P, Pansini GC, Liboni A. Early oral feeding after colorectal resection: a randomized controlled study. *ANZ J Surg* 2004; **74**: 298-301

8 **Soop M**, Carlson GL, Hopkinson J, Clarke S, Thorell A, Nygren J, Ljungqvist O. Randomized clinical trial of the effects of immediate enteral nutrition on metabolic responses to major colorectal surgery in an enhanced recovery protocol. *Br J Surg* 2004; **91**: 1138-1145

9 **DiFronzo LA**, Yamin N, Patel K, O'Connell TX. Benefits of early feeding and early hospital discharge in elderly patients undergoing open colon resection. *J Am Coll Surg* 2003; **197**: 747-752

10 **Rios G**, Conrad A, Cole D, Adams D, Leveen M, O'Brien P, Baron P. Trends in indications and outcomes in the Whipple procedure over a 40-year period. *Am Surg* 1999; **65**: 889-893

11 **Brooks AD**, Marcus SG, Gradek C, Newman E, Shamamian P, Gouge TH, Pachter HL, Eng K. Decreasing length of stay after pancreatoduodenectomy. *Arch Surg* 2000; **135**: 823-830

12 **Gouma DJ**, van Geenen RC, van Gulik TM, de Haan RJ, de Wit LT, Busch OR, Obertop H. Rates of complications and death after pancreaticoduodenectomy: risk factors and the impact of hospital volume. *Ann Surg* 2000; **232**: 786-795

13 **Gianotti L**, Braga M, Gentilini O, Balzano G, Zerbi A, Di Carlo V. Artificial nutrition after pancreaticoduodenectomy. *Pancreas* 2000; **21**: 344-351

14 **Lillemoe KD**, Cameron JL, Yeo CJ, Sohn TA, Nakeeb A, Sauter PK, Hruban RH, Abrams RA, Pitt HA. Pancreaticoduodenectomy. Does it have a role in the palliation of pancreatic cancer? *Ann Surg* 1996; **223**: 718-25; discussion 725-8

15 **Yeo CJ**, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA, Hruban RH, Ord SE, Sauter PK, Coleman J, Zahurak ML, Grochow LB, Abrams RA. Six hundred fifty consecutive pancr eaticoduodenectomies in the 1990s: pathology, complications, and outcomes. *Ann Surg* 1997; **226**: 248-57; discussion 257-60

16 **Pedrazzoli S**, DiCarlo V, Dionigi R, Mosca F, Pederzoli P, Pasquali C, Klöppel G, Dhaene K, Michelassi F. Standard versus extended lymphadenectomy associated with pancreatoduodenectomy in the surgical treatment of adenocarcinoma of the head of the pancreas: a multicenter, prospective, randomized study. Lymphadenectomy Study Group. *Ann Surg* 1998; **228**: 508-517

17 **Martignoni ME**, Friess H, Sell F, Ricken L, Shrikhande S, Kulli C, Büchler MW. Enteral nutrition prolongs delayed gastric emptying in patients after Whipple resection. *Am J Surg* 2000; **180**: 18-23

18 **Mack LA**, Kaklamanos IG, Livingstone AS, Levi JU, Robinson C, Sleeman D, Franceschi D, Bathe OF. Gastric

decompression and enteral feeding through a double-lumen gastrojejunostomy tube improves outcomes after pancreaticoduodenectomy. *Ann Surg* 2004; **240**: 845-851
19 **Rai J**, Flint LM, Ferrara JJ. Small bowel necrosis in association with jejunostomy tube feedings. *Am Surg* 1996; **62**: 1050-1054
20 **Sonawane RN**, Thombare MM, Kumar A, Sikora SS, Saxena R, Kapoor VK, Kaushik SP. Technical complications of feeding jejunostomy: a critical analysis. *Trop Gastroenterol* 1997; **18**: 127-128
21 **Harris JA**, Benedict FG. Biometric studies of basal metabolism in man. Washington: Carnegie Institute, 1919
22 **Gouillat C**, Chipponi J, Baulieux J, Partensky C, Saric J, Gayet B. Randomized controlled multicentre trial of somatostatin infusion after pancreaticoduodenectomy. *Br J Surg* 2001; **88**: 1456-1462
23 **Nakatsuka A**, Yamaguchi K, Chijiiwa K, Tanaka M. Octreotide inhibits pancreatic exocrine secretion and prevents pancreatoenterostomy leakage. *Int Surg* 2000; **85**: 124-129
24 **Berberat PO**, Friess H, Uhl W, Büchler MW. The role of octreotide in the prevention of complications following pancreatic resection. *Digestion* 1999; **60 Suppl 2**: 15-22
25 **Crane CH**, Beddar AS, Evans DB. The role of intraoperative radiotherapy in pancreatic cancer. *Surg Oncol Clin N Am* 2003; **12**: 965-977
26 **Ma HB**, Di ZL, Wang XJ, Kang HF, Deng HC, Bai MH. Effect of intraoperative radiotherapy combined with external beam radiotherapy following internal drainage for advanced pancreatic carcinoma. *World J Gastroenterol* 2004; **10**: 1669-1771
27 **Willett CG**, Del Castillo CF, Shih HA, Goldberg S, Biggs P, Clark JW, Lauwers G, Ryan DP, Zhu AX, Warshaw AL. Long-term results of intraoperative electron beam irradiation (IOERT) for patients with unresectable pancreatic cancer. *Ann Surg* 2005; **241**: 295-299
28 **Seiler CA**, Wagner M, Bachmann T, Redaelli CA, Schmied B, Uhl W, Friess H, Büchler MW. Randomized clinical trial of pylorus-preserving duodenopancreatectomy versus classical Whipple resection-long term results. *Br J Surg* 2005; **92**: 547-556
29 **Miedema BW**, Sarr MG, van Heerden JA, Nagorney DM, McIlrath DC, Ilstrup D. Complications following pancreaticoduodenectomy. Current management. *Arch Surg* 1992; **127**: 945-99; discussion 945-99
30 **Cameron JL**, Pitt HA, Yeo CJ, Lillemoe KD, Kaufman HS, Coleman J. One hundred and forty-five consecutive pancrea ticoduodenectomies without mortality. *Ann Surg* 1993; **217**: 430-45; discussion 430-45
31 **van Berge Henegouwen MI**, van Gulik TM, DeWit LT, Allema JH, Rauws EA, Obertop H, Gouma DJ. Delayed gastric emptying after standard pancreaticoduodenectomy versus pylorus-preserving pancreaticoduodenectomy: an analysis of 200 consecutive patients. *J Am Coll Surg* 1997; **185**: 373-379
32 **Horstmann O**, Becker H, Post S, Nustede R. Is delayed gastric emptying following pancreaticoduodenectomy related to pylorus preservation? *Langenbecks Arch Surg* 1999; **384**: 354-359
33 **Balcom JH**, Rattner DW, Warshaw AL, Chang Y, Fernandez-del Castillo C. Ten-year experience with 733 pancreatic resections: changing indications, older patients, and decreasing length of hospitalization. *Arch Surg* 2001; **136**: 391-398
34 **Okabayashi T**, Kobayashi M, Sugimoto T, Namikawa T, Okamoto K, Hokimoto N, Araki K. Postoperative pancreatic fistula following distal pancreatectomy for pancreatic neoplasm; can pancreatic fistula be prevented? *Hepatogastroenterology* 2004; **51**: 1838-1841
35 **van Berge Henegouwen MI**, De Wit LT, Van Gulik TM, Obertop H, Gouma DJ. Incidence, risk factors, and treatment of pancreatic leakage after pancreaticoduodenectomy: drainage versus resection of the pancreatic remnant. *J Am Coll Surg* 1997; **185**: 18-24
36 **Hashimoto N**, Ohyanagi H. Pancreatic juice output and amylase level in the drainage fluid after pancreatoduodenectomy in relation to leakage. *Hepatogastroenterology* 2002; **49**: 553-555
37 **Grobmyer SR**, Rivadeneira DE, Goodman CA, Mackrell P, Lieberman MD, Daly JM. Pancreatic anastomotic failure after pancreaticoduodenectomy. *Am J Surg* 2000; **180**: 117-120
38 **Kubota K**, Makuuchi M, Takayama T, Sakamoto Y, Harihara Y, Kimura W. Appraisal of two-staged pancreatoduodenectomy: its technical aspects and outcome. *Hepatogastroenterology* 2000; **47**: 269-274

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 94-98
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

L1 is a potential marker for poorly-differentiated pancreatic neuroendocrine carcinoma

Jussuf T Kaifi, Ulrich Zinnkann, Emre F Yekebas, Paulus G Schurr, Uta Reichelt, Robin Wachowiak, Henning C Fiegel, Susann Petri, Melitta Schachner, Jakob R Izbicki

Jussuf T Kaifi, Ulrich Zinnkann, Emre F Yekebas, Paulus G Schurr, Robin Wachowiak, Jakob R Izbicki, Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Uta Reichelt, Susann Petri, Institute for Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Henning C Fiegel, Department of Pediatric Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Melitta Schachner, Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
Supported by research grants from the ´Hamburger Krebsgesellschaft e. V.´
Correspondence to: Jussuf T Kaifi MD, Department of General, Visceral and Thoracic Surgery, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany. jkaifi@uke.uni-hamburg.de
Telephone: +49-40-42803-5408 Fax: +49-40-42803-9049
Received: 2005-05-17 Accepted: 2005-07-08

Abstract

AIM: To determine the expression of L1 in pancreatic neuroendocrine tumor and to correlate it with WHO classification of this tumor.

METHODS: We retrospectively analyzed L1 expression in 63 cases of pancreatic neuroendocrine tumor by immunohistochemistry on paraffin sections of primary tumors or metastases. Staining was performed by peroxidase technique with monoclonal antibody UJ127.11 against human L1. All tumors were classified according to WHO classification as well-differentiated neuroendocrine tumors and carcinomas or poorly-differentiated neuroendocrine carcinomas.

RESULTS: L1 was detected in 5 (7.9%) of 63 pancreatic neuroendocrine tumors. Four (44.4%) of 9 poorly-differentiated carcinomas expressed L1. In contrast, only 1 (1.9%) of 54 well-differentiated tumors or carcinomas was positive for L1. No expression was found in Langerhans islet cells of normal pancreatic tissue. Cross table analysis showed a significant association between L1 expression and classification of neuroendocrine tumors of the pancreas (P<0.01).

CONCLUSION: L1 is specifically expressed in poorly-differentiated pancreatic neuroendocrine carcinomas that are known to have the worst prognosis. L1 might be a marker for risk prediction of patients diagnosed with pancreatic neuroendocrine carcinomas.

Key words: Neuroendocrine pancreatic tumor; Tumor markers; Cell adhesion molecules; L1

Kaifi JT, Zinnkann U, Yekebas EF, Schurr PG, Reichelt U, Wachowiak R, Fiegel HC, Petri S, Schachner M, Izbicki JR. L1 is a potential marker for poorly-differentiated pancreatic neuroendocrine carcinoma. *World J Gastroenterol* 2006; 12(1): 94-98

http://www.wjgnet.com/1007-9327/12/94.asp

INTRODUCTION

Neuroendocrine tumors of the gastroenteropancreatic axis are rare and characterized by significant phenotypic differences. They can present themselves as benign or highly malignant and their clinical behavior is very heterogeneous. They are considered to originate from cells of the disseminated neuroendocrine cell system[1]. Most endocrine tumors of the pancreas are well-differentiated neuroendocrine tumors or carcinomas. Frequently, they appear to be malignant with the exception of insulinoma [2]. Fifty percent to sixty percent of these tumors are functionally active and secrete insulin, gastrin, vasoactive intestinal polypeptide (VIP), glucagon or other rare hormones and consequently cause characteristic syndromes. The most important criteria of malignancy include a tumor size of more than 2 cm, angioinvasion and proliferative activity of more than 2% of the tumor cells apart from metastases to the regional lymph nodes and the liver or invasion of adjacent organs [3,4]. Neuroendocrine tumors of the pancreas are classified according to the WHO classification into well-differentiated tumors and carcinomas or poorly-differentiated carcinomas[4,5]. Poorly-differentiated neuroendocrine carcinomas of the pancreas are highly malignant with a bad prognosis[3].

Neoplastic cells frequently re-express adhesion molecules involved in cell migration during tissue morphogenesis and fetal development[6]. The L1 cell adhesion molecule (CD171) is a 200-220 ku type I glycoprotein of the immunoglobulin superfamily and plays a role in development of the nervous system by regulating cell interactions, includ-

ing neuronal migration[7,8]. L1 also mediates neuron-neuron adhesion, neurite outgrowth on Schwann cells, neurite fasciculation and myelination[7]. L1 undergoes homophilic L1-L1 binding and heterophilic interactions with several ligands such as integrins[9,10]. L1 is expressed also in hematopoietic and certain epithelial cells as well as in a variety of tumors, such as of neuroblastomas, melanomas, small cell lung cancer and breast carcinomas[11-15]. Metalloproteinase (ADAM10) also triggers cell migration and cleaves L1 from the tumor cell surface[8,12,16-18]. Recently, it was reported that expression of L1 has a prognostic significance in ovarian and uterine carcinomas and is associated with metastasis of melanomas[19,20]. Furthermore, L1 is expressed in neuroendocrine tumors of the skin[21]. Up-regulation of L1 expression has also been observed in malignant pleural mesotheliomas and malignant peripheral nerve sheath tumors by microarray expression profiling[22,23].

The aim of this study was to determine the expression of L1 in neuroendocrine tumors of the pancreas and its relation to tumor stage of this heterogeneous cancer type. We detected the expression of L1 in 4 (44.4%) of 9 poorly differentiated pancreatic neuroendocrine carcinomas. However, only 1 (1.9%) of 54 well-differentiated tumors or carcinomas was positive for L1. Cross table analysis showed a significant correlation between L1 expression and poorly-differentiated neuroendocrine carcinomas. Our data indicate that L1 is a specific marker for malignant phenotype of pancreatic neuroendocrine carcinomas.

MATERIALS AND METHODS

Study design and patients

The study was approved by the Ethics Committee of the Chamber of Physicians in Hamburg, Germany. Written informed consent was obtained from all patients for use of the resected samples. For this study, 63 patients with pancreatic neuroendocrine tumors were chosen retrospectively. We selected patients on the basis of availability of tissues and did not stratify them due to rare occurrence and different treatment strategies. Forty-seven primary tumors of the pancreas and 38 metastases (21 from liver, 16 from lymph nodes and 1 from spleen) were available. All tumors were categorized into 3 groups according to WHO classification of 2 000 into well-differentiated tumors (grade 1a) and carcinomas (grade 1b) or poorly-differentiated neuroendocrine carcinomas (grade 2)[4]. Briefly, this classification was based on tumor size, angioinvasion, proliferating activity, histological differentiation and hormonal activity. All data including sex, histology, depth of tumor invasion, lymph node metastasis, tumor type and disease stage were obtained from the clinical and pathological records.

Immunohistochemical staining and evaluation of expression

Immunohistochemical staining was performed for 5-μm thick sections of formalin-fixed and paraffin-embedded tissues placed on pre-coated slides with 3-triethoxysilylpropylamin (Merck, Darmstadt, Germany). After deparaffinization with Rotihistole (Merck) and rehydration in ethanol and TBS (0.05mol/L, pH 7.6) containing 10 g/L Tween 20 (Sigma, Deisenhofen, Germany), tissue sections were pre-treated for 30 min in 10 g/L ammonium chloride (NH_4Cl) in TBS, for 15 min in 0.05 mol/L glycine/TBS and then boiled with ChemMate® target retrieval solution (Dako, Hamburg, Germany) in a microwave oven according to the manufacturer´s instructions. Staining was performed with the peroxidase method (HRP-AEC System, Cell and Tissue Staining Kit; R&D Systems, Minneapolis, MN, USA). The primary antibody, a murine anti-human L1 monoclonal antibody (IgG_1, clone UJ127) (NeoMarkers, Fremont, CA, USA) binding to the extracellular domain of this molecule, was diluted at 1:50 in antibody diluent (Dako) and slides were incubated overnight in a humidity chamber at 4 °C [24]. For each sample one slide, a control section was incubated with irrelevant murine monoclonal IgG_1 (MOPC21; Sigma) as a negative control to determine the unspecific binding. All washing steps were done with TBS containing 10 g/L Tween 20. Counterstaining was performed with Haemalaun Mayer (Merck) for 30 s followed by Mayer´s haematoxylin solution (Merck) for 7 min. At last, slides were covered with coverslips with aqueous mounting medium (Aquatex®; Merck). Specimens were considered immunopositive for L1 when >20% of the tumor cells had clear evidence of immunostaining. Peripheral nerves present in almost all sections served as internal positive controls. Langerhans islet cells were negative for L1 in normal pancreatic tissue. Immunohistochemical analysis and scoring of the sections were performed by two independent investigators and one pathologist in a blinded fashion. Two sections were scored differently and in these cases the opinion of the pathologist was decisive.

Statistical analysis

We used SPSS for Windows (SPSS Inc., Chicago, IL USA) for statistical analysis. The immunostaining results of L1 and WHO classification of neuroendocrine tumors of the pancreas were calculated using a cross table and statistical analysis was performed with *F*-test. $P<0.05$ was considered statistically significant.

RESULTS

Characteristics of the patients

Sixty-three patients suffering from pancreatic neuroendocrine tumor were included in the study. Characteristics of the patients are listed in Table 1. Briefly, the median age of the study population was 57 years, 32 (50.8%) patients were male and 31 (49.2%) female. According to WHO classification for neuroendocrine tumors of the gastroenteropancreatic axis, 50 (79.4%) were classified as well-differentiated neuroendocrine tumors (grade 1a), 4 (6.3%) as well-differentiated carcinomas (grade 1b) and 9 (14.3%) as poorly-differentiated neuroendocrine carcinomas (grade 2), being the most malignant phenotype. Eleven (17.5%) tumors showed hormone production and 6 (9.5%) of 63 patients suffered from endocrine neoplasia (MEN) type I.

Immunohistochemical analysis of L1 in pancreatic neuroendocrine tumors

L1 expression was determined by immunohistochemical analysis in samples from 63 pancreatic neuroendocrine tu-

Table 1 Characteristics of the patients and levels of L1 expression *n*(%)

Variable	Patients	L1-positive tumors
Total	63	5 (7.9)
Male	32 (50.8)	4 (12.5)
Female	31 (49.2)	1 (3.2)
WHO classification of neuroendocrine pancreatic tumour		
Well-differentiated neuroendocrine tumour (grade 1a)	50 (79.4)	1 (2.0)
Well-differentiated neuroendocrine carcinoma (grade 1b)	4 (6.3)	0
Poorly-differentiated neuroendocrine carcinoma (grade 2)	9 (14.3)	4 (44.4)
Hormone production		
Yes	11 (17.5)	0
No	52 (82.5)	5 (9.6)
Multiple endocrine neoplasia (MEN)-I		
MEN-I	6 (9.5)	0
No MEN-I	57 (90.5)	5 (8.8)

Table 2 Correlation of L1 expression with WHO classification

WHO classification	L1-negative	L1-positive	Total
Well-differentiated neuroendocrine tumors and Well-differentiated neuroendocrine carcinomas (grade 1a and 1b)	53	1	54
Poorly-differentiated neuroendocrine carcinomas (grade 2)	5	4	9
Total	58	5	63

$P<0.01$ by Fisher's test (two-sided)

mor patients. Forty-seven primary tumors of the pancreas and 38 metastases (21 from liver, 16 from lymph nodes and 1 from spleen) were available and immunostained. In 18 patients, both primary tumor and metastases (12 from lymph nodes, 3 from liver, 2 from both lymph nodes and liver, 1 from liver and spleen) were investigated. In 16 patients, only metastases were available (14 from liver, 1 from lymph nodes, 1 from both lymph nodes and liver). No differences in terms of positivity or negativity of L1 expression were detected between primary tumor and metastases in any patient. Figure 1 shows the representative negative and positive staining patterns for L1 of pancreatic neuroendocrine tumors. Staining was not detected in normal pancreatic islet cells.

Five (7.9%) of 63 cases were L1-positive (Table 1). were stained. The remaining 58 (92.1%) patients were negative for L1. According to the WHO classification, 4 (44.4%) of 9 poorly-differentiated neuroendocrine carcinomas (grade 2) were L1-positive. Only 1 (1.9%) of 54 well-differentiated neuroendocrine tumor samples (grade 1a) was L1-positive. None of the 4 well-differentiated neuroendocrine carcinomas (grade 1b) was positive for L1. Although only 9 poorly-differentiated pancreatic neuroendocrine carcinomas (grade 2) were available, these results showed that L1 was specifically expressed in poorly- differentiated tumors. Forty-four point four percent of these most highly malignant tumors were positive for L1 compared to 1.9% in the group of well-differentiated neuroendocrine tumors or carcinomas (grade 1a and 1b).

Correlation between L1 expression and WHO classification of tumor

A significant correlation between L1 expression and well-differentiated neuroendocrine tumor (grade 1a) and (grade 1b) or poorly-differentiated neuroendocrine carcinoma (grade 2) was found by Fisher's exact test ($P<0.01$, Table 2).

DISCUSSION

Pancreatic neuroendocrine tumors or carcinomas are rare and clinically very heterogeneous. Neuroendocrine-specific molecules are positive markers of endocrine differentiation in tumor cells[1]. Little is known about the molecular differences between benign and malignant phenotypes of neuroendocrine tumors of the pancreas. Cell adhesion molecules, such as L1, have been repeatedly implicated in tumor progression and metastasis. In this study, we determined L1 expression in 7.9% of 63 cases of pancreatic neuroendocrine tumor. Since L1 is not expressed in normal Langerhans islet cells, which are believed to be the precursor cells of pancreatic neuroendocrine tumors, an up-regulation of L1 expression in tumor cells may be associated with tumorigenesis in this tumor type.

We used immunohistochemical analysis for detection of L1 in tumor cells. Because the optimal cutpoint approach has some limitations in statistical evaluation of prognostic factors, we chose a cutpoint of 20% L1-positive tumor cells in the analysed cell population, achieving an easy discrimination of immunostained tumor tissue as proposed by Altman and colleagues[25].

In our study, there were 9 patients with poorly-differentiated neuroendocrine carcinoma according to WHO classification (grade 2). Out of these, 4(44.4%) had positive L1 expression. L1 In contrast, only 1 (1.9%) of 54 well-differentiated tumors and carcinomas (grade 1a and 1b) was positive for L1. Statistical analysis showed a significant correlation between L1 expression and poor differentiation of pancreatic neuroendocrine tumors (grade 2), suggesting that L1 is a marker for poorly differentiated and highly malignant pancreatic neuroendocrine carcinomas. Although the number of L1-positive tumors in our study was too low for a final conclusion, the number of L1-negative tumors supports our notion that L1 is a marker for malignancy of this tumor entity.

These observations are in agreement with previous studies correlating expression of L1 in different tumors of neuroectodermal origin, such as melanomas or uterine and ovarian carcinomas, with malignancy and poor prognosis[15,19-21,26]. Our results prove that expression of L1 can also be found in another neuroendocrine tumor, namely the neuroendocrine tumor of the pancreas.

Further studies are needed to determine the potential prognostic value of L1 expression in patients suffering from pancreatic neuroendocrine carcinomas. Our data also indicate that downregulation of L1 expression by antisense technologies may be used as a therapeutic method.

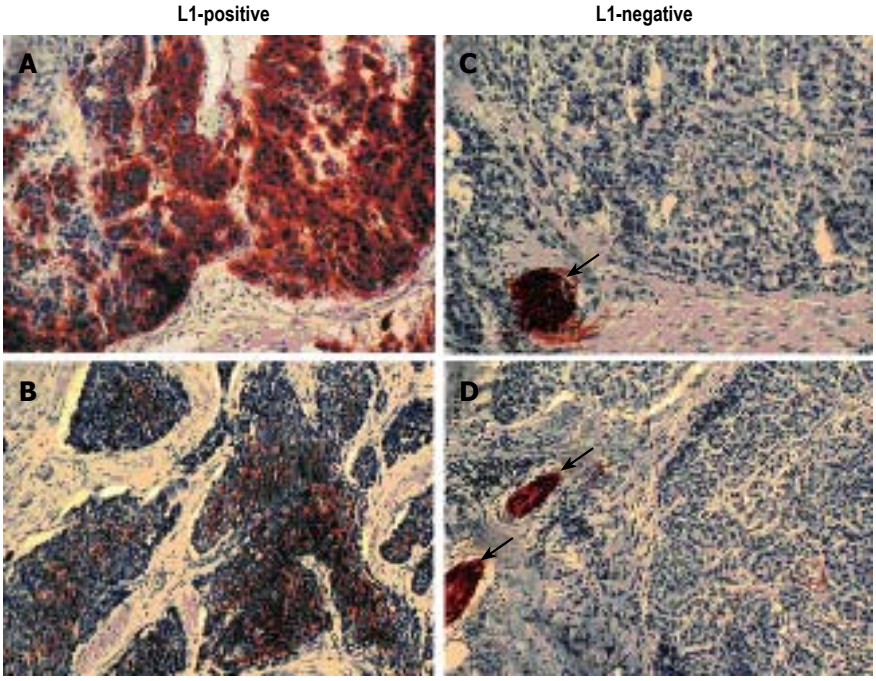


Figure 1 L1 expression in pancreatic neuroendocrine tumours or carcinomas. Immunohistochemical staining was performed by peroxidase method using monoclonal antibody UJ.127 against L1. Poorly-differentiated L1-positive pancreatic neuroendocrine carcinomas (grade 2; A and B) were shown in comparison to well-differentiated L1-negative tumours (grade 1a; C and D). Peripheral nerves (*arrows*) stained in (C, D) served as internal positive controls (Magnification ×200 (A and C) and ×400 (B and C)).

ACKNOWLEDGEMENT

The authors thank Antje Heinecke for excellent technical assistance.

REFERENCES

1 **Rindi G**, Klöppel G. Endocrine tumors of the gut and pancreas tumor biology and classification. *Neuroendocrinology* 2004; **80 Suppl 1**: 12-15
2 **Rindi G**, Villanacci V, Ubiali A. Biological and molecular aspects of gastroenteropancreatic neuroendocrine tumors. *Digestion* 2000; **62 Suppl 1**: 19-26
3 **Heymann MF**, Joubert M, Nemeth J, Franc B, Visset J, Hamy A, le Borgne J, le Neel JC, Murat A, Cordel S, le Bodic MF. Prognostic and immunohistochemical validation of the capella classification of pancreatic neuroendocrine tumours: an analysis of 82 sporadic cases. *Histopathology* 2000; **36**: 421-432
4 **Solcia E**, Kloppel G, Sobin LH. Histological typing of endocrine tumors. In: World Health Organization International Histological Classification of Endocrine Tumors. 2 ed. New York: Springer, 2000: 38-74
5 **Klöppel G**, Perren A, Heitz PU. The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. *Ann N Y Acad Sci* 2004; **1014**: 13-27
6 **Fidler IJ**. Critical determinants of metastasis. *Semin Cancer Biol* 2002; **12**: 89-96
7 **Schachner M**. Neural recognition molecules and synaptic plasticity. *Curr Opin Cell Biol* 1997; **9**: 627-634
8 **Ohnishi T**, Matsumura H, Izumoto S, Hiraga S, Hayakawa T. A novel model of glioma cell invasion using organotypic brain slice culture. *Cancer Res* 1998; **58**: 2935-2940
9 **De Angelis E**, MacFarlane J, Du JS, Yeo G, Hicks R, Rathjen FG, Kenwrick S, Brümmendorf T. Pathological missense mutations of neural cell adhesion molecule L1 affect homophilic and heterophilic binding activities. *EMBO J* 1999; **18**: 4744-4753
10 **Oleszewski M**, Beer S, Katich S, Geiger C, Zeller Y, Rauch U, Altevogt P. Integrin and neurocan binding to L1 involves distinct Ig domains. *J Biol Chem* 1999; **274**: 24602-24610
11 **Gil OD**, Sakurai T, Bradley AE, Fink MY, Cassella MR, Kuo JA, Felsenfeld DP. Ankyrin binding mediates L1CAM interactions with static components of the cytoskeleton and inhibits retrograde movement of L1CAM on the cell surface. *J Cell Biol* 2003; **162**: 719-730
12 **Gutwein P**, Oleszewski M, Mechtersheimer S, Agmon-Levin N, Krauss K, Altevogt P. Role of Src kinases in the ADAM-mediated release of L1 adhesion molecule from human tumor cells. *J Biol Chem* 2000; **275**: 15490-15497
13 **Linnemann D**, Raz A, Bock E. Differential expression of cell adhesion molecules in variants of K1735 melanoma cells differing in metastatic capacity. *Int J Cancer* 1989; **43**: 709-712
14 **Miyahara R**, Tanaka F, Nakagawa T, Matsuoka K, Isii K, Wada H. Expression of neural cell adhesion molecules (polysialylated form of neural cell adhesion molecule and L1-cell adhesion molecule) on resected small cell lung cancer specimens: in relation to proliferation state. *J Surg Oncol* 2001; **77**: 49-54
15 **Fogel M**, Mechtersheimer S, Huszar M, Smirnov A, Abu-Dahi A, Tilgen W, Reichrath J, Georg T, Altevogt P, Gutwein P. L1 adhesion molecule (CD 171) in development and progression of human malignant melanoma. *Cancer Lett* 2003; **189**: 237-247
16 **Mechtersheimer S**, Gutwein P, Agmon-Levin N, Stoeck A, Oleszewski M, Riedle S, Postina R, Fahrenholz F, Fogel M, Lemmon V, Altevogt P. Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J Cell Biol* 2001; **155**: 661-673
17 **Montgomery AM**, Becker JC, Siu CH, Lemmon VP, Cheresh DA, Pancook JD, Zhao X, Reisfeld RA. Human neural cell adhesion molecule L1 and rat homologue NILE are ligands for integrin alpha v beta 3. *J Cell Biol* 1996; **132**: 475-485
18 **Kalus I**, Schnegelsberg B, Seidah NG, Kleene R, Schachner M. The proprotein convertase PC5A and a metalloprotease are involved in the proteolytic processing of the neural adhesion molecule L1. *J Biol Chem* 2003; **278**: 10381-10388
19 **Fogel M**, Gutwein P, Mechtersheimer S, Riedle S, Stoeck A, Smirnov A, Edler L, Ben-Arie A, Huszar M, Altevogt P. L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. *Lancet* 2003; **362**: 869-875
20 **Thies A**, Schachner M, Moll I, Berger J, Schulze HJ, Brunner G, Schumacher U. Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma. *Eur J Cancer* 2002; **38**: 1708-1716
21 **Deichmann M**, Kurzen H, Egner U, Altevogt P, Hartschuh W. Adhesion molecules CD171 (L1CAM) and CD24 are expressed by primary neuroendocrine carcinomas of the skin (Merkel cell carcinomas). *J Cutan Pathol* 2003; **30**: 363-368
22 **Kettunen E**, Nicholson AG, Nagy B, Wikman H, Seppänen JK, Stjernvall T, Ollikainen T, Kinnula V, Nordling S, Hollmén J, Anttila S, Knuutila S. L1CAM, INP10, P-cadherin, tPA and

ITGB4 over-expression in malignant pleural mesotheliomas revealed by combined use of cDNA and tissue microarray. *Carcinogenesis* 2005; **26**: 17-25

23 **Watson MA**, Perry A, Tihan T, Prayson RA, Guha A, Bridge J, Ferner R, Gutmann DH. Gene expression profiling reveals unique molecular subtypes of Neurofibromatosis Type I-associated and sporadic malignant peripheral nerve sheath tumors. *Brain Pathol* 2004; **14**: 297-303

24 **Patel K**, Kiely F, Phimister E, Melino G, Rathjen F, Kemshead JT. The 200/220 kDa antigen recognized by monoclonal antibody (MAb) UJ127.11 on neural tissues and tumors is the human L1 adhesion molecule. *Hybridoma* 1991; **10**: 481-491

25 **Altman DG**, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst* 1994; **86**: 829-835

26 **Fogel M**, Huszar M, Altevogt P, Ben-Arie A. L1 (CD171) as a novel biomarker for ovarian and endometrial carcinomas. *Expert Rev Mol Diagn* 2004; **4**: 455-462

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 99-104
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Risk factors for immediate post-operative fatal recurrence after curative resection of hepatocellular carcinoma

Bong-Wan Kim, Young-Bae Kim, Hee-Jung Wang, Myung-Wook Kim

Bong-Wan Kim, Hee-Jung Wang, Myung-Wook Kim, Department of Surgery, Ajou University School of Medicine, Wonchon dong, Youngtong ku, Suwon, Kyounggi Province, South Korea
Young-Bae Kim, Department of Pathology, Ajou University School of Medicine, Wonchon dong, Youngtong ku, Suwon, Kyounggi Province, South Korea
Correspondence to: Hee-Jung Wang, MD, PhD, Professor, Department of Surgery, Ajou University School of Medicine, San-5 442-749, Wonchon dong, Youngtong ku, Kyounggi Province, Suwon, South Korea. wanghj@ajou.ac.kr
Telephone: +82-31-219-5011 Fax: +82-31-219-5755
Received: 2005-05-07 Accepted: 2005-06-18

Abstract

AIM: To investigate the clinicopathological risk factors for immediate post-operative fatal recurrence of hepatocellular carcinoma (HCC), which may have practical implication and contribute to establishing high risk patients for pre- or post-operative preventive measures against HCC recurrence.

METHODS: From June 1994 to May 2004, 269 patients who received curative resection for HCC were reviewed. Of these patients, those who demonstrated diffuse intra-hepatic or multiple systemic recurrent lesions within 6 mo after surgery were investigated (fatal recurrence group). The remaining patients were designated as the control group, and the two groups were compared for clinicopathologic risk factors.

RESULTS: Among the 269 patients reviewed, 30 patients were enrolled in the fatal recurrence group. Among the latter, 20 patients showed diffuse intra-hepatic recurrence type and 10 showed multiple systemic recurrence type. Multivariate analysis between the fatal recurrence group and control group showed that pre-operative serum alpha-fetoprotein (AFP) level was greater than 1 000 μg/L ($P = 0.02$; odds ratio = 2.98), tumor size greater than 6.5 cm ($P = 0.03$; OR = 2.98), and presence of microvascular invasion ($P = 0.01$; OR = 4.89) were the risk factors in the fatal recurrence group. The 48.1% of the patients who had all the three risk factors and the 22% of those who had two risk factors experienced fatal recurrence within 6 mo after surgery.

CONCLUSION: Three distinct risk factors for immediate post-operative fatal recurrence of HCC after curative resection are pre-operative serum AFP level > 1 000 μg/L, tumor size > 6.5 cm, and microvascular invasion. The high risk patients with two or more risk factors should be the candidates for various adjuvant clinical trials.

Key words: Hepatocellular carcinoma; Hepatectomy; Early recurrence; Risk factors

Kim BW, Kim YB, Wang HJ, Kim MW. Risk factors for immediate post-operative fatal recurrence after curative resection of hepatocellular carcinoma. *World J Gastroenterol* 2006; 12(1): 99-104

http://www.wjgnet.com/1007-9327/12/99.asp

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the common causes of cancer death among Koreans, and it is also one of the frequently occurring cancers worldwide. Surgical resection of the liver has been one of the mainstays in the curative treatment of this cancer. Recent advances in anatomical knowledge of liver, surgical skills and instruments, intra- and post-operative management techniques have led to a marked reduction in post-operative mortality rates. However, recurrence after curative partial hepatectomy for HCC occurs in approximately 70% of patients[1-3], and even after careful selection of relatively early disease patients for liver transplantation (OLT) according to the Milan selection criteria. It has been reported that the recurrence rate is about 20%[4]. This finding is considered to be the most significant risk factor for the survival of the patient.

The pattern of recurrence after curative surgery for HCC is variable. Among these diverse patterns of recurrence, diffuse intra-hepatic recurrence and multiple systemic recurrences are thought to be fatal not only because there is no effective treatment strategy, but also these recurrence patterns inevitably mean a short remaining survival time. Moreover, the development of such fatal recurrences soon after surgical resection may indicate the presence of multiple intra-hepatic micro-metastases or systemic dissemination and colonization of HCC cells at the time of surgery. But present clinical technology does not allow pre-operative determination of micro-metastatic lesions, and therefore the available

approach at present is the determination of risk factors for immediate post-operative fatal recurrence.

Numerous studies have investigated the recurrence of HCC after partial hepatectomy and have reported a number of risk factors for recurrence. But the application of such risk factors in the practical field for surgeons is difficult, which may be due to the fact that the limitation of application of these risk factors is confusing, and sometimes too broad in perspective. In this study, we attempted to elucidate the risk factors involved in the recurrence of HCC soon after partial hepatectomy, which entail more practical implications for the liver surgeon.

MATERIALS AND METHODS

Patients

From June 1994 to May 2004 for a period of 10 years, the medical records of 322 HCC patients who received partial hepatectomy at the Department of Surgery, Ajou University Hospital were reviewed. These patients were divided into the fatal recurrence group and the control group. The inclusion criteria of the fatal recurrence group were the patients who had diffuse intra-hepatic recurrence or multiple systemic recurrence within 6 mo after curative surgical resection of HCC. The rest were designated as the control group, and risk factors for the fatal recurrence group were analyzed.

Among the total 322 patients, the following were excluded from this study: patients who died within 6 mo after surgery due to reasons other than recurrence; patients who were lost to follow-up within 6 mo after surgery; patients whose histopathologic examination showed fibrolamellar or combined cholangiohepatocellular carcinoma pathologies; and patients who received non-curative resection.

Methods

The criteria for curative liver resection followed by the authors were the General Rules for the Study of Primary Liver Cancer Guidelines set up by the Korean Liver Cancer Study Group[5]. In these guidelines, the definition of curative surgery for HCC is classified as A1, A2, and B: "A1 = tumor size < 2 cm and no residual tumor after resection, without vascular or ductal invasion; A2 - tumor size 2 - 5 cm and no residual tumor after resection, without vascular or ductal invasion; and B = no residual tumor after resection, but not included in A1 or A2". Those who were regarded as non-curative resection were the patients with gross evidence of residual tumor, tumor invasion into the 1st order branch of the portal vein or main portal vein, tumor invasion of the left, right, middle, and inferior right or short hepatic vein and tumor invasion into 2nd or 1st order branches of the intra-hepatic bile duct or common hepatic duct.

Pre-operative radiological evaluation included abdominal ultrasonography, abdominal computerized tomography (CT) scans and hepatic angiography. Magnetic resonance imaging (MRI) and/or positron emission tomography (PET) scan were also conducted when deemed necessary by the physician in charge. To evaluate the residual liver function, all patients received the dye retention test using indocyanine green (ICG) in addition to the general chemistry tests. Also, to accurately assess the degree of curative surgery, all patients underwent intra-operative ultrasonography evaluation.

Post-operative follow-up consisted of monthly serum alpha-fetoprotein (AFP) level examination, and abdominal ultrasonography and plain chest films at every 3 mo. Abdominal CT was performed every 6 mo. The follow-up continued until the patient died of disease. If the serum AFP level did not decrease to normal level or abnormally increased or if one or more suspicious recurrent lesions were detected on ultrasonography during the post-operative follow-up, the results were then further confirmed by abdominal CT and/or chest CT and/or PET scan and hepatic angiography.

Diffuse intra-hepatic recurrence was defined as five or more recurrent lesions in the remnant liver as demonstrated by hepatic angiography[6], and multiple systemic recurrence was defined as two or more systemic recurrent lesions without evidence of intra-hepatic recurrence, as shown by imaging studies.

Twenty-four variables were compared between the fatal recurrence group and control group. The patient factors were: age, sex, presence of symptoms, hepatitis B antigen status, hepatitis C antibody status, Child's classification, pre-operative serum AST and ALT levels, pre-operative ICG R-15 and serum AFP levels, the grade of hepatitis, and stage of cirrhosis in non-tumor liver tissues. The tumor factors that were investigated were: size of tumor, single or multiple lesions, tumor growth patterns, presence of tumor capsule and invasion of tumor capsule, gross evidence of vascular or bile duct tumor involvement, formation of intra-tumor septum, tumor invasion of the Glisson's capsule, presence of microvascular invasion and the Edmond-Steiner grade of tumor cells. Successful anatomic resection and tumor margins of more than 1 cm were defined as surgical factors. The anatomical resection was defined as complete segmental resection of the Couinaud's segment system. The pathological review was performed by an experienced pathologist (Kim) in the Department of Pathology in our institute.

Statistical analysis

Univariate statistical analysis was performed using χ^2 test and Student's *t*-test, and multivariate analysis was carried out using logistic regression analysis.

RESULTS

Among the 322 patients, 53 patients were excluded from this study: 7 patients who died within 6 mo after surgery due to reasons other than recurrence (3 of 7 patients were post-operative in-hospital mortality); 3 patients who were lost to follow-up within 6 mo after surgery; 10 patients whose histopathologic examination showed fibrolamellar or combined cholangiohepatocellular carcinoma pathologies; and 33 patients who were regarded as non-curative resection.

Among the 269 patients who were included in this study, the mean follow-up period was 32.2 ± 25.6 mo (range, 4 - 107 mo). Of the 269 patients, 42 (15.6%) experienced

Table 1 Univariate analysis of host factors for immediate post-operative diffuse intra-hepatic recurrence or multiple distant recurrence after curative resection for HCC

Host factor	Patients (%) or (mean ± SD) Fatal recurrence group	Control group	*P*
Sex, male (%)	76	74	NS
Mean age(yr)	49.2±9.9	52.1±10.4	NS
HCC-related symptom	50	31	0.04
Child's classification A/B/C	90/10/0	90/9/1	NS
ICG R-15 (%)	14.6±11.3	13.4±8.6	NS
Serum ALT (nkat/L)	1185±1285	1034±944	NS
Serum AST (nkat/L)	1649±1259	1125±1539	NS
HBs Ag positivity	77	74	NS
HCV Ab positivity	13	8.4	NS
Serum AFP level (μg/L)	10 447.4±12 931.9	23045±6 660	0.00
Histologic grade of hepatitis 0, 1, 2/3, 4[1]	86.7/13.3	92.8/7.2	NS
Histologic stage of cirrhosis 0, 1, 2/3, 4[1]	15.8/84.2	19.2/80.8	NS

[1]Data in each parameter showed less than 2% missing rate, but histologic grade of hepatitis and histologic stage of cirrhosis showed 9% and 10.5% data missing rate, respectively.

Table 2 Univariate analysis of tumor and surgical factors for immediate post-operative diffuse intra-hepatic recurrence or multiple distant recurrence after curative resection of HCC

	Patients(%) or (mean ± SD) Fatal recurrence group	Control group	*P*
Tumor factors			
Tumor size (cm)	10.1±6.2	4.8±3.2	0.00
Tumor growth pattern, Eg[1]/Ig[2]	36.7/63.3	7.9/92.1	0.00
Multiple tumors	60.0	28.5	0.01
Tumor capsule formation	66.7	81.4	NS
Tumor capsule infiltration	45.0	29.6	NS
Intra-tumor septum formation	6.7	6.7	NS
Gross vascular or duct invasion	23.3	6.2	0.01
Microvascular invasion	86.6	33.9	0.00
Glisson's capsule invasion	70.0	29.8	0.00
Edmond-Steiner grade[3] I, II/III, IV	47.4/52.6	40.7/59.3	NS
Surgical factors			
Anatomical resection	93.3	87.8	NS
Resection margin ≥ 1 cm	66.7	42.3	0.02

[1]Expanding growth pattern; [2]Infiltrative growth pattern; [3]Data in each parameter showed less than 2% missing data rate, but there was 11.5% missing rate in the Edmond-Steiner grade.

Table 3 Multivariate analysis of significant risk factors for immediate postoperative diffuse intrahepatic recurrence or multiple distant recurrence after curative resection of HCC

Risk factors	Odds ratio	Standard error	*P*
Microvascular invasion	4.89	0.62	0.01
AFP ≥ 1 000 ng/mL	2.98	0.46	0.02
Tumor size ≥ 6.5 cm	2.98	0.50	0.03

tumor recurrence within 6 mo after curative resection. Among these 42 patients, 20 (47%) patients demonstrated diffuse intra-hepatic recurrence and 10 (24%) patients showed multiple systemic recurrence without evidence of intra-hepatic recurrence. Thus, the fatal recurrence group included 30 patients (11.2%, 30/269).

The mean disease-free survival time until recurrence of HCC of the patients in the fatal recurr-ence group was 3.9 ± 1.7 mo, and the mean survival time after recurrence was 6.7 ± 6.1 mo. The remaining 12 patients who were not in the fatal recurrence group but had tumor recurrence or recurrences within 6 mo after resection had the mean disease-free survival time until recurrence at 3.4 ± 1.8 months after surgery; however, the mean survival time after recurrence was 25.4 ± 29 mo, and this was significantly longer when compared to the fatal recurrence group ($P < 0.05$).

Of the 269 patients, 41 (15%) patients died within 1 year of surgery. Among these 41 patients, 35 (13%, 35/269) patients died of recurrence.

The fatal recurrence group consisted of 23 males and 7 females with the mean age of 49.2 ± 9.9 years. In the 239 control group patients, there were 178 males and 61 females with the mean age of 52.1 ± 10.4 years, showing no significant difference of gender and age between the two groups.

Univariate analysis of patient factors between the two groups showed that there were significant differences with regard to the presence of pre-operative tumor-related symptoms ($P = 0.042$) and serum AFP levels ($P = 0.00$) (Table 1). The tumor-related symptoms included right upper quadrant pain, radiating shoulder pain, weight loss, and palpable abdominal mass. Univariate analysis showed that significant tumor and surgical risk factors between the two groups were tumor size, infiltrative growth pattern, multiple tumors, gross vascular or ductal invasion, micro-vascular invasion, Glisson's capsule invasion, and less than 1 cm resection margin (Table 2).

Multivariate analysis showed that there were three major risk factors for early post-operative fatal recurrence: microvascular invasion ($P = 0.01$, OR = 4.89); tumor size > 6.5 cm ($P = 0.03$, OR = 2.98); and pre-operative serum AFP levels > 1 000 μg/L ($P = 0.02$, OR = 2.98) (Table 3).

Among the 269 patients, 27 patients had all the three risk factors, and of them 13 (48.1%) patients experienced actually fatal recurrence within 6 mo after curative surgery. There were 50 patients who had two risk factors, among them 11 (22%) patients experienced fatal recurrence. In addition, 71 patients were found to have only one risk

Table 4 Predictive values of risk factors for fatal recurrence after curative resection of HCC

	n	Number of fatal recurrence[1]	PPV (%)	NPV (%)	Test efficiency (%)	*P*[2]
All three risk factors present	27	13	48.1	92.9	88.5	0.023
Only two risk factors present	50	11	22.0	91.3	78.4	0.023
Microvascular invasion and tumor size ≥ 6.5 cm	26	6	23.1	90.1	83.6	0.027
Microvascular invasion and AFP ≥ 1 000 ng/mL	21	4	19.0	89.5	84.0	0.027
Tumor size ≥ 6.5 cm and AFP ≥ 1 000 ng/mL	3	1	33.3	89.1	88.5	0.027
Only one risk factor present	71	5	7.0	87.4	66.2	0.027
Micro-vascular invasion	33	3	9.1	88.6	78.8	0.027
Tumor size ≥ 6.5 cm	15	1	6.7	88.6	84.0	0.027
AFP ≥ 1 000 μg/L	23	1	4.3	88.2	81.1	0.027
No risk factor present	121	1	0.8	80.4	44.6	0.027
Number of total patients	269	30				

PPV: positive predictive value; NPV: negative predictive value; [1]Number of patients with fatal recurrence within 6 mo of surgery; [2]Statistical difference of PPV between patients with fatal recurrence within 6 months of surgery according to number of risk factors in each group.

factor, among whom 5 (7%) patients experienced fatal recurrence within 6 mo after surgery.Table 4 illustrates the statistical difference of positive predictive value between the different numbers of risk factors.

DISCUSSION

It has been known that risk factors which affect the pattern and timing of recurrence are different. In general, tumor factors have been considered as significant risk factors in early recurrence, while liver function factors have been reported more significant in late recurrent disease[7-9]. Recurrence of HCC is the major risk factor for the survival of the patient after surgery. Previous studies have showed that early death within 1 year of surgery due to recurrence of the disease was approximately 10%[7], which was in consistent with our study.

Curative treatment modalities for HCC are composed by surgical resection, liver transplantation, and percutaneous ablation[10]. Among these modalities, surgical resection has been traditionally known to be the most effective mode of therapy for HCC[11,12], but some serious problems, such as post-operative liver failure due to underlying cirrhosis and post-operative recurrence, still remain. To overcome these problems, alternative curative methods, such as local ablation therapy and liver transplantation, are being widely employed[4,13].

Local cauterization therapies for HCC include injection of chemical agents, such as alcohol, and the use of heat energy, such as radiofrequency or microwave techniques. These are applicable for early HCC patients even with poor hepatic functional reserve who are not suitable for surgery. Among these alternatives, radiofrequency ablation is considered to be more efficacious compared to alcohol injection methods in terms of cellular necrosis at the tumor margins or destruction of intra-tumoral septum. But, complete response rates have been reported to be very low for tumors that are more than 5 cm in diameter, and the tumor may not be easily accessible due to its difficult location in the liver in some instances[14,15].

Liver transplantation has been shown to result in a favorable outcome in selected HCC patients with uncompensated or compensated liver function[4,16]. But, in patients with advanced HCC, high rate of recurrence after OLT is a major problem of this mode of treatment. Possibly, accompanying immunosuppression may contribute to accelerating recurrence after OLT. Also, even if liver transplantation is indicated, the lack of donors and high cost of the procedure limits the wide employment of this modality.

According to a series reported by Llovet *et al*[10], surgical resection can be a more effective modality, rather than OLT or local ablative treatment, especially in early stage HCC with good hepatic functional reserve. However, because of the limitations of the aforementioned local therapy and liver transplantation, it is a common practice to perform partial hepatectomy with curative intention in patients even with intermediate or advanced HCC, if surgery can completely remove the tumor and as long as the functional liver reserve allows the procedure in hopes of long-term survival[11]. Recent advances in pre-operative liver function reserve assessment, surgical techniques and intra- and post-operative management have contributed to lowering the post-operative mortality rate after partial hepatectomy to below 5%. Subsequently, it is thought that hepatectomy has become safer than before and therefore, the indications for resection have been accordingly broadened. Therefore, in the practical field, it is thought that the efficacy of treatment is higher in advanced HCC than local ablation therapy or OLT.

When recurrent HCC appears immediately after partial hepatectomy for HCC, it is thought that metastases of occult cancer cells that were not detected either grossly or by imaging techniques were already present at the time of surgery. If it is possible to detect these occult micro-metastases in the pre-operative evaluation stage, then surgical resection of main tumor may be contraindicated. And it has been shown in previous studies that the liver regeneration process after partial hepatectomy may enhance the growth of occult metastases which rapidly develops into overt metastasis[17-19]. Therefore, the effort to predict these micro-metastasis and systemic dissemination of cancer cells should not be indolent.

In this study, using multivariate analysis, we were able to observe that the characteristic risk factors in the fatal recurrence group were tumor size > 6.5 cm, serum AFP

levels > 1 000 μg/L and microvascular invasion. It has been well documented that large tumors are a risk factor for recurrence after surgery, and that larger the tumor size is the earlier the recurrences is after curative resection. One of the reasons for this has been postulated as the frequent presence of micro-metastases of tumor cells beyond the resection margins at the time of surgery. Lai *et al*[20] reported that intra-hepatic micro-satellite lesions are found at a greater distance from the primary tumors > 4 cm in size. In addition, intra-hepatic micro-satellites at greater distances from the primary tumor are observed frequently in HCC with multiple tumors, presence of vascular invasion, and microvascular invasion, thus making low-actual curability by partial hepatectomy . The mechanism for such intra-hepatic microscopic metastasis is due to vascular invasion, which is considered to be a risk factor for early post-operative recurrence. This has been confirmed by reports that have identified microvascular invasion or gross portal vein invasion as significant risk factors for recurrence after liver transplantation or partial hepatectomy for HCC[9,21-23]. In previous studies, vascular invasion by the tumor has been regarded as microscopic intra-hepatic metastasis[6,24].

The rate of recurrence is high after OLT for HCC that is above the Milan criteria. The only mechanism of the recurrence after OLT is due to vascular invasion of HCC cells that leads to extensive systemic dissemination and circulation of the cancer cells at the time of transplantation. This has been suggested as the mechanism responsible for early post-operative intra-hepatic recurrence or distant metastasis after partial hepatectomy[25].

Previous studies have demonstrated that there is no significant difference in post-transplantation recurrence between the patients with solitary lesion of up to 6.5 cm in size and the patients with lesions of less than 5 cm (Milan criteria)[16]. Interestingly, although the presence of HCC lesions of more than 5 cm is traditionally considered as advanced disease, our results could not show the tumor size > 5 cm to be a significant risk factor of the fatal recurrence group in the multivariate analysis. The statistical difference appeared when the lesion size of 6.5 cm was compared, indicating it as a significant risk factor. This result was in agreement with the previous data by Yao *et al* [16] who reported that the upper limit of HCC lesion size for transplantation was 6.5 cm, since the greater sized lesions led to significantly high rates of recurrence. These observations not only imply that when the size of tumor is greater than 6.5 cm, there is a greater possibility of systemic dissemination, but also that tumor greater than 6.5 cm may require strict control with adjuvant therapy against early post-operative recurrence as a consequence of circulating cancer cells or micro-metastatic lesion after partial hepatectomy for HCC.

Systemic adjuvant chemotherapy is the mainstay treatment modality for controlling systemic dissemination of cancer cells after surgical resection of HCC. Although many studies have failed to show clear benefits of adjuvant systemic chemotherapy for HCC in randomized control trials, Yamamoto *et al* [26] Suggested that post-operative adjuvant oral 5-fluorouracil may be beneficial for stage II HCC patients with relatively favorable liver function and there are randomized control trials of immunotherapy and interferon therapy with positive results for the prevention of recurrence after resection of HCC[27,28]. Needless to say, further more in-depth studies are required to clarify efficacy of adjuvant therapy, because advanced HCC may be potentially curable surgically, tumor recurrence from systemic dissemination of cancer cells is a frequent outcome of the disease. Therefore, in order to enhance surgical curability theoretically, it is essential to initially remove all gross disease while implementing pre- or post-operative chemotherapy or immunotherapy to control disseminated cancer cells or micro-metastasis.

Previous data have shown that high pre-operative AFP level is a significant risk factor for early post-operative recurrence[29-31]. In this study, we also observed the similar result that serum AFP level > 1 000 μg/L is one of the significant risk factors. Also, the pre-operative serum AFP level is thought to be closely correlated with vascular invasion of tumor and dissemination of HCC cells[25], and thus it may provide valuable prognostic information for the pre-operative evaluation.

In conclusion, the high risk patients who have two or three risk factors mentioned in this study (serum AFP > 1 000 μg/L, microvascular invasion, tumor size > 6.5cm) should be the candidates of various adjuvant clinical trials against early post-operative fatal recurrence. Furthermore, our results may provide a control for comparing effectiveness of adjuvant clinical trial against early post-operative fatal recurrence after curative resection of high risk HCC patients.

REFERENCES

1 **Nagasue N**, Uchida M, Makino Y, Takemoto Y, Yamanoi A, Hayashi T, Chang YC, Kohno H, Nakamura T, Yukaya H. Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology* 1993; **105**: 488-494

2 **Chen MF**, Hwang TL, Jeng LB, Wang CS, Jan YY, Chen SC. Postoperative recurrence of hepatocellular carcinoma. Two hundred five consecutive patients who underwent hepatic resection in 15 years. *Arch Surg* 1994; **129**: 738-742

3 **Lin TY**, Lee CS, Chen KM, Chen CC. Role of surgery in the treatment of primary carcinoma of the liver: a 31-year experience. *Br J Surg* 1987; **74**: 839-842

4 **Bismuth H**, Majno PE, Adam R. Liver transplantation for hepatocellular carcinoma. *Semin Liver Dis* 1999; **19**: 311-322

5 The general rules for the clinical and pathological study of primary liver cancer. Liver Cancer Study Group of Japan. *Jpn J Surg* 1989; **19**: 98-129

6 **Matsumata T**, Kanematsu T, Takenaka K, Yoshida Y, Nishizaki T, Sugimachi K. Patterns of intrahepatic recurrence after curative resection of hepatocellular carcinoma. *Hepatology* 1989; **9**: 457-460

7 **Regimbeau JM**, Abdalla EK, Vauthey JN, Lauwers GY, Durand F, Nagorney DM, Ikai I, Yamaoka Y, Belghiti J. Risk factors for early death due to recurrence after liver resection for hepatocellular carcinoma: results of a multicenter study. *J Surg Oncol* 2004; **85**: 36-41

8 **Ercolani G**, Grazi GL, Ravaioli M, Del Gaudio M, Gardini A, Cescon M, Varotti G, Cetta F, Cavallari A. Liver resection for hepatocellular carcinoma on cirrhosis: univariate and multivariate analysis of risk factors for intrahepatic recurrence. *Ann Surg* 2003; **237**: 536-543

9 **Poon RT**, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic

recurrence after resection of hepatocellular carcinoma. *Cancer* 2000; **89**: 500-507
10 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917
11 **Fong Y**, Sun RL, Jarnagin W, Blumgart LH. An analysis of 412 cases of hepatocellular carcinoma at a Western center. *Ann Surg* 1999; **229**: 790-79; discussion 790-79
12 **Takayama T**, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, Kosuge T, Okada S, Takayasu K, Yamasaki S. Early hepatocellular carcinoma as an entity with a high rate of surgical cure. *Hepatology* 1998; **28**: 1241-1246
13 **Buscarini L**, Buscarini E, Di Stasi M, Vallisa D, Quaretti P, Rocca A. Percutaneous radiofrequency ablation of small hepatocellular carcinoma: long-term results. *Eur Radiol* 2001; **11**: 914-921
14 **Livraghi T**, Giorgio A, Marin G, Salmi A, de Sio I, Bolondi L, Pompili M, Brunello F, Lazzaroni S, Torzilli G. Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. *Radiology* 1995; **197**: 101-108
15 **Lencioni R**, Pinto F, Armillotta N, Bassi AM, Moretti M, Di Giulio M, Marchi S, Uliana M, Della Capanna S, Lencioni M, Bartolozzi C. Long-term results of percutaneous ethanol injection therapy for hepatocellular carcinoma in cirrhosis: a European experience. *Eur Radiol* 1997; **7**: 514-519
16 **Yao FY**, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, Ascher NL, Roberts JP. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33**: 1394-1403
17 **Schindel DT**, Grosfeld JL. Hepatic resection enhances growth of residual intrahepatic and subcutaneous hepatoma, which is inhibited by octreotide. *J Pediatr Surg* 1997; **32**: 995-97; discussion 995-97
18 **Panis Y**, Ribeiro J, Chrétien Y, Nordlinger B. Dormant liver metastases: an experimental study. *Br J Surg* 1992; **79**: 221-223
19 **Picardo A**, Karpoff HM, Ng B, Lee J, Brennan MF, Fong Y. Partial hepatectomy accelerates local tumor growth: potential roles of local cytokine activation. *Surgery* 1998; **124**: 57-64
20 **Lai EC**, You KT, Ng IO, Shek TW. The pathological basis of resection margin for hepatocellular carcinoma. *World J Surg* 1993; **17**: 786-90; discussion 791
21 **Marsh JW**, Dvorchik I, Bonham CA, Iwatsuki S. Is the pathologic TNM staging system for patients with hepatoma predictive of outcome? *Cancer* 2000; **88**: 538-543
22 **Vauthey JN**, Klimstra D, Franceschi D, Tao Y, Fortner J, Blumgart L, Brennan M. Factors affecting long-term outcome after hepatic resection for hepatocellular carcinoma. *Am J Surg* 1995; **169**: 28-34; discussion 34-5
23 **Okada S**, Shimada K, Yamamoto J, Takayama T, Kosuge T, Yamasaki S, Sakamoto M, Hirohashi S. Predictive factors for postoperative recurrence of hepatocellular carcinoma. *Gastroenterology* 1994; **106**: 1618-1624
24 **Yamamoto J**, Kosuge T, Takayama T, Shimada K, Yamasaki S, Ozaki H, Yamaguchi N, Makuuchi M. Recurrence of hepatocellular carcinoma after surgery. *Br J Surg* 1996; **83**: 1219-1222
25 **Jeng KS**, Sheen IS, Tsai YC. Does the presence of circulating hepatocellular carcinoma cells indicate a risk of recurrence after resection? *Am J Gastroenterol* 2004; **99**: 1503-1509
26 **Yamamoto M**, Arii S, Sugahara K, Tobe T. Adjuvant oral chemotherapy to prevent recurrence after curative resection for hepatocellular carcinoma. *Br J Surg* 1996; **83**: 336-340
27 **Takayama T**, Sekine T, Makuuchi M, Yamasaki S, Kosuge T, Yamamoto J, Shimada K, Sakamoto M, Hirohashi S, Ohashi Y, Kakizoe T. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. *Lancet* 2000; **356**: 802-807
28 **Kubo S**, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Yamazaki O, Shiomi S, Tamori A, Oka H, Igawa S, Kuroki T, Kinoshita H. Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med* 2001; **134**: 963-967
29 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207
30 **Hanazaki K**, Kajikawa S, Koide N, Adachi W, Amano J. Prognostic factors after hepatic resection for hepatocellular carcinoma with hepatitis C viral infection: univariate and multivariate analysis. *Am J Gastroenterol* 2001; **96**: 1243-1250
31 **Ikeda Y**, Kajiyama K, Adachi E, Yamagata M, Shimada M, Yanaga K. Early recurrence after surgery of hepatocellular carcinoma. *Hepatogastroenterology* 1995; **42**: 469-472

S- Editor Kumar M and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 105-109
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Detection of gelatinase B activity in serum of gastric cancer patients

Vesna V Dragutinović, Nebojša S Radovanović, Lidija T Izrael-Živković, Miroslav M Vrvić

Vesna V Dragutinović, Lidija T Izrael-Živković, Institute of Chemistry, School of Medicine, University of Belgrade, Višegradska 26, 11 000 Belgrade, Serbia and Montenegro
Nebojša S Radovanović, Institute of Digestive Diseases, Center of Esophagogastric Surgery, Clinical Center of Serbia, Koste Todorovića 6, 11 000 Belgrade, Serbia and Montenegro
Miroslav M Vrvić, Faculty of Chemistry, University of Belgrade, Studentski trg 16, 11 001 Belgrade, Serbia and Montenegro
Supported by the Ministry of Science and Environment Protection of the Republic of Serbia, No. 1740, TR-6845B and TD-7032B
Correspondence to: Vesna V. Dragutinović, Institute of Chemistry, School of Medicine, University of Belgrade, Višegradska 26, 11 000 Belgrade, Serbia and Montenegro. vdragu@ptt.yu
Telephone: +381-113615773 Fax: +381-113615764
Received: 2005-05-18 Accepted: 2005-06-18

Abstract

AIM: To determine the proteolytic activity and expression of gelatinase B in serum of gastric cancer patients and their correlation with the stage of the tumor.

METHODS: Sera from 23 patients who underwent surgery for primary gastric cancer as the experimental group and from 11 as the control group were used to determine the proteolytic activity and its inhibition by EDTA and 1,10-phenanthroline. Gelatinase B activity was detected by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and SDS-PAGE zymography.

RESULTS: Proteolytic enzyme activity was increased in gastric cancer patients when compared to the control group ($P<0.05$). The proteinases were determined to be metalloproteinases upon inhibition test with specific metalloproteinase inhibitors 1,10-phenanthroline ($P<0.05$) and EDTA ($P<0.01$). SDS-PAGE and SDS-PAGE zymography revealed gelatinase B (proMMP-9) activity and its molecular mass of 92 ku.

CONCLUSION: Proteinase activity is overexpressed in serum of gastric cancer patients. Gelatinase B in serum plays an important role in the progression of gastric cancer. ProMMP-9 can be used as a marker for invasiveness of gastric cancer.

Key words: Matrix metalloproteinase-9; Gastric cancer; Proteolytic activity; Inhibition

Dragutinović VV, Radovanović NS, Izrael-Živković LT, Vrvić MM. Detection of gelatinase B activity in serum of gastric cancer patients. *World J Gastroenterol* 2006; 12(1): 105-109

http://www.wjgnet.com/1007-9327/12/105.asp

INTRODUCTION

Proteolysis occurs in normal tissue but is limited in duration. A general aspect of malignant neoplasms may be an unbalance of proteolysis, which favors invasion[1].

Tumor progression is a step-wise process. Multiple alterations in normal cells can lead to a localized tumor that can finally invade the surrounding tissues and metastasize. Tumor cell invasion involves attachment of tumor cells to the underlying basement membrane, local proteolysis and migration of tumor cells through the proteolytically modified region[2]. Local proteolysis is facilitated by proteinases outside the tumor cells, perhaps bound to the cell surface and/or secreted from the tumor cells. Recent data suggest that proteinases inside the tumor cells also participate in local proteolysis by digesting phagocytic extracellular matrix. In order to metastasize, cells must be able to move into the vasculature (intravasation), survive in the circulation, move out of the vasculature (extravasation), invade the surrounding tissues and grow. All these steps involve interactions among tumor cells, stromal cells, invading lymphocytic cells, endothelial cells, and extracellular matrix. Proteinases expressed in these cells are believed to participate in many of these steps[3-7].

Matrix metalloproteinases (MMPs) are extracellular enzymes capable of degrading many extracellular matrix proteins. They are classified into five groups according to their structure and substrate specificity: collagenases, gelatinases, stromelysins, matrilysins and membrane-type MMPs. There is considerable evidence that MMPs play a major role in diverse physiologic processes and pathologic processes, including aspects of embryonic development, tissue morphogenesis, wound repair, inflammatory diseases and cancer. Overexpression and activation of MMPs have been linked with a variety of diseases[8-10].

In the matrix metalloproteinase of MMP family, including a 72 ku enzyme resembling matrix metalloproteinase-2 (MMP-2) known as gelatinase A and

a 92 ku enzyme resembling matrix metalloproteinase-9 (MMP-9) known as gelatinase B, have been demonstrated to be closely associated with several tumor systems and to invasive potential of tumor cells[11-13]. Type IV collagenase can degrade not only interstitial matrix but also the basement membrane. Malignant ascites[14] is the direct and prominent manifestation of advanced malignant diseases associated with invasion and metastasis of peritoneal cavity by tumor cells. In the present study, we detected the gelatinase B activity in the sera from patients with gastric cancer by gelatin zymography in order to provide the scientific basis for clinical diagnosis of gastric cancer.

MATERIALS AND METHODS

Reagents

N^{α}-benzoyl-arginine p-nitroanilide hydrochloride (BAPNA), EDTA and 1,10-phenanthroline were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The chemicals used for electrophoresis were from Merck (Darmstadt, Germany). Gelatin was purchased from Difco (Detroit, MN, USA). The mini gel electrophoresis equipment SE260 was from Hoefer Scientific Instruments (San Francisco, CA, USA).

Clinical specimens

In this study, we used 23 patients with gastric cancer as the experimental group and 11 patients as the control group. All patients with gastric cancer underwent surgery in the Institute for Digestive Diseases, Clinical Center of Serbia, from June 2002 to January 2004 and received neither chemotherapy nor radiation therapy before surgery. Of these patients, 15 (65%) were men and 8 (35%) were women with a mean age of 58 years (range: 38-75 years).

We had preoperative pathological diagnoses for all patients. Eleven patients underwent abdominal exploration or feeding jejunostomy because of liver metastases, peritoneal dissemination or malignant ascites. Twelve patients underwent radical surgery. In these patients, pathological examinations including depth of the tumor invasion, vascular invasion, lymphatic permeation, and lymph node metastasis were made according to the general rules of gastric cancer outlined by the Japanese Research Society for Gastric Cancer. In the control group, all the 11 patients were diagnosed to have groin hernia.

According to the TNM Classification System of the UICC, there were 2 stage 1, 3 stage 2, 5 stage 3, and 2 stage 4 tumors. According to their histological differentiation, there were 3 well, 4 moderately, and 5 poorly differentiated tumors. The clinicopathological features were found by reviewing all HE stained tissue sections.

Proteolytic activity

Proteolytic activity was determined using the method described by Ebeling *et al* [15].

Metalloproteinase inhibition test

The effect of EDTA and 1,10-phenanthroline (in concentration of 5 mmol/L) on proteolytic activity of the serum was examined. The serum was incubated at 37 ℃ for 30 min and the remaining proteolytic activity was determined under standard conditions.

SDS-PAGE

SDS-PAGE was performed with 75 g/L polyacrylamide gel[16] under no reduction conditions using a solution mixture of protein markers containing ovalbumin (45 ku), bovine serum albumin (BSA, 67 ku), β-galactosidase (116 ku) and myosin (200 ku). Serum was diluted in 200 g/L sucrose to prepare the samples. The samples were analyzed by SDS-PAGE to determine the molecular mass.

SDS-PAGE zymography

Samples were analyzed by SDS-PAGE zymography according to the method of Kleiner and Stetler-Stevenson[17] to determine the molecular mass and relative abundance of the gelatinases present. Samples were incubated for 40 min at 37 ℃ and electrophoreses were performed without reduction of 75 g/L polyacrylamide gels copolymerized with 0.01 g/L gelatin at 4 ℃ at a constant current of 15 mA. When the tracking dye at the front reached the bottom of the gel, the gel was removed and shaken gently for 45 min in 0.25 g/L Triton x-100 to remove SDS. Then the gel slabs were transferred to a bath (without Triton x-100) and washed for 20 min to remove Triton x-100. The above procedure was repeated twice at 4 °C. Then the gels were incubated and shaken for 60 h in 0.1 mol/L glycine, 50 mmol/L Tris-HCl, 5 mmol/L $CaCl_2$, 1 μmol/L $ZnCl_2$, 0.5 mol/L NaCl, pH 8.3, at 37℃. Regions of proteolytic activity were visualized as clear zones against a blue background after 3-h staining with Coomassie brilliant blue.

MMP inhibition test on zymography

In order to verify that the clear zones resembling matrix metalloproteinase, 5 mmol/L EDTA was added into the samples before incubation to inhibit MMP activities on gelatin zymography.

Statistical analysis

Mann-Whitney test and Wilcoxon signed rank test were used for statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS

Proteolytic activity

Proteolytic activity was increased ($P<0.05$) in gastric cancer patients compared to the control group (Tables 1 and 2). On the other hand, there was no significant correlation in proteolytic activity among the patients after radical or palliative surgery.

Metalloproteinase inhibition test

EDTA and 1,10-phenanthroline inhibited proteolytic activity on BAPNA superstrate in the sera of patients with gastric cancer. 1,10-phenanthroline ($P<0.05$) showed less inhibition on proteolytic activity than EDTA ($P<0.01$) (Table 1).

Detection of gelatinase B in serum

The samples of gastric cancer patients were shown on

Table 1 Proteolytic activity and inhibition of metalloproteinase activity in serum of patients with gastric cancer

Sample	BAPNA (mU)	EDTA (mU)	Inhibition in presence of EDTA (%)	1,10-Phen (mU)	Inhibition in presence of 1,10-phen (%)	TNM stage
1	8.6	1.4	83.7	0.9	89.5	–
2[1]	3.1	1.7	45.2	1.6	51.6	II
3[1]	9.0	2.3	74.4	1.7	81.1	IV
4[1]	4.0	2.8	30.0	4.2	–	III
5[1]	8.8	0.0	100.0	2.2	75.0	IV
6	4.5	5.2	–	1.4	68.9	–
7	0.7	2.1	–	3.5	–	–
8	4.9	5.3	–	8.0	–	–
9[1]	7.0	3.3	52.9	5.7	18.6	III
10	6.2	0.0	100.0	3.8	38.7	–
11[1]	2.8	5.6	–	4.2	–	II
12[1]	1.7	1.5	11.8	6.1	–	II
13	6.9	0.6	91.3	0.7	89.9	–
14	8.0	0.3	96.2	1.9	76.2	–
15[1]	5.2	0.1	98.1	1.5	71.2	III
16[1]	1.8	0.7	61.1	2.5	–	I
17	0.8	0.7	12.5	2.3	–	–
18	8.8	1.1	87.5	5.0	43.2	–
19[1]	4.7	1.9	59.6	3.6	23.4	III
20[1]	4.8	1.5	68.8	1.9	60.4	III
21	5.3	0.0	100.0	2.4	54.7	–
22	4.1	0.6	88.7	2.4	41.7	–
23[1]	1.3	0.5	61.5	1.1	15.4	I

[1]Radical surgery.

Table 2 Proteolytic activity and inhibition of metalloproteinase activity in serum of control group

Control	BAPNA (mU)	EDTA (mU)	Inhibition in presence of EDTA (%)	1,10-Phen (mU)	Inhibition in presence of EDTA (%)
1	1.3	4.3	-	0.4	69.2
2	0.7	3.2	-	4.3	-
3	0.4	2.3	-	1.7	-
4	4.4	6.8	-	0.9	79.5
5	0.3	1.3	-	1.7	-
6	2.2	0.9	59.1	-	-
7	0.9	1.6	-	2.5	-
8	1.0	0.0	100.0	3.2	-
9	0.3	0.9	-	2.6	-
10	0.3	1.1	-	2.7	-
11	1.7	0.0	100.0	0.0	100.0

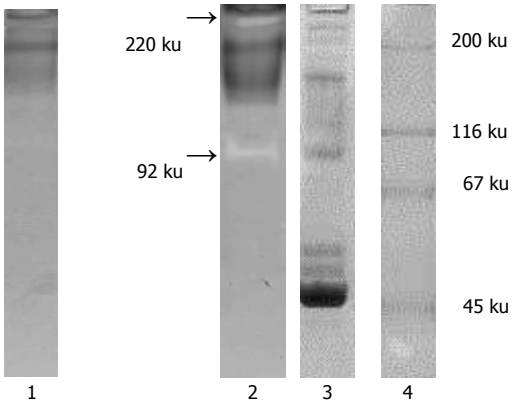


Figure 1 Results of SDS-PAGE and SDS-PAGE zymography. 1: Serum of gastric cancer patients with EDTA inhibitor; 2: serum of control group; 3: molecular mass determination; 4: Protein marker mixture.

SDS-PAGE Coomassie brilliant blue staining bands at the mass position of 92 ku. The protein molecular mass of 92 ku was detected in 82% of patients with gastric cancer. Molecular mass of 92 ku indicated MMP-9 protein (Figure 1). In the control group, MMP-9 protein was not detected.

The gelatinase B activity was detected by SDS-PAGE zymography as the clear bands against the blue background (Figure 1) in the sera of gastric cancer patients. There were no clear bands in the control group. The clear bands detected by gelatin zymography were characterized by the activity of gelatinases A (72 ku) and B (92 ku). The reaction was positive for band migrating at approximately 220 and 92 ku and for bands at 200 and 116 ku in some samples. The 220-ku band was strongly positive for gelatinase B, suggestive of homodimer. The 200- and 116-ku bands were interpreted as proMMP-9/TIMP-1 complexes.

Metalloproteinase inhibition test by zymography

Gelatinase B activity in the serum of gastric cancer patients was inhibited by EDTA.

DISCUSSION

Proteolytic activity in the sera of patients with gastric cancer was higher than that in the control group, indicating that proteolysis can be degraded by ECM[18-20]. For the occurrence of metastasis, tumor cells must repeatedly cross over the basement membrane barrier, a process for which proteolysis of ECM components is required[21,22]. Some of the proteins associated with invasion and metastases of tumors are produced by tumor cells. Then, the proteins (whole or fragments) may accumulate in blood or urine of patients.

According to the inhibition test with EDTA and 1,10-phenanthroline, proteinases are found to be metalloproteinases and the inhibition is an additional biochemical parameter for correlation of proteolytic activity and gastric cancer. Increased levels of metalloproteinase have been implicated in the invasive potential of tumors[23-26]. These results suggest that overexpression of metalloproteinases in the serum plays an important role in the progression of gastric cancer.

Overexpression of type IV collagenase has been demonstrated in a variety of cancers including colorectal cancer[27], gastric cancer[28], and breast cancer. There is evidence that type IV collagenase activity or concentration is increased in the plasma of patients with advanced carcinoma[29-33]. It was reported that type IV collagenase activity is increased in urine[34] and ascites[14] of cancer patients.

In the present study, we initially measured the gelatinase B activities in the serum of gastric cancer patients. The

results demonstrated that proteolytically active proMMP-9 was significantly associated with cancer. Proteolytic activity was shown in tumor patients. On the basis of molecular size and inhibition by EDTA, the bands were respectively interpreted as proMMP-9 (92 ku) and its putative dimmer 220 ku and proMMP-9/TIMP-1 complexes (200 and 116 ku). The activated form of gelatinase B (83 ku) was not detected in the serum of cancer patients. Gelatinase A activity was not detected in the serum of gastric cancer patients. The gelatinases, particularly gelatinase A, seem to be important in the initial stage of tumor invasion[12] as they degrade the components of the basement membrane, while other MMPs contribute to the later stages of tumor invasion[35]. In some reports[36], gelatinase B in gastric carcinoma is positively correlated with the existence of vessel permeation, lymph node metastasis or the depth of tumor invasion.

In conclusion, gelatinase B protein may serve as a marker for invasiveness and metastasis of gastric cancer[37-39]. ProMMP-9 can be used for the detection of primary or recurrent cancer and for the estimation of tumor extent.

ACKNOWLEDGMENT

Prof Ivanka Karadžić from the Institute of Chemistry, School of Medicine, University of Belgrade for her suggestions and friendly support.

REFERENCES

1 **Liotta LA**, Stetler-Stevenson WG. Principles of Molecular Cell: Biology of Cancer: Cancer Metastasis. In: De Vita T., Hellman S, Rosenberg S, eds. Cancer, principles and practice of oncology. Philadelphia: J.B. Lippincot Com, 1993: 134-149
2 **Liotta LA**, Rao CN, Barsky SH. Tumor invasion and the extracellular matrix. *Lab Invest* 1983; **49**: 636-649
3 **Chambers AF**, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 1997; **89**: 1260-1270
4 **Noël A**, Gilles C, Bajou K, Devy L, Kebers F, Lewalle JM, Maquoi E, Munaut C, Remacle A, Foidart JM. Emerging roles for proteinases in cancer. *Invasion Metastasis* 1997; **17**: 221-239
5 **Kim J**, Yu W, Kovalski K, Ossowski L. Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. *Cell* 1998; **94**: 353-362
6 **Parsons SL**, Watson SA, Brown PD, Collins HM, Steele RJ. Matrix metalloproteinases. *Br J Surg* 1997; **84**: 160-166
7 **Ray JM**, Stetler-Stevenson WG. The role of matrix metalloproteases and their inhibitors in tumour invasion, metastasis and angiogenesis. *Eur Respir J* 1994; **7**: 2062-2072
8 **Itoh T**, Tanioka M, Yoshida H, Yoshioka T, Nishimoto H, Itohara S. Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. *Cancer Res* 1998; **58**: 1048-1051
9 **Liotta LA**. Tumor invasion and metastases--role of the extracellular matrix: Rhoads Memorial Award lecture. *Cancer Res* 1986; **46**: 1-7
10 **Liotta LA**, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 1980; **284**: 67-68
11 **Sonnante AM**, Correale M, Linsalata M, Di Leo A, Guerra V. Circulating Levels of Matrix Metalloproteinase-9 in Patients with Colorectal Cancer. *Scand J Gastroenterol* 2000; **35**: 671-672
12 **Tryggvason K**, Höyhtyä M, Pyke C. Type IV collagenases in invasive tumors. *Breast Cancer Res Treat* 1993; **24**: 209-218
13 **Zucker S**, Lysik RM, Zarrabi MH, Moll U. M(r) 92,000 type IV collagenase is increased in plasma of patients with colon cancer and breast cancer. *Cancer Res* 1993; **53**: 140-146
14 **Sun XM**, Dong WG, Yu BP, Luo HS, Yu JP. Detection of type IV collagenase activity in malignant ascites. *World J Gastroenterol* 2003; **9**: 2592-2595
15 **Ebeling W**, Hennrich N, Klockow M, Metz H, Orth HD, Lang H. Proteinase K from Tritirachium album Limber. *Eur J Biochem* 1974; **47**: 91-97
16 **Laemmli UK**. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227**: 680-685
17 **Kleiner DE**, Stetler-Stevenson WG. Quantitative zymography: detection of picogram quantities of gelatinases. *Anal Biochem* 1994; **218**: 325-329
18 **Meyer T**, Hart IR. Mechanisms of tumour metastasis. *Eur J Cancer* 1998; **34**: 214-221
19 **Kleiner DE**, Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. *Cancer Chemother Pharmacol* 1999; **43 Suppl**: S42-S51
20 **Conway JG**, Trexler SJ, Wakefield JA, Marron BE, Emerson DL, Bickett DM, Deaton DN, Garrison D, Elder M, McElroy A, Willmott N, Dockerty AJ, McGeehan GM. Effect of matrix metalloproteinase inhibitors on tumor growth and spontaneous metastasis. *Clin Exp Metastasis* 1996; **14**: 115-124
21 **Ara T**, Fukuzawa M, Kusafuka T, Komoto Y, Oue T, Inoue M, Okada A. Immunohistochemical expression of MMP-2, MMP-9, and TIMP-2 in neuroblastoma: association with tumor progression and clinical outcome. *J Pediatr Surg* 1998; **33**: 1272-1278
22 **Ellenrieder V**, Alber B, Lacher U, Hendler SF, Menke A, Boeck W, Wagner M, Wilda M, Friess H, Büchler M, Adler G, Gress TM. Role of MT-MMPs and MMP-2 in pancreatic cancer progression. *Int J Cancer* 2000; **85**: 14-20
23 **Aznavoorian S**, Murphy AN, Stetler-Stevenson WG, Liotta LA. Molecular aspects of tumor cell invasion and metastasis. *Cancer* 1993; **71**: 1368-1383
24 **Nagase H**, Woessner JF. Matrix metalloproteinases. *J Biol Chem* 1999; **274**: 21491-21494
25 **Stetler-Stevenson WG**, Aznavoorian S, Liotta LA. Tumor cell interactions with the extracellular matrix during invasion and metastasis. *Annu Rev Cell Biol* 1993; **9**: 541-573
26 **Ellenrieder V**, Adler G, Gress TM. Invasion and metastasis in pancreatic cancer. *Ann Oncol* 1999; **10 Suppl 4**: 46-50
27 **Mook OR**, Frederiks WM, Van Noorden CJ. The role of gelatinases in colorectal cancer progression and metastasis. *Biochim Biophys Acta* 2004; **1705**: 69-89
28 **Torii A**, Kodera Y, Uesaka K, Hirai T, Yasui K, Morimoto T, Yamamura Y, Kato T, Hayakawa T, Fujimoto N, Kito T. Plasma concentration of matrix metalloproteinase 9 in gastric cancer. *Br J Surg* 1997; **84**: 133-136
29 **Sato H**, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M. A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature* 1994; **370**: 61-65
30 **Fishman DA**, Bafetti LM, Banionis S, Kearns AS, Chilukuri K, Stack MS. Production of extracellular matrix-degrading proteinases by primary cultures of human epithelial ovarian carcinoma cells. *Cancer* 1997; **80**: 1457-1463
31 **Costiner E**, Ghiea D, Simionescu L, Oprescu M. Modified technique of perfusion of isolated rat pancreas tested by insulin release after glucose administration. *Endocrinol Exp* 1975; **9**: 197-204
32 **Gohji K**, Fujimoto N, Hara I, Fujii A, Gotoh A, Okada H, Arakawa S, Kitazawa S, Miyake H, Kamidono S, Nakajima M. Serum matrix metalloproteinase-2 and its density in men with prostate cancer as a new predictor of disease extension. *Int J Cancer* 1998; **79**: 96-101
33 **Waas ET**, Lomme RM, DeGroot J, Wobbes T, Hendriks T. Tissue levels of active matrix metalloproteinase-2 and -9 in colorectal cancer. *Br J Cancer* 2002; **86**: 1876-1883
34 **Moses MA**, Wiederschain D, Loughlin KR, Zurakowski D, Lamb CC, Freeman MR. Increased incidence of matrix metalloproteinases in urine of cancer patients. *Cancer Res* 1998;

58: 1395-1399
35 **Stetler-Stevenson WG**, Liotta LA, Kleiner DE. Extracellular matrix 6: role of matrix metalloproteinases in tumor invasion and metastasis. *FASEB J* 1993; **7**: 1434-1441
36 **Endo K**, Maehara Y, Baba H, Yamamoto M, Tomisaki S, Watanabe A, Kakeji Y, Sugimachi K. Elevated levels of serum and plasma metalloproteinases in patients with gastric cancer. *Anticancer Res* 1997; **17**: 2253-2258
37 **Zhang S**, Li L, Lin JY, Lin H. Imbalance between expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in invasiveness and metastasis of human gastric carcinoma. *World J Gastroenterol* 2003; **9**: 899-904
38 **Turpeenniemi-Hujanen T**. Gelatinases (MMP-2 and -9) and their natural inhibitors as prognostic indicators in solid cancers. *Biochimie* 2005; **87**: 287-297
39 **Vihinen P**, Ala-aho R, Kähäri VM. Matrix metalloproteinases as therapeutic targets in cancer. *Curr Cancer Drug Targets* 2005; **5**: 203-220

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 110-113
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis

Judit Bene, Katalin Komlósi, Viktória Havasi, Gábor Talián, Beáta Gasztonyi, Krisztina Horváth, Gyula Mózsik, Béla Hunyady, Béla Melegh, Mária Figler

Judit Bene, MTA PTE Clinical Genetics Research Group of Hungarian Academy of Sciences at the University of Pécs, Pécs, Hungary
Judit Bene, Katalin Komlósi, Viktória Havasi, Gábor Talián, Béla Melegh, Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary
Beáta Gasztonyi, Krisztina Horváth, Gyula Mózsik, Béla Hunyady, Mária Figler, 1st Department of Medicine, School of Medicine, University of Pécs, Pécs, Hungary
Supported by the grant of Ministry of Health, No. ETT 325/2003 and 595/2003; the grant of Hungarian Science Foundation, No. OTKA T 35026 and T 49589; and from the National grant No. NKFP-4/005/2002
Correspondence to: Dr Béla Melegh, Professor of Medical Genetics and Pediatrics, Department of Medical Genetics and Child Development, University of Pécs, H-7624 Pécs, Szigeti 12, Hungary. bela.melegh@aok.pte.hu
Telephone: +36-72-536427 Fax: +36-72-536427
Received: 2005-05-23 Accepted: 2005-06-18

Abstract

AIM: To determine the plasma carnitine ester profile in adult patients with ulcerative culitis (UC) and compared with healthy control subjects.

METHOD: Using ESI triple quadrupole tandem mass spectrometry, the carnitine ester profile was measured in 44 patients with UC and 44 age- and sex-matched healthy controls.

RESULTS: There was no significant difference in the fasting free carnitine level between the patients with UC and the healthy controls. The fasting propionyl- (0.331 ± 0.019 *vs* 0.392 ± 0.017 μmol/L), butyryl- (0.219 ± 0.014 *vs* 0.265 ± 0.012), and isovalerylcarnitine (0.111 ± 0.008 *vs* 0.134 ± 0.008) levels were decreased in the UC patients. By contrast, the level of octanoyl- (0.147 ± 0.009 *vs* 0.114 ± 0.008), decanoyl- (0.180 ± 0.012 *vs* 0.137 ± 0.008), myristoyl- (0.048 ± 0.003 *vs* 0.039 ± 0.003), palmitoyl- (0.128 ± 0.006 *vs* 0.109 ± 0.004), palmitoleyl- (0.042 ± 0.003 *vs* 0.031 ± 0.002) and oleylcarnitine (0.183 ± 0.007 *vs* 0.163 ± 0.007; $P < 0.05$ in all comparisons) were increased in the patients with UC.

CONCLUSION: Our data suggest selective involvement of the carnitine esters in UC patients, probably due to their altered metabolism.

Key words: Carnitine; Carnitine ester profile; Ulcerative colitis

Bene J, Komlósi K, Havasi V, Talián G, Gasztonyi B, Horváth K, Mózsik G, Hunyady B, Melegh B, Figler M. Changes of plasma fasting carnitine ester profile in patients with ulcerative colitis. *World J Gastroenterol* 2006; 12(1): 110-113

http://www.wjgnet.com/1007-9327/12/110.asp

INTRODUCTION

Ulcerative colitis (UC) is a disorder of the idiopathic and chronic inflammation of the colonic mucosa. The etiology and the pathogenesis of the disease are yet unknown; a classic study on isolated colonic epithelial cells demonstrated decreased utilization of n-butyrate. Since the major energy sources of the epithelial cells of the distal colon are the short-chain fatty acids (SCFAs), these cells are able to metabolize other fuels, such as glucose and glutamine, only at a much lower rate[1]. SCFAs are generated from carbohydrates by bacterial degradation and they are readily absorbed by the colon and represent energy fuels for the colonocytes and other tissues, such as the skeletal muscle[2,3]. Patients with distal UC may have increased or moderately decreased stool SCFA concentrations, reflecting their altered absorption[4,5]. UC can, therefore, be regarded essentially as an SCFA oxidation failure-associated disease, where the energy deficiency is a primary event in the development of the disease[1].

L-carnitine plays an essential role in the energy metabolism, since it enables the transport of activated long-chain fatty acids (LCFA) as carnitine esters across the inner mitochondrial membrane. Moreover, it is able to form esters with several medium- and SCFAs of both endogenous and exogenous origins[6,7]. The impact of altered SCFA metabolism in UC prompted us to study the circulating carnitine ester profile in the UC patients.

MATERIALS AND METHODS

Patients

We examined 44 patients with UC (25 males, 19 females,

Table 1 Some major clinical and laboratory parameters in patients with ulcerative colitis and control subjects (mean ± SE)

Parameters	UC patients, n = 44	Controls, n = 44
Females/males	19/25	24/20
CRP (mg/L)	12.2 ± 4.5	2.6 ± 0.5
Albumin (g/L)	44.6 ± 0.7	50.2 ± 0.8
Iron (μmol/L)	16.1 ± 1.2	23.7 ± 1.6
Hb (g/dL)	131.3 ± 2.5	159 ± 1.2
MCV (L)	86.2 ± 1.2	94.8 ± 2.5
WBC (G/L)	7.6 ± 0.4	9.2 ± 0.6
BMI (kg/m^2)	24.6 ± 0.6	24.1 ± 0.5
PLT (G/L)	298.5 ± 13.5	228.3 ± 10.4
Both ileum and colon localization	5/44 (11.4%)	
Rectosigmoid localization only	8/44 (18.2%)	
Colon localization	31/44 (70.4%)	

mean age: 39.7 years, range: 17-65 years), and 44 carefully selected clinically healthy age-, sex-, weight-, and height-matched control subjects (20 males, 24 females, mean age: 37.0 years, range: 23-60 years). The control subjects did not receive any drug medication, while the UC patients were treated with either sulfasalazine or 5-aminosalicylic acid. We assumed that these drugs do not have any influence on the carnitine status since there were no such data available in the literature.

Diagnosis of the disease relied upon the history of the patients, clinical symptoms, negative stool examination for bacteria and parasites, and histologic results of rectal and/or colonic biopsy. Exclusion criteria in both groups were as follows: secondary causes of colonic disease, systemic diseases, any malformations, evidence of intestinal bacterial infection, history or evidence of any inherited metabolic disease, hepatic or renal disease, and pregnancy. (Table 1) shows the clinical parameters of the UC patients.

The clinical and laboratory data were the results of measurements performed from sample aliquots of blood collected after an overnight fasting precisely between 08:00 a.m. and 08:30 a.m., both in the UC patients and in the healthy control subjects. This strict post-alimental time scheduling was introduced to prevent the diet or fasting time-induced dynamic changes of carnitine esters in the circulation[8].

Informed consent was obtained from each participant of the study and the study design was approved by the Departmental Ethics Committee.

Methods

Plasma albumin, iron, and C-reactive protein levels were determined by routine methods. The hemoglobin (Hb), mean corpuscular volume (MCV), white blood cells (WBC) and platelet (PLT) were measured by automated analysis (sysmex XE 2100, Japan). The body mass index (BMI) was calculated as body weight/height^2 (in kg/m^2).

Acylcarnitines were measured after derivatization as butyl esters using isotope dilution mass spectrometry method in a Micromass Quattro Ultima ESI triple-quadrupole mass spectrometer. The procedure was principally the method described previously[9]. Essentially, 10 μL of plasma was spotted and dried onto a filter paper and prepared by extraction with 200 μL of methanol containing internal deuterium-labeled standards (0.76 μmol/L [^{2}H$_3$] -free carnitine, 0.04 μmol/L [^{2}H$_3$] -propionylcarnitine, 0.04 μmol/L [^{2}H$_3$] -octanoylcarnitine and 0.08 μmol/L [^{2}H$_3$] -palmitoylcarnitine). After 20 min of agitation, the supernatant was evaporated to dryness under nitrogen at 40 ℃ and then 100 μL of 3 mol/L butanolic HCl was added. The solution was incubated at 65 ℃ for 15 min and evaporated to dryness again under nitrogen at 40 ℃ and re-dissolved in 100 μL of the mobile phase (acetonitrile:water 80:20). A total of 10 μL of sample aliquots were introduced to the ESI cone by using Waters 2795 HPLC system. The free carnitine and all acylcarnitines were determined by ESI-MS/MS analysis using positive precursor ion scan of m/z 85; scan range was 200-550 m/z. The capillary voltage was 2.50 kV, while the cone voltage was 55 V, and the collision energy was 25 eV. The flow rate was 100 μL/min and the total analysis time was 4 min per sample. Each sample was measured in triplicates starting with the injection step, and the results were the means of the three determinations.

Statistical analysis

Student's t test for unpaired samples was used for the statistical analysis. The values were expressed as mean ± SE, in three decimals for the carnitine esters with respect to the low levels in the case of the long-chain carnitine esters. $P < 0.05$ was considered statistically significant.

RESULTS

The plasma circulating carnitine ester profiles are shown in Table 2. The plasma level of free carnitine and acetyl carnitine did not differ between the UC patients and the controls. By contrast, significant decreases were observed in fasting propionyl-, butyryl-, and isovalerylcarnitine ester levels in UC patients as compared with the controls. The level of total short-chain carnitine esters was markedly lower in the patients with UC (9.855 ± 0.094 μmol/L) than in the healthy controls (11.003 ± 0.100 μmol/L, $P < 0.01$).

The levels of octanoyl-, and decanoylcarnitine were decreased in the healthy subjects. The levels of total medium-chain acylcarnitines were obviously higher in the UC patients (0.629 ± 0.007 μmol/L) than in the control subjects (0.548 ± 0.007 μmol/L, $P < 0.01$).

In the long-chain acylcarnitine group, the plasma levels of myristoyl-, palmitoyl-, palmitoleyl- and oleylcarnitine were significantly decreased in the healthy group. The levels of total long-chain carnitine esters were markedly higher in the patients with UC (0.596 ± 0.005 μmol/L) than in the controls (0.515 ± 0.009 μmol/L, $P < 0.01$).

In addition, the level of total carnitine esters was significantly decreased in the UC patients (11.080 ± 0.035 μmol/L) as compared with the healthy controls (12.066 ± 0.037 μmol/L, $P < 0.01$).

DISCUSSION

Carnitine [β-hydroxy-γ(trimethylamino) butyric acid]

Table 2 Plasma carnitine ester profiles in ulcerative colitis patients and controls (μmol/L, mean ± SE)

	UC patients, n = 44	Controls, n = 44
Free carnitine (C0)	31.595 ± 1.454	31.431 ± 1.042
Short-chain acylcarnitines		
Acetylcarnitine (C2)	9.164 ± 0.426	10.179 ± 0.461
Propionylcarnitine (C3)	0.331 ± 0.019[a]	0.392 ± 0.017
Butyrylcarnitine (C4)	0.219 ± 0.014[a]	0.265 ± 0.012
Isovalerylcarnitine (C5)	0.111 ± 0.008[a]	0.134 ± 0.008
Tiglylcarnitine (C5:1)	0.030 ± 0.004	0.033 ± 0.002
Medium-chain acylcarnitines		
Hexanoylcarnitine (C6)	0.071 ± 0.006	0.081 ± 0.005
Octenoylcarnitine (C8)	0.147 ± 0.009[a]	0.114 ± 0.008
Octenoylcarnitine (C8:1)	0.064 ± 0.005	0.062 ± 0.007
Decanoylcarnitine (C10)	0.180 ± 0.012[a]	0.137 ± 0.008
Cecenoylcarnitine (C10:1)	0.113 ± 0.006	0.104 ± 0.008
Lauroylcarnitine (C12)	0.054 ± 0.003	0.050 ± 0.003
Long-chain acylcarnitines		
Myristoylcarnitine (C14)	0.026 ± 0.001	0.024 ± 0.001
Myristoleylcarnitine (C14:1)	0.048 ± 0.003[a]	0.039 ± 0.003
Palmitoylcarnitine (C16)	0.128 ± 0.006[a]	0.109 ± 0.004
Palmitoylcarnitine (C16:1)	0.042 ± 0.003[a]	0.031 ± 0.002
Stearoylcarnitine (C18)	0.085 ± 0.003	0.079 ±0.003
Oleylcarnitine (C18:1)	0.183 ± 0.007[a]	0.163 ± 0.007
Hydroxymyristoylcarnitine (C14OH)	0.007 ± 0.001	0.006 ± 0.001
Hydroxypalmitoylcarnitine (C16OH)	0.026 ± 0.002	0.023 ± 0.001
Hydroxypalmitoleylcarnitine (C16:1OH)	0.033 ± 0.002	0.029 ± 0.002
Hydroxyoleylcarnitine (C18:1OH)	0.018 ± 0.002[a]	0.012 ± 0.001

[a]$P < 0.05$ *vs* controls.

is known as a carrier for transporting activated LCFA into the mitochondrial matrix for β-oxidation. With this function the L-carnitine plays an essential role in the energy metabolism[6]. Moreover, the carnitine molecule is able to form esters with several medium- and short-chain fatty acids of both endogenous and exogenous origins[6,7]. The circulating carnitine ester spectrum can reflect affected cellular metabolism of the short-, medium-, and long-chain fatty acids. Therefore, the monitoring of the carnitine ester composition is a widespread tool for the diagnosis of several inborn errors of metabolism. Besides the complete metabolic blockage caused by the inherited lack of enzyme activities, influences on carnitine ester spectra may be the consequence of only partially affected flux of metabolites via the carnitine acyltransferases.

In the present study, significant decrease was found in the fasting plasma levels of propionyl-, butyryl-, and isovalerylcarnitine esters, leading to the decrease of SCFA carnitine esters. Although the pathogenesis of UC is still unknown, a widely accepted hypothesis focuses on the pivotal role of the diminished availability of SCFAs for the enteral cells. Normally, SCFAs are rapidly absorbed from the colon and have many properties, as they represent an energy source for colonocytes and if they are exported to other tissues. Moreover, they affect lipid metabolism, colonic mucosal blood flow, motility, and mucus secretion[2]. In the normal case, the major energy source of the epithelial cells of the distal colon derives from the metabolism of the SCFAs[10], which is impaired in UC[1]. In addition to the SCFA metabolism, the influenced coenzyme A esterification has been reported to be associated with UC[11]. In the cells, the fatty acyl moieties are transferred from coenzyme A to the beta hydroxyl group of the carnitine via the short-, medium, and long-chain carnitine acyltransferases[6]. These events separately or in combination, can explain the decrease of the circulating SCFA carnitine esters.

The circulating plasma carnitine profile is determined by the balance of the release and uptake mechanisms. Carnitine releases into the circulation by the liver primarily as acylcarnitine[12]. While in the hepatic vein, the ester proportion is relatively high, approximately half of the total carnitine is esterified. The actual ester pattern detected in the peripheral blood is a result of the uptake/release action of the peripheral tissues; and in a peripheral venous blood, much less (approximately 1/3-1/4 of the total carnitine) is esterified. Whereas, the decrease of the SCFA carnitine esters found in the UC patients could be explained as discussed earlier. Based on the current knowledge, it is much more difficult due to the limited nature of the data, to explain the opposite change of the medium- or long-chain carnitine esters. Only a few studies are available reporting alterations of fatty acid metabolism. However, the data are inconsistent, but suggest the involvement of LCFA metabolism in UC[13-15]. Further studies are required to clarify these issues.

After the positive results on topical SCFA treatment in UC[16], Gasbarrini *et al* [3] studied propionyl-L-carnitine administration as rectal irrigation and found that improved clinical picture and histological status of the bowel are improwed. In the light of the current findings, the likely decreased tissue reserves could be corrected by administration of the drug and the positive outcome could be a consequence of the successful replacement therapy. Whether the already known beneficial therapeutic effects of special LCFA containing or supplemented with diets[14,15,17-20] are due to at least in part, a similar replacement phenomenon, also remains to be elucidated.

ACKNOWLEDGMENT

Ferenc Pakodi, Áron Vincze, and Ilona Szántó for their help in the technical management of the study.

REFERENCES

1 **Roediger WE**. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* 1980; **2**: 712-715
2 **Cummings JH**, Rombeau JL, Sakata T (eds). Physiological and clinical aspects of short chain fatty acids. Cambridge: Cambridge University Press, 1995
3 **Gasbarrini G**, Mingrone G, Giancaterini A, De Gaetano A, Scarfone A, Capristo E, Calvani M, Caso V, Greco AV. Effects of propionyl-L-carnitine topical irrigation in distal ulcerative colitis: a preliminary report. *Hepatogastroenterology* 2003; **50**: 1385-1389
4 **Roediger WE**, Heyworth M, Willoughby P, Piris J, Moore A, Truelove SC. Luminal ions and short chain fatty acids as markers of functional activity of the mucosa in ulcerative colitis. *J Clin Pathol* 1982; **35**: 323-326
5 **Scheppach W**, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S, Richter F, Dusel G, Kasper H. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology* 1992; **103**: 51-56
6 **Bieber LL**. Carnitine. *Annu Rev Biochem* 1988; **57**: 261-283
7 **Melegh B**, Kerner J, Bieber LL. Pivampicillin-promoted

excretion of pivaloylcarnitine in humans. *Biochem Pharmacol* 1987; **36**: 3405-3409
8 **Costa CC**, de Almeida IT, Jakobs C, Poll-The BT, Duran M. Dynamic changes of plasma acylcarnitine levels induced by fasting and sunflower oil challenge test in children. *Pediatr Res* 1999; **46**: 440-444
9 **Bene J**, Komlósi K, Gasztonyi B, Juhász M, Tulassay Z, Melegh B. Plasma carnitine ester profile in adult celiac disease patients maintained on long-term gluten free diet. *World J Gastroenterol* 2005; **11**: 6671-6675
10 **Scheppach W**, Müller JG, Boxberger F, Dusel G, Richter F, Bartram HP, Christl SU, Dempfle CE, Kasper H. Histological changes in the colonic mucosa following irrigation with short-chain fatty acids. *Eur J Gastroenterol Hepatol* 1997; **9**: 163-168
11 **Ellestad-Sayed JJ**, Nelson RA, Adson MA, Palmer WM, Soule EH. Pantothenic acid, coenzyme A, and human chronic ulcerative and granulomatous colitis. *Am J Clin Nutr* 1976; **29**: 1333-1338
12 **Sandor A**, Kispal G, Melegh B, Alkonyi I. Ester composition of carnitine in the perfusate of liver and in the plasma of donor rats. *Eur J Biochem* 1987; **170**: 443-445
13 **Siguel EN**, Lerman RH. Prevalence of essential fatty acid deficiency in patients with chronic gastrointestinal disorders. *Metabolism* 1996; **45**: 12-23
14 **Esteve-Comas M**, Ramírez M, Fernández-Bañares F, Abad-Lacruz A, Gil A, Cabré E, González-Huix F, Moreno J, Humbert P, Guilera M. Plasma polyunsaturated fatty acid pattern in active inflammatory bowel disease. *Gut* 1992; **33**: 1365-1369
15 **Kinsella JE**, Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutrition* 1990; **6**: 24-44; discussion 59-62
16 **Harig JM**, Soergel KH, Komorowski RA, Wood CM. Treatment of diversion colitis with short-chain-fatty acid irrigation. *N Engl J Med* 1989; **320**: 23-28
17 **Stenson WF**, Cort D, Rodgers J, Burakoff R, DeSchryver-Kecskemeti K, Gramlich TL, Beeken W. Dietary supplementation with fish oil in ulcerative colitis. *Ann Intern Med* 1992; **116**: 609-614
18 **Lorenz R**, Weber PC, Szimnau P, Heldwein W, Strasser T, Loeschke K. Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease--a randomized, placebo-controlled, double-blind cross-over trial. *J Intern Med Suppl* 1989; **731**: 225-232
19 **Loeschke K**, Ueberschaer B, Pietsch A, Gruber E, Ewe K, Wiebecke B, Heldwein W, Lorenz R. n-3 fatty acids only delay early relapse of ulcerative colitis in remission. *Dig Dis Sci* 1996; **41**: 2087-2094
20 **Salomon P**, Kornbluth AA, Janowitz HD. Treatment of ulcerative colitis with fish oil n--3-omega-fatty acid: an open trial. *J Clin Gastroenterol* 1990; **12**: 157-161

S-Editor Kumar M and Guo SY **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 114-118
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Effect of percutaneous endoscopic gastrostomy on gastro-esophageal reflux in mechanically-ventilated patients

Emmanuel E Douzinas, Andreas Tsapalos, Antonios Dimitrakopoulos, Evanthia Diamanti-Kandarakis, Alexandros D Rapidis, Charis Roussos

Emmanuel E Douzinas, Andreas Tsapalos, Charis Roussos, Department of Critical Care, Evangelismos Hospital, University of Athens, Medical School, Athens 10675, Greece
Antonios Dimitrakopoulos, Eginition Hospital, University of Athens, Medical School, Athens 11528, Greece
Evanthia Diamanti-Kandarakis, 1st Department of Internal Medicine, Laiko Hospital, University of Athens, Medical School, Athens 115 27, Greece
Alexandros D Rapidis, Head and Neck Unit, Greek Anticancer Institute, Athens 11522, Greece
Correspondence to: Emmanuel E Douzinas, Department of Critical Care, Evangelismos Hospital, Athens 10675 Greece. edouzin@cc.uoa.gr
Telephone: +30-210-7243320
Received: 2005-05-26 Accepted: 2005-07-27

Abstract

AIM: To investigate the effect of percutaneous endoscopic gastrostomy (PEG) on gastroesophageal reflux (GER) in mechanically-ventilated patients.

METHODS : In a prospective, randomized, controlled study 36 patients with recurrent or persistent ventilator-associated pneumonia (VAP) and GER > 6% were divided into PEG group ($n = 16$) or non-PEG group ($n = 20$). Another 11 ventilated patients without reflux (GER < 3%) served as control group. Esophageal pH-metry was performed by the "pull through" method at baseline, 2 and 7 d after PEG. Patients were strictly followed up for semi-recumbent position and control of gastric nutrient residue.

RESULTS: A significant decrease of median (range) reflux was observed in PEG group from 7.8 (6.2 - 15.6) at baseline to 2.7 (0 - 10.4) on d 7 post-gastrostomy ($P < 0.01$), while the reflux increased from 9 (6.2 - 22) to 10.8 (6.3 - 36.6) ($P < 0.01$) in non-PEG group. A significant correlation between GER (%) and the stay of nasogastric tube was detected ($r = 0.56$, $P < 0.01$).

CONCLUSION: Gastrostomy when combined with semi-recumbent position and absence of nutrient gastric residue reduces the gastroesophageal reflux in ventilated patients.

Key words: Nasogastric tube; Gastroesophageal reflux; Semi-recumbency; Gastric residue;Percutaneous endoscopic gastrostomy.

Douzinas EE, Tsapalos A, Dimitrakopoulos A, Diamanti-Kandarakis E, Rapidis AD, Roussos C. Effect of percutaneous endoscopic gastrostomy on gastro-esophageal reflux in mechanically-ventilated patients. *World J Gastroenterol* 2006; 12(1): 114-118

http://www.wjgnet.com/1007-9327/12/114.asp

INTRODUCTION

Life threatening nosocomial pneumonia secondary to aspiration of gastric contents is frequent in intubated mechanically-ventilated patients[1,2]. A number of causes have been implicated in the development of ventilator-associat-ed pneumonia (VAP) during mechanic-al ventilation, namely the oropharyngeal colonization[2], body position[3], nasogastric tube (NGT)[4], and its size[5]. Supine position and the length of patient's permanence in this position are other potential risk factors for aspiration[3]. Though the semi-recumbent position reduces the risk of pulmonary aspiration, gastroesophageal reflux (GER) is still possib e[6,7]. However, GER occurs with a significantly higher incidence in semi-recumbent mechanically-ventilated patients with NGT than without (74% *vs* 35%)[8]. According to other studies[9,10], large-bore tubes do not cause more reflux than small-bore ones.

If the duration of nasogastric intubation correlates with the degree of GER, NGT removal after gastrostomy should normally decrease both GER and aspiration pneumonia rates. Percutaneous endoscopic gastrostomy (PEG), however, is considered neither as a non-pharmacological measure for the prevention of VAP, nor as an adjunctive measure to its treatment because it elicits an increase in GER, aspiration, and incidence of pneumonia, at least in the early post-gastrostomy period[8,11]. Nevertheless, in these studies the body position was not specified and the gastric content was not controlled after gastrostomy.

The aim of the present study was to investigate if long-standing presence of NGT for feeding is associated with increased incidence of GER and if PEG combined with semi-recumbent position and avoidance of gastric nutrient retention lead to decreased incidence of GER in mechanically-ventilated patients.

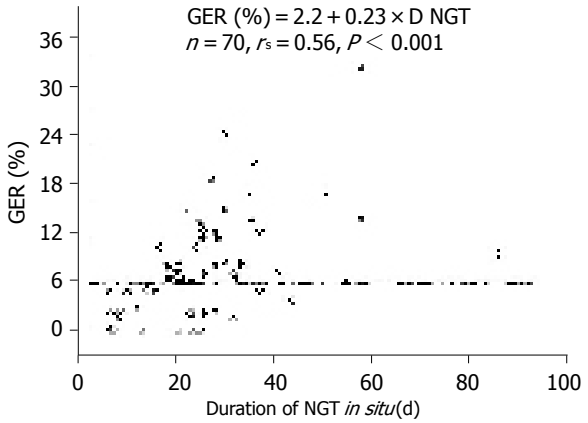


Figure 1 Correlation between duration of NGT and mechanical ventilation standing period to the GER rate measured in 70 patients.

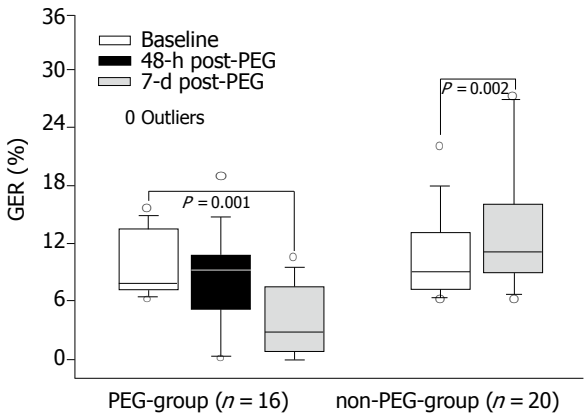


Figure 2 Variation of the median GER (%) together with 10%, 25%, 75%, and 90% from pH-metries originated from the sensor located at 20 cm proximally to the lower esophageal sphincter. The symbol in the 48-h period is lacking in patients of non-PEG-group since no pH-metry was performed in that period in this group of patients.

Table 1 Characteristics of patients in the study [median (range)]

	PEG (*n* = 16)	Non-PEG (*n* = 20)	Control (*n* = 11)	*P*
Age	53 (20-82)	58 (25-85)	56 (34-76)	NS
Sex (M/F)	10/6	12/8	7/4	NS
Weight (kg)	75 (55-85)	79 (56-95)	83(68-90)	NS
APACHE II	17 (12-23)	17 (9-28)	15 (5-26)	
Primary disease				
Head injury	7	5	1	
Spinal cord injury	3	4	-	
Multiple trauma	5	8	3	NS
Stroke	4	2	5	
COPD	5	4	3	
Post-operative resuscitation	4	3	-	
Days of MV and NGT	25 (19-36)	27 (17-56)	24 (12 – 32)	NS
Days of VAP before study	14 (8-19)	13 (4-36)	-	NS

MATERIALS AND METHODS

Pilot study

Simultaneous measurement of gastric and esophageal pH was performed during a 24-h period[12] in 23 adult intensive care patients, who were mechanically-ventilated and had a NGT in place for a duration of 3-90 d. Exclusion criteria from the study included unstable hemodynamic state, administration of morphine, atropine, theophylline, barbiturates, and cisapride, and a past history of GER or hiatal hernia. GER was expressed as percentage of the time when the esophageal pH was less than 4 in a given 24-h period of time GER (%). Values of GER less than 3% were considered as normal. In these 23 patients, there was a positive correlation of the duration of nasogastric intubation in mechanically-ventilated patients to the degree of GER (%) ($r = 0.78$, $P < 0.01$). GER mainly occurred after a 10-d period of NGT and mechanical ventilation. Based on these results, only patients who were on mechanical ventilation with NGT for more than 10 d were enrolled in the main study.

Main study and patient selection

The institutional review board approved the study protocol and informed consent was obtained from patient's kin in each case. Over a 28-mo period, 39 consecutive mechanically-ventilated patients with NGT in place, suffering from persistent or recurrent VAP and presenting a GER above 6% constituted the study population. The diagnosis of persistent or recurrent VAP was established according to the criteria previously described[13,14]. The exclusion criteria mentioned in the pilot study were maintained. Nineteen patients were randomly allocated to receive PEG, but three among them were excluded because of hiatal hernia (2 cases) and intestinal bloating (1 case). Finally, 16 patients received PEG (PEG group) and 20 did not (non-PEG group). In the non-PEG group patients the eventual presence of hiatal hernia was possibly missed, since no endoscopy was performed for PEG placement. Twelve patients in the PEG group and 15 patients in the non-PEG group presented persistent pneumonia. The rest of the patients in both groups had recurrent pneumonia. Eleven mechanically-ventilated patients with acute respiratory failure and NGT for more than 15 d and GER lower than 3% were used as controls. Patients enrolled in the study had comparable severity scores of VAP or acute respiratory failure, radiographic scores and ventilation parameters (data not shown). The characteristics of patients are presented in (Table 1).

Gastrostomy was performed using the pull through (Ponsky) technique[15] after feeding was stopped for 24 h. Patients of all groups were on continuous drip NGT or PEG-feeding at 60 mL/h with a polymeric diet of energy content of 1 000 kcal/L. Thereafter, they were placed in a semi-recumbent position (30°) and the volume of the gastric nutrient residue was measured with a syringe at 8-h intervals. If the nutrient volume exceeded 200 mL, feeding was withheld and restarted when the volume decreased. These two measures were followed for the subsequent 20-d period during which conventional treatment for pneumonia was applied.

All pH-metries were performed on a 24 h basis. Baseline pH-metries and those performed on d 7 in non-PEG patients were carried out as follows: with the patient in semi-recumbent position a single crystal antimony multi use electrode was used which disposes three sensors

located at the tip, 15 and 30 cm, respectively connected to a portable recorder (Digitrapper Mk III, Synectics Medical AB, Stockholm, Sweden). The electrode had a diameter of 2.1 mm at the level of the sensors and was attached via an adhesive material to a new 14 bore NGT so that the distal pH-meter sensor corresponded at 10 cm proximally to the tip of the NGT. In this way, reflux associated with the presence of NGT could be detected. An *in vitro* calibration of the whole system was carried out with buffer solutions of pH 1 and pH 7 before each pH-metry.

After ordinary NGT removal, the new NGT with the sensor probe attached was introduced via the nose into the stomach until acid pH was recorded with the distal and middle sensors as previously described[12]. The electrode was then slowly withdrawn until the middle sensor channel detected a sudden pH change from acid (< 4) to above 5. The electrode was then withdrawn for further 5 cm. In this way, the distal sensor was located into the stomach and the middle and proximal sensors were located at 5 and 20 cm above the lower esophageal sphincter, respectively. The correct positioning of the electrode was verified at the end of pH-metry and before its withdrawal by a chest x-ray. The recording device measured pH values every 4 s and stored the mean of 20 values every 80 s.

Patients received sucralfate, 2 g twice daily via NGT at least 72 h before the study for gastric mucosa protection. Antacids, H-2 blocking agents or omeprazole that could interfere with pH neutralization were not used and for the same reason feeding was stopped 6 h before and during the period of pH-metry. Patients were not sedated nor paralyzed.

In PEG group patients, two additional pH-metries were carried out: the first at an early (48 h) post-PEG period and the second at a late (7 d) post-PEG period. The first pH-metry investigated the described increased incidence of reflux and/or aspiration at that period[8]. The second pH-metry also performed in the non-PEG group of patients was carried out in order to estimate and evaluate the effectiveness of PEG on reflux. These additional pH-metries were performed in the same manner with the following modifications: a two-sensor probe was used and positioned in such a way that the distal and proximal sensors were located at 5 and 20 cm over the lower esophageal sphincter, respectively. With the two-sensor probe the lower esophageal sphincter was free from the presence of any catheter. Patients were fed through PEG and the degree of reflux was assessed in the absence of NGT.

PEG was arbitrarily considered effective if within a 7-d post PEG period the GER (%) decreased by more than 60% compared to the pre-PEG value? PEG and non-PEG patients were followed up for 20 d for pneumonia healing and all patients for weaning from mechanical ventilation and intensive care discharge.

Statistical analysis

To evaluate the differences in GER (%) between the three study periods (pre-PEG *vs* early and late post-PEG) in each group, Wilcoxon's signed-rank test was used. To assess the changes in the number of days of mechanical ventilation and NGT standing, pneumonia occurrence before PEG, weaning after PEG, days from PEG to discharge between the two groups, Wilcoxon's rank sum test was performed. Spearman's *r* was used to detect if there was any correlation between the standing-period of NGT and GER (%). Statistical significance was defined as a *P* value of 0.05 or less.

RESULTS

The correlation between GER (%) and duration of NGT permanence in the 23 patients from the pilot study along with the values obtained from the first pH-metry in the 47 patients of the main study is shown in Figure 1. After 20 d of NGT in situ, 38 out of 50 patients (76%) presented a reflux rate of above 6% while all the 14 patients with NGT in situ for less than 15 d had a reflux rate of less than 6%.

The pH-metry from the lower esophageal sensor consistently recorded 15 - 20% higher GER than that from the upper sensor. However, since the importance of reflux detection in the upper part of esophagus is greater in relation to aspiration, only data from the upper sensor were presented. The median (range) GER (%) in the PEG group was 7.8 (6.2 - 15.6) at baseline. Forty-eight hours post-PEG, though there was no significant change in the median value [8.7 (0.1 - 19)], an increase in GER (%) was observed in 5 out of 16 patients. On d 7, post-PEG, GER (%) decreased to 2.7 (0 - 10.4) ($P < 0.01$). In contrast, in the non-PEG group, GER (%) increased from 9 (6.2 - 22) at baseline to 10.8 (6.3 - 36.6) on d 7 ($P < 0.01$) (Figure 2).

In the following 20-d period, the weaned, discharged, and died patients were respectively 11 and 4 ($P = 0.006$), 10 and 2 ($P < 0.01$), and 3 and 5 in PEG and non-PEG groups. The respective values for patients in the control group were 8, 8, and 1. The outcome of the PEG-group was similar to that of the control group.

DISCUSSION

This study showed that the degree of GER correlated with the duration of NGT *in situ*. Removal of NGT and feeding through PEG with the patients in semi-recumbent position along with the volume control of the nutrient gastric residue resulted in a decrease or even elimination of the reflux in almost two-thirds of the patients. Additionally, PEG seemed to exert a favorable effect on pneumonia healing rate, weaning period, and patient discharge from ICU.

There are several reasons for frequent GER occurrence in the critically ill patients. Drugs depressing the function of lower esophageal sphincter and/or delaying gastric emptying, such as morphine, atropine, theophylline, and barbiturates, are frequently administered in the critically ill patients[16]. The presence of NGT is an important cause of reflux, since it may induce lower esophageal sphincter relaxation[17]. In patients undergoing elective laparotomy and nasogastric intubation, a significant increase in the mean number of reflux episodes has been observed during the perioperative period in contrast to those without NGT (137 *vs* 8, $P < 0.01$)[18]. The mean lower esophageal sphincter pressures are also lower in the same patients, and reflux occurs within 24 h after NGT insertion at the

induction of anesthesia. Similarly, in patients undergoing cardiac surgery with simultaneous esophageal and tracheal pH-metry, reflux is seen more frequently in those with a NGT in place than in those without ($P < 0.001$)[19].

The above observations lead to the notion that NGT removal after PEG should theoretically eliminate or decrease the incidence of GER. However, data in the literature paradoxically support the opposite. There is evidence that PEG or percutaneous endoscopic jejunostomy provides no benefit in terms of reflux and/or incidence of aspiration pneumonia[8,11,19-23]. In a study of 64 patients, 9 developed aspiration pneumonia within 3 d of the procedure and four died[8]. Aspiration occurred in 11% of 79 patients with either neurologic disorders or cancer, whose PEG or percutaneo-us endoscopic jejunostomy was performed[11]. In another study comprising 20 malnourished patients, aspiration was the most common adverse event following percutaneous endoscopic jejunostomy, accounting for 50% of deaths[20]. Similarly, GER seems to be a frequent disorder following PEG in children[21-23]. Yet, in 58 neurologically disabled patients who had clinical evidence of aspiration pneumonia, 17 demonstrated pneumonia after gastrostomy[24]. As a result of the negative outcomes, the use of PEG has declined during recent years. However, in all these studies the body position following PEG was not reported and the volume of gastric nutrient residue was not controlled.

The positive results found in our PEG group compared to those reported in the literature have to be attributed to the volume control of the nutrient gastric residue and the semi-recumbent position during the whole 20-dobservation period. The semi-recumbent position has been previously shown to reduce aspiration of gastric contents[6]. Gastric distension is also an important cause of lower esophageal sphincter transient relaxation, thus permitting GER[25]. It seems that owing to gravity, semi-recumbency, and avoidance of gastric retention prevent reflux of gastric juice into the esophagus.

The results of the present study describe the pathophysiological sequence of PEG-induced lower esophageal sphincter restoration. In 11/16 patients in the PEG group, the reflux rate did not decrease during the first 48 post-PEG hours. Moreover, in 5 of them GER (%) increased in this period. These results indicate that "PEG was arbitrarily considered effective if within a 7-day post PEG period the GER (%) decreased by more than 60% compared to the pre-PEG value". Thus, a period of at least 7 d seems to be required after NGT removal before lower esophageal sphincter returns to its normal function. The same findings also provide an explanation for the high incidence of aspiration pneumonia encountered during the early post-PEG period[7,8,17,18].

The role of GER in the pathogen-esis of VAP is still controversial[26-28]. All patients in the present study suffering from persistent or recurrent pneumonia were severely ill and failed to respond to the usual therapeutic measures. However, reflux was eliminated in 10 out of 16 patients undergoing PEG who weaned from the respirator and discharged from ICU within the 20-d period of observation. In contrast, among the 20 non-PEG patients reflux increased through time and only 4 and 2 patients were weaned and discharged, respectively. The curative rate of PEG-group was similar to that of control group with the GER being lower than 3%. Among the 11 patients with GER < 3% only one presented VAP. Therefore, it is concluded that GER is implicated in the pathogenesis of VAP and the elimination of reflux results in a more favorable outcome, possibly because the repetitive instillation of infective materials to the trachea that occurs during reflux, is halted.

In conclusion, NGT presence seems to promote reflux of gastric contents, resulting in aspiration and/or pneumonia. The NGT replacement by PEG combined with semi-recumbent position and control of gastric residue can decrease GER in the majority of patients. However, there is persistence of GER and aspiration in some patients, which may be due to the functional alteration of the lower esophageal sphincter rather than ineffectiveness of PEG *per se*. Gastrostomy combined with semi-recumbency and control of gastric residue should be taken into consideration for the effective management and prevention of VAP.

ACKNOWLEDGMENT

Dr J. Milic-Emili for his editorial assistance and useful criticism as well as L. Haziroglou, BSc, for language review.

REFERENCES

1 **Kingston GW**, Phang PT, Leathley MJ. Increased incidence of nosocomial pneumonia in mechanically ventilated patients with subclinical aspiration. *Am J Surg* 1991; **161**: 589-592
2 **Pingleton SK**, Hinthorn DR, Liu C. Enteral nutrition in patients receiving mechanical ventilation. Multiple sources of tracheal colonization include the stomach. *Am J Med* 1986; **80**: 827-832
3 **Torres A**, Serra-Batlles J, Ros E, Piera C, Puig de la Bellacasa J, Cobos A, Lomeña F, Rodríguez-Roisin R. Pulmonary aspiration of gastric contents in patients receiving mechanical ventilation: the effect of body position. *Ann Intern Med* 1992; **116**: 540-543
4 **Bullock TK**, Waltrip TJ, Price SA, Galandiuk S. A retrospective study of nosocomial pneumonia in postoperative patients shows a higher mortality rate in patients receiving nasogastric tube feeding. *Am Surg* 2004; **70**: 822-826
5 **Ibáñez J**, Peñafiel A, Marsé P, Jordá R, Raurich JM, Mata F. Incidence of gastroesophageal reflux and aspiration in mechanically ventilated patients using small-bore nasogastric tubes. *JPEN J Parenter Enteral Nutr* 2000; **24**: 103-106
6 **Orozco-Levi M**, Torres A, Ferrer M, Piera C, el-Ebiary M, de la Bellacasa JP, Rodriguez-Roisin R. Semirecumbent position protects from pulmonary aspiration but not completely from gastroesophageal reflux in mechanically ventilated patients. *Am J Respir Crit Care Med* 1995; **152**: 1387-1390
7 **Ibáñez J**, Peñafiel A, Raurich JM, Marse P, Jordá R, Mata F. Gastroesophageal reflux in intubated patients receiving enteral nutrition: effect of supine and semirecumbent positions. *JPEN J Parenter Enteral Nutr* 1992; **16**: 419-422
8 **Jarnagin WR**, Duh QY, Mulvihill SJ, Ridge JA, Schrock TR, Way LW. The efficacy and limitations of percutaneous endoscopic gastrostomy. *Arch Surg* 1992; **127**: 261-264
9 **Dotson RG**, Robinson RG, Pingleton SK. Gastroesophageal reflux with nasogastric tubes. Effect of nasogastric tube size. *Am J Respir Crit Care Med* 1994; **149**: 1659-1662
10 **Ferrer M**, Bauer TT, Torres A, Hernández C, Piera C. Effect of nasogastric tube size on gastroesophageal reflux and microaspiration in intubated patients. *Ann Intern Med* 1999;

130: 991-994
11 **Kadakia SC**, Sullivan HO, Starnes E. Percutaneous endoscopic gastrostomy or jejunostomy and the incidence of aspiration in 79 patients. *Am J Surg* 1992; **164**: 114-118
12 **Schindlbeck NE**, Heinrich C, König A, Dendorfer A, Pace F, Müller-Lissner SA. Optimal thresholds, sensitivity, and specificity of long-term pH-metry for the detection of gastroesophageal reflux disease. *Gastroenterology* 1987; **93**: 85-90
13 **Malangoni MA**. Single versus combination antimicrobial therapy for ventilator-associated pneumonia. *Am J Surg* 2000; **179**: 58S-62S
14 **Bonten MJ**, Bergmans DC, Ambergen AW, de Leeuw PW, van der Geest S, Stobberingh EE, Gaillard CA. Risk factors for pneumonia, and colonization of respiratory tract and stomach in mechanically ventilated ICU patients. *Am J Respir Crit Care Med* 1996; **154**: 1339-1346
15 **Ponsky JL**, Gauderer MW, Stellato TA. Percutaneous endoscopic gastrostomy. Review of 150 cases. *Arch Surg* 1983; **118**: 913-914
16 **Ogorek CP**, Cohen S. Gastroesophageal reflux disease: new concepts in pathophysiology. *Gastroenterol Clin North Am* 1989; **18**: 275-292
17 **Nagler R,** Spiro HM. Persistent gastrroesophaged reflux induced during prolonged gastric intubation. *N Engl J Med* 1963; **269**: 495-500
18 **Manning BJ**, Winter DC, McGreal G, Kirwan WO, Redmond HP. Nasogastric intubation causes gastroesophageal reflux in patients undergoing elective laparotomy. *Surgery* 2001; **130**: 788-791
19 **Russell GN**, Yam PC, Tran J, Innes P, Thomas SD, Berry PD, Fox MA, Fabri BM, Jackson M, Weir WI. Gastroesophageal reflux and tracheobronchial contamination after cardiac surgery: should a nasogastric tube be routine? *Anesth Analg* 1996; **83**: 228-232
20 **DiSario JA**, Foutch PG, Sanowski RA. Poor results with percutaneous endoscopic jejunostomy. *Gastrointest Endosc* 1990; **36**: 257-260
21 **Grunow JE**, al-Hafidh A, Tunell WP. Gastroesophageal reflux following percutaneous endoscopic gastrostomy in children. *J Pediatr Surg* 1989; **24**: 42-4; Discussion 44-5
22 **Mollitt DL**, Golladay ES, Seibert JJ. Symptomatic gastroesophageal reflux following gastrostomy in neurologically impaired patients. *Pediatrics* 1985; **75**: 1124-1126
23 **Berezin S**, Schwarz SM, Halata MS, Newman LJ. Gastroesophageal reflux secondary to gastrostomy tube placement. *Am J Dis Child* 1986; **140**: 699-701
24 **Hassett JM**, Sunby C, Flint LM. No elimination of aspiration pneumonia in neurologically disabled patients with feeding gastrostomy. *Surg Gynecol Obstet* 1988; **167**: 383-388
25 **Coben RM**, Weintraub A, DiMarino AJ, Cohen S. Gastroesophageal reflux during gastrostomy feeding. *Gastroenterology* 1994; **106**: 13-18
26 **Ewig S**, Torres A. Prevention and management of ventilator-associated pneumonia. *Curr Opin Crit Care* 2002; **8**: 58-69
27 **Kollef MH**. The prevention of ventilator-associated pneumonia. *N Engl J Med* 1999; **340**: 627-634
28 **Fiddian-Green RG**, Baker S. Nosocomial pneumonia in the critically ill: product of aspiration or translocation? *Crit Care Med* 1991; **19**: 763-769

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M and Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 119-122
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Self-expandable metallic stents for malignant biliary obstruction: Efficacy on proximal and distal tumors

Jui-Hao Chen, Cheuk-Kay Sun, Chao-Sheng Liao, Chain-Smoke Chua

Jui-Hao Chen, Cheuk-Kay Sun, Chao-Sheng Liao, Chain-Smoke Chua, Department of Gastroenterology , Shin-Kong Wu-Ho-Su Memorial Hospital, Taipei, Taiwan
Correspondence to: Jui-Hao Chen, Department of Gastroenterology, Shin-Kong Wu-Ho-Su Memorial Hospital, 95 Weng Chang Road, Shih-Lin District,
Taipei, Taiwan. m000723@ms.skh.org.tw
Telephone: +886-2-28332211 Fax: +886-2-28389335
Received: 2005-05-25 Accepted: 2005-07-01

Abstract

AIM: To compare the efficacy of self-expandable metallic stents (EMS) in the treatment of distal and proximal stricture of malignant biliary tumors.

METHODS: From March 1995 to June 2004, 61 patients (40 males, 21 females) with malignant biliary obstruction who received self-expandable metallic stent implantation were reviewed retrospectively. The stents were inserted by an endoscopic or percutaneous transhepatic method. We tried to place two stents in the biliary system in T or Y configuration in cases of hilar tumors with bilateral hepatic duct obstruction. The end points of the study were stent occlusion or patient death.

RESULTS: The mean time of stent patency was 421 ± 67 d in the group of proximal stricture(group I) and 168 ± 18 d in the group of distal stricture (group II). The difference was significant in borderline between the two groups ($P = 0.0567$). The mean survival time was 574 ± 76 d in group I and 182 ± 25 d in group II. There was a significant difference between the two groups ($P = 0.0005$).

CONCLUSION: EMS implantation is a feasible, palliative method for unresectable malignant biliary obstruction. The clinical efficacy of EMS in patients with proximal hilar tumors is better than that in patients with distal tumors.

Key words: Metallic stent; Biliary malignancy

Chen JH, Sun CK, Liao CS, Chua CS. Self-expandable metallic stents for malignant biliary obstruction: Efficacy on proximal and distal tumors. *World J Gastroenterol* 2006; 12(1): 119-122

http://www.wjgnet.com/1007-9327/12/119.asp

INTRODUCTION

Biliary stent placenrent is the treatment of choice for malignant biliary obstruction caused by unresectable neoplasms[1,2].Although self-expandable metallic stents (EMS) are much more expensive than plastic stents, EMS is claimed to be superior to plastic stents in long-term stent patency[3]. At first, when EMS is uncovered, the tumor often invades the stent via meshes of the metallic stent, resulting in stent obstruction[1]. To overcome the problem of tumor ingrowth in uncovered metallic stents, covered EMS have been developed in the 1990s[1,4,5]. However, complications of covered EMS, such as cholecystitis and pancreatitis, should be noted[1,5].

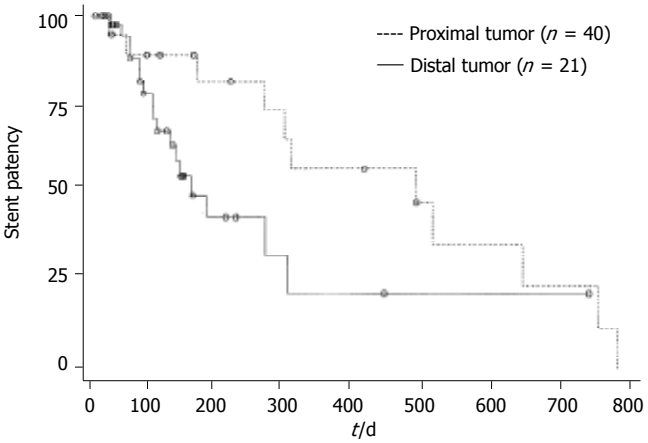
Uncovered EMS are introduced into Taiwan in the 1990s to overcome the weak points of plastic stents [2]. In our hospital, we bave begun to use uncovered EMS for the treatment of unresectable malignant biliary obstruction since 1995 and covered EMS in selective cases since 2002.

Lee *et al* [6] found that the clinical efficacy of EMS in patients with hilar tumor is superior in those with common bile duct obstruction. Rieber and Brambs[7] demonstrated that worse results are seen in patients with pancreatic tumors and with lymph nodes metastases of the colon and gastric cancers. We have found similar trends in our practice. Therefore, we performed this study to compare the efficacy of EMS in the treatment of distal and proximal stricture of malignant biliary tumors.

MATERIALS AND METHODS

From March 1995 to June 2004, 61 patients (40 males, 21 females) with malignant biliary obstruction who received EMS implantation were reviewed retrospectively. Neoplasms were unresectable and the diagnosis was based on pathological examination or clinical and imaging findings.

The patients received endoscopic retrograde cholangiopancreaticography (ERCP) or percutaneous transhepatic cholangiography (PTC) initially. Plastic stent drainage, nasobiliary drainage or PTCD was set up. when the neoplasms were cnfirmed to be unresectable, the patients were assigned to insertion of EMS if they agreed. Wallstent (Schneider, Switzerland) and Ultraflex diamond stent (Microvasive; Boston Scientific Corporation, MA, USA) were used in our patients. EMS were inserted either by therapeutic duodenoscopy (TJF 200, Olympus, Tokyo, Japan) or by the percutaneous transhepatic approach. We tried to place two stents in



Figuer 1 Kaplan-Meier graph showing cumulative stent patency. The difference was borderline significant between the two groups.

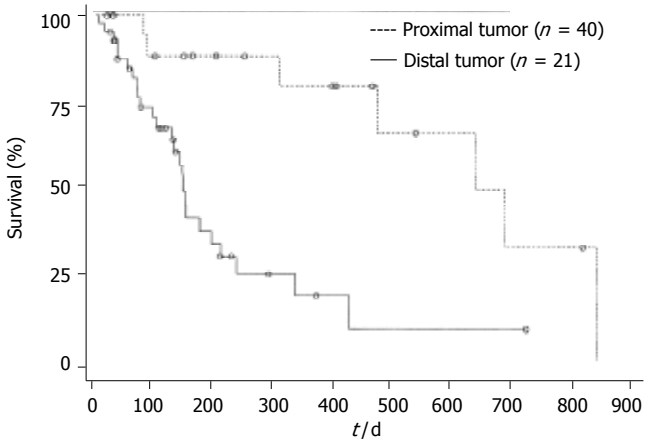


Figure 2 Kaplan-Meier graph showing survival of the patients. There was a significant difference observed between the proximal tumor and distal tumor.

the biliary system in T or Y configuration in cases of hilar tumors with bilateral hepatic duct obstruction. Covered EMS were inserted only in patients with distal stricture.

The lesions were defined as distal stricture if the tumors were located at or below the orifice of the cystic duct. The lesions were defined as proximal stricture if the tumors were located above the orifice of the cystic duct.

Stent occlusion was defined as recurrence of jaundice or cholangitis with evidence of stent stenosis requiring biliary intervention after successful insertion of EMS. The stent patency period was calculated as the time between stent placement and its occlusion or patient death. Cumulative stent patency and patient survival were evaluated by the Kaplan-Meier technique. The end points of the study were stent occlusion or patient death.

RESULTS

Patient enrollment and characteristics

Sixty-one patients were enrolled in this study. The patients were divided into two groups according to their obstruction level.

Group I (21 patients) was consisted of proximal stricture patients. The obstruction level was above the orifice of the cystic duct. The group included 19 patients with hilar cholangiocarcinoma and two patients with hepatocellular carcinoma. Twelve patients with hilar cholangiocarcinoma received two stents (in T or Y configuration) for drainage of bilateral hepatic ducts.

Group II (40 patients) was consisted of distal stricture patients. The obstruction level was at or below the orifice of the cystic duct. The group included 9 patients with cholangiocarcinoma, 17 patients with pancreatic cancers, 3 patients with ampulla of Vater cancers, 2 patients with gall bladder cancers, and 9 patients with lymph node metastases of colon cancer (2/9), gastric cancer (3/9), lung cancer (1/9), nasopharyngeal cancer (1/9), hepatocellular cancer (1/9) and laryngeal cancer (1/9).

Eight patients in group I (8/21) and 24 patients (24/40) in group II died at the time of evaluation. Covered EMS were inserted in seven patients with distal stricture and the other 53 patients received uncovered stents.

If stent stenosis was noted during follow-up, either a second EMS (six patients), or a plastic stent through an original EMS (three patients) or PTCD (one patient) or nasobiliary drainage (three patients) was set up. However, some patients chose conservative treatment after stent occlusion.

Stent patency and survival

The mean time of stent patency was 421 ± 67 d in group I and 168 ± 18 d in group II. The difference was significant in borderline between the two groups ($P = 0.0567$). The mean survival time was 574 ± 76 d in group I and 182 ± 25 d in group II. There was a significant difference between the two groups ($P = 0.0005$). Cumulative stent patency and patient survival according to the Kaplan-Meier life table are shown in Figures 1 and 2.

Early complications

Early complications were defined as "complications occurring within 30 d after EMS placement". Nine cases had early complications. Seven of them belonged to distal stricture and two belonged to proximal stricture. The clinical features of early complications are listed in Table 1.

Late complications

Late complications were defined as "complications occurring after 30 d of EMS placement". A patient with hilar cholangiocarcinoma suffered from common bile duct stones 175 d after stent placement. Endoscopic sphinterotomy was performed and the stones were extracted. Gallbladder empyema was in two patients. One of them received covered EMS due to cholangiocarcinoma near the orifice of the cystic duct and symptoms occurred 66 d after stent placement. The other patient received uncovered EMS due to hilar cholangiocarcinoma and symptoms occurred 37 d after stent placement. Percutaneous transhepatic gallbladder drainage (PTGBD) relieved their symptoms. Three patients with pancreatic cancers suffered from gastric outlet obstruction (on days 80, 93 and 270 respectively) due to tumor invasion into the duodenum. Bypass surgery relieved their outlet

Table 1 Early complications after insertion of metallic stents

Case	Complications	Type of stent	Timing of complication	Management	Result
1	Acute pancreatitis	Covered stent	Immediately	Conservative	Recovered
2	Acute pancreatitis	Covered stent	Immediately	Conservative	Recovered
3	Acute pancreatitis with pseudocyst	Uncoverd stent	Immediately	Percutaneous catheter drainage	Recovered
4	Inadequate expansion of stent	Uncoverd stent	3 d	Balloon dilatation	Good
5	Inadequate expansion of stent	Uncoverd stent	3 d	Balloon dilatation	Good
6	Acute cholangitis without Stent stenosis	Uncoverd stent	22 d	Antibiotics	Recovered
7	Peritonitis	Uncoverd stent	30 d	Antibiotics	Recovered
8	Stent occlusion	Uncoverd stent	22 d	PTCD	Good
9	Subcapsular liver abscess	Uncoverd stent	1 d	Percutaneous catheter drainage	Recovered

Case 1-7: distal stricture.
Case 8-9: proximal stricture.

obstructions.

Complications of covered EMS

It seemed that more complications occurred in patients who received covered EMS. However, we could not arrive at any final conclusion due to the limited number of cases in our series. Acute pancreatitis occurred immediately after stent placement in two cases (2/8). Fortunately, they recovered uneventfully after conservative treatment. Stent migration (1/8) was found in a patient with an ampulla of Vater tumor 85 d after stent placement. He received conservative treatment only because of tumor infiltration in the entire second portion of the duodenum and the patient expired soon after. One patient developed gallbladder empyema (1/8) 66 d after stent placement.Her symptoms were relieved after PTGBD.

DISCUSSION

Endoscopic or percutaneous transhepatic stentplacement in the biliary tree has become a main stream in the treatment of inoperable malignant obstructive jaundice[1]. The major drawback of plastic stents is early stent clogging and migration in spite of various modifications in the design[2,3]. The use of EMS apparently improves the weak points of plastic stents. Although EMS is much more expensive than plastic stents, it is a cost-effective strategy [3,8]. EMS improves patient compliance due to prolonged stent patency and less complications[3].

According to Lee *et al*[6], patients with hilar obstruction have better clinical efficacy than those with common bile duct obstruction. In our study, we demonstrated similar results. Stent patency and patient survival were better in our patients with proximal stricture than in those with distal stricture. Twelve of 21 patients with proximal stricture received bilateral biliary drainage in our series. If one of the two stents were occluded, jaundice would rarely develop. However, stent occlusion would cause immediate jaundice in distal strictures.

Our study demonstrated that most of early complications were related to the effect of stents or manipulation procedures. Acute pancreatitis(3/9) might be due to occlusion of the pancreatic duct by covered stents or secondary to the ERCP procedure. Liver abscess (1/9) might be due to the contamination of the procedure. The inadequate expansion of EMS (2/9) might be due to poor function of the metallic wires.

Most late complications were related to tumor progression. The first case with gallbladder empyema in our study might be due to the dual effects of covered stents and tumor progression. The second case with gallbladder empyema might be due to tumor progression with cystic duct occlusion. The gastric outlet obstruction in patients with pancreatic cancers was, surely due to tumor extension. Almost all stenoses of the stent and/or cholangitis are caused by tumor growth with occluded ducts, but cholangitis unrelated to stent occlusion can be noted [11].

Although the patency of EMS is longer, there are many drawbacks after their placement, such as tumor ingrowth or overgrowth, mucosa hyperplasia induced by chronic inflammatory reaction to the stent meshes, biliary sludge and food impaction in transpapillary stents[9]. Covered stents are significantly superior to uncovered stents by preventing tumor ingrowth[1,4,5]. However covered stents are risky for occlusion of branch ducts (such as side branches of bile ducts, cystic ducts or pancreatic ducts), stent migration and sludge formation. Only eight of our patients with distal stricture received covered stents, and complications occurred in four of eight. Complications included acute pancreatitis (2/8), gallbladder empyema (1/8) and stent migration (1/8). A higher rate of migration is another possible disadvantage of covered stents[10]. Due to the limited number of covered stents in our series, further studies are needed to determine the frequency of side effects in covered stents.

Because of the high cost of EMS, selection of patients and types of stents are important. Life expectancy shorter than 6 mo[8] or tumors with liver metastases[12] are not cost-effective for EMS placement. Although many

types of EMS are now available, which type can best improve the cost-effectiveness and quality of life remains unknown[13-16].

In conclusion, EMS implantation is a feasible, palliative method for unresectable malignant biliary obstruction. The clinical efficacy of EMS in patients with proximal hilar tumors is superior to that in patients with distal tumors. Covered EMS is risky in regard to the complications due to pancreatitis although stent patency may be longer.

REFERENCES

1 **Isayama H**, Komatsu Y, Tsujino T, Sasahira N, Hirano K, Toda N, Nakai Y, Yamamoto N, Tada M, Yoshida H, Shiratori Y, Kawabe T, Omata M. A prospective randomised study of "covered" versus "uncovered" diamond stents for the management of distal malignant biliary obstruction. *Gut* 2004; **53**: 729-734

2 **Tsai CC**, Mo LR, Lin RC, Kuo JY, Chang KK, Yeh YH, Yang SC, Yueh SK, Tsai HM, Yu CY. Self-expandable metallic stents in the management of malignant biliary obstruction. *J Formos Med Assoc* 1996; **95**: 298-302

3 **Schmassmann A**, von Gunten E, Knuchel J, Scheurer U, Fehr HF, Halter F. Wallstents versus plastic stents in malignant biliary obstruction: effects of stent patency of the first and second stent on patient compliance and survival. *Am J Gastroenterol* 1996; **91**: 654-659

4 **Thurnher SA**, Lammer J, Thurnher MM, Winkelbauer F, Graf O, Wildling R. Covered self-expanding transhepatic biliary stents: clinical pilot study. *Cardiovasc Intervent Radiol* 1996; **19**: 10-14

5 **Bezzi M**, Zolovkins A, Cantisani V, Salvatori FM, Rossi M, Fanelli F, Rossi P. New ePTFE/FEP-covered stent in the palliative treatment of malignant biliary obstruction. *J Vasc Interv Radiol* 2002; **13**: 581-589

6 **Lee BH**, Choe DH, Lee JH, Kim KH, Chin SY. Metallic stents in malignant biliary obstruction: prospective long-term clinical results. *AJR Am J Roentgenol* 1997; **168**: 741-745

7 **Rieber A**, Brambs HJ. Metallic stents in malignant biliary obstruction. *Cardiovasc Intervent Radiol* 1997; **20**: 43-49

8 **Arguedas MR**, Heudebert GH, Stinnett AA, Wilcox CM. Biliary stents in malignant obstructive jaundice due to pancreatic carcinoma: a cost-effectiveness analysis. *Am J Gastroenterol* 2002; **97**: 898-904

9 **Hatzidakis AA**, Tsetis D, Chrysou E, Sanidas E, Petrakis J, Gourtsoyiannis NC. Nitinol stents for palliative treatment of malignant obstructive jaundice: should we stent the sphincter of Oddi in every case? *Cardiovasc Intervent Radiol* 2001; **24**: 245-248

10 **Kim HS**, Lee DK, Kim HG, Park JJ, Park SH, Kim JH, Yoo BM, Roe IH, Moon YS, Myung SJ. Features of malignant biliary obstruction affecting the patency of metallic stents: a multicenter study. *Gastrointest Endosc* 2002; **55**: 359-365

11 **Cheng JL**, Bruno MJ, Bergman JJ, Rauws EA, Tytgat GN, Huibregtse K. Endoscopic palliation of patients with biliary obstruction caused by nonresectable hilar cholangiocarcinoma: efficacy of self-expandable metallic Wallstents. *Gastrointest Endosc* 2002; **56**: 33-39

12 **Kaassis M**, Boyer J, Dumas R, Ponchon T, Coumaros D, Delcenserie R, Canard JM, Fritsch J, Rey JF, Burtin P. Plastic or metal stents for malignant stricture of the common bile duct? Results of a randomized prospective study. *Gastrointest Endosc* 2003; **57**: 178-182

13 **Mosca S**. Choosing the metal biliary stent type for palliation of neoplastic stenosis during endoscopic retrograde cholangio pancreatography. *Endoscopy* 2003; **35**: 629-630

14 **Ahmad J**, Siqueira E, Martin J, Slivka A. Effectiveness of the Ultraflex Diamond stent for the palliation of malignant biliary obstruction. *Endoscopy* 2002; **34**: 793-796

15 **Ferro C**, Perona F, Ambrogi C, Barile A, Cianni R. Malignant biliary obstruction treated by Wallstents and Strecker tantalum stents: a retrospective review. *Cardiovasc Intervent Radiol* 1995; **18**: 25-29

16 **Lee BH**, Do YS, Lee JH, Kim KH, Chin SY. New self-expandable spiral metallic stent: preliminary clinical evaluation in malignant biliary obstruction. *J Vasc Interv Radiol* 1995; **6**: 635-640

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 123-126
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Evaluation of contrast-enhanced computed tomographic colonography in detection of local recurrent colorectal cancer

Yau-Tong You, Chung-Rong Chang Chien, Jeng-Yi Wang, Koon-Kwan Ng, Jinn-Shiun Chen, Reiping Tang, Jy-Ming Chiang, Chien-Yuh Yeh, Pao-Shiu Hsieh

Yau-Tong You, Chung-Rong Chang Chien, Jinn-Shiun Chen, Reiping Tang, Jy-Ming Chiang, Chien-Yuh Yeh, Pao-Shiu Hsieh, Division of Colon and Rectal Surgery, Chang Gung Memorial Hospital, Lin-Kou, Taiwan
Jeng-Yi Wang, Division of Colon and Rectal Surgery, Chang Gung Memorial Hospital, Jia-Yi, Taiwan
Koon-Kwan Ng, Department of Radiology, Chang Gung Memorial Hospital, Kee-Lung, Taiwan
Correspondence to: Yau-Tong You, MD, Division of Colon and Rectal Surgery, Chang Gung Memorial Hospital, 5, Fu-Hsing Street, Kuei-Shan, Tao-Yuan, Taiwan. proct@adm.cgmh.org.tw
Telephone: +886-3-3281200 ext.2101 Fax: +886-3-3278355
Received: 2005-05-09 Accepted:2005-06-18

Key words: Computed tomographic colonography; Recurrent colorectal cancer

You YT, Chang Chien CR, Wang JY, Ng KK, Chen JS, Tang R, Chiang JM, Yeh CY, Hsieh PS. Evaluation of contrast-enhanced computed tomographic colonography in detection of local recurrent colorectal cancer. *World J Gastroenterol* 2006; 12(1): 123-126

http://www.wjgnet.com/1007-9327/12/123.asp

Abstract

AIM: To evaluate the diagnostic accuracy, sensitivity, specificity of contrast-enhanced computed tomographic colonography in detecting local recurrence of colorectal cancer.

METHODS: From January 2000 to December 2004, 434 patients after potentially curative resection for invasive colorectal cancer were followed up for a period ranging from 20 to 55 mo. Eighty of the four hundred and thirty-four patients showing strong clinical evidence for recurring colorectal cancer during the last follow-up were enrolled in this study. Each patient underwent contrast-enhanced computed tomographic colonography and colonoscopy on the same day. Any lesions, biopsies, identified during the colonoscopic examination, immediate complications and the duration of the procedure were recorded. The results of contrast-enhanced computed tomographic colonography were evaluated by comparing to those of colonoscopy, surgical finding, and clinical follow-up.

RESULTS: Contrast-enhanced computed tomographic colonography had a sensitivity of 100%, a specificity of 83% and an overall accuracy of 94% in detecting local recurrent colorectal cancer.

CONCLUSION: Conventional colonoscopy and contrast-enhanced tomographic colonography can complement each other in detecting local recurrence of colorectal cancer.

INTRODUCTION

Colorectal cancer has the third highest incidence of all cancers worldwide. Approximately 70% of colorectal cancer patients can undergo potentially curative surgical resection. Unfortunately, colorectal cancer recurs in 30% of these patients. With the advent of more aggressive surgical resection for recurrent colorectal cancer[1], early detection of recurrent cancers while they are still limited to a local site is important to improve the patient's survival. If radical resection of locally recurrent colorectal cancer is performed before distant metastatic or unresectable disease develops, one-third to one-half of patients can increase their survival time. However, potentially curative surgery is followed by a period of uncertainty as to whether the operation has successfully cured the cancer. Treatment failure is usually apparent during the first 3 years after surgery. The precise post-operative surveillance procedures[2] are based on clinical assessment, CEA, colonoscopy, ultrasound and computerized tomography depending on the site of primary tumor. The role of follow-up in the early diagnosis of recurrent colorectal cancer in patients having undergone resection has been investigated extensively. A large array of screening tests is available for detecting recurrent colorectal cancer, but each test has its particular limitations. Computed tomographic colonography is a new method to exploit recent developments in image acquisition which applies algorithms of virtual-reality systems to build three-dimensional models of the inner surface of the colon tube thereby simulating the conventional colonoscopic view[3-5]. The colon wall and pericolonic structures can also be detected at the same time. Computed tomographic colonography has a high accuracy in detecting colonic neoplasia[6-8]. Like computed tomography, contrast-

enhanced computed tomography is performed after a patient receives an air enema, and uses a narrow collimation and reconstruction interval to detect colonic lesions. Contrast-enhanced computed tomographic colonography theoretically has the ability to detect local cancer recurrence by examining both the colonic mucosa and the pericolonic tissue. The use of IV contrast material in contrast-enhanced computed tomographic colonography facilitates a thorough examination of metastatic disease in solid organs. Contrast-enhanced computed tomographic colonography can display both mucosa and extramucosal local recurrence, metachronous polyps and cancers, hepatic and peritoneal metastasis[9]. This study aimed to assess the diagnostic accuracy, sensitivity, and specificity of contrast-enhanced computed tomographic colonography in detecting local recurrence of colorectal cancer following curative resection.

MATERIALS AND METHODS

Patients

From January 2000 to December 2004, 434 patients who underwent potentially curative resection for invasive colorectal cancer (181 stage B, 253 stage C) were followed up for a period ranging from 20 to 55 mo. Eighty of the four hundred and thirty-four patients showed strong clinical evidence for recurring colorectal cancer at the last follow-up. Patients with an end or diverting colostomy or those who had contraindications for IV contrast dye were excluded.

Methods

Eighty patients who were sent for conventional colonoscopy and agreed to receive contrast-enhanced computed tomographic colonography were enrolled in this study. Written informed consent forms for both conventional colonoscopy and contrast-enhanced computed tomographic colonography were obtained from each patient. The average age of the patients was 64 years (range, 28-82 years). The ratio of male to female was 43:37. The clinical manifestations of local recurrence included bloody stools, increased serum CEA level ($\geq$ 50 ng/mL), abdominal mass and colonic obstruction. Previous resection for invasive colorectal cancer included stages B and C of rectal cancer and colon cancer. Each patient underwent contrast-enhanced computed tomographic colonography and colonoscopy. The endoscopist was not informed of the radiological results on the same day. The average time between previous resection and contrast-enhanced computed tomographic colonography was 32 mo (range, 20-55 mo). Twenty-four hours before contrast-enhanced computed tomographic colonography, each patient received a standard bowel preparation[10,11] consisting of 4 L of polyethylene glycol solution and 25 mg bisacodyl tablets. Prior to computed tomographic scanning, patients were placed in a left lateral decubitus position on the computed tomographic table for the introduction of a rectal enema tube. After insertion of the rectal tube, the colon was inflated with room air to patient tolerance. To reduce bowel peristalsis and colon spasms, 20 mg of buscopan was administered intravenously immediately before air insufflation. Patient's tolerance with regard to the volume of insufflated air was measured (range, 1 500-2 000 cm^3). Adequate colonic distention was checked with a computed tomographic scout. If inadequate distention developed at any colon segment, idiosyncratic positioning or additional air insufflation was performed to improve colonic distention in the collapsed regions. Contrast-enhanced computed tomographic colonography was performed employing 150 mL of Isovue-300 (Bracco Diagnostics, Princeton, NJ, USA) IV contrast medium injected at a rate of 3-5 mL/s. Images were acquired 70 s after the injection.

Computed tomographic examinations were conducted with Sightspeed Plus and Sightspeed QX/i computed tomographic scanners (General Electric Medical Systems). The patient was first examined in the supine position, and then in the prone position. Images were acquired with a 5-mm beam collimation (table speed of 10 mm/s, and reconstruction slice overlap of 2.5 mm, 230-260 mA, 120 kV). A gastrointestinal radiologist with experience in computed tomographic colonography analyzed the volumetric computed tomographic datasets using a Sun Advantage Windows (General Electric Medical Systems)[12,13] and the General Electric Navigator program that reformats the axial two-dimensional multiplanar and three-dimensional endoluminal images[14,15] and allowed for comparison of supine and prone datasets. Local recurrence was rated as either present, absent or indeterminate. Local recurrence was recorded as present when the characteristic appearance of an enhancing, primary extracolonic mass on intraluminal masses at or near the surgical anastomosis with or without adjacent adenopathy was identified by the contrast-enhanced computed tomography. Predominantly intraluminal abnormalities at the anastomosis were considered indeterminate for local recurrence. The liver, peritoneum, retroperitoneum, lung bases, and lymph nodes were also evaluated for the presence of metastatic disease. The colonoscopic examination was performed 2 h after contrast-enhanced computed tomographic colonography. The incidence of lesions, immediate complications, and the overall duration of the colonoscopic examination were recorded. Examination reports being indeterminate for local recurrence on contrast-enhanced computed tomographic colonography but negative colonoscopic examinations were counted as false positive examinations in statistical analysis. The sensitivity, specificity, and accuracy of contrast-enhanced computed tomographic colonography for post-operative detection of local recurrence and metastatic colorectal cancer were estimated.

RESULTS

All the 80 patients completed the contrast-enhanced computed tomographic colonography successfully. No patients had pain or complications during the procedures. Table 1 presents the findings of the contrast-enhanced computed tomographic colonography. Examination results were as follows. Local recurrence was found in 51 patients. Seventy-five of the eighty patients had adequate colonic inflation throughout the entire colon. Two of the five remaining patients had inadequate transverse colon distention and three had inadequate sigmoid colon distention though additional

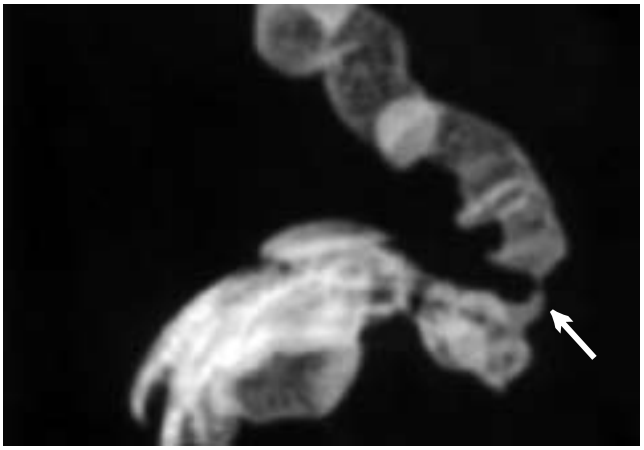


Figure 1 Virtual double contrast of the colon in a patient with local recurrence at previous anastomotic site.

air was insufflated and the positions of the patient were changed. In contrast-enhanced computed tomographic colonography, all the five patients showing thickened segmental colon wall and external luminal tumor mass compression (Figure 1) were classified as present local recurrence. The colonoscopic findings in these corresponding segmental regions showed only lumen stenosis, but no mass or mucosal lesions were found in the lumen in all the five patients. All the five patients received laparotomy for local recurrence based on a clinical presentation of three abdominal palpable masses and two colon obstructions. Surgical findings showed external colon lumen recurrent masses at previous anastomotic sites in all the five patients. Two of the five patients also had peritoneal metastasis. They all received resection of the local recurrent tumor and the colon segment with or without colostomy diversion.

Of the 51 patients with local recurrence, colonoscopic findings showed a tumor or a stricture with friable mucosa at the anastomosis, prompting a biopsy for recurrent adenocarcinoma. All the 51 patients with positive findings on both contrast-enhanced computed tomographic colonography and colonoscopy received laparotomy for local recurrence. Surgical findings showed local recurrence in all 51 patients, 35 of the 51 patients underwent segmental resection of the recurrent colorectal cancer with anastomosis, the remaining 16 patients underwent segmental resection of the colorectal cancer with colostomy diversion. All the 51 patients with local recurrence with or without liver metastasis or peritoneal metastasis received adjuvant chemotherapy after surgery.

The colonoscopic findings in the five patients which were classified as indeterminate by contrast-enhanced computed tomographic colonography revealed mucosa swelling, erythema in two patients and multiple ulcers at anastomotic site in three patients.

All the five patients underwent both contrast-enhanced computed tomographic colonography and colonoscopy 6 months later. No local recurrence or distant metastases were found, and their anastomotic sites were normal.

The colonoscopic finding in one patient, whose contrast-enhanced computed tomographic colonography showed no local recurrence but a metachronous mass at the ascending colon, revealed a tumor at the ascending colon. The patient with metachronous cancer underwent right hemicolectomy with anastomosis. No distant metastasis or local recurrence was found in this case. The remaining 23 patients whose contrast-enhanced computed tomographic colonography did not show local recurrence were negative for colonoscopy. All the 23 patients were routinely followed up. There was no true false-negative local recurrent cancer on contrast-enhanced computed tomographic colonography. However, the five patients classified as indeterminate for local recurrence in contrast-enhanced computed tomographic colonography reports were false positive. Contrast-enhanced computed tomographic colonography had a sensitivity of 100%, a specificity of 83%, and an overall accuracy of 94% in detecting local recurrent colorectal cancer.

Table 1 Performance-based contrast-enhanced computed tomographic colonography findings in 80 patients

	Patients (*n*)
Local recurrence	
Present	51
Indeterminate	5
Not present	24
Metachronous cancer	1
Distant metastasis	
Liver	8
Peritoneal	5

DISCUSSION

In patients who have undergone potentially curative colonic resections for invasive colorectal cancer, hematogenous metastases and local recurrence are the most important factors influencing prognosis. After surgery, however, there is a period of uncertainty as to whether the operation has cured the cancer or not. Treatment failure will usually be apparent during the first 2-3 years after surgery. Precise post-operative surveillance procedures, including clinical assessment, colonoscopy, abdominal computed tomography, are employed to detect recurrence of colorectal cancer. Although colonoscopy can detect intraluminal local recurrence, some local recurrences are not intraluminal and are endoscopically obscure. Abdominal computed tomography can detect hepatic and peritoneal metastases, but it is not reliable for detecting local recurrence except in those patients with a previous abdominoperineal resection. Unlike these two tests, contrast-enhanced computed tomographic colonography directly displays the anastomosis, luminal surface, colon wall and pericolonic tissues. It has, therefore, a potential to detect mucosal, intramural and extracolonic local recurrences. In our study, the overall accuracy was 94%, which is similar to that in the study by Fletcher *et al* [16]. At the same time, it is also advantageous over the colonoscopy for detecting extracolonic local recurrence and peritoneal metastasis. In this study, 46 of the 51 local recurrences developed from the extralumen soft tissue and local lymph nodes, nearly previous anastomotic area. At the same time, 40 of the 51 cases were rectal can-

cer and 47 of stage C at their original primary cancer. It may be the reasons that were related to high local recurrent rate of our samples. As shown in our study, 5 of the 51 patients (10%) who had local recurrence detected by contrast-enhanced computed tomographic colonography had no intraluminal recurrence by colonoscopic examination.

However, all the five patients received laparotomy for local recurrence of abdominal mass and intestinal obstruction. External colon lumen local recurrence with or without peritoneal metastasis was found during surgery. Contrast-enhanced computed tomographic colonography may also show the structure of the colon when colonoscopy is incomplete[17]. Although contrast-enhanced computed tomographic colonography has a high sensitivity (100%) for local recurrent colorectal cancer, its specificity is only 83% as shown in our study. This may be due to the inability of contrast-enhanced computed tomographic colonography to distinguish local recurrence from inflammation when enhancing soft tissue is present. Our results showed that five patients, classified as indeterminate by contrast-enhanced computed tomographic colonography, had colonoscopic findings of mucosa swelling, erythema or multiple ulcers. No local recurrence was identified in any of these five patients during the subsequent follow-up. These indeterminate conditions were then considered as false positive examinations. Another significant difference in this technique in comparison to colonoscopy is that a biopsy cannot be taken during contrast-enhanced computed tomographic colonography. However, it is recognized that contrast-enhanced computed tomographic colonography is more accurate in detecting extraluminal recurrent tumor than conventional colonoscopy.

In conclusion, contrast-enhanced computed tomographic colonography has several advantages over alternative tests in detecting local recurrent colorectal cancer. It can be a very helpful adjuvant method to colonoscopy in detecting extraluminal local recurrence, peritoneal carcinomatosis and distant metastasis. With regard to the threat of colorectal cancer and the early detection of local recurrence and distant metastasis in patients who have undergone potentially curative colonic resections for invasive colorectal cancers, conventional colonoscopy and contrast-enhanced tomographic colonography can complement each other.

REFERENCES

1 **Bozzetti F**, Bertario L, Rossetti C, Gennari L, Andreola S, Baratti D, Gronchi A. Surgical treatment of locally recurrent rectal carcinoma. *Dis Colon Rectum* 1997; **40**: 1421-1424

2 **Hall NR**, Finan PJ, Stephenson BM, Purves DA, Cooper EH. The role of CA-242 and CEA in surveillance following curative resection for colorectal cancer. *Br J Cancer* 1994; **70**: 549-553

3 **Mendelson RM**, Forbes GM. Computed tomography colonography (virtual colonoscopy): review. *Australas Radiol* 2002; **46**: 1-12

4 **Tarján Z**, Zágoni T, Györke T, Mester A, Karlinger K, Makó EK. Spiral CT colonography in inflammatory bowel disease. *Eur J Radiol* 2000; **35**: 193-198

5 **Rex DK**. Virtual colonoscopy: time for some tough questions for radiologists and gastroenterologists. *Endoscopy* 2000; **32**: 260-263

6 **Miao YM**, Amin Z, Healy J, Burn P, Murugan N, Westaby D, Allen-Mersh TG. A prospective single centre study comparing computed tomography pneumocolon against colonoscopy in the detection of colorectal neoplasms. *Gut* 2000; **47**: 832-837

7 **Kay CL**, Kulling D, Hawes RH, Young JW, Cotton PB. Virtual endoscopy--comparison with colonoscopy in the detection of space-occupying lesions of the colon. *Endoscopy* 2000; **32**: 226-232

8 **Macari M**, Bini EJ, Xue X, Milano A, Katz SS, Resnick D, Chandarana H, Krinsky G, Klingenbeck K, Marshall CH, Megibow AJ. Colorectal neoplasms: prospective comparison of thin-section low-dose multi-detector row CT colonography and conventional colonoscopy for detection. *Radiology* 2002; **224**: 383-392

9 **Lefere PA**, Gryspeerdt SS, Dewyspelaere J, Baekelandt M, Van Holsbeeck BG. Dietary fecal tagging as a cleansing method before CT colonography: initial results polyp detection and patient acceptance. *Radiology* 2002; **224**: 393-403

10 **Hara AK**, Johnson CD, Reed JE. Colorectal lesions: evaluation with CT colography. *Radiographics* 1997; **17**: 1157-167; discussion 1157-167

11 **Yoshida H**, Näppi J, MacEneaney P, Rubin DT, Dachman AH. Computer-aided diagnosis scheme for detection of polyps at CT colonography. *Radiographics* 2002; **22**: 963-979

12 **Spinzi G**, Belloni G, Martegani A, Sangiovanni A, Del Favero C, Minoli G. Computed tomographic colonography and conventional colonoscopy for colon diseases: a prospective, blinded study. *Am J Gastroenterol* 2001; **96**: 394-400

13 **Hara AK**, Johnson CD, MacCarty RL, Welch TJ, McCollough CH, Harmsen WS. CT colonography: single- versus multi-detector row imaging. *Radiology* 2001; **219**: 461-465

14 **Rogalla P**, Meiri N, Rückert JC, Hamm B. Colonography using multislice CT. *Eur J Radiol* 2000; **36**: 81-85

15 **Hara AK**, Johnson CD, Reed JE, Ehman RL, Ilstrup DM. Colorectal polyp detection with CT colography: two- versus three-dimensional techniques. Work in progress. *Radiology* 1996; **200**: 49-54

16 **Fletcher JG**, Johnson CD, Krueger WR, Ahlquist DA, Nelson H, Ilstrup D, Harmsen WS, Corcoran KE. Contrast-enhanced CT colonography in recurrent colorectal carcinoma: feasibility of simultaneous evaluation for metastatic disease, local recurrence, and metachronous neoplasia in colorectal carcinoma. *AJR Am J Roentgenol* 2002; **178**: 283-290

17 **Neri E**, Giusti P, Battolla L, Vagli P, Boraschi P, Lencioni R, Caramella D, Bartolozzi C. Colorectal cancer: role of CT colonography in preoperative evaluation after incomplete colonoscopy. *Radiology* 2002; **223**: 615-619

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 127-129
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Influence of HBcAg in liver cell plasma on expression of transforming growth factor-beta 1 in liver tissue of low-grade chronic hepatitis B patients

Yong-Gang Liu, Jian-Hua Lu, Xin-Xin Wang, Jian-Lin Yang, Zhen-Wei Lang, Xin Meng, Li-Jie Zhang, Lin Sun, Shi-Jie Zhang, Jun-Qiang Li, Chen-Zhao Song

Yong-Gang Liu, Department of Clinical Hepatitis, Tianjin Infectious Disease Hospital, Tianjin 300192, China
Jian-Hua Lu, Laboratory of Pathology, Shijiazhuang No. 5 Hospital, Shijiazhuang 050021, Hebei Province, China
Xin-Xin Wang, Zhen-Wei Lang, Xin Meng, Li-Jie Zhang, Lin Sun, Shi-Jie Zhang, Jun-Qiang Li, Chen-Zhao Song, Laboratory of Pathology, Capital Medical University Affiliated Youan Hospital, Beijing 100054, China
Jian-Lin Yang, Laboratory of Pathology, Eerduosi No. 2 People's Hospital, Eerduosi 017000, Inner Mongolia Autonomous Region, China
Supported by the Beijing Science and Technology Commission, No. H020920020290
Co-first-author: Jian-Hua Lu
Correspondence to: Dr. Yong-Gang Liu, Tianjin Infectious Disease Hospital, Tianjin 300192, China. ygwx@public.tpt.tj.cn
Telephone: +86-22-23004472
Received: 2005-05-25 Accepted: 2005-06-18

Abstract

AIM: To study the influence of HBcAg on the expression of transforming growth factor-beta 1 (TGF-β1) in liver tissue of low-grade chronic hepatitis B (CHB) patients.

METHODS: The expression of TGF-β1 and HBcAg in liver samples from 93 low-grade CHB patients was detected by immunohistochemistry and valuated by semi-quantitative scoring.

RESULTS: In the 93 low-grade CHB patients, HBcAg was expressed in cell plasma but not in the liver tissue. There was no significant difference between the two groups.

CONCLUSION: The expression of TGF-β1 is not related with HBcAg expressed as plasma type in the tissues of low-grade CHB patients.

Key words: HBcAg; Factor-beta 1; Chronic hepatitis B

Liu YG, Lu JH, Wang XX, Yang JL, Lang ZW, Meng X, Zhang LJ, Sun L, Zhang SJ, Li JQ, Song CZ. Influence of HBcAg in liver cell plasma on expression of transforming growth factor-beta 1 in liver tissue of low-grade chronic hepatitis B patients. *World J Gastroenterol* 2006; 12(1): 127-129

http://www.wjgnet.com/1007-9327/12/127.asp

INTRODUCTION

The incidence rate of chronic hepatitis B (CHB) is high in China. The status of virus replication and the process of hepatic fibrosis are regarded as important. At present, TGF-β1 is known not only as a cytokine which adjusts proliferation, development, conversion, and differentiation of cells, but also as an important transmitter of hepatic fibrosis. It plays an important role in the formation of cirrhosis[1-3]. The synthesis and degradation of extracellular matrix (ECM) are adjusted by it[4,5]. This study was to observe the influence of HBcAg on the expression of TGF-β1 in liver tissue of low-grade CHB patients.

MATERIALS AND METHODS

Reagents

Mouse anti-human TGF-β1 antibody was purchased from Fuzhou Maxim Biotechnology Co., Ltd (Lot No.: 30212238L). The PV-9000 kit was provided by Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Rabbit anti-human HBcAg was purchased from Fuzhou Maxim Biotechnology Co., Ltd.

Clinical data

A total of 93 low-grade CHB patients (68 males and 25 females, mean age 33.3 years, ranging from 17 to 56 years) were analyzed. HBcAg was expressed as plasma type in the liver tissue of 50 cases and no HBcAg was expressed in 43 cases. The diagnosis of all cases was coincident with the program of prevention and cure for viral hepatitis[6].

Liver biopsy

The liver biopsy was taken from 93 cases under B ultrasound guidance. The liver tissue longer than 1.0 cm and without break was fixed by formaldehyde solution and embedded in paraffin. Six serial sections (4 μm thick) were prepared for HE, Masson, Gordon Sweet, HBsAg, HBcAg, and TGF-β1.

Table 1 Influence of HBcAg in liver cell plasma on the expression of TGF-β1 in liver tissue of low-grade CHB patients

Groups	Expression of TGF-β1 protein by semi-quantitative scoring(Score)					Total
	0	1	2	3	4	
HBcAg expression in cell plasma	2	2	19	23	4	50
No HBcAg expression in liver tissue	4	2	13	20	4	43

Immunohistochemistry

TGF-β1 antigen was repaired by microwave in pH 6.0 citrate solutions. The next procedure was performed according to the instructions of PV-9000 kit. TGF-β1 was observed randomly at least in five portal areas under 200 light microscope and the expression of TGF-β1 was evaluated with semi-quantitative scoring method: score 0: no stain or no cell was hyperchromatic or the positive cells were less than 1% of total liver tissues; score 1-4: the areas of positive cells in hepatic lobules, hepatic sinusoid, portal areas, and fibrous plate were 1%-9%, 10%-15%, 16%-20% and more than 20% of total liver tissues, respectively[7].

Statistical analysis

Statistical analyses were carried out with the rank test.

RESULTS

TGF-β1 expression

The positive cells of TGF-β1 were mainly distributed over the focal necrosis and the active fibrosis areas. They were mainly expressed in the interstitial cells of hepatic sinusoid and the inflammatory cells of portal areas. Some bile duct cells and plasma hepatocytes were also expressed.

HBcAg expression

In the 93 low-grade CHB patients, HBcAg was expressed in cell plasma but not in the liver tissue. There was no significant difference between the two groups ($H = 0.004$, $P > 0.05$, Table 1).

DISCUSSION

TGF-β1 is a cluster of active polypeptides with closely correlative structures and similar functions. Five isomers (TGF-β1-5) have been found though TGF-β1 is the main content of TGF-β in human liver and has important functions. It mainly comes from the Kupffer cells (KCs) though hepatic stellate cells (HSCs) can autocrine TGF-β1. TGF-β1 can transfer anti-signals of cell cycle with Smad molecules and inhibit gene transcription of cell cycle correlative proteins[8]. It can inhibit the expression of cyclin related to $P70^{s6k}$ through $P70^{s6k}$ (serine/threonine kinase)[9]. The upregulation of connective tissue growth factor (CTGF) expression is related to TGF-β1[10] and it may be the core of activation in HSCs. TGF-β1 can inhibit the proliferation of quiescent HSCs but cannot inhibit the activated HSCs[11]. In CHB, its serum level is increased[12-15] and its expression in the liver is reinforced[16-18]. TGF-β1 is one of the network cytokines related to hepatic fibrosis and can accelerate the synthesis of ECM and inhibit the degradation of ECM[19]. Following the expression of TGF-β1, the proliferating cell nuclear antigen (PCNA) decreases in the liver[20]. TGF-β1 can inhibit regeneration of hepatocytes[21] and accelerate apoptosis of hepatocytes[22-24], but not HCC cells[25]. Powell *et al* [26] showed that the risk of developing cirrhosis is higher in hyper-expression than in hypo-expression of TGF-β1.

Some scholars have found that the expression of TGF-β1 in the liver tissues is not related with HBeAg and HBV DNA in the serum of CHB patients[27,28]. In our study, TGF-β1 in the liver tissue of low-grade CHB patients did not influence the status of inflammation and fibrosis with the comparability improved. We found that the expression of TGF-β1 evaluated by semi-quantitative scoring was not related with HBcAg expression in liver cell plasma of low-grade chronic hepatitis B. We suppose that the expression of some cytokines is not related with hepatitis B virus possibly due to the role of virus replication and body immune response.

REFERENCES

1 **Friedman SL**. Seminars in medicine of the Beth Israel Hospital, Boston. The cellular basis of hepatic fibrosis. Mechanisms and treatment strategies. *N Engl J Med* 1993; **328**: 1828-1835

2 **Border WA**, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994; **331**: 1286-1292

3 **Gressner AM**. Cytokines and cellular crosstalk involved in the activation of fat-storing cells. *J Hepatol* 1995; **22**: 28-36

4 **Gressner AM**, Weiskirchen R, Breitkopf K, Dooley S. Roles of TGF-beta in hepatic fibrosis. *Front Biosci* 2002; **7**: d793-d807

5 **Roberts AB**, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci U S A* 1986; **83**: 4167-4171

6 The programme of prevention and cure for viral hepatitis. *Chin J Infect Dis* 2001; **19**: 56-62

7 **Peter J**, Richard A, Amar P. Scoring of chronic hepatitis. *Clin Liver Dis* 2002; **6**: 335-347

8 **Massagué J**. TGF-beta signal transduction. *Annu Rev Biochem* 1998; **67**: 753-791

9 **Petritsch C**, Beug H, Balmain A, Oft M. TGF-beta inhibits p70 S6 kinase via protein phosphatase 2A to induce G(1) arrest. *Genes Dev* 2000; **14**: 3093-3101

10 **Paradis V**, Dargere D, Bonvoust F, Vidaud M, Segarini P, Bedossa P. Effects and regulation of connective tissue growth factor on hepatic stellate cells. *Lab Invest* 2002; **82**: 767-774

11 **Liu CH**, Xan HP. [Effects of transforming growth factor beta1 on the proliferation and type I collagen expression at different differential rat hepatic stellate cells]. *Zhonghua Gan Zang Bing Za Zhi* 2003; **11**: 731-734

12 **Liu F**, Li BS, Man YM, Li SM, Zhang HF, Wang CQ. The effect of serum TGF-β1 of patients with chronic hepatitis B in liver fibrosis formation. *Zhonghua Ganbing Zazhi* 1999; **7**: 196-198

13 **Sun Y**, Xuan S, Xin Y, Lu W, Chu L. [Relationship between serum TGF beta-1 with chronic hepatitis B involving in liver cell function and liver biopsy fibrosis]. *Zhonghua Gan Zang Bing Za Zhi* 2002; **10**: 221-222

14 **Li HZ**, Zhou XP, Mi JP, Zheng MD, Li AY, Chen LF. Relationship between levels of TGF-β1, TNF-α, HA, LN, PCIII in serum of patients with chronic hepatitis B and fibrosis degree analysis of liver tissue. *Zhonghua Linchuang Ganbing Zazhi* 2002; **18**: 86-88

15 **Liu F**, Liu JX, Cao ZC, Li BS, Pei X, Wang LS, Zhang JG. The serum TGF-β1 in the patients with chronic hepatitis B involving in liver cell damage and liver fibrosis formation. *Zhonghua Chuanranbing Zazhi* 1999; **17**: 241-244

16 **Xin SJ**, Wang LJ, Wang SS, Mao YL, Zhang SL, Chen LM. Study on the relationshio between the expression of transforming growth factor-β1 in the liver tissues of viral hepatitis and liver fibrosis. *Zhonghua Ganbing Zazhi* 1997; **5**: 66-68

17 **Zhang M**, Zhang LX, Long JY, Zhao JM, Xin SJ, Wang SS, Xi DP, Cheng Y, Wang LJ. Expression of TGF-β1 and its mRNA in liver tissiues of patients with chronic viral hepatitis. *Zhonghua Ganbing Zazhi* 1999; **7**: 193-195

18 **Zhang M**, Zhang LX, Xin SJ, Zhao JM, Wang SS, Wang LJ. The relationship between transforming growth factor 1 and liver fibrosis. *Zhonghua Linchuang Ganbing Zazhi* 2000; **16**: 32-33

19 **Shi DL**, Savona C, Gagnon J, Cochet C, Chambaz EM, Feige JJ. Transforming growth factor-beta stimulates the expression of alpha 2-macroglobulin by cultured bovine adrenocortical cells. *J Biol Chem* 1990; **265**: 2881-2887

20 **Delhaye M**, Louis H, Degraef C, Le Moine O, Devière J, Peny MO, Adler M, Galand P. Hepatocyte proliferative activity in human liver cirrhosis. *J Hepatol* 1999; **30**: 461-471

21 **Fausto N**. Hepatocyte apoptosis: is transforming growth factor-B1 the kiss of death? *Hepatology* 1993; **18**: 1536-1537

22 **Xin S**, Zhao J, Wang L. [Study on relationship between the apoptosis of hepatocytes and liver fibrosis of chronic viral hepatitis]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 2000; **14**: 31-33

23 **Lang ZW**, Meng X, Wang HJ, Luo ZX, Li JQ, Zhang SJ. The relationship between the expression of TGF-β1 and apoptosis in the liver tissue of viral hepatitis. *Weichangbingxue He Ganbingxue Zazhi* 1998; **7**: 383

24 **Oberhammer FA**, Pavelka M, Sharma S, Tiefenbacher R, Purchio AF, Bursch W, Schulte-Hermann R. Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. *Proc Natl Acad Sci U S A* 1992; **89**: 5408-5412

25 **Bedossa P**, Peltier E, Terris B, Franco D, Poynard T. Transforming growth factor-beta 1 (TGF-beta 1) and TGF-beta 1 receptors in normal, cirrhotic, and neoplastic human livers. *Hepatology* 1995; **21**: 760-766

26 **Powell EE**, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, Purdie DM, Jonsson JR. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000; **31**: 828-833

27 **Cui X**, Wang L, Fu CS, Lang ZW. The expression and significance of TGF-β1, α-SMA in the liver tissues of chronic hepatitis B . *Shandong Yiyao* 2003; **43**: 18-20

28 **Liu YG,** Lu JH, Wang XX, Lang ZW, Yang JL, Meng X, Zhang LJ, Sun L, Zhang SJ, Li JQ, Song CZ. The influence of HBV replication on the expression of transforming growth factor-beta1 in the liver tissue of low grade CHB patients. *Tianjin Yixue Zazhi* 2004; **32**: 602-603

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 130-133
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Clinical application of plasma shock wave lithotripsy in treating impacted stones in the bile duct system

Zhi Xu, Li-Xin Wang, Neng-Wei Zhang, Chun-Sheng Hou, Xiao-Feng Ling, Yao Xu, Xiao-Si Zhou

Zhi Xu, Li-Xin Wang, Neng-Wei Zhang, Chun-Sheng Hou, Xiao-Feng Ling, Xiao-Si Zhou, Department of Surgery, Third Hospital of Peking University, 49 North Garden Road, Haidian District, Beijing 100083, China
Yao Xu, Institute of Physics, Chinese Academy of Sciences, Beijing 100037, China
Correspondence to: Zhi Xu, Department of Surgery, Third Hospital of Peking University, 49 North Garden Road, Haidian District, Beijing 100083, China. xuzhi123456@sohu.com
Telephone: +86-10-62017691-2575 Fax: +86-10-62017700
Received: 2005-05-30 Accepted: 2005-07-29

Abstract

AIM: To verify the safety and efficacy of plasma shock wave lithotripsy (PSWL) in fragmenting impacted stones in the bile duct system.

METHODS: From September 1988 to April 2005, 67 patients (26 men and 41 women) with impacted stones underwent various biliary operations with tube (or T-tube) drainage. Remnant and impacted stones in the bile duct system found by cholangiography after the operation were fragmented by PSWL and choledochofiberscopy. A total of 201 impacted stones were fragmented by PSWL setting the voltage at 2.5-3.5 kV, and the energy output at 2-3 J for each pulse of PSWL. Then the fragmented stones were extracted by choledochofiberscopy. The safety and efficacy of PSWL were observed during and after the procedure.

RESULTS: One hundred and ninety-nine of 201 impacted stones (99.0%) in the bile duct system were successfully fragmented using PSWL and extracted by choledochofiberscopy. The stone clearance rate for patients was 97% (65/67). Ten patients felt mild pain in the right upper quadrant of the abdomen, and could tolerate it well. Eleven patients had a small amount of bleeding from the mucosa of the bile duct. The bleeding was transient and stopped spontaneously within 2 min of normal saline irrigation. There were no significant complications during and after the procedure.

CONCLUSION: PSWL is a safe and effective method for fragmenting impacted stones in the bile duct system.

Key words: Impacted stone; Plasma shock wave lithotripsy; Choledochofiberscopy

Xu Z, Wang LX, Zhang NW, Hou CS, Ling XF, Xu Y, Zhou XS. Clinical application of plasma shock wave lithotripsy in treating impacted stones in the bile duct system. *World J Gastroenterol* 2006; 12(1): 130-133

http://www.wjgnet.com/1007-9327/12/130.asp

INTRODUCTION

Primary bile duct stones, especially intrahepatic ones, are common findings in Asian patients and challenging problems encountered in biliary surgery[1,2]. Retained and recurrent stones represent the two main problems in the surgical treatment of stones. With the application of intra- and postoperative choledochofiberscopy, the incidence of retained stones after surgery has been markedly reduced. Though many postoperative remnant stones can be extracted via choledochofiberscopy, it remains difficult to extract impacted stones or very large stones[3]. Impacted stones preclude insertion of the Dormia basket and cannot be captured with conventional techniques via choledochofiberscopy. Since large stones cannot pass through the T-tube fistula, it is both time consuming and frustrating to remove such stones. In order to solve this problem, we combined plasma shock wave lithotripsy (PSWL) with choledochofiberscopy. Both *in vitro* and *in vivo* studies have been conducted since the 1980s to test the safety and efficacy of this technique and the combination is widely used in clinical practice[4].

MATERIALS AND METHODS

Patients

From September 1988 to April 2005, 67 patients (26 men and 41 women) with impacted stones or very large stones in the bile duct system underwent PSWL in combination with choledochofiberscopy in our hospital. The mean age of the patients involved was 53 ± 3 years (range, 26-83 years). Of the 67 patients, 3 had a diagnosis of acute cholecystitis with cholelithiasis and previously underwent cholecystostomy with tube drainage, 36 patients had choledocholithiasis and underwent choledocholithotomy and common bile duct exploration with T-tube drainage, and 28 patients had a diagnosis of hepatolithiasis with or without stones in the extra-hepatic bile duct and received common bile duct exploration, intrahepatic bile duct stone removal via the common bile duct with the use of stone

Table 1 Sites of impacted or huge remnant stones

Sites	Impacted stones, *n* (%)
Distal end of common bile duct	22 (10.9)
Common bile duct	14 (7.0)
Cyst duct	3 (1.5)
Hilus of hepatic duct	10 (5.0)
Left hepatic duct	24 (11.9)
Left internal hepatic duct	71 (35.3)
Left external hepatic duct	8 (4.0)
Right hepatic duct	14 (7.0)
Right anterior hepatic duct	3 (1.5)
Right posterior hepatic duct	32 (15.9)
Total	201 (100)

forceps and partial hepatectomy as well as T-tube drainage when necessary. No cholangiocarcinoma was encountered in these patients. In our study, 39 patients were transferred from other hospitals. About eight attempts or so were made to extract choledochofiberscopic stones. Twenty-eight patients were admitted to our hospital at the beginning of their treatment.

Methods

All the patients underwent PSWL combined with choledochofiberscopic stone extraction without any anesthesia or sedation. The drainage tube was removed from the gallbladder or from the common bile duct, and a flexible choledochofiberscope (model CHF-T_{20}), 6 mm in diameter with a 2.6-mm working channel (Olympus, Tokyo, Japan), was inserted through the drainage fistula into the gallbladder or the bile duct system. Once the impacted or large remnant stones were found, the PSWL probe (co-designed by the Institute of Physics at the Chinese Academy of Sciences and the Department of Surgery at the Third Hospital of Peking University) was inserted into the sites of the stones through the working channel of a choledochofiberscope. The tip of the PSWL probe (length, 100 cm; diameter 2 mm, flexibility similar to that of the catheter of Dormia basket) was targeted at the impacted stones and kept approximately 5 mm away. On the basis of the build-in PSWL circuit, the voltage switch was set at 2.5 - 3.5 kV and the energy output at 2 - 3 J for each pulse of PSWL. The number of PSWL pulses sufficient to break down a stone varied in each case. The treatment was continued until the impacted stones were fragmented sufficiently by PSWL to permit extraction with the Dormia basket (Olympus, SCOP Medicine, Tokyo, Japan) or passage into the duodenum via the sphincter of Oddi with normal saline perfusion. The bile ducts were irrigated during the procedure with normal saline and gentamycin, 4 U per 500 mL of normal saline, through the working channel of a choledochofiberscope[5].

RESULTS

In the 67 patients, 201 impacted or very large remnant stones were found in the bile ducts. The locations of stones are shown in Table 1. The gross appearance of the stones (39/201, 19.4%) found in the extrahepatic bile duct was consistent with that of the cholesterol stones. The stones found in the intrahepatic bile duct (162/201, 80.6%) resembled the pigment stones. We measured the size of impacted stones by direct visualization on cholangiogram. No difference in stone size was found between cholesterol and pigment stone groups. The size ranged from 5 to 50 mm in diameter, with 16 stones smaller than 10 mm, 167 stones between 10 and 20 mm, 16 between 21 and 30 mm, and 2 larger than 30 mm in diameter, respectively.

In our study, 199 of the 201 stones in the extra- and intrahepatic bile ducts of 67 patients were fragmented successfully by PSWL, and then extracted under a choledochofiberscope. Each PSWL procedure took several minutes to half an hour. The success rate of fragmentation with PSWL was 99.0% (199/201). Twenty-one stones required fewer than 10 pulses of PSWL sparks for fragmentation, 65 stones 11 - 50 pulses, 78 stones 51 - 100 pulses and 37 stones more than 100 pulses. The maximum number of PSWL sparks required was 700 pulses and the minimum was only two. The average number of pulses used was 52 ± 151.

Of the 201 stones, two were not fragmented by PSWL, one remained unfragmented though four procedures of PSWL totaling 1 063 pulses were carried out. A repeat operation was necessary for this failed PSWL. A huge impacted hard stone, 50 mm in diameter, was found in the left internal hepatic duct, which was not amenable to extraction with stone forceps. The left hepatic duct was opened and the large pigment stone was eventually extracted via a bile duct incision. Another impacted stone in the neck of the gallbladder was not fragmented by PSWL because the patient refused to fragment it by PSWL. Cholecystectomy was performed for this patient at last and the tightly impacted cholesterol stone was extracted from an incision at the neck of the gallbladder.

In 65 of the 67 patients, the fragmented stones were extracted successfully using a Dormia basket which was inserted into the bile duct through the working channel of a choledochofiberscope. The stone clearance rate was 97% (65/67). In 35 patients, only one choledochofiberscope procedure was needed to achieve clearance of remnant stones, whereas 22 patients required 2 to 5 procedures, 7 patients 6 to 10 procedures and 1 patient 18 procedures. The average number of procedures was 2 ± 41. A total of 171 procedures were performed.

In the process of fragmenting stones with PSWL via choledochofiberscopy, all the patients felt vibration. Ten patients felt mild pain in the right upper quadrant of the abdomen and could tolerate it well. Eleven patients had a small amount of bleeding from the mucosa of the extra- and intrahepatic bile ducts. It was thought that the bleeding was induced by the pulse of PSWL sparks. The bleeding was transient and stopped spontaneously within 2 min of normal saline irrigation. No other serious PSWL-related complications occurred during and after the procedure.

DISCUSSION

Impacted stone is one of the challenging problems in biliary surgery. Before the advent of PSWL, impacted stones or very large stones were removed using biopsy forceps through the working channel of a choled-

ochofiberscope. The procedure is time-consuming and often frustrating. To solve this problem, we designed the PSWL in 1980s and have conducted a series of experiments both *in vitro* and *in vivo* to test its safety and efficacy before its application in clinical practice. Fresh cholesterol and pigment stones can be fragmented effectively both *in vitro* and *in vivo* by PSWL[4].

Plasma shock wave lithotripsy uses magnetic pressure ($F = B^2/8\pi$) exerted on plasma. The plasma shock wave is derived from the magnetic pressure. The total magnetic energy is constant. Since magnetic field B can be increased by decreasing the area in which B exists, a stronger wave can be achieved with low energy. PSWL has three advantages. First, there is no impulse to luminal wall when PSWL is used to break down gallstones within the lumen. Second, when PSWL is combined with choledochofiberscopy, there is no heat injury and no vapor to obscure the visual field of a choledochofiberscope due to its low energy. Third, PSWL has its selection when it acts on an elastic buffer. Fragmentation of gallstones is achieved by impulsion of PSWL. When impulsion acts on elastomer, the fragmentation is selective. Therefore, PSWL can effectively break down nonelastic stones, while leaving the elastic soft tissue intact[5].

In our study, 38 impacted or large cholesterol stones and 161 pigment stones were fragmented successfully by PSWL in the extra- and intra-hepatic bile ducts. The success rate of fragmentation was 99.0% (199/201), and the success rate of stone clearance was 97% (65/67).

Only two stones were not fragmented by PSWL. One impacted stone in the intrahepatic bile duct could not be fragmented by multiple procedures of PSWL with a total of 1 063 pulses of PSWL sparks delivered. Re-operation was performed for the involved patient, and the stone was too hard and too large (diameter, 50 mm) to be fragmented and extracted with stone forceps. The intrahepatic bile duct was opened and the stone was removed manually. Another cholesterol stone at the neck of the gallbladder was not fragmented by two procedures of PSWL with 84 pulses of PSWL sparks delivered. Cholecystectomy was performed for this patient, and a large hard cholesterol stone (diameter, 30 mm) at the neck of the gallbladder was removed.

In the present study, we successfully fragmented 199 impacted stones in extra- and intrahepatic bile ducts using PSWL when the conventional methods failed to remove them. The voltage switch was set at 2.5 - 3.5 kV, and the energy output was controlled within the range of 2 - 3 J at each pulse of PSWL. It may be very difficult to put a choledochofiberscope at the site of an impacted stone due to branches, strictures and angles of intrahepatic bile ducts. Therefore, it is unavoidable to spark directly on the impacted stone and the wall of bile duct in performing lithotripsy. In our study, 11 sites of intrahepatic bile ducts were sparked directly using the PSWL probe. Minor bleeding from the inflammatory mucosa of the bile duct occurred, and the bleeding stopped spontaneously within 2 min. No serious complications were found during and after the treatment, indicating that PSWL is a very safe method for breaking down stones *in vivo*.

The PSWL probe is flexible and can be easily placed at the site of impacted stones to fragment the stones through the working channel of a choledochofiberscope. The position of the probe tip can be adjusted by pulling it back and forth through the working channel. The best position is 5 mm away from the stone. At this position, the PSWL probe can release energy most effectively.

Electrohydraulic shock wave lithotripsy (ESWL) and PSWL have their similarities and differences. Using discharge in fluid to induce high-amplitude hydraulic pressure waves of varying wavelengths, ESWL can fragment stones extracorporeally or intracorporeally. The extracorporeal lithotriptor uses the ellipsoid reflector to reflect the shock wave into the intracorporeal site to break down the stones. The ellipsoid reflector has two focus points. One is extracorporeal, where the shock wave is emitted by discharge. The other is intracorporeal, where stones are located. Stone fragmentation and clearance rates of 76%[6] and 92%[7] have been achieved. Our study showed that PSWL was more effective than ESWL.

It was reported that the overall complication rate for ESWL is 13.2% - 22%[7,8]. Bleeding and perforation are the main problems. Perhaps the power of ESWL is strong enough not only to fragment stones, but also to damage the bile duct wall. Harrison *et al* [9] reported that to avoid grave complications, the ESWL probe should not directly contact the bile duct wall. However, PSWL may safely break down the stones without damaging the bile duct wall when the PSWL probe is in contact with the bile duct wall, suggesting that the safety of PSWL is superior to that of ESWL.

Laser has been used to fragment stones in common bile duct[10] and intrahepatic bile duct[11,12]. Orii *et al* [11] reported that yttrium-aluminum laser has enough power to crush pigment stones, but its efficacy on cholesterol stones is not satisfactory. Prat *et al* [13] reported that bile duct stones can be fragmented by laser lithotripsy. The overall success rate for stone clearance is 87.5% and the complication rate is 18.8%. Harris *et al* [14] reported that the success rate for fragmentation of stones by laser lithotripsy is 96%, whereas the complication rate is 28%. The complications may be due to the impact of laser fiber tip on the bile duct wall[13]. Therefore, care must be taken to advance the laser filament to the end of the scope, with the scope straight outside the patient. The relatively rigid, sharp filament may perforate the side wall of the working channel if it is advanced with force through a bent scope. Firing the laser, while the tip of the filament is inside the working channel can also damage the lining of the channel[14]. These shortcomings limit the efficacy of laser lithotripsy. Hochberger *et al* [10] have strongly suggested that laser can be used in the gallbladder and common bile duct, but not in intrahepatic duct. In terms of safety, PSWL is also superior to laser. The use of mechanical lithotripsy is limited to the treatment of stones in the common bile duct and in the gallbladder. It cannot be used to treat intrahepatic bile duct stones[15]. Ultrasound lithotripsy is limited to break down stones in the gallbladder because it cannot reach the bile duct[16].

In conclusion, PSWL is a very safe and effective method for *in vivo* fragmentation of impacted stones or large remnant stones. The PSWL combined with

choledochofiberscopy, can fragment and clear most stones when a choledochofiberscope is inserted into the bile duct system.

REFERENCES

1 **Hwang JH**, Yoon YB, Kim YT, Cheon JH, Jeong JB. Risk factors for recurrent cholangitis after initial hepatolithiasis treatment. *J Clin Gastroenterol* 2004; **38**: 364-367
2 **Mahadeva S**, Prabakharan R, Goh KL. Endoscopic intervention for hepatolithiasis associated with sharp angulation of right intrahepatic ducts. *Gastrointest Endosc* 2003; **58**: 279-282
3 **Leckie PA**, Schmidt N, Taylor R. Impacted common bile duct stones. *Am J Surg* 1982; **143**: 540-541
4 **Deng SQ**, Zhang NW, Wang BT, Zhou XS, Xu Y, Li BH, He FJ. Plasma shock wave lithotripsy of gallstones in vitro. Animal experiments and clinical application. *Chin Med J (Engl)* 1991; **104**: 858-862
5 **Xu Z**, Wang L, Zhang N, Deng S, Xu Y, Zhou X. Clinical applications of plasma shock wave lithotripsy in treating postoperative remnant stones impacted in the extra- and intrahepatic bile ducts. *Surg Endosc* 2002; **16**: 646-649
6 **Hui CK**, Lai KC, Ng M, Wong WM, Yuen MF, Lam SK, Lai CL, Wong BC. Retained common bile duct stones: a comparison between biliary stenting and complete clearance of stones by electrohydraulic lithotripsy. *Aliment Pharmacol Ther* 2003; **17**: 289-296
7 **Bonnel DH**, Liguory CE, Cornud FE, Lefebvre JF. Common bile duct and intrahepatic stones: results of transhepatic electrohydraulic lithotripsy in 50 patients. *Radiology* 1991; **180**: 345-348
8 **Sheen-Chen SM**, Cheng YF, Chen FC, Chou FF, Lee TY. Ductal dilatation and stenting for residual hepatolithiasis: a promising treatment strategy. *Gut* 1998; **42**: 708-710
9 **Harrison J**, Morris DL, Haynes J, Hitchcock A, Womack C, Wherry DC. Electrohydraulic lithotripsy of gall stones--in vitro and animal studies. *Gut* 1987; **28**: 267-271
10 **Hochberger J**, Tex S, Maiss J, Hahn EG. Management of difficult common bile duct stones. *Gastrointest Endosc Clin N Am* 2003; **13**: 623-634
11 **Orii K**, Ozaki A, Takase Y, Iwasaki Y. Lithotomy of intrahepatic and choledochal stones with Yag laser. *Surg Gynecol Obstet* 1983; **156**: 485-488
12 **Uchiyama K**, Onishi H, Tani M, Kinoshita H, Ueno M, Yamaue H. Indication and procedure for treatment of hepatolithiasis. *Arch Surg* 2002; **137**: 149-153
13 **Prat F**, Fritsch J, Choury AD, Frouge C, Marteau V, Etienne JP. Laser lithotripsy of difficult biliary stones. *Gastrointest Endosc* 1994; **40**: 290-295
14 **Harris VJ**, Sherman S, Trerotola SO, Snidow JJ, Johnson MS, Lehman GA. Complex biliary stones: treatment with a small choledochoscope and laser lithotripsy. *Radiology* 1996; **199**: 71-77
15 **Cipolletta L**, Costamagna G, Bianco MA, Rotondano G, Piscopo R, Mutignani M, Marmo R. Endoscopic mechanical lithotripsy of difficult common bile duct stones. *Br J Surg* 1997; **84**: 1407-1409
16 **Qian ZP**. Clinical application of percutaneous cholecystolithotripsy. *Zhonghua Waike Zazhi* 1991; **29**: 619-622, 653

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 134-136
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Interventional therapy for acute hemorrhage in gastrointestinal tract

Hong-Hui Wang, Bin Bai, Kai-Bing Wang, Wei Xu, Yuan-Shu Ye, Wei-Feng Zhang

Hong-Hui Wang, Bin Bai, Kai-Bing Wang, Wei Xu, Yuan-Shu Ye, Department of Radiology, the Second Affiliated Hospital of Harbin Medical University, Harbin 150086, Heilongjiang Province, China
Wei-Feng Zhang, Department of Thyroid Surgery, the Second Affiliated Hospital of Harbin Medical University, Harbin 150086, Heilongjiang Province, China
Correspondence to: Dr Hong-Hui Wang, Department of Radiology, the Second Affiliated Hospital of Harbin University, Harbin 150086, Heilongjiang Province, China. honghuiwang@sina.com
Telephone: +86-451-86605419 Fax: +86-451-86605413
Received: 2005-05-12 Accepted: 2005-06-06

Abstract

AIM: To evaluate the diagnostic angiography and therapy for acute massive hemorrhage in gastrointestinal tract.

METHODS: Twenty-five cases of acute hemorrhage in gastrointestinal tract admitted between April 2002 and September 2004 were reviewed and analyzed by angiography and embolotherapy.

RESULTS: Fifteen patients were men and ten patients were women. The Seldinger technique and method of coaxial duct were used to get access to the bleeding region. PVA particles, gelfoam, and coils were used for embolism. All bleeding sites could be confirmed and were successfully embolized. Hemostasis was achieved in all the patients without bleeding again. The cure rate was 100%.

CONCLUSION: Interventional therapy can not only ascertain the bleeding site, but also stop the bleeding .The method is simple and the effect is certain.

Key words: Intervention; Acute gastrointestinal bleeding; Angiography; Embolization

Wang HH, Bai B, Wang KB, Xu W, Ye YS, Zhang WF. Interventional therapy for acute hemorrhage in gastrointestinal tract. *World J Gastroenterol* 2006; 12(1): 134-136

http://www.wjgnet.com/1007-9327/12/134.asp

INTRODUCTION

Great achievements have been made in interventional therapy in China since 1980s. The method of diagnosis and therapy for hemorrhage in gastrointestinal tract have made great progress[1]. Transcutaneous catheter embolization is widely used in the treatment of acute massive hemorrhage in gastrointestinal tract. Selective angiography can confirm the bleeding site in gastrointestinal tract. We carried out selective or superselective embolotherapy to achieve quick hemostasis. Selective angiography is the most effective measure to detect hemorrhage[2].

MATERIALS AND METHODS

Patients

Twenty-five patients with hemorrhage in the gastrointestinal tract admitted between April 2002 and September 2004 were treated with interventional therapy. Fifteen patients were men and ten patients were women. There were 10 cases of gastric hemorrhage, 1 case of duodenal hemorrhage, 9 cases of small intestinal hemorrhage, 3 cases of colonic hemorrhage, and 2 cases of liver disruption. The major clinical manifestation was substantive bloody stools. Some cases had hematemesis. Hemorrhagic shock occurred in five cases. All patients received treatment but hemorrhage could not be controlled. The diastolic blood pressure was lower than 40 mmHg in four patients.

Equipment and materials

Angiostar Plus-type DSA machine was purchased from Siemens Company. Catheter 4F and RADISITE ® SP catheter 3 F were purchased from Cook Company. The contrast medium ultravist® 370 was obtained from Schering Company. PVA particles and coils were bought from Cook Company. All patients received celiac arteriography as well as superior and inferior mesenteric arteriography to identify the bleeding artery.

Methods

The patients underwent celiac arteriography as well as superior and inferior mesenteric arteriography. Eight patients received superselective catheterization to get access to the corresponding site of feeding artery and then embolotherapy was carried out with the corresponding materials.

RESULTS

A total of 25 patients underwent angiography for

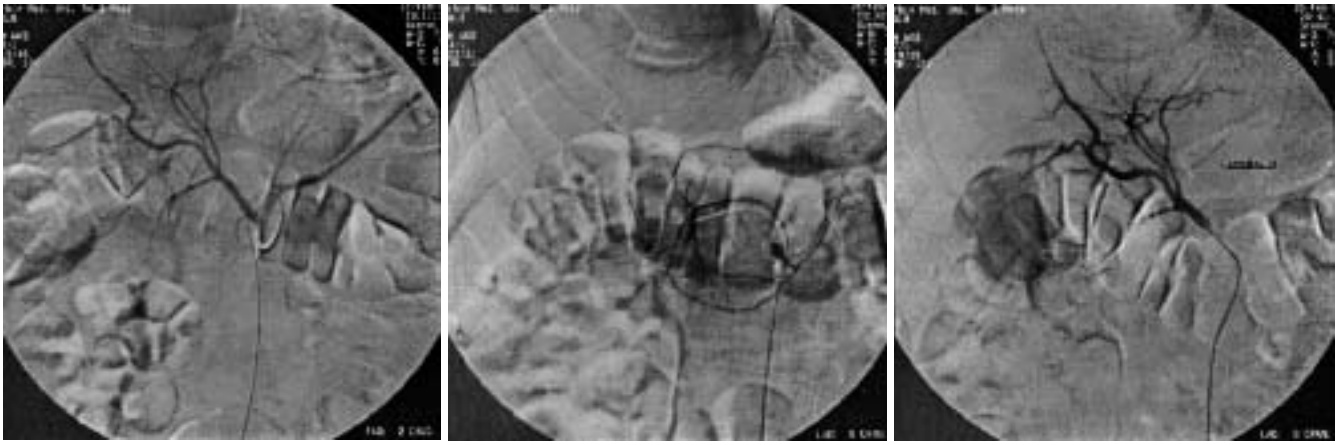


Figure 1 A 25-year-old patient with acute life-threatening gastrointestinal bleeding. **A:** Extravasation was made from a ramification of gastrointestinal artery. **B:** Superselective angiography of the bleeding artery as an aneurysm sign of a ramification belonging to gastrointestinal artery. **C:** Control angiography after embolization with 500-710 μm PVA particles and coils demonstrating complete hemostasis.

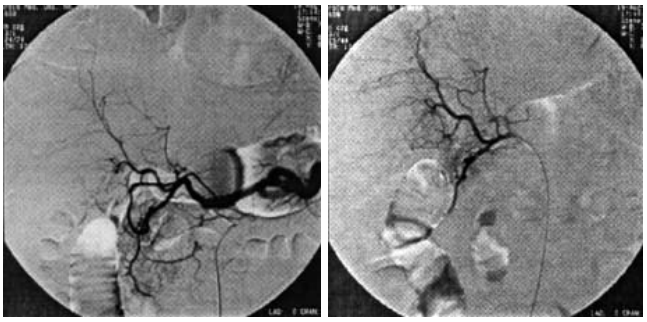


Figure 2 A 51-year-old patient with acute gastrointestinal bleeding. **A:** Contrast medium extravasation from a ramification of gastrointestinal artery. **B:** Complete hemostasis after embolization with 500-710 μm PVA particles and coils.

acute gastrointestinal bleeding. There were 15 men (60%) and 10 women (40%) (mean age 54 years, range 34-74 years). Among the 25 patients, 3 were accompanied with hematemesis, 3 had shock, 15 had bleeding from mesenteric superior artery confirmed by angiography, 2 had hemorrhage from intestinum rectum, 6 had gastric hemorrhage, and 2 had liver arteriorrhexis. Eighteen cases underwent embolism with PVA particles or PVA particles plus gelfoam and seven cases underwent coil embolism. Angiographic embolization was successful in 25 patients with gastrointestinal bleeding, and the success rate was 100%. There were no intestinal parva necrosis and other severe complications in this group (Figure 1 and 2).

DISCUSSION

Acute massive hemorrhage in gastrointestinal tract is one of the most acute abdomen[3]. The mortality of emergency surgery is about 10%[4]. It is difficult to identify the bleeding site and cause of hemorrhage. The treatment of hemorrhage in gastrointestinal tract includes non-operative treatment, exploratory laparotomy and interventional embolotherapy[5,6].

Though endoscopy has been used universally, it still has some limitations in diagnosis[7,8]. Antishock and hemostasis can decrease hemorrhage but cannot achieve permanent hemostasis.

Intervention embolotherapy for gastrointestinal hemorrhage is a convenient and efficient microinvasive therapy[9]. When acute hemorrhage in gastrointestinal tract occurs, hemorrhage is often massive[10,11]. According to the leakage location of the contrast medium, we performed superselective catheterization for feeding artery embolism, which achieved hemostasis immediately, suggesting that it is a practical and effective method for old and weak patients and those who cannot tolerate operation. Embolotherapy for lower digestive tract hemorrhage is a choice of treatment[12]. We proved that it could prevent intestinal tract ischemia.

Examination of DSA has the most important clinical value and can prevent other tissue overlapping and dynamically observe the status of artery ramification, capillaries and refluxing veins, particularly for intestinal parva as well as ascending, transverse, and descending colon. The major cause of hemorrhage is tumor and vascular malformation. Our study proved DSA could show tumor blood vessel malformation and precise image for further embolotherapy and exairesis.

Gelfoam is safer and PVA particles may be better for vascular malformation because they can achieve permanent embolism. With regard to the magnitude of PVA particles and coils, we prefer to use larger particles instead of the smaller ones.

When gastrointestinal tract hemorrhage occurs, the body constitution of patients is possibly weak and the patients usually have hemorrhagic shock[13]. Angiography can find out the source of hemorrhage and is an important treatment modality[14,15].

In conclusion, angiography with embolization can successfully control acute massive gastrointestinal bleeding. Embolotherapy can stop acute bleeding and prolong the life of patients.

REFERENCES

1 **Ledermann HP**, Schoch E, Jost R, Decurtins M, Zollikofer CL. Superselective coil embolization in acute gastrointestinal

hemorrhage: personal experience in 10 patients and review of the literature. *J Vasc Interv Radiol* 1998; **9**: 753-760
2 **Ledermann HP**, Schoch E, Jost R, Zollikofer CL. Embolization of the vasa recta in acute lower gastrointestinal hemorrhage: A report of five cases. *Cardiovasc Intervent Radiol* 1999; **22**: 315-320
3 **Xu ZB**, Zhou XY, Peng ZY, Xu SL, Ruan LX. Evaluation of selective hepatic angiography and embolization in patients with massive hemobilia. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 254-258
4 **Lefkovitz Z**, Cappell MS, Kaplan M, Mitty H, Gerard P. Radiology in the diagnosis and therapy of gastrointestinal bleeding. *Gastroenterol Clin North Am* 2000; **29**: 489-512
5 **Walsh RM**, Anain P, Geisinger M, Vogt D, Mayes J, Grundfest-Broniatowski S, Henderson JM. Role of angiography and embolization for massive gastroduodenal hemorrhage. *J Gastrointest Surg* 1999; **3**: 61-5; discussion 66
6 **Han YM**, Lee JM, Jin KY, Lee SY, Kim CS. Embolization of superior mesenteric artery branches in dogs. Ischemic bowel changes depend on location of vessel occlusion and embolic materials. *Invest Radiol* 1999; **34**: 629-635
7 **Moreno C**, Arvanitakis M, Devière J, Van Gossum A. Capsule endoscopy examination of patients with obscure gastrointestinal bleeding: evaluation of clinical impact. *Acta Gastroenterol Belg* 2005; **68**: 10-14
8 **Narula HS**, Kotru A, Nejim A. Hepatic artery aneurysm: an unusual cause for gastrointestinal haemorrhage. *Emerg Med J* 2005; **22**: 302
9 **Arasaradnam RP**, Donnelly MT. Acute endoscopic intervention in non-variceal upper gastrointestinal bleeding. *Postgrad Med J* 2005; **81**: 92-98
10 **Strate LL**, Syngal S. Predictors of utilization of early colonoscopy vs. radiography for severe lower intestinal bleeding. *Gastrointest Endosc* 2005; **61**: 46-52
11 **Nikolopoulou VN**, Thomopoulos KC, Katsakoulis EC, Vasilopoulos AG, Margaritis VG, Vagianos CE. The effect of octreotide as an adjunct treatment in active nonvariceal upper gastrointestinal bleeding. *J Clin Gastroenterol* 2004; **38**: 243-247
12 **Kuo WT**, Lee DE, Saad WE, Patel N, Sahler LG, Waldman DL. Superselective microcoil embolization for the treatment of lower gastrointestinal hemorrhage. *J Vasc Interv Radiol* 2003; **14**: 1503-1509
13 **Akhtar AJ**. Lower gastrointestinal bleeding in elderly patients. *J Am Med Dir Assoc* 2003; **4**: 320-322
14 **Huang IF**, Wu TC, Wang KS, Hwang B, Hsieh KS. Upper gastrointestinal endoscopy in children with upper gastrointestinal bleeding. *J Chin Med Assoc* 2003; **66**: 271-275
15 **Lundell L**. Upper gastrointestinal hemorrhage--surgical aspects. *Dig Dis* 2003; **21**: 16-18

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 137-140
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Effect of resveratrol on pancreatic oxygen free radicals in rats with severe acute pancreatitis

Zhen-Dong Li, Qing-Yong Ma, Chang-An Wang

Zhen-Dong Li, Qing-Yong Ma, Department of Hepatobiliary Surgery, First Hospital of Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Chang-An Wang, Department of Pathology, Xi'an Municipal Center Hospital, Xi'an 710068, Shaanxi Province, China
Correspondence to: Dr Zhen-Dong Li, Department of Hepatobiliary Surgery, First Hospital of Xi'an Jiaotong University, 1 Jiankang Road, Xi'an 710061, Shaanxi Province
China. victor7922@163.com
Telephone: +86-29-85324009 Fax: +86-29-85323201
Received: 2005-05-09 Accepted: 2005-06-06

Abstract

AIM: To investigate the therapeutic effects of resveratrol (RESV) as a free radical scavenger on experimental severe acute pancreatitis (SAP).

METHODS: Seventy-two male Sprague–Dawley rats were divided randomly into sham operation group, SAP group, and resveratrol-treated group. Pancreatitis was induced by intraductal administration of 0.1 mL/kg 4% sodium taurocholate. RESV was given intravenously at a dose of 20 mg/kg body weight. All animals were killed at 3, 6, 12 h after induction of the model. Serum amylase, pancreatic superoxide dismutase (SOD), malondialdehyde (MDA), and myeloperoxidase (MPO) were determined. Pathologic changes of the pancreas were observed under optical microscope.

RESULTS: The serum amylase, pancreatic MPO and the score of pathologic damage increased after the induction of pancreatitis, early (3, 6 h) SAP samples were characterized by decreased pancreatic SOD and increased pancreatic MDA. Resveratrol exhibited a protective effect against lipid peroxidation in cell membrane caused by oxygen free radicals in the early stage of SAP. This attenuation of the redox state impairment reduced cellular oxidative damage, as reflected by lower serum amylase, less severe pancreatic lesions, normal pancreatic MDA levels, as well as diminished neutrophil infiltration in pancreas.

CONCLUSION: RESV may exert its therapeutic effect on SAP by lowering pancreatic oxidative free radicals and reducing pancreatic tissue infiltration of neutrophils.

Key words: Severe acute pancreatitis; Resveratrol; Oxygen free radical; Neutrophil

Li ZD, Ma QY, Wang CA. Effect of resveratrol on pancreatic oxygen free radicals in rats with severe acute pancreatitis. *World J Gastroenterol* 2006; 12(1): 137-140

http://www.wjgnet.com/1007-9327/12/137.asp

INTRODUCTION

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring phytoalexin present in grapes, fruits, and a variety of medicinal plants[1]. It is the major active component of Rhubarb and Giant Knotweed Rhizome, etc., in traditional Chinese medicine. In *in vitro, ex vivo,* and *in vivo* experiments, RESV displays diverse pharmacological effects including modulation of lipoprotein metabolism and cardiovascular protection[2], anti-inflammation[3], platelet antiaggregatory activity[2], antimicrobial activity[4], antiallergic activity[5], anticancer properties[6,7], and most notably, antioxidant properties[8]. In the present study, the sodium taurocholate-induced model of SAP was used to investigate the effects of RESV on SOD, MDA, MPO, serum amylase, and pancreatic pathological change to assess the role of oxidative stress in SAP and the therapeutic effects of RESV on SAP.

MATERIALS AND METHODS

Materials

RESV was obtained from Huike Botanical Development Co, stocked solution of RESV was made in Tween-80 at the concentration of 10 mg/mL and kept frozen. Sodium taurocholate was purchased from Sigma Chemical Co. SOD, MDA, and MPO assay reagents were from Nanjing Jiangcheng Bioengineering Institute.

Animals

Male Sprague-Dawley rats weighing 250 - 300 g, purchased from Laboratory Animal Center attached to Medical College of Xi'an Jiaotong University, were used. All animals were housed in a macroion cage at 22 - 24 °C in a 12/12-h light/dark cycle. The animals were given a standard rat chow and fasted overnight with free access to water before the experiment. Care was provided in accordance with the "Guide for the care and use of laboratory animals" (NIH publication No. 85 - 23, revised in 1996). The study was approved by the Subcommittee on Experimental Animal Care of our institution.

Table 1 Pancreatic histopathologic scoring in rats (mean ± SE)

	n	Sham operation	SAP	RESV-treated
3 h	8	0.283 ± 0.112	9.236 ± 0.624[b]	5.283 ± 0.646[d]
6 h	8	0.219 ± 0.171	11.357 ± 0.535[b]	5.598 ± 0.417[d]
12 h	8	0.112 ± 0.051	13.559 ± 0.636[b]	6.003 ± 0.717[d]

[b]$P<0.01$ *vs* Sham operation; [d]$P<0.01$ *vs* SAP group.

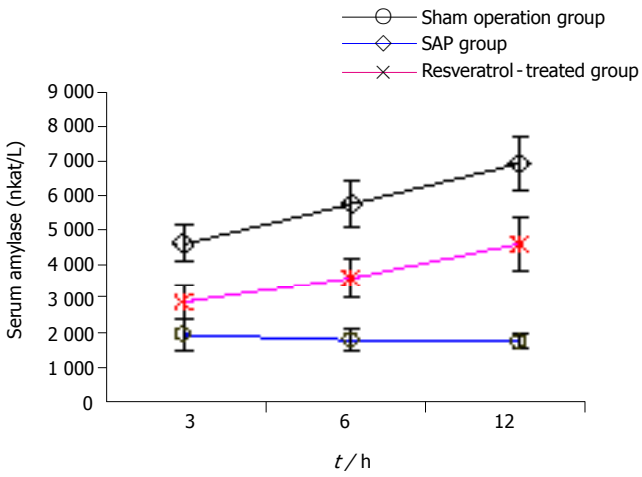


Figure 1 Comparison of serum amylase in rats.

Induction of severe acute pancreatitis

The rats were anesthetized by an intraperitoneal injection of pentobarbital (30 mg/kg, Sigma). Through a midline incision, the duodenum and the pancreatic bile duct were identified, and the duodenal wall was punctured with a 24-gauge Teflon catheter. The catheter was advanced into the common pancreatic bile duct. A microvascular clamp was placed on the duct at the hilum of the liver, a microtube clamp was placed around the catheter and the wall of the duct close to the duodenum to prevent reflux. Four percent sodium taurocholate (1 mL/kg body weight, Sigma T-0750) was injected into the pancreatic bile duct for 60 s. The clamp remained in place throughout the intraductal infusion to prevent misdirected flow into the biliary system.

Experimental design

Seventy-two rats were randomly divided into sham operation group: laparotomy followed by tipping of the pancreas without any infusion, SAP group: receiving infusion of 40 g/L sodium taurocholate into the pancreatic bile duct, RESV-treated group: perfused with RESV at a dose of 20 mg/kg body weight through vena dorsalis penis 10 min after the induction of SAP. After 3, 6, and 12 h, eight rats from each group were killed and blood was taken from the left ventricle of the heart. The samples were centrifuged (3 000 r/min, 10 min, 4 °C) and serum was derivatized and immediately stored at -70 °C for amylase determination. The pancreas was removed; the head of pancreas was fixed in 40 g/L paraformaldehyde for histologic analysis. Caudal pancreatic tissue was powdered using a mortar and pestle on dry ice and immediately stored at -70 °C for determining pancreatic SOD, MDA, and MPO.

Histopathologic analysis

Tissue samples of the pancreas were fixed in 40 g/L paraformaldehyde and embedded with paraffin. Five-micrometer thick sections were stained with hematoxylin/eosin and examined and graded as previously described[9]. The total surface of the slides was scored by one blinded pathologist for four different variables (edema, acinar necrosis, hemorrhage and fat necrosis, inflammation and perivascular infiltrate) to determine the severity of pancreatic injury.

Detection of serum amylase and measurement of pancreatic SOD, MDA, and MPO

Amylase activity in serum was determined using an automatic biochemistry analyzer (Hitachi 7170). Pancreas was homogenized in physiological saline or 5 g/L HTAB using ultrasonication. The SOD content was measured using the xanthine oxidase technique based on the spectrophotometric monitoring of SOD-mediated reduction of DTNB at 550 nm. The concentration of MDA was quantified by thiobarbituric acid reaction and MPO contents were determined as described by Bhatia *et al*[10].

Statistical analysis

Results were expressed as mean ± SE. Statistical analysis was done using the SPSS10.0 software package. One-way analysis of variance was used to establish whether the difference among the three groups was statistically significant. $P<0.05$ was considered statistically significant.

RESULTS

Histopathology

There was no or a small amount of clear ascitic fluid in sham operation group. More than 8 mL turbid hemorrhagic ascites could be seen in all rats of SAP group. No obvious change or slight edema could be seen in sham operation group. Pancreas in SAP group displayed disparate edema with punctiform or lamellar hemorrhage and necrosis. Saponified spots could be seen at pancreas, epiploon, mesentery, peritoneum, and perinephric fat. Pancreatic tissue was normal in sham operation group. In SAP group, pancreatic tissue displayed interstitial edema, widened lobula interspace, inflammatory cell infiltration, hemorrhage and necrosis. Microthromb could be found inside the small vessels around focal necrosis of the pancreas. In contrast, in RESV-treated group, the ascitic fluid diminished significantly and turbidity was lower than that in SAP group. Saponified spots, pancreatic edema, necrosis, inflammatory cell infiltration decreased significantly in RESV-treated group (Table 1).

Serum amylase

Compared to sham operation group, the serum amylase in SAP group increased at all time points ($P<0.01$), decreased significantly in RESV-treated group when compared to SAP group at the corresponding time points ($P<0.01$, Figure 1).

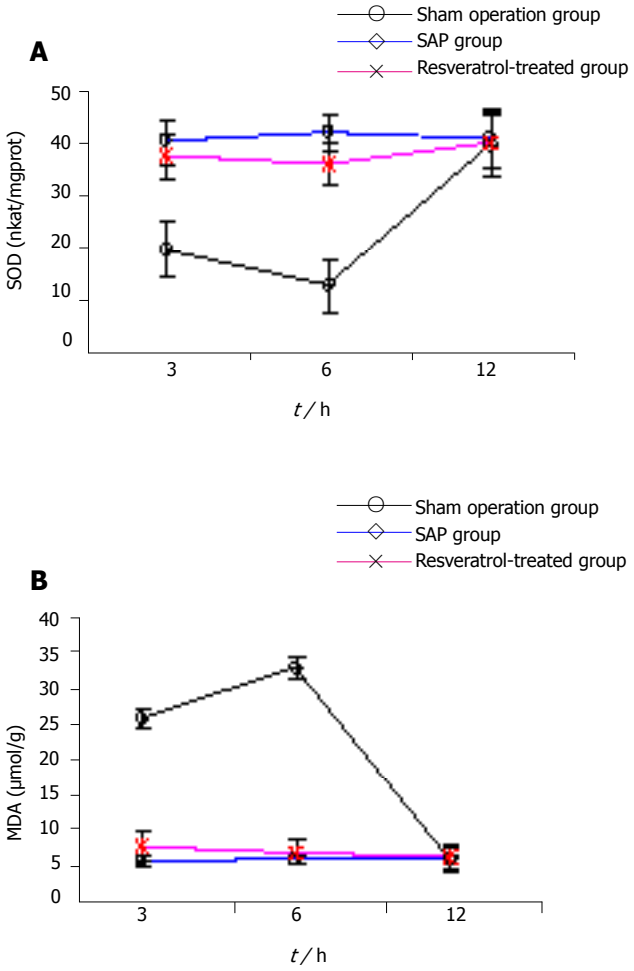


Figure 2 Comparison of pancreatic SOD(**A**) and MDA(**B**) at different time points.

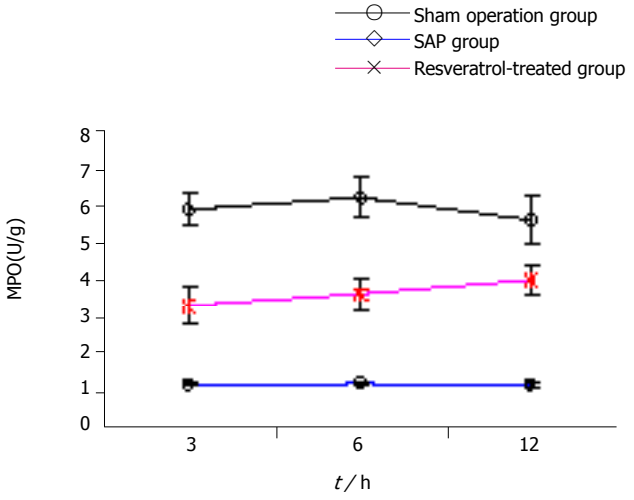


Figure 3 Comparison of pancreatic MPO at different time points.

SOD and MDA in pancreatic tissue

Compared to sham operation group, pancreatic SOD descended and MDA increased in SAP group at 3 and 6 h ($P < 0.01$), but there was no difference between the two groups at 12 h in SOD and MDA. In contrast, pancreatic SOD increased and MDA descended in RESV-treated group at 3 and 6 h when compared to SAP group ($P < 0.01$), but there was no difference in SOD and MDA at 12 h between two groups (Figures 2A and 2B).

MPO in pancreatic tissue

Compared to sham operation group, the pancreatic MPO in SAP group increased at all time points ($P < 0.01$), but decreased significantly in RESV-treated group when compared to SAP group at the corresponding time points ($P < 0.01$, Figure 3).

DISCUSSION

The pathogenesis and therapeutics of SAP are constantly emphasized in general surgery. Sanfey *et al* [11] have suggested a possible involvement of oxygen free radicals (OFRs) in acute pancreatitis. In 1995, Kishimoto *et al*.[12] detected pancreatic OFRs in acute pancreatitis using the technique of chemiluminescence probe and high sensitive photon counting and found that OFRs emerge 2 - 3 h after the induction of acute pancreatitis, demonstrating that there exists peroxidation in acute pancreatitis. OFRs can attack polyunsaturated fatty acid's aldehyde group inside the biomembrane, initiating lipid peroxidation and accordingly forming lipid peroxidation products, as such MDA, which result in the loss of membrane stability and release of acinar cell enzyme precursors, and activate phospholipase A1 which can decompose lecithinum inside cellular membrane, further causing tissue damage. SOD is an internal antioxidase. OFRs *in vivo* are augmented when acute pancreatitis develops, which results in the consumption of antioxidant, SOD activity decrease. Therefore, it is difficult to prevent damage to the pancreas and other organs by lipid peroxidation. Detection of pancreatic SOD and MDA can reflect the peroxidation of pancreatic acinar cells and indirectly reflect the damage due to OFRs.

A number of antioxidant therapies can improve pancreatitis induced by the administration of cerulein[13] and infusion of taurocholate[14]. Lasztity *et al*.[15] found that when enteral formula enriched with n-3 polyunsaturated fatty acids is used in the treatment of acute pancreatitis, the erythrocyte SOD activity is elevated significantly. Leonard *et al*.[8] showed that RESV can scavenge OFRs as measured by spin trapping competitions using sodium formate as a second free radical scavenger, and is effective in inhibiting lipid peroxidation of cellular membranes. In the present study, when compared to sham operation group, 3 h after the induction of SAP, pancreatic SOD decreased significantly, reaching perigee at 6 h, and returned to the level of sham operation group at 12 h. In contrast, 3 h after the induction of SAP, pancreatic MDA increased significantly, reaching to apogee at 6 h, and returned to the level of sham operation group at 12 h. Simultaneously, the serum amylase and pancreatic histopatho-logic score increased gradually. The results indic-ate that overproduction of OFRs occurs in early SAP and is a significant factor for aggravating pathogenetic condition. This is coincident with the research by Reinheckel *et al* [16]. When compared to SAP group, pancreatic SOD in RESV-treated group increased significantly at 3 and 6

h ($P < 0.01$), whereas pancreatic MDA in RESV-treated group decreased significantly at 3 and 6 h ($P < 0.01$). On the other hand, compared to SAP group, both serum amylase and pancreatic histopathologic score in RESV-treated group decreased at all three time points ($P < 0.01$) indicating that RESV can depress earlier OFR production and lipid peroxidation of cellular membrane, diminish enzyme precursor release and necrosis of acinar cells, thus ameliorating pancreatic pathological lesions.

Neutrophils are the other major cellular source of OFRs during acute pancreatitis[17,18], and can directly release several inflammatory cytokines, evoking systemic inflammatory reactive syndrome (SIRS). since OFRs can exert a chemoattractant effect, thereby promoting accumulation of leukocytes in the inflamed gland[17]. Decreased acinar OFR production after RESV treatment may contribute to the reduced neutrophil infiltration, further ameliorating SIRS in SAP. In our study neutrophil sequestration within the pancreas was estimated by measuring tissue MPO activity. When compared to SAP group, the pancreatic MPO decreased at all the time points in RESV-treated group ($P < 0.01$). Moreover, studies showed that RESV can suppress the activation of NF-κB. Thus, RESV treatment might lead to the suppression of NF-κB activation and the subsequent prevention of several inflammatory mediator genes from being actively expressed[19-21]. This mechanism may also help to reduce the sequestration of neutrophils in the pancreas and the associated OFR generation, thus effectively attenuating pancreatic damage.

In conclusion, overproduction of OFRs takes place in early SAP, and is a significant factor for aggravating pathogenetic condition. RESV can ameliorate pathological lesions in the pancreas by lowering pancreatic OFRs and reducing pancreatic tissue infiltration of neutrophils. It may have certain therapeutical effects on acute pancreatitis.

REFERENCES

1 **Kollár P**, Hotolová H. [Biological effects of resveratrol and other constituents of wine]. *Ceska Slov Farm* 2003; **52**: 272-281
2 **Hung LM**, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc Res* 2000; **47**: 549-555
3 **Jang DS**, Kang BS, Ryu SY, Chang IM, Min KR, Kim Y. Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production. *Biochem Pharmacol* 1999; **57**: 705-712
4 **Docherty JJ**, Fu MM, Stiffler BS, Limperos RJ, Pokabla CM, DeLucia AL. Resveratrol inhibition of herpes simplex virus replication. *Antiviral Res* 1999; **43**: 145-155
5 **Cheong H**, Ryu SY, Kim KM. Anti-allergic action of resveratrol and related hydroxystilbenes. *Planta Med* 1999; **65**: 266-268
6 **Fontecave M**, Lepoivre M, Elleingand E, Gerez C, Guittet O. Resveratrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett* 1998; **421**: 277-279
7 **Jang M**, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 1997; **275**: 218-220
8 **Leonard SS**, Xia C, Jiang BH, Stinefelt B, Klandorf H, Harris GK, Shi X. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. *Biochem Biophys Res Commun* 2003; **309**: 1017-1026
9 **Schmidt J**, Rattner DW, Lewandrowski K, Compton CC, Mandavilli U, Knoefel WT, Warshaw AL. A better model of acute pancreatitis for evaluating therapy. *Ann Surg* 1992; **215**: 44-56
10 **Bhatia M**, Brady M, Zagorski J, Christmas SE, Campbell F, Neoptolemos JP, Slavin J. Treatment with neutralising antibody against cytokine induced neutrophil chemoattractant (CINC) protects rats against acute pancreatitis associated lung injury. *Gut* 2000; **47**: 838-844
11 **Sanfey H**, Bulkley GB, Cameron JL. The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis. *Ann Surg* 1984; **200**: 405-413
12 **Kishimoto W**, Nakao A, Nakano M, Takahashi A, Inaba H, Takagi H. Detection of superoxide free radicals in rats with acute pancreatitis. *Pancreas* 1995; **11**: 122-126
13 **Demols A**, Van Laethem JL, Quertinmont E, Legros F, Louis H, Le Moine O, Devière J. N-acetylcysteine decreases severity of acute pancreatitis in mice. *Pancreas* 2000; **20**: 161-169
14 **Rau B**, Poch B, Gansauge F, Bauer A, Nüssler AK, Nevalainen T, Schoenberg MH, Beger HG. Pathophysiologic role of oxygen free radicals in acute pancreatitis: initiating event or mediator of tissue damage? *Ann Surg* 2000; **231**: 352-360
15 **Lasztity N**, Hamvas J, Biró L, Németh E, Marosvölgyi T, Decsi T, Pap A, Antal M. Effect of enterally administered n-3 polyunsaturated fatty acids in acute pancreatitis--a prospective randomized clinical trial. *Clin Nutr* 2005; **24**: 198-205
16 **Reinheckel T**, Nedelev B, Prause J, Augustin W, Schulz HU, Lippert H, Halangk W. Occurrence of oxidatively modified proteins: an early event in experimental acute pancreatitis. *Free Radic Biol Med* 1998; **24**: 393-400
17 **Poch B**, Gansauge F, Rau B, Wittel U, Gansauge S, Nüssler AK, Schoenberg M, Beger HG. The role of polymorphonuclear leukocytes and oxygen-derived free radicals in experimental acute pancreatitis: mediators of local destruction and activators of inflammation. *FEBS Lett* 1999; **461**: 268-272
18 **Wisner J**, Green D, Ferrell L, Renner I. Evidence for a role of oxygen derived free radicals in the pathogenesis of caerulein induced acute pancreatitis in rats. *Gut* 1988; **29**: 1516-1523
19 **Tsai SH**, Lin-Shiau SY, Lin JK. Suppression of nitric oxide synthase and the down-regulation of the activation of NFkappaB in macrophages by resveratrol. *Br J Pharmacol* 1999; **126**: 673-680
20 **Manna SK**, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. *J Immunol* 2000; **164**: 6509-6519
21 **Holmes-McNary M**, Baldwin AS. Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. *Cancer Res* 2000; **60**: 3477-3483

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 141-145
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Known and probable risk factors for hepatitis C infection: A case series in north-eastern Poland

Sławomir Chlabicz, Robert Flisiak, Anna Grzeszczuk, Oksana Kovalchuk, Danuta Prokopowicz, Lech Chyczewski

Sławomir Chlabicz, Department of Family Medicine and Community Nursing, Medical University of Białystok, Poland
Robert Flisiak, Anna Grzeszczuk, Danuta Prokopowicz, Department of Infectious Diseases, Medical University of Białystok, Poland
Oksana Kovalchuk, Lech Chyczewski, Department of Clinical Molecular Biology, Medical University of Białystok, Poland
Supported by the grant from the Medical University of Białystok, Poland, No. 3-56876L
Correspondence to: Dr Sławomir Chlabicz, Department of Family Medicine and Community Nursing, Mieszka I 4 B, 15-054 Białystok, Poland. schlabicz@poczta.onet.pl
Telephone: +48-85-7326820 Fax: +48-85-7327848
Received: 2005-05-03 Accepted: 2005-06-09

Abstract

AIM: To describe the risk profile of patients in hospital with hepatitis C virus (HCV) infection in Poland.

METHOD: Using a structured questionnaire, all patients with confirmed HCV infection were interviewed about the risk factors.

RESULTS: Among the 250 patients studied, transfusion before 1993 was the primary risk factor in 26%, intravenous drug use setting in 9% and occupational exposure in health-care in 9%. Women were more likely to have a history of occupational exposure or transfusion before 1993 and less likely to undergo minor surgery. Known nosocomial risk factors (transfusion before 1993, dialysis) were responsible for 27% of infections, probable nosocomial factors (transfusions after 1992, minor surgery) for 14% and further 9% were occupationally acquired infections.

CONCLUSION: A careful history investigation can identify a known or probable risk factor for HCV acquisition in 59% of patients with HCV infection. Preventive activities in Poland should focus on infection control measures in health-care setting.

Key words: Hepatitis C; Risk factors

Chlabicz S, Flisiak R, Grzeszczuk A, Kovalchuk O, Prokopowicz D, Chyczewski L. Known and probable risk factors for hepatitis C infection: A case series in north-eastern Poland. *World J Gastroenterol* 2006; 12(1): 141-145

http://www.wjgnet.com/1007-9327/12/141.asp

INTRODUCTION

Hepatitis C virus (HCV) is the most common chronic blood-borne infection in developed countries and the major cause of chronic liver disease, cirrhosis and hepatocellular cancer. Since no effective vaccine against HCV infection is available, reducing the spread of the disease relies on primary prevention activities that can cut the transmission routes and reduce or eliminate the risk of acquiring infection. Since its discovery in 1989, much has been learnt about the ways in which HCV is transmitted. Well-known and common modes of transmission involve transfusions received before the routine screening of blood donors was implemented (in Poland since July 1992), intravenous drug use (IVDU), hemodialysis, and occupational exposure to the infected blood in health-care facilities[1-3]. Sexual transmission of HCV has also been demonstrated, but it is known to occur with less frequency compared to hepatitis B or HIV. Other risk factors are considered, but their role has not been established convincingly. Some case-control studies linked HCV infection to surgical or dental procedures, endoscopies, tattooing, body piercing, acupuncture, household contact with an anti-HCV person, and intranasal cocaine use. The results of the studies are, however, conflicting and some expert groups have found no associations between those exposures and HCV infections. In fact, there may be geographical differences in predominance of certain routes of transmission over others. Although the data on HCV epidemiology in Eastern Europe are scarce, the available literature and experts' opinions indicate that surgical and parenteral procedures (independent from blood transfusions) account for 40%-71% of HCV infections[4].

The precise data on HCV prevalence in the general population in Poland is lacking since no population-based study was carried out. According to WHO estimates about 1.4% of general Polish population may be infected with HCV, which means about 560 000 persons in the whole country[5]. At the same time, statistics of the National Institute of Hygiene in Warsaw registered about 13 000 infections in the years 1997-2003 (compulsory registration of HCV started in 1997)[6]. It is clear that most people with HCV infection in Poland are unaware of their status, even if we assume under-reporting by medical and laboratory services.

In Poland, in contrast to USA or UK, where drug use prevails, many HCV cases are presumed to be nosocomial infections. Previous studies based on the samples of patients hospitalized for acute or chronic hepatitis C linked as many as 59%-71% of HCV infections to medical procedures[7]. Our recent case-controlled study aiming at identifying medical procedures associated with exposure to HCV found that transfusions (OR = 3.7, 95%CI = 2.2-6.3), minor surgery (OR = 3.2, 95%CI = 1.5-6.7) and dental care (OR = 2.3, 95%CI = 1.4-4.0) were independently associated with HCV infection[8]. In Poland, hepatitis B virus (HBV) infection, which spreads in a similar way to HCV, is also frequently a medically linked disease[9].

Identifying risk factors is important in order to plan preventive activities and is also necessary to target screening for people with higher pre-screening probability of the disease.

We undertook this study in order to describe the risk profile in a population of patients seeking care in a tertiary care level hospital in a defined region of Poland.

Table 1 Prevalence of all known and probable risk factors among 250 chronic hepatitis C patients (*n*, %)

Risk factors	All *n* = 250 (100%) *n* (%)	F *n* = 92 (36.8%) *n* (%)	M *n* = 158 (63.2%) *n* (%)
Known risk factors			
IVDU	22 (8.8)	4 (4.4)	18 (11.4)
Transfusion <1993	67 (26.8)	31 (33.7)	36 (22.8)
Hemodialysis	5 (2.0)	3 (3.3)	2 (1.3)
Occupational exposure - health-care	34 (13.6)	21 (22.8)[1]	13 (8.2)[1]
Sexual exposure to HCV	2 (0.8)	0 (0.0)	2 (1.3)
Probable risk factors			
Transfusions after 1992	17 (6.8)	6 (6.5)	11 (7.0)
Minor surgery	36 (14.4)	5 (5.4)[2]	31 (19.6)[2]

[1]$P<0.05$, F *vs* M in occupational exposure - health-care group; [2]$P<0.05$, F *vs* M in minor surgery group.

MATERIALS AND METHODS

Patients

The study took place in the Department of Infectious Diseases, Medical University of Bialystok (north-eastern Poland) between June 1, 1998 and December 31, 2004. All consecutive adult patients with acute or chronic hepatitis C admitted to the department were invited to participate. This department is the biggest hepatologic center in Podlaskie Region (1 200 000 inhabitants, north-eastern Poland), where the majority of patients with chronic viral hepatitis from the whole region are referred for evaluation and antiviral treatment.

The diagnosis of hepatitis C was based on the presence of anti-HCV antibodies (ELISA, third generation test, IMx MEIA, Abbott, Chicago, USA) and was confirmed by means of HCV-RNA testing (qualitative nested RT-PCR). The standard procedures with a suspected case of chronic hepatitis C include initial testing for anti-HCV and determination of ALT levels, and repeating the tests for anti-HCV and ALT levels after 6 mo. If anti-HCV is repeatedly positive and ALT levels remain elevated above the normal range, patients are tested for HCV-RNA, and liver biopsy is performed. Since confirmatory tests using immunoblotting were not available, only the patients who were positive for anti-HCV and HCV-RNA were included in the present study.

Methods

All patients were interviewed extensively by one of the two doctors with the use of a structured questionnaire. The questionnaire covered demographic data (age, sex, education, job, place of living) and information about the possible risk factors. The risky exposures considered in our study were as follows: (1) known risk factors, such as IVDU, transfusions of blood or blood products before 1993, employment as a health-care worker with exposure to blood or other fluids, hemodialysis, and sexual contact with an anti-HCV positive person; (2) probable risk factors, such as household (non-sexual) contact with an anti-HCV positive person, transfusion after 1992, and minor surgery; and (3) other potential risk factors for HCV infection, such as surgeries, endoscopies, tattoos, previous hospitalizations, and acupuncture. In further analysis, the patients with more than one risk factor were classified as having only the risk factor according to the hierarchy. The hierarchy of risk factors used in our study was based on the data from medical literature as well as on the results of our previous study indicating the link between history of minor surgery, transfusion after 1992 and increased risk for HCV infection in Poland. We did not include dental care (which had also been associated with HCV infection) into probable risk factors because of the low specificity of that exposure with nearly 90% of the study group providing history of dental treatment. For comparisons between groups, the patients were stratified by age (<45 or ≥ 45 years) and by gender.

Statistical analysis

The statistical calculations were performed with the use of statistical package, Statistica Pl. Fisher's exact test was used for the analysis of differences in risk factors between groups (males *vs* females, younger *vs* older and younger *vs* older patients of the same sex). A P value < 0.05 was considered statistically significant.

RESULTS

A total of 420 anti-HCV positive individuals were evaluated during the study period and 250 were eligible for the study. In the remaining 170 cases, the results of HCV-RNA testing were either negative or unavailable. Among the study group, there were 92 females (36.8%) and 158 males (63.2%). Patients' age ranged from 18 to 70 years with the mean age of 39.7 (± 2.8) years. Females were found to be obviously older compared to males (mean age, 43.4 *vs* 38.4 years, $P < 0.05$). Majority of the patients came from urban setting (219 patients, 87.6%) and had secondary (120, 48.0%) or elementary (80, 32.0%) education.

Table 1 presents the overall prevalence of the considered

Table 2 Distribution of known and probable primary risk factors (one per person according to the hierarchy) stratified by age and gender

Risk factors	All *n* (%) 250 (100%)	F *n* (%) 92 (36.8)	M *n* (%) 158 (63.2)	F < 45 *n* (%) 43 (46.7)	M < 45 *n* (%) 109 (69.0)	F > 44 *n* (%) 49 (53.3)	M > 44 *n* (%) 49 (31.0)
Known risk factors	112 (44.8)	49 (53.3)[a]	63 (39.9)[a]	26 (60.5)[c]	43 (39.4)[c]	23 (46.9)	20 (40.8)
IVDU	22 (8.8)	4 (4.4)	18 (11.4)	3 (7.0)[e]	18 (16.5)[e]	1 (2.0)[e]	0 (0.0)[e]
Transfusion before 1993	65 (26.0)	31 (33.7)[g]	34 (21.5)[g]	18 (41.9)[i]	19 (17.4)[i]	13 (26.5)	15 (30.6)
Occupational exposure – health-care	22 (8.8)	13 (14.1)[k]	9 (5.7)[k]	5 (11.6)	6 (5.5)	8 (16.3)	3 (6.1)
Dialysis	3 (1.2)	1 (1.1)	2 (1.3)	0 (0.0)	0 (0.0)	1 (0.0)	2 (4.1)
Sexual contact with HCV	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Probable risk factors	35 (14.0)	6 (6.5)[m]	29 (18.4)[m]	4 (9.3)	17 (15.6)	2 (4.1)[o]	12 (24.5)[o]
Transfusion after 1992	13 (5.2)	4 (4.4)	9 (5.7)	2 (4.7)	3 (2.8)	2 (4.1)	6 (12.2)
Minor surgery	22 (8.8)	2 (2.2)[a]	20 (12.7)[a]	2 (4.7)	14 (12.8)	0 (0.0)[s]	6 (12.2)[s]
No known or probable risk factors	103 (41.2)	37 (40.2)	66 (41.8)	13 (30.2)	49 (45.0)	24 (49.0)	17 (34.7)

[a]$P < 0.05$, known risk factors F *vs* M; [c]$P < 0.05$, known risk factors F < 45 *vs* M < 45; [e]$P < 0.05$, IVDU all < 45 *vs* all > 44; [g]$P < 0.05$, transfusions before 1993 F *vs* M; [i]$P < 0.05$, transfusions before 1993 F < 45 *vs* M < 45; [k]$P < 0.05$, occupational health-care F *vs* M; [m]$P<0.05$, probable risk factors F *vs* M; [o]$P < 0.05$, probable risk factors F>44 *vs* M>44; [q]$P<0.05$, minor surgery F *vs* M; [s]$P < 0.05$, minor surgery F > 44 *vs* M > 44

Table 3 Potential sources of exposure to HCV among 103 persons without known or probable risk factors

Risk factors	All *n* (%) 103 (100%)	F *n* (%) 37 (35.9)	M *n* (%) 66 (64.1)
Surgery (other than minor)	49 (47.6)	24 (64.9)[a]	25 (37.9)[a]
Endoscopies	52 (50.5)	22 (59.5)	30 (45.5)
Hospitalizations (more than 5)	22 (21.4)	15 (40.5)[c]	7 (10.6)
Tattoo	11 (10.7)	0 (0.0)	11 (16.7)
Dental care	91 (88.3)	33 (89.2)	58 (87.9)
None of above	1 (1.0)	0 (0.0)	1 (1.5)

[a]$P < 0.05$, surgery (other than minor) F *vs* M; [c]$P < 0.05$, hospitalizations (more than 5) F *vs* M; [c]$P < 0.05$, tattoo F *vs* M.

known and probable risk factors. In the studied hepatitis C group, there were no cases of household (non-sexual) exposures to HCV and that factor is not presented in the table. The most prevalent reason was transfusion before 1993 in 67 (26.8%) cases, followed by minor surgeries in 36 (14.4%) cases, occupational exposure in 34 (13.6%) cases during health-care and IVDU in 22 (8.8%) cases. Table 2 displays hierarchical distribution of primary risk factors for HCV infection (each case is represented only once). Overall known risk factors could be identified in nearly 45% of patients and probable in further 14%. About 41.0% had no known or probable risk factors according to the criteria established in our study. Three factors, such as history of transfusion before 1993, occupational exposure and minor surgery, were significantly associated with gender. The first two factors occurred more frequently in females while minor surgery was more common among males. There was a clear tendency towards more frequent occurrence of IVDU among males, but the difference did not reach statistical significance.

Overall females were more probable than males to have known risk factors (53.3% *vs* 39.9%, $P < 0.05$). The prevalence of drug use in the younger group (21/152, 13.8%) was significantly higher than that in the older group (1/98, 1.0%, $P < 0.05$). IVDU in our study was almost limited to males under the age of 45 with 82% (18/22) of patients.

In our study, females ≥45 years were the group with the highest frequency (49%) of unidentified (without known or probable risk factors) source of HCV infection, but the difference was not statistically significant.

Table 3 depicts the overall prevalence of other potential exposures to HCV among 103 patients without known or probable risk factors. Only one person denied all of the considered risk factors.

Known nosocomial risk factors (hemodialysis, transfusion before 1993) were responsible for 27% of all infections, while probable nosocomial risk factors (transfusion after 1992, minor surgery) were responsible for 14% of infections. Further 9% were occupationally acquired infections in health-care workers. Altogether at least 50% of all HCV infections in our study were associated with a health-care sector.

DISCUSSION

Hepatitis C infection affects approximately 560 000 people in Poland. Difficulties in identifying risky exposures result from the fact that most cases are clinically silent and remain undiagnosed for many years. In addition, some potential risk factors (e.g., dental care, hospitalizations) are very common and their non-specific nature hinders

establishing their role in HCV transmission and makes targeting prevention measures difficult.

The demographic characteristics of our sample were comparable to the data collected by National Institute of Hygiene in Warsaw for all 2 255 new hepatitis C cases registered in Poland in 2003 (incidence 5.90/100 000 inhabitants)[6]. In our group, males constituted 63% of cases and males under the age of 45 made up nearly 44% of all HCV infections. The national statistics for 2003 indicate that 57% of HCV infections occurred in males, and younger males between 20 and 24 years of age had the highest incidence of infection (10.8/100 000 inhabitants). Similarly, as in our findings, majority of the registered new cases came from urban setting (80.0%). Currently, there are no national reports on the distribution of risk factors among registered HCV cases in Poland.

In our study, known risk factors could be identified in nearly 45% of HCV infections. As expected, transfusions before 1993 represented the most prevalent known exposure. Transfusion before 1993 could be documented in more than 60% of HCV infected females under the age of 45. In a majority of cases, the infection was the iatrogenic effect of postpartum iron-deficiency anemia treatment. Blood transfusion was frequently used to raise the hemoglobin levels and to allow earlier discharge from hospital.

Primary prevention activities have been already undertaken and current procedures have virtually eliminated the risk of HCV infection from blood transfusion. The knowledge of history of transfusion is important for secondary prevention, which means target screening for HCV infection. Testing should be routinely offered to the persons with the history of transfusions before 1993, accompanied by appropriate counseling and medical management.

IVDU was responsible for 9% of infections and was almost exclusively limited to males under the age of 45. At the same time, younger males made the group with high frequency of no known or probable risk factors. It may be speculated that, at least in some of those cases, incidental drug use could be responsible. Many of American blood donors found to be positive for HCV infection revealed the history of drug use, despite initial denial of such exposure[10]. In our study, the rate of HCV infections resulting from drug use among males <45 years of age (17%) was still much lower than that reported in similar groups in the United States (59%-60%)[11,12]. This finding confirms the primary role of nosocomial HCV spread in Poland and may reflect lower numbers of drug users in Poland compared to USA or other Western European countries. Estimates of the prevalence of problem drug use (defined as injecting drug use or long duration, regular use of opiates, cocaine and/or amphetamines) range in European Union countries between 2 and 10 cases per 1 000 of the population aged between 15 and 64 years, and Poland remains in the low range[13]. As in many Central and Eastern European countries, the major problem regarding drug use in Poland concerns heroin. Opiate users represent the biggest proportion of persons admitted to residential treatment due to drug addiction. Shared usage of contaminated needles and syringes results in high prevalence of HCV antibodies. Among 100 parenteral drug users in Warsaw, 76% were found to be seropositive for anti-HCV[14]. Another possible explanation for relatively low percentage of intravenous drug users in our study might be failing secondary prevention of HCV infection among drug-dependant population in Poland. In this respect, it is possible that drug users are under-represented in the hospital samples because of the hindered access to screening procedures, health-care services and long-term antiviral treatment.

Occupational infections constituted nearly 10% of primary risk factors in our study and occurred mostly in women in both age groups. Nurses are the predominant occupational group injured by needles and other sharp-edged instruments, because they are the largest segment of the workforce in health-care, and also because they may have a higher rate of injury. This group sustains about 50% of all needle-stick injuries. The accidents typically happen when workers are recapping needles, transferring body fluids from one container to another, or when they do not dispose the used needles properly[15,16]. In 2001, hepatitis C constituted the major cause of all occupational blood-borne infections in health-care workers in Poland[17].

In this study, known nosocomial and probable nosocomial risk factors were responsible for 27% and 14% of all infections, respectively. Fortunately, the major nosocomial risk factor, transfusion of contaminated blood, has been virtually eliminated. After the introduction of screening of all blood donations for HCV-RNA, the calculated risk of HCV infection resulting from the transfusion of blood during window period is about 1/1 000 000 blood units[18].

In 102 out of 103 HCV-infected individuals without known or probable risk factors, other potential exposures could be found and most of those were medically linked.

Our study confirms that hospital setting remains as an important source of infection. This seems to be a common feature in Eastern European countries. Nosocomial transmission of HCV is possible if infection-control techniques or disinfection procedures are inadequate and contaminated equipment is shared among the patients. Diagnostic or treatment procedures (surgical or parenteral procedures without blood transfusions) in hospitals were indicated as the source of infection in approximately 59%-65% cases in Poland, 59% in Latvia and 46% in Hungary[4].

Our study had certain limitations as follows: the patients with IVDU or hemodialysis were under-represented in the sample; the percentage of patients with probable risk factors was related to our definition of those factors; and we did not include certain medical procedures or events into that category because previous case-control study did not confirm their relation to HCV infection and because some of them could not be the cause but the result of HCV infection (endoscopies, hospitalizations). In our previous study, we found an increased risk associated with minor surgery[8]. Including that exposure into probable risk factors category could overestimate the percentage of patients in our study with known or probable risk factors. The possible overestimation was almost limited to men.

In our experience, a careful history can elicit a risk factor in nearly 60% of hepatitis C infections. The study reveals that overall of at least 50% of HCV infections are associated with health-care sector (transfusions, occupational exposure, other nosocomial exposures). Among the remaining 40% of patients without known or probable risk factors, almost all individuals provided a history of contact with health-care sector preceding diagnosis of hepatitis C. There is a clear tendency towards association of risk factors with age and gender. IVDU occurs mainly in younger males, whereas transfusion before 1993 was more common in younger females. Females are more probable to acquire the infection performing occupational activities in health-care. The two groups with the highest rates of risk factors were women > 44 years and men < 45 years. We speculate that hospitalization and surgery are responsible for some of those infections in older females and IVDU in the younger males.

We conclude that preventive activities against HCV spread in Poland should focus on infection control measures in health-care setting.

REFERENCES

1 **Alter MJ**. Hepatitis C virus infection in the United States. *J Hepatol* 1999; **31**: Suppl 1 88-91
2 **Heintges T**, Wands JR. Hepatitis C virus: epidemiology and transmission. *Hepatology* 1997; **26**: 521-526
3 **Yen T**, Keeffe EB, Ahmed A. The epidemiology of hepatitis C virus infection. *J Clin Gastroenterol* 2003; **36**: 47-53
4 **Naoumov NV**. Hepatitis C virus infection in Eastern Europe. *J Hepatol* 1999; **31 Suppl 1**: 84-87
5 **World Health Organization**. Weekly Epidemiological Record 1999; 74: 421-428. Available from: URL: http: //www.who.int/docstore/wer/pdf/1999/wer7449.pdf
6 **National Institute of Hygiene**. Infectious diseases and poisonings in Poland (annual report). Reports of Polish Institute of Hygiene 1997-2003. Available from: URL: http: //www.pzh.gov.pl/epimeld/index_a.html
7 **Mazurek J**. [Hospital acquired infections caused by hepatitis C virus]. *Przegl Epidemiol* 2000; **54**: 315-324
8 **Chlabicz S**, Grzeszczuk A, Prokopowicz D. Medical procedures and the risk of iatrogenic hepatitis C infection: case-controlled study in north-eastern Poland. *J Hosp Infect* 2004; **58**: 204-209
9 **Chlabicz S**, Grzeszczuk A, Pytel B, Prokopowicz D. Is acute hepatitis B still a nosocomially-acquired infection in Poland? A case-controlled study of transmission routes in a hospital sample. *Scand J Infect Dis* 2002; **34**: 808-810
10 **Murphy EL**, Bryzman SM, Glynn SA, Ameti DI, Thomson RA, Williams AE, Nass CC, Ownby HE, Schreiber GB, Kong F, Neal KR, Nemo GJ. Risk factors for hepatitis C virus infection in United States blood donors. NHLBI Retrovirus Epidemiology Donor Study (REDS) *Hepatology* 2000; **31**: 756-762
11 **Flamm SL**, Parker RA, Chopra S. Risk factors associated with chronic hepatitis C virus infection: limited frequency of an unidentified source of transmission. *Am J Gastroenterol* 1998; **93**: 597-600
12 **Yee LJ**, Weiss HL, Langner RG, Herrera J, Kaslow RA, van Leeuwen DJ. Risk factors for acquisition of hepatitis C virus infection: a case series and potential implications for disease surveillance. *BMC Infect Dis* 2001; **1**: 8
13 **European Monitoring Centre for Drugs and Drug Addiction.** The state of the drugs problem in the European Union and Norway. Annual Report 2004. Available from: URL: http: //annualreport.emcdda.eu.int/en/home-en.html
14 **Laskus T**, Radkowski M, Lupa E, Slusarczyk J, Cianciara J, Halama G, Nowicka R. Prevalence of markers of hepatotropic viruses among drug addicts in Warsaw, Poland. *J Hepatol* 1992; **15**: 114-117
15 **Department of Health Guidance for Clinical Healthcare workers**. Protection against infection with blood-borne viruses. Recommendations for the Expert Advisory Group on AIDS and the Advisory Group on Hepatitis 1998. Available from: URL: http: //www.dh.gov.uk/assetRoot/04/01/44/74/04014474.pdf
16 **Trim JC**, Elliott TS. A review of sharps injuries and preventative strategies. *J Hosp Infect* 2003; **53**: 237-242
17 **Pepłońska B**, Szeszenia-Dabrowska N. [Occupational diseases among personnel of Polish hospitals, 2001]. *Med Pr* 2002; **53**: 369-374
18 **Pillonel J**, Laperche S; Groupe "Agents Transmissibles par Transfusion" de la Société française de transfusion sanguine; Etablissement français du sang; Centre de transfusion sanguine des armées. [Trends in residual risk of transfusion-transmitted viral infections (HIV, HCV, HBV) in France between 1992 and 2002 and impact of viral genome screening (Nucleic Acid Testing)]. *Transfus Clin Biol* 2004; **11**: 81-6

S- Editor Kumar M **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 146-149
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Sigmoidorectal intussusception of adenoma of sigmoid colon treated by laparoscopic anterior resection after sponge-on-the-stick-assisted manual reduction

Ki-Jae Park, Hong-Jo Choi, Sung-Heun Kim, Sang-Young Han, Sook-Hee Hong, Jin-Han Cho, Hyung-Ho Kim

Ki-Jae Park, Hong-Jo Choi, Sung-Heun Kim, Department of Surgery, Dong-A University College of Medicine, Pusan, South Korea
Sang-Young Han, Department of Internal Medicine, Dong-A University College of Medicine, Pusan, South Korea
Sook-Hee Hong, Department of Pathology, Dong-A University College of Medicine, Pusan, South Korea
Jin-Han Cho, Department of Radiology, Dong-A University College of Medicine, Pusan, South Korea
Hyung-Ho Kim, Department of Surgery, Seoul National University Bundang Hospital, Bundang, Kyunggi-Do, South Korea
Correspondence to: Dr Hong-Jo Choi, Department of Surgery, Dong-A University College of Medicine, 3-1 Dongdaeshin-Dong, Seo-Gu, Pusan 602-714, South Korea.colonch@donga.ac.kr
Telephone: +82-51-240-5146 Fax: +82-51-247-9316
Received: 2005-05-13 Accepted:2005-06-02

Abstract

We present herein a case report of sigmoidorectal intussusception as an unusual case of sigmoid adenomatous polyp. The patient was a 56-year-old man who suffered from rectal bleeding for one day. He initially visited his general practitioner and was diagnosed as having an intraluminal mass of 15 cm from the anal verge. Several hours after admission to our coloproctology clinic, he suddenly presented with lower abdominal cramping pain with rectal bleeding during his bowel preparation using polyethylene glycol electrolyte solution. An emergency colonoscopy revealed that the invaginated colon with polypoid mass was protruded to the lower rectum. Gastrograffin enema showed that the invaginated bowel segment was 3 cm from the anal verge. CT scan showed the typical finding of intussusception. We performed laparoscopic anterior resection and anastomosis after the sponge-on-the-stick-assisted manual reduction. The permanent pathologic finding showed villotubular adenoma of the sigmoid colon.

Key words: Sigmoidorectal intussusception; Adenomatous polyp; Laparoscopic resection

Park KJ, Choi HJ, Kim SH, Han SY, Hong SH, Cho JH, Kim HH. Sigmoidorectal intussusception of adenoma of sigmoid colon treated by laparoscopic anterior resection after sponge-on-the-stick-assisted manual reduction. *World J Gastroenterol* 2006; 12(1): 146-149

http://www.wjgnet.com/1007-9327/12/146.asp

INTRODUCTION

Adenomatous polyp of the colon is quite a common disease, but its presentation as intussusception is very rare. The diagnosis of the underlying lesion in adult intussusception is often difficult, and it is commonly established only after surgical exploration. However, a modern imaging technique, CT, can be helpful in the precise preoperative identification of the etiology of the intussusception[1,2]. Although surgical resection is generally indicated in most adult intussusceptions, it is still controversial whether or not the sigmoidorectal intussusception should be reduced before resection. We present here a rare case of large villotubular adenoma of the sigmoid colon as a cause of sigmoidorectal intussusception, and it was treated by laparoscopic anterior resection after a manual reduction using our own technique.

CASE REPORT

A 56-year-old male was referred by his general practitioner to our Coloproctology Clinic for further evaluation and management of an intraluminal rectal mass with intermittent rectal bleeding for one day. The sodium phosphate enema (Fleet® Enema; CB Fleet Co, Lynchburg, VA, USA) was used for bowel preparation for sigmoidoscopy in the local clinic. The local practitioner finished the sigmoidoscopy without any complications and gave a presumptive diagnosis of rectal mass with partial downward displacement of involved bowel. The rectal mass was detected at 15 cm from anal verge upon flexible sigmoidoscopy (Figure 1A), and the local practitioner did not explore the entire colon beyond the lesion. Several hours after his admission, he suddenly had lower abdominal cramping pain with rectal bleeding during bowel preparation using polyethylene glycol electrolyte solution. A digital rectal examination palpated a protuberant mass-like lesion with a smooth surface. An emergency colonoscopy revealed a sausage-like protrusion at the rectum about 3 cm proximal to the anal verge (Figure 1B),

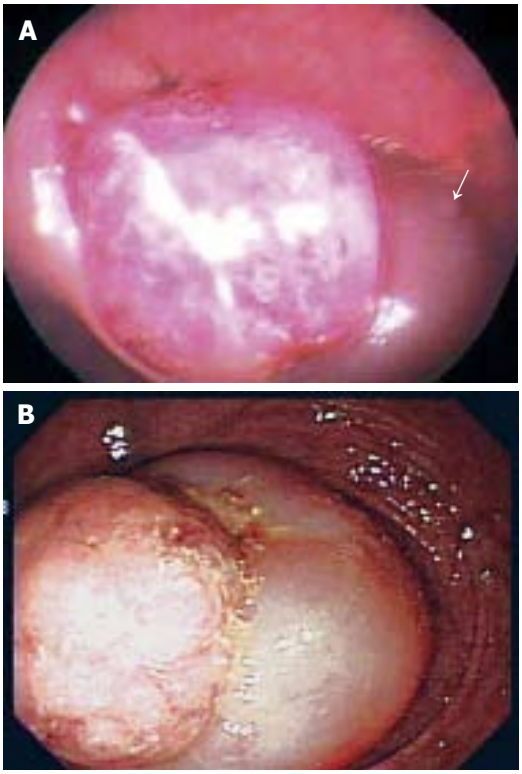


Figure 1 Endoscopic findings. **A:** Flexible sigmoidoscopy at a local clinic showing the mass lesion 15 cm from the anal verge, and the partial downward displacement of involved bowel (arrow); **B:** Colonoscopy at our clinic showing the invaginated bowel with a round mass lesion about 3 cm from the anal verge.

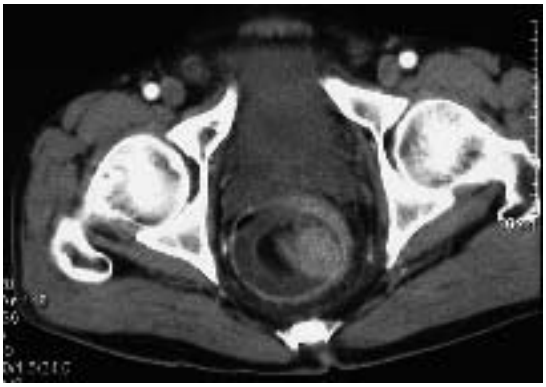


Figure 2 Contrast enhanced CT scan of pelvis showing a homogenously well enhancing mass at the intussuscepted bowel tip.

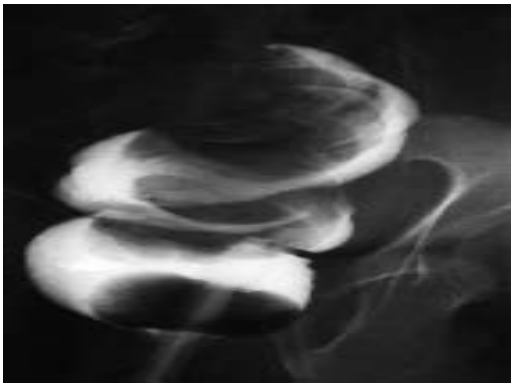


Figure 3 Gastrograffin study showing a large smooth-invaginated mass intussuscepting into the rectum.

and our initial endoscopic diagnosis was nongangrenous sigmoidorectal intussusception that was caused by a polyp. A CT scan of the pelvis was performed to obtain a detailed delineation of the lesion. The CT showed a round target-shaped mass lesion consistent with intussusception, and the leading lesion was thought to be a benign mass with a low likelihood of malignancy (Figure 2). Our clinical impression was sigmoidorectal intussusception secondary to benign adenomatous polyp of the sigmoid colon. For the next step, we tried a contrast study using gastrograffin for the reduction as well as for the diagnostic imaging of the intussusception. This also showed an invaginated bowel segment at the lower rectum about 3 cm proximal to the anal verge (Figure 3), and a hydrostatic pressure was given via the rectum for reduction, but it was in vain.

After the general anesthesia, he was placed supine in the modified lithotomy position using Dan Allen stirrups. After the insertion of the umbilical port for the establishment of pneumoperitoneum, surgery was begun in the Trendelenburg position. On laparoscopic exploration, the neck or commencement of the intussusception was located at the rectosigmoid junction, but the distal end was not identified in the peritoneal cavity. The rectal wall of the involved segment was mildly edematous, but it was otherwise healthy on gross examination. No other intra-abdominal pathologic findings were detected. For the manual reduction of the intussusception, we tried to push up the distal end of invaginated segment with a lubricated sponge-on-the-stick from the anus while monitoring by a laparoscope. This maneuver made it possible to laparoscopically visualize the distal end of the intussusception in the pelvic cavity. We then pulled the intussuscepted segment out proximally using a laparoscopic bowel grasper, but this trial failed. We next tried manual reduction again by grasping the upper ring fold of the intussusception steadily by the index and middle fingers of the surgeon through a suprapubic vertical midline incision 4 cm in length while simultaneously pushing the lowest segment up using a lubricated sponge-on-the-stick via the anus; in this fashion we were successful in reducing the intussuscepted segment. The leading point of the intussusception was located in the mid-sigmoid colon. We performed laparoscopic anterior resection on an oncologic basis because the pathology of the mass had not been confirmed as being benign. After the resection of the segment, the specimen was extracted via the previous suprapubic vertical midline incision. The bowel continuity was safely restored using a standard double-stapling technique between the descending colon and midrectum. The final pathologic diagnosis was villotubular adenoma of the sigmoid colon measuring 2.5 cm × 2 cm in size (Figure 4). The patient was discharged from the hospital 8 d after the operation without any complications.

DISCUSSION

Intussusception is a quite different entity in adults from

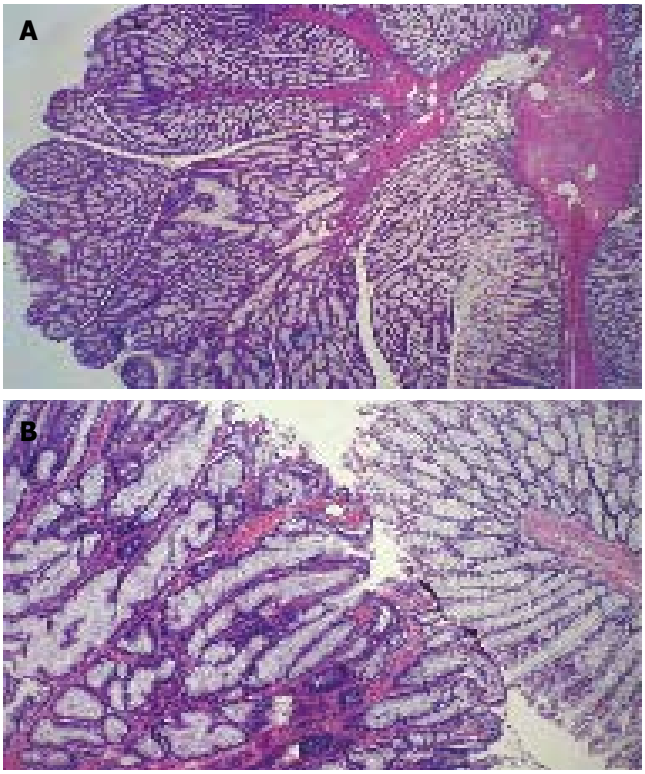


Figure 4 Hematoxylin and eosin staining of the lesions. **A:** The polypoid mass of the colon showing branching papillary projections composed of proliferated glands and thin fibrous stalks (× 10); **B:** The proliferated glands of the polyp (left) lined by single or pseudostratified hyperchromatic columnar cells as compared with normal colonic mucosa (right), but without any malignant changes (× 40).

that found in children. An organic etiology is found in 70%-90% of adult intussusceptions, and primary or secondary malignant neoplasia are documented in about 20%-50% of adult intussusceptions[3-5]. In particular, intussusception occurring in large bowel is more likely to have a malignant etiology[4]. Large polypoid adenomas and lipomas are the most common benign leading points found in adult colocolic intussusception[5,6]. Most reported cases of sigmoido-recto-anal intussusception in the literature are caused by adenoma or carcinoma[7-10], as compared to our case where the lowest intussuscepted segment was located in the lower rectum without protrusion through the anus.

In pathogenesis of intussusception, peristalsis and ingested food push the lesion with the adjacent bowel, which telescopes into the relaxed intestinal segment distal to it[3]. We initially planned to perform colonoscopy after bowel preparation using the polyethylene glycol electrolyte solution, not only to inspect the entire colon but to make a more specific diagnosis of the intussusception. But based on the findings that the patient suddenly complained of lower abdominal pain during bowel preparation and that the intussusceptum was detected 3 cm apart from anal verge at our clinic contrary to the sigmoidoscopic finding that the mass was reported to be 15 cm proximal to the anal verge at previous clinic, this bowel preparation might be, at least, an accelerating factor of intussusception by increased peristalsis. On the ground of clinical course in our case, whole gut irrigation as a bowel preparation should be used with caution because it may accelerate intussusception.

Early diagnosis and appropriate treatment are essential for intussusception because the mesentery of the involved segment is trapped between the overlapping layers of the bowel and its vascularity may be compromised. Unfortunately, a precise preoperative diagnosis is established in only less than half of the cases[3,5,11]. Two factors may be responsible for the low accuracy of the preoperative diagnosis in adult intussusception. One reason is the rare incidence of the intussusception itself and the other reason is the vague or nonspecific symptomatology. This condition is often misdiagnosed as a large polyp[9,12] or rectal prolapse[7,8] because of the wide variety of nonspecific symptoms that include abdominal pain, nausea and/or vomiting. It is noteworthy that, in most reports, the experience of the adult intussusception at each institution is limited to one or two cases per year[3-6,11]. Accordingly, the surgeon must keep in mind the possibility of intussusception even though the clinical signs of the patient may be very subtle.

Radiologic investigations may be helpful to distinguish intussusception from other common causes of intestinal obstruction, and for precisely identifying the etiologies of the intussusception preoperatively[13]. The characteristic finding of intussusception on a contrast study is a cup-shaped filling defect that is often accompanied with an additional filling defect representing the leading tumor[3]. Hydrostatic reduction under radiologic control, as employed in children, is less effective in adults and risk of perforation during the procedure is not negligible[10]. Generally, CT scan is acknowledged to be the most useful radiologic method that may provide additional preoperative information including the possible extension and/or dissemination of a malignant tumor[2,5,11,14]. The most common CT finding is a thickened segment of bowel with an eccentrically placed crescent-like fatty area; this represents the intussusception and the intussuscepted mesentery. This appears either as a round target mass or as a long sausage-shaped mass[14].

The appropriate management of adult intussusception is not always clear cut. Most authors agree that operative management of adult intussusception is almost always indicated because of high likelihood of neoplasm as a leading point, particularly if this occurs in the colon. There is controversy about the reduction before resection in cases of sigmoidorectal intussusception, even though most surgeons agree that primary surgical resection without a prior attempt at reduction is the treatment of choice in colocolic adult intussusception[3,5,13]. Several aspects to consider for reduction prior to resection are the reduction of externally viable bowel despite mucosal necrosis, intraluminal or transperitoneal seeding, venous embolization of malignant cells, and spillage of succus through inadvertent perforation[5]. For patients with sigmoidorectal intussusception, however, the decision regarding the operative procedure may be changed according to tumor involvement of the lower rectum. If the lower rectum is involved, an abdominoperineal resection is indicated. However, without evidence of distal disease, an initial reduction may permit a sphincter-saving procedure instead of the abdominoperineal resection as

advocated by Matsuda *et al* [15]. In this sense, we would like to emphasize the significance of an attempt at manual reduction of the sigmoidorectal intussusception prior to resection, especially for those cases caused by benign lesion whereby an unnecessary abdominoperineal resection can be avoided. Because most colonic intussusceptions do not lead to complete obstruction, adequate preoperative bowel preparation is generally possible and this allows for a primary anastomosis[4].

There are good reasons for laparoscopic anterior resection in this case. The first reason is that the wide resection of intussuscepted colon and its mesentery dictates the most important technical consideration, because two-thirds of colocolic intussusceptions are associated with a malignant lesion[4]. Although preoperative findings were compatible to benign lesion, definite histopathologic diagnosis could not be made in an emergency situation. Moreover, the size of the sessile polyp was large enough (2 cm×2.5 cm on pathologic measurement) for surgeons to consider the possibility of a malignant polyp. In these emergency circumstances, choice of surgical procedure should be judged assuming a possibility of malignancy. The second is the danger in the reduction of externally viable bowel with mucosal necrosis. The third is that intra-abdominal exploration is possible to exclude dissemination in benign-appearing but malignancy-undeniable polyp in an emergency situation. Validities of laparoscopic oncologic colon surgery has been already established in the randomized multicenter trial[16].

In summary, the sigmoidorectal intussusception caused by sigmoid villotubular adenoma can be reduced by the push-up reduction technique, and safely treated by laparoscopic anterior resection. Our own technique using the sponge-on-the-stick is very useful in the situation that an abdominoperineal resection is, at first, deemed to be inevitable because the intussuscepted segment is very close to the anus. Therefore, this prior reduction technique followed by a resection can be considered for the sigmoidorectal intussusception to avoid inadvertent abdominoperineal resection. In conclusion, our principle of prior reduction technique followed by a laparoscopic resection may be of use in the management of benign sigomoidorectal intussusception involving lower rectum to avoid permanent stoma. Besides, it may also be applicable to instances of proven malignant intussusception involving the rectum provided that the lesion is confirmed to be localized without intra-abdominal dissemination or metastasis on the laparoscopic exploration before reduction and bowel resection.

REFERENCES

1 **Huang BY**, Warshauer DM. Adult intussusception: diagnosis and clinical relevance. *Radiol Clin North Am* 2003; **41**: 1137-1151
2 **Takeuchi K**, Tsuzuki Y, Ando T, Sekihara M, Hara T, Kori T, Kuwano H. The diagnosis and treatment of adult intussusception. *J Clin Gastroenterol* 2003; **36**: 18-21
3 **Begos DG**, Sandor A, Modlin IM. The diagnosis and management of adult intussusception. *Am J Surg* 1997; **173**: 88-94
4 **Nagorney DM**, Sarr MG, McIlrath DC. Surgical management of intussusception in the adult. *Ann Surg* 1981; **193**: 230-236
5 **Azar T**, Berger DL. Adult intussusception. *Ann Surg* 1997; **226**: 134-138
6 **Carter CR**, Morton AL. Adult intussusception in Glasgow, UK. *Br J Surg* 1989; **76**: 727
7 **Lynn M**, Agrez M. Management of sigmoid colon intussusception presenting through the anus. *Aust N Z J Surg* 1998; **68**: 683-685
8 **Nelson TM**, Pearl RK, Prasad ML, Abcarian H. Perineal sigmoidectomy for sigmoid procidentia: report of a case. *Am Surg* 1995; **61**: 320-321
9 **Brozzetti S**, Papa O, Polistena A, Mosiello G, Annessi P, Alessi G, Cavallaro A. Recurrent intussusception of the sigmoid colon caused by a transanal protruding sessile tubulo-villous malignant polyp. *Anticancer Res* 2000; **20**: 4697-4700
10 **Nesbakken A**, Haffner J. Colo-recto-anal intussusception. Case report. *Acta Chir Scand* 1989; **155**: 201-204
11 **Eisen LK**, Cunningham JD, Aufses AH. Intussusception in adults: institutional review. *J Am Coll Surg* 1999; **188**: 390-395
12 **Fujino Y**, Fujio Y, Shimada E, Okazaki A. Intussusception due to vanishing colon cancer with metastasis of the regional lymph nodes: report of a case. *Surg Today* 2000; **30**: 188-190
13 **Weilbaecher D**, Bolin JA, Hearn D, Ogden W. Intussusception in adults. Review of 160 cases. *Am J Surg* 1971; **121**: 531-535
14 **Gayer G**, Apter S, Hofmann C, Nass S, Amitai M, Zissin R, Hertz M. Intussusception in adults: CT diagnosis. *Clin Radiol* 1998; **53**: 53-57
15 **Matsuda K**, Suda K, Tamura K, Deguchi T, Yamazaki E, Yago H, Inaba T, Takeshima T, Adachi M, Okinaga K. Surgical management of adult sigmoid colon intussusception caused by a malignant tumor: report of a case. *Surg Today* 2003; **33**: 768-771
16 **The Clinical Outcomes of Surgical Therapy Study Group**. A comparison of laparoscopically assisted and open colectomy for colon cancer. *N Engl J Med* 2004; **350**: 2050-2059

S- Editor Kumar M **L- Editor** Elsevier HK **E- Editor** Li HY

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 150-153
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Spontaneous resolution of systemic sarcoidosis in a patient with chronic hepatitis C without interferon therapy

Tae-Hun Kim, Jong-Eun Joo

Tae-Hun Kim, Departments of Internal Medicine and Medical Research Institute, Ewha Womans University College of Medicine, Seoul, South Korea
Jong-Eun Joo, Department of Pathology, Eulji University School of Medicine, Seoul, South Korea
Correspondence to: Tae-Hun Kim, MD, PhD, Department of Internal Medicine, Ewha Womans University Hospital, 911-1 Mok-Dong, Yangchun-Gu, Seoul 158-710, Mokdong South Korea. thkm@ewha.ac.kr
Telephone: +82-2-2650-2724 Fax: +82-2-2655-2076
Received: 2005-05-09 Accepted: 2005-06-02

Abstract

A 39-year-old male patient complaining of bilateral hand joint arthralgia was evaluated and found to have chronic hepatitis C and systemic sarcoidosis involving lung, skin, liver, and spleen. Hepatic and cutaneous sarcoidoses were confirmed by the presence of numerous noncaseating granulomas on histological examination. Pulmonary and splenic involvements were diagnosed by imaging studies.

Fifteen months later, the sarcoidotic lesions in lung, liver, and spleen were resolved by radiological studies and a liver biopsy showed no granuloma but moderate to severe inflammatory activity. systemic sarcoidosis is a rare comorbidity of chronic hepatitis C which may spontaneously resolve.

Key words: Sarcoidosis; Hepatitis C; Spontaneous resolution

Kim TH, Joo JE. Spontaneous resolution of systemic sarcoidosis in a patient with chronic hepatitis C without interferon therapy. *World J Gastroenterol* 2006; 12(1): 150-153

http://www.wjgnet.com/1007-9327/12/150.asp

INTRODUCTION

Sarcoidosis is a granulomatous disorder of unknown etiology, and is characterized by widespread noncaseating granulomas in many organs. Most commonly sarcoidosis affects the lungs, followed by mediastinal, hilar lymph nodes, and skin. Hepatic involvement of systemic sarcoidosis is frequently demonstrated[1]. In fact, sarcoidosis is the most common cause of hepatic granulomas in the West, and infectious disorders resulting from HCV or HIV infections are possible comorbidities of hepatic sarcoidosis. Most reported cases of HCV associated-sarcoidosis are not the consequence of HCV infection *per se*, but are the results of interferon antiviral therapy[2,3]. However, cases of HCV associated-sarcoidosis without interferon therapy have also been recently reported and the possibility of HCV infection *per se* inducing sarcoidosis by activating host immune systems has been suggested[3,4].

Active sarcoidosis and active viral hepatitis are somewhat incompatible because sarcoidosis is a reflection of activated host cellular immunity to an unknown stimulant, while an augmented cellular immunity is one of the main mechanisms by which HCV replication is suppressed by antiviral agents. Thus, changes in the activities of viral hepatitis *vs* sarcoidosis are worth observing when they simultaneously affect a patient.

We experienced a case of systemic sarcoidosis in a patient with chronic hepatitis C who did not receive interferon therapy, and observed the spontaneous resolution of sarcoidotic lesions with more aggravated hepatitis activities during the natural course of this rare comorbidity.

CASE REPORT

A 39-year-old male patient was admitted because of bilateral hand joint arthralgia, who developed suddenly and impaired hand grip. His serum rheumatoid factor titer (102.7 kU/L) was elevated but other associated clinical features were not compatible with a diagnosis of rheumatoid arthritis. Moreover, his joint symptom improved in 2 d without specific medication.

The patient had suffered from diabetes and chronic hepatitis C for 20 years and received treatment with oral hypoglycemic agents and subcutaneous insulin injections. He was told that his hepatitis activities were mild and need not receive antiviral therapy for chronic hepatitis C. He was in a relatively good condition until 4 mo prior to admission, when he developed fatigue, myalgia, and a mild dry cough. He visited other hospitals for these symptoms and was found to have small pulmonary nodules, which were attributed to past tuberculous infection. His respiratory symptoms improved over the intervening 4 mo, but fatigue and weakness persisted.

A physical examination disclosed no remarkable findings except for three oval brownish papular skin rashes

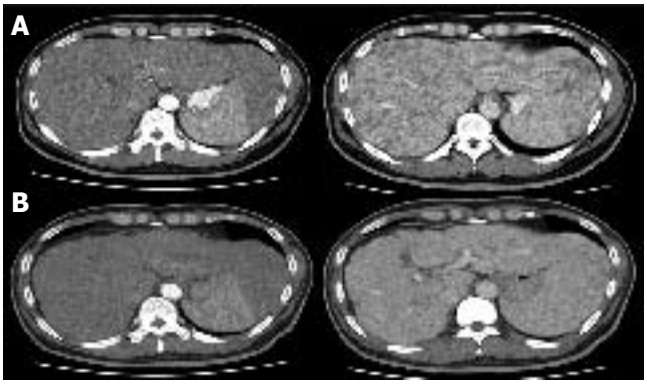


Figure 1 Multiple low-attenuating nodular lesions in the liver and an enlarged spleen (**A**) and their disappearance after 15 mo (**B**) on CT images.

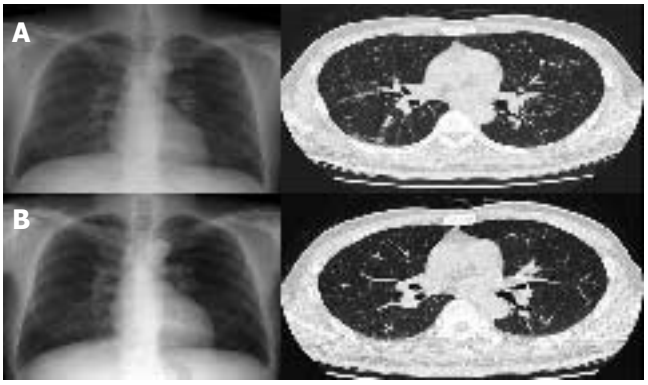


Figure 3 Scattered reticulonodular opacities and multiple fine parenchymal nodules in the case of sarcoidosis (**A**) and their absence after 15 mo (**B**).

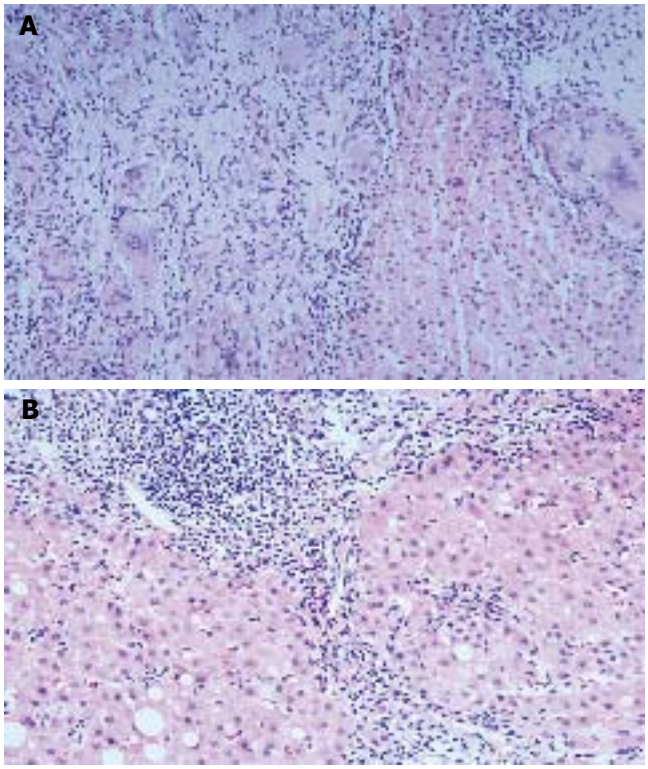


Figure 2 Broad areas of granulomatous inflammation (**A**) and their disappearance (**B**) with portal and periportal inflammation, and porto-portal fibrous septa accompanying piecemeal necrosis in hepatic lobules after 15 mo (HE ×200).

on the periumbilical and lower back areas. Chest X-ray revealed tiny nodules scattered throughout the lung fields and maxillary sinus haziness was found on skull X-ray. Complete blood cell counts with differential counts were all within the normal ranges. Serological markers for HBV infection (HBsAg and anti-HBs) were negative but anti HCV antibody was positive. A sensitive polymerase chain reaction (PCR) detected serum HCV RNA and identified the genotype of the HCV as 1b. Blood chemistry tests revealed 23 IU/L aspartate aminotransferase (AST), 29 IU/L alanine aminotransferase (ALT), 237 U/L alkaline phosphatase, 8.3 g/dL total protein, 3.8 g/dL albumin, 0.8 mg/dL total bilirubin, 9.4 mg/dL total calcium, 3.0 mg/dL phosphorus, 7.6% hemoglobin A1C, 201 mg/dL fasting blood sugar, 90% (11.6 s, INR 1.05) prothrombin time, 3.3 ng/mL alpha-fetoprotein, 17.3 mg/dL BUN, 0.9 mg/dL creatinine, 1.34 mg/dL free T4 (normal: 0.9-1.8 mg/dL), and 2.84 mIU/L TSH (normal: 0.3-6.5 mIU/L). Cryoglobulin was negative and serum angiotensin converting enzyme concentration was in the upper normal range, 51.8 U/L (normal: 8-52 U/L).

Abdominal ultrasonography showed multiple ill-defined low echoic nodular lesions in the liver and an enlarged spleen implying the presence of liver cirrhosis and hepatocellular carcinoma, but no evidence of enhancement was identified among the numerous low-attenuation nodules scattered throughout the liver and spleen on his abdominal CT scan (Figure 1A). Upper gastrointestinal endoscopic findings were normal and there was no clinical evidence of portal hypertension. A liver biopsy was performed as a basal evaluation of the chronic HCV infection and as a diagnostic procedure for the multiple hepatic nodules, which revealed numerous granulomas with multinucleated giant cells and occasional asteroid bodies in addition to the features of chronic hepatitis such as a mild inflammatory reaction and moderate fibrosis (Figure 2A). Special stains for tuberculosis and fungus, using acid fast bacillus (AFB) and periodic acid Schiff (PAS) were negative, and Mycobacterium tuberculosis nucleic acid was not detected by PCR in the liver tissue.

A high-resolution chest CT scan showed multiple fine nodules distributed in peribronchial areas, especially in the upper and middle lobes, without lymph node enlargement, which was compatible with type III pulmonary sarcoidosis (Figure 3A). Lung tissues obtained by transbronchial lung biopsy revealed a patchy distribution of mild interstitial and perivascular fibrosis, without distinctive granulomas or significant inflammatory cell infiltrations, and stains for AFB and PAS and PCR was negative for Mycobacterium tuberculosis nucleic acids. An analysis of cell types obtained by bronchoalveolar lavage showed that 42% were lymphocytes that consisted of CD4+ cells (63%) and CD8+ cells (37%), a CD4/CD8 ratio of 1.7. Cytologic analysis of bronchial washing fluid for malignant cells was negative and his pulmonary function test was normal.

Granulomatous inflammation with asteroid bodies and multinucleated giant cells were also found in skin biopsy specimens of the periumbilical lesions. Echocardiographic

and ophthalmologic evaluations and a thyroid function test, performed to determine the presence of indolent organ involvement, were all within the normal ranges. With a diagnosis of systemic sarcoidosis involving lung, liver, skin, spleen, and mild chronic hepatitis C, no specific therapies for either sarcoidosis or chronic hepatitis C were adopted, because there was no definitive evidence for significant organ dysfunction and disease progression.

Radiologic and biochemical examinations after 2 months showed no significant changes. After then, he went abroad and was not followed up for a year. Fifteen months later, the hepatosplenic and pulmonary sarcoidotic lesions were remarkably improved on radiologic examination (Figures 1B and 3B) and a liver biopsy showed no remnant granulomas, but moderate to severe inflammatory activities in the lobular and periportal areas, and grade 3 septal fibrosis (Figure 2B). Blood chemistry showed a markedly elevated serum transaminase level (AST 392 IU/mL, ALT 608 IU/mL) with a high serum viral load (serum HCV RNA 2.6×10^5 IU/mL).

DISCUSSION

Liver involvement in sarcoidosis is reported in 40%-70% of patients, but significant hepatic dysfunction is rare[5]. The histologic features of hepatic sarcoidosis are variable. Granulomas are identified in all the patients and cholestasis presenting as ductal or periductal inflammation and ductopenia are evident in more than 50% of patients. Necroinflammatory changes and vascular changes are found in 41% and 29%, respectively, and hepatic fibrosis is also found in about 20% of patients[6]. Asteroid bodies are found in about 10% of hepatic sarcoidosis cases[1]. The hepatic histologic findings in the present case are compatible with those of hepatic sarcoidosis, although chronic HCV infection may also have contributed. Hypodense nodular lesions in the liver and spleen as seen in the CT scans of this patient have been reported to be a characteristic feature of hepatosplenic sarcoidosis[7]. Splenic sarcoidosis appears as a nonspecific splenomegaly with retroperitoneal lymphadenopathies in most cases of abdominal sarcoidosis, and approximately 15% of cases present as multiple focal low-attenuating nodules[8,9]. The constitutional symptoms, arthritis, paranasal sinusitis, pulmonary nodules, and noncaseating granulomas in the liver and skin tissues of this patient, are all consistent with the characteristic features of systemic sarcoidosis.

Reported comorbidities of sarcoidosis include infectious diseases, neoplastic disorders, and immunologic-inflammatory diseases such as lupus erythematosus, myasthenia, and primary biliary cirrhosis. HCV infection is reported to be the most commonly associated infectious disease of sarcoidosis, however most cases are associated with interferon antiviral therapy[2,3]. Treatment-associated cases of sarcoidosis have been reported in many disorders such as chronic hepatitis C, chronic myelogenous leukemia, renal cell carcinoma and lymphoma, in which the beneficial effect of interferon therapy has been established. Importantly, in cases of chronic hepatitis C, interferon can both induce sarcoidosis[10] and reactivate sarcoidosis[11], and interferon-based combination antiviral regimens cannot eliminate the occurrence of sarcoidosis[12].

Interferon not only has direct antiviral activity but also has potent immune stimulating activities especially on T helper (Th1) immune response[10,13], which is also involved in the pathogenesis of sarcoidosis[14,15]. Granulomas in sarcoidosis have an abundance of CD4+ T lymphocytes and mononuclear phagocytes, which are thought to be a result of cytokine stimulation and immunologic dysregulation[16]. Moreover, ribavirin, an antiviral agent which augments the anti-HCV effect of interferon in chronic hepatitis C, also enhances Th1 cytokine response, while inhibiting Th2 cytokine response[17,18]. Thus it appears that treatment-associated sarcoidosis in chronic hepatitis C is mediated by an augmentation of Th1 immune response.

Cases of sarcoidosis and chronic hepatitis C with no history of interferon therapy are rarely reported. Bonnet *et al* [4] reported two cases of pulmonary sarcoidosis associated with untreated chronic hepatitis C, one presented with respiratory and cutaneous symptoms that responded to corticosteroid therapy, the other manifested as cervical lymph adenopathies with pulmonary symptoms and responded poorly to corticosteroids. However, sarcoidosis hepatic involvement was absent in both cases, liver biopsies showed features of chronic hepatitis but no granulomas. Our case is unique in that treatment-unrelated hepatic sarcoidosis and its spontaneous resolution were proven histologically in this chronic hepatitis C patient.

Interferon-based antiviral therapy is the standard treatment for chronic hepatitis C, but careful evaluations are required before starting the treatment because many adverse events can be induced by interferon. Flu like symptoms, cytopenia, depression, hyperglycemia, and thyroid dysfunctions are commonly encountered during interferon therapy. Interstitial lung disease, cardiomyopathy, and retinopathy rarely develop. Thus severe depression, autoimmune disorders, and uncontrolled diabetes contraindicate interferon therapy[19]. In addition to these disorders, sarcoidosis must also be considered, not only during interferon therapy but also before antiviral therapy, since sarcoidosis may preclude interferon administration.

The clinical course of pulmonary sarcoidosis is variable with a spontaneous remission rate of 40% over a 6-mo observation period before steroid therapy[20]. In addition, the clinical course of treatment-associated sarcoidosis in chronic viral hepatitis is also variable. The discontinuation of interferon with or without corticosteroid therapy usually improves sarcoidotic lesions[11], but the remission of pulmonary sarcoidosis has also been reported though interferon therapy is continued[21]. However, the natural course of treatment-unrelated sarcoidosis in chronic hepatitis C has not been previously reported.

Although corticosteroid therapy can suppress the active inflammatory reaction of sarcoidosis, its effect on clinical outcome has not been fully established. Moreover, it often provokes serious adverse events, such as hepatic functional deterioration and enhanced viral replication[22]. In a few cases of hepatic sarcoidosis, corticosteroid therapy could normalize liver enzymes but could not achieve histologic improvements[23-25]. Due to the potential provocation of viral replication and uncertain efficacy in a

clinically stable condition, the patient was simply observed without corticosteroid therapy

In view of the fact that sarcoidosis is a manifestation of an augmented (Th1) immune response to an unidentified stimulant and that interferon-induced immune activation is an important mechanism of viral replication suppression in chronic hepatitis C, hepatitis activities may be suppressed when chronic hepatitis C is combined with active sarcoidosis. In fact, the present case provides clinical evidence that supports this postulation, because serum ALT levels and histologic hepatitis activities were mild when the patient initially presented with active systemic sarcoidosis; whereas 15 mo later when the sarcoidotic lesions resolved, hepatitis activities were aggravated with a higher serum ALT level and a high serum HCV RNA titer. In conclusion, it is prudent to keep in mind that systemic sarcoidosis is a rare comorbidity of chronic hepatitis C which may spontaneously resolve.

REFERENCES

1 **Bilir M**, Mert A, Ozaras R, Yanardag H, Karayel T, Senturk H, Tahan V, Ozbay G, Sonsuz A. Hepatic sarcoidosis: clinicopathologic features in thirty-seven patients. *J Clin Gastroenterol* 2000; **31**: 337-338

2 **Bonnet F**, Dubuc J, Morlat P, Delbrel X, Doutre MS, de Witte S, Bernard N, Lacoste D, Longy-Boursier M, Beylot J. [Sarcoidosis and comorbidity: retrospective study of 32 cases]. *Rev Med Interne* 2001; **22**: 619-623

3 **Ramos-Casals M**, Mañá J, Nardi N, Brito-Zerón P, Xaubet A, Sánchez-Tapias JM, Cervera R, Font J. Sarcoidosis in patients with chronic hepatitis C virus infection: analysis of 68 cases. *Medicine (Baltimore)* 2005; **84**: 69-80

4 **Bonnet F**, Morlat P, Dubuc J, De Witte S, Bonarek M, Bernard N, Lacoste D, Beylot J. Sarcoidosis-associated hepatitis C virus infection. *Dig Dis Sci* 2002; **47**: 794-796

5 **Newman LS**, Rose CS, Maier LA. Sarcoidosis. *N Engl J Med* 1997; **336**: 1224-1234

6 **Devaney K**, Goodman ZD, Epstein MS, Zimmerman HJ, Ishak KG. Hepatic sarcoidosis. Clinicopathologic features in 100 patients. *Am J Surg Pathol* 1993; **17**: 1272-1280

7 **Thanos L**, Zormpala A, Brountzos E, Nikita A, Kelekis D. Nodular hepatic and splenic sarcoidosis in a patient with normal chest radiograph. *Eur J Radiol* 2002; **41**: 10-11

8 **Robertson F**, Leander P, Ekberg O. Radiology of the spleen. *Eur Radiol* 2001; **11**: 80-95

9 **Scott GC**, Berman JM, Higgins JL. CT patterns of nodular hepatic and splenic sarcoidosis: a review of the literature. *J Comput Assist Tomogr* 1997; **21**: 369-372

10 **Hoffmann RM**, Jung MC, Motz R, Gössl C, Emslander HP, Zachoval R, Pape GR. Sarcoidosis associated with interferon-alpha therapy for chronic hepatitis C. *J Hepatol* 1998; **28**: 1058-1063

11 **Li SD**, Yong S, Srinivas D, Van Thiel DH. Reactivation of sarcoidosis during interferon therapy. *J Gastroenterol* 2002; **37**: 50-54

12 **Pérez-Alvarez R**, Pérez-López R, Lombraña JL, Rodríguez M, Rodrigo L. Sarcoidosis in two patients with chronic hepatitis C treated with interferon, ribavirin and amantadine. *J Viral Hepat* 2002; **9**: 75-79

13 **Brinkmann V**, Geiger T, Alkan S, Heusser CH. Interferon alpha increases the frequency of interferon gamma-producing human CD4+ T cells. *J Exp Med* 1993; **178**: 1655-1663

14 **James DG**. Sarcoidosis 2001. *Postgrad Med J* 2001; **77**: 177-180

15 **Hunninghake GW**, Crystal RG. Pulmonary sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. *N Engl J Med* 1981; **305**: 429-434

16 **Fireman EM**, Topilsky MR. Sarcoidosis: an organized pattern of reaction from immunology to therapy. *Immunol Today* 1994; **15**: 199-201

17 **Tam RC**, Pai B, Bard J, Lim C, Averett DR, Phan UT, Milovanovic T. Ribavirin polarizes human T cell responses towards a Type 1 cytokine profile. *J Hepatol* 1999; **30**: 376-382

18 **Hultgren C**, Milich DR, Weiland O, Sällberg M. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. *J Gen Virol* 1998; **79 (Pt 10)**: 2381-2391

19 **Sievert W**. Management issues in chronic viral hepatitis: hepatitis C. *J Gastroenterol Hepatol* 2002; **17**: 415-422

20 **Gibson GJ**, Prescott RJ, Muers MF, Middleton WG, Mitchell DN, Connolly CK, Harrison BD. British Thoracic Society Sarcoidosis study: effects of long term corticosteroid treatment. *Thorax* 1996; **51**: 238-247

21 **Luers C**, Sudhop T, Spengler U, Berthold HK. Improvement of sarcoidosis under therapy with interferon-alpha 2b for chronic hepatitis B virus infection. *J Hepatol* 1999; **30**: 347

22 **Gruber A**, Lundberg LG, Björkholm M. Reactivation of chronic hepatitis C after withdrawal of immunosuppressive therapy. *J Intern Med* 1993; **234**: 223-225

23 **Nores JM**, Chesneau MC, Charlier JP, de Saint-Louvent P, Nenna AD. [A case of hepatic sarcoidosis. Review of the literature]. *Ann Gastroenterol Hepatol* (Paris) 1987; **23**: 257-260

24 **Brailski Kh**, Dimitrov B, Damianov B, Dinkov L, Todorov D. [Case of sarcoidosis with predominant involvement of the liver and lymph nodes]. *Vutr Boles* 1986; **25**: 83-91

25 **Mueller S**, Boehme MW, Hofmann WJ, Stremmel W. Extrapulmonary sarcoidosis primarily diagnosed in the liver. *Scand J Gastroenterol* 2000; **35**: 1003-1008

S- Editor Wang XL **L- Editor** Elsevier HK **E- Editor** Wang J

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 154-156
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Spontaneous chylous peritonitis mimicking acute appendicitis: A case report and review of literature

Fu-Chi Fang, Sheng-Der Hsu, Chuang-Wei Chen, Teng-Wei Chen

Fu-Chi Fang, Sheng-Der Hsu, Chuang-Wei Chen, Teng-Wei Chen, Division of General Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, China
Supported by the National Natural Science Foundation of China, No. 30224801
Correspondence to: Dr. Teng-Wei Chen, Division of General Surgery, Department of Surgery, Tri-Service General Hospital, 325 Section 2, Cheng-Kung Road, Neihu 114, Taipei, Taiwan, China. arjie@pchome.com.tw
Telephone: +886-2-8792-7191
Received: 2005-05-03 Accepted: 2005-06-09

Abstract

Acute abdominal pain with signs and symptoms of peritonitis due to sudden extravasation of chyle into the peritoneal cavity is a rare condition that is often mistaken for other disease processes. The diagnosis is rarely suspected preoperatively. We report a case of spontaneous chylous peritonitis that presented with typical symptoms of acute appendicitis such as intermittent fever and epigastric pain radiating to the lower right abdominal quadrant before admission.

Key words: Acute abdominal pain; Chyle; Appendicitis

Fang FC, Hsu SD, Chen CW, Chen TW. Spontaneous chylous peritonitis mimicking acute appendicitis: A case report and review of literature. *World J Gastroenterol* 2006; 12(1): 154-156

http://www.wjgnet.com/1007-9327/12/154.asp

INTRODUCTION

Chylous ascites is the accumulation of a milky or creamy peritoneal fluid rich in triglycerides due to the presence of lymph. It is a rare clinical condition that results from the disruption of the abdominal lymphatic system. Multiple causes have been described including abdominal malignancy, cirrhosis, inflammation, congenital and postoperative or traumatic causes, and miscellaneous disorders. Management is based on identifying and treating the underlying cause. A sudden outpouring of chyle into the peritoneal cavity may produce acute chylous peritonitis. Patients with such defect usually present with the features of acute abdomen. Very few cases of acute chylous peritonitis have been described in the literature. We have discussed the clinical features and management of acute chylous peritonitis, and its rarity and presentation compared to common surgical emergencies, such as acute appendicitis.

CASE REPORT

A 22-year-old male presented with intermittent fever and abdominal pain for two days before admission. He first presented at a clinic, where acute appendicitis was suspected. He was referred by the clinic to our hospital for further management. The symptoms of abdominal distension and pain were continuous and aggravated by any movement. The patient did not have any history of previous trauma or similar pain, and there was nothing relevant in his past medical history. He did not smoke cigarettes or consume alcohol, and denied any recent long distance or overseas travel. On examination, his temperature was 38.2 ºC, pulse rate was 80/min, and blood pressure was 116/70 mmHg. The systemic examination was unremarkable. The abdomen was soft and ovoid in shape, and there was tenderness over the periumbilical and right lower quadrant regions. Bowel sounds were audible. Blood analysis gave the following values: total leukocyte count, 11.6×10^9/μL; Hb, 143 g/L; platelet count, 31.4×10^9/μL; neutrophils, 64.3%; lymphocytes, 22.7%; Na, 141 mmol/L; K, 3.9 mmol/L; urea, 3.9mmol/L; creatinine, 70.7μmol/L; glucose, 5.3mmol/L; AST, 367nkat/L; and ALT, 133nkat/L.

The chest X-ray was normal, but the abdominal X-ray showed nonspecific gas-filled loops of the large intestine. Computed tomography of the abdomen with contrast showed lobulated fluid accumulation over the retroperitoneal space extending from the level of the renal hilum into the right side pelvis and right inguinal area (Figure 1). Suspecting a ruptured appendix, we performed an exploratory laparotomy. When entering the peritoneum, we noted a large amount of milky fluid in the peritoneal cavity. Chylous ascites was first considered because of the obvious lymphatic leakage from the thoracic duct after we opened the retroperitoneum (Figure 2). We performed suture ligation of the lymphatic leakage and drainage of the retroperitoneal lymphocele. Postoperative drainage was < 100 mL in the first week. The patient commenced a low-fat diet, and was recovering well after a 6-month follow-up.

DISCUSSION

Ascites is the presence of excess fluid in the peritoneal

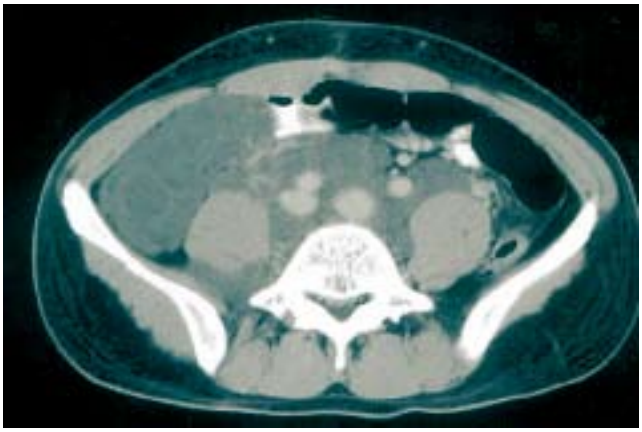


Figure 1 Accumulation of lobulated fluid over the retroperitoneal space and at the para-cecum region shown by computed tomography with contrast to the abdomen.

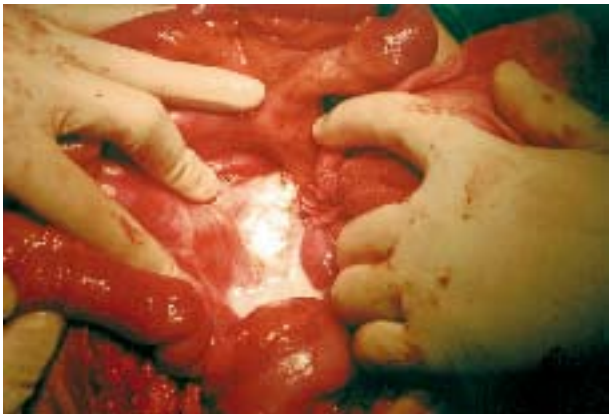


Figure 2 Obvious lymphatic leakage from the thoracic duct after the retroperitoneum was opened.

cavity and is a common clinical finding with a wide range of causes, which can require varied treatment. Acute chylous peritonitis is defined as an acute abdomen with all the signs of acute peritoneal irritation resulting from free chyle in the peritoneal cavity, without any underlying disease. Acute chylous peritonitis can also involve extravasation of milky or creamy peritoneal fluid that is rich in triglycerides with small amounts of cholesterol and phospholipids, caused by the presence of thoracic or intestinal lymph in the abdominal cavity. Chylous ascites occurs in one in 20 000 patients admitted to the hospital[1].

The lymphatic system is an accessory route through which fluids and protein flow from the interstitial spaces to the vascular system. Almost all tissues of the body have lymphatic channels composed of one-way valves that drain the excess fluid from the interstitial spaces of tissue. These channels play a pivotal role in clearing the interstitium of debris and bacteria, which are carried to lymph nodes where they are opsonized and phagocytized. Gastrointestinal tract lymphatics also transport absorbed water and lipids to the circulatory system[2]. In the gut, long chain triglycerides are converted into monoglycerides, free fatty acids, and absorbable chylomicrons, which explain the high content of triglycerides and the milky, cloudy appearance of lymph. In our case, the triglyceride concentration in the ascites fluid was 5.48 mmol/L, much higher than the blood triglyceride concentration of 102 mg/dL. The basal flow rate of lymphatic fluid through the thoracic duct averages about 1.0 mL/kg per an hour, with a total volume of 1 500 - 1 700 mL/d. These volumes increase markedly with the ingestion of fats, and fluid rates as high as 200 mL/h have been reported[3].

Chylous ascites may result from many pathological conditions, including congenital defects of the lymphatic system; nonspecific bacterial, parasitic, and tuberculous peritoneal infections; liver cirrhosis; malignant neoplasm; and blunt abdominal trauma. These etiologies may be categorized into distinct mechanism-based groups[4]. The most common etiological factors are abdominal malignancy and congenital lymphatic abnormalities in adults and children, respectively[5]. We have presented an unusual case of spontaneous chylous ascites-related peritonitis mimicking acute appendicitis in a young adult. Abdominal surgery is another common cause that is most frequently associated with the resection of an abdominal aortic aneurysm or retroperitoneal lymph node dissection[6]. Various vascular surgical procedures such as aorta-to-femoral artery bypass may cause chylous complications[7]. It has been suggested that overloading of the lymphatic channels with chyle after a heavy fatty meal may cause extravasation of chyle intraperitoneally and retroperitoneally[8].

Abdominal distension is the most common symptom in patients with chylous ascites. Other clinical features include abdominal pain, anorexia, weight loss, edema, weakness, nausea, dyspnea, weight gain, lymphadenopathy, early satiety, fever, and night sweats. The clinical presentation of acute abdomen is less common. Free chyle is relatively nonirritating to the serosal surface, but pain may result from the stretching of the retroperitoneum and the mesenteric serosa[8]. The pain is severe and a physical examination may lead to the misdiagnosis of appendicitis, cholecystitis, mesenteric arterial embolism, or a perforated viscus. The unusual case we present here is spontaneous chylous ascites-related peritonitis mimicking acute appendicitis in a young adult. Just like a similar case reported by Lamblim *et al* [9] the diagnosis was established by the celiotomy.

The diagnosis of chylous ascites is confirmed by analyzing the ascites fluid, which is only possible if such a diagnosis is suspected preoperatively. The chief characteristics of chylous effusions include a milky appearance, separation into a creamy layer on standing, lacking an odor, alkaline chemical properties, specific gravity greater than 1.012, bacteriostatic properties, 3% total protein, staining of fat globules with Sudan red stain, fat content of 0.4% - 4%, and total solids > 4%[10]. The triglyceride level is an important diagnostic tool, and concentration in the chylous ascites is typically two to eight times that of plasma[11]. True chylous ascites must be distinguished from "chyliform" and "pseudochylous" effusions, in which the turbid appearance is due to cellular degeneration from bacterial peritonitis or neoplasm.

However, the triglyceride concentration is low in these effusions. Other diagnostic tests such as computed tomography, lymphangiography, lymphoscintigraphy, and laparotomy have the highest yield of diagnostic information[4].

The optimal management of true chylous peritonitis depends upon the underlying etiology. In patients with symptoms of an acute abdominal process, immediate exploration should be performed. Laparotomy usually allows a definitive diagnosis and provides an opportunity to address the underlying cause. The source of chylous extravasation can be corrected by ligation of the leaking lymphatics or removal of the offending lesion, the cause of many congenital and all traumatic cases. The goals of nonsurgical therapy for chylous ascites include maintaining or improving nutrition, and decreasing the rate of chyle formation. Dietary intervention involves a diet that is rich in protein, and low in fat and medium-chain triglycerides to decrease lymph flow in the major lymphatic tracts and to facilitate the closure of chylous fistulas[12]. Total parenteral nutrition can be used to achieve complete bowel rest and might allow resolution of the chylous ascites. Somatostatin improves chylous ascites by inhibiting lymph fluid excretion through specific receptors found in the normal intestinal wall of lymphatic vessels[13]. In patients with a large amount of ascites, a total paracentesis to relieve discomfort and dyspnea can be performed and repeated as needed. However, one should note the risk of infection and fat emboli. If the patients are poor surgical candidates and refractory to nonsurgical treatment, peritoneovenous shunting may be an option, although these shunts carry a high rate of complications[14].

In conclusion, we have described a rare case of acute chylous peritonitis that mimicked acute appendicitis, but could not identify the cause. This case suggests that diagnostic laparoscopy may play an important role in the initial management of this condition[15].

REFERENCES

1 **Press OW**, Press NO, Kaufman SD. Evaluation and management of chylous ascites. *Ann Intern Med* 1982; **96**: 358-364
2 **Aalami OO**, Allen DB, Organ CH. Chylous ascites: a collective review. *Surgery* 2000; **128**: 761-778
3 **Madding GF**, McLaughlin RF, McLaughlin RF. Acute chylous peritonitis. *Ann Surg* 1958; **147**: 419-422
4 **Cárdenas A**, Chopra S. Chylous ascites. *Am J Gastroenterol* 2002; **97**: 1896-1900
5 **Browse NL**, Wilson NM, Russo F, al-Hassan H, Allen DR. Aetiology and treatment of chylous ascites. *Br J Surg* 1992; **79**: 1145-1150
6 **Combe J**, Buniet JM, Douge C, Bernard Y, Camelot G. [Chylothorax and chylous ascites following surgery of an inflammatory aortic aneurysm. Case report with review of the literature]. *J Mal Vasc* 1992; **17**: 151-156
7 **Pabst TS**, McIntyre KE, Schilling JD, Hunter GC, Bernhard VM. Management of chyloperitoneum after abdominal aortic surgery. *Am J Surg* 1993; **166**: 194-18; discussion 194-18
8 **Weichert RF**, Jamieson CW. Acute chylous peritonitis. A case report. *Br J Surg* 1970; **57**: 230-232
9 **Lamblin A**, Mulliez E, Lemaitre L, Pattou F, Proye C. [Acute peritonitis: a rare presentation of chylous ascites]. *Ann Chir* 2003; **128**: 49-52
10 **Hibbeln JF**, Wehmueller MD, Wilbur AC. Chylous ascites: CT and ultrasound appearance. *Abdom Imaging* 1995; **20**: 138-140
11 **Ward PC**. Interpretation of ascitic fluid data. *Postgrad Med* 1982; **71**: 171-13, 171-13
12 **Hashim** SA, Roholt HB, Babayan VK, Vanitallie TB. Treatment of chyluria and chylothorax with medium chain triglyceride. *N Engl J Med* 1964; **270**: 756-761
13 **Shapiro AM**, Bain VG, Sigalet DL, Kneteman NM. Rapid resolution of chylous ascites after liver transplantation using somatostatin analog and total parenteral nutrition. *Transplantation* 1996; **61**: 1410-1411
14 **Voros D**, Hadziyannis S. Successful management of postoperative chylous ascites with a peritoneojugular shunt. *J Hepatol* 1995; **22**: 380
15 **Sanna A**, Adani GL, Anania G, Donini A. The role of laparoscopy in patients with suspected peritonitis: experience of a single institution. *J Laparoendosc Adv Surg Tech A* 2003; **13**: 17-19

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 157-158
World Journal of Gastroenterology ISSN 1007-9327

Percutaneous local therapies for hepatocellular carcinoma impair gastric function

Fumihiko Kinekawa, Kazuya Matsuda, Tsutomu Masaki, Kazutaka Kurokohchi, Hirohito Yoneyama, Hideyuki Inoue, Hirohide Kurata, Yoshihito Uchida, Seishiro Watanabe, Shigeki Kuriyama

Fumihiko Kinekawa, Tsutomu Masaki, Kazutaka Kurokohchi, Hirohito Yoneyama, Hideyuki Inoue, Hirohide Kurata, Seishiro Watanabe, Shigeki Kuriyama, Third Department of Internal Medicine, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan
Kazuya Matsuda, Department of Internal Medicine, Matsuda Clinic, 1-3-20 Fujitsuka-cho, Takamatsu-city, Kagawa 760-0071, Japan
Yoshihito Uchida, Department of Internal Medicine, Takuma Municipal National Health Insurance Eikoh Hospital, 1381 Takuma, Takuma-cho, Mitoyo-gun, Kagawa 769-1101, Japan
Correspondence to: Dr. Shigeki Kuriyama, Third Department of Internal Medicine, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0793, Japan. skuriyam@med.kagawa-u.ac.jp
Telephone: +81-87-891-2156 Fax: +81-87-891-2158
Received: 2005-06-22 Accepted: 2005-08-25

Key words: Electrogastrography; Gastric myenteric activity; Hepatocellular carcinoma; Percutaneous ethanol injection; Radiofrequency ablation

Kinekawa F,Matsuda K,Masaki T,Kurokohchi K,Yoneyama H,Inoue H,Kurata H,Uchida Y,Watanabe S,Kuriyama S Percutaneous local therapies for hepatocellular carcinoma impair gastric function. *World J Gastroenterol* 2006; 12(1): 157-158

http://www.wjgnet.com/1007-9327/12/157.asp

TO THE EDITOR

Percutaneous local therapies, such as percutaneous ethanol injection (PEI), microwave coagulation and radiofrequency ablation (RFA), are frequently used worldwide for the treatment of hepatocellular carcinoma (HCC) because of their high effectiveness. Although these treatment modalities can induce effectively coagulated tumor necrosis in the liver, they may cause adverse effects on extrahepatic abdominal organs. There are, however, no published reports on the influence of percutaneous local therapies on the gastric myenteric activity. Therefore, it is unclear whether or not gastric function is affected by percutaneous local therapies. In this study, to make clear the effect of PEI and RFA on the gastric function, we continuously recorded the gastric myoelectric activity by electrogastrography (EGG) and estimated the effect of percutaneous local therapies for HCC on gastric function.

Five patients with HCC (3 males and 2 females; age ranging from 66 to 81 years) were enrolled in the present study. Written informed consent was obtained from each patient. We have developed several novel percutaneous local therapies for HCC. The first is the combination of PEI and RFA (PEI-RFA). In this treatment modality, RFA is performed immediately after PEI. The second is percutaneous ethanol-lipiodol injection (PELI). In this modality, mixture of pure ethanol and lipiodol, a lipid-based contrast medium, at a ratio of 10:1, is injected percutaneously into HCC. The last is the combination of PELI and RFA (PELI-RFA). Usefulness of these new treatment modalities has been reported elsewhere[1-7]. In the present study, two patients with HCC underwent PELI and three underwent PELI-RFA.

We recorded EGG before and 3 d after therapy, and the results were compared. EGG was recorded with a portable electrogastrographic recorder (NIPRO; Tokyo, Japan). Five electrodes were affixed to the abdomen as shown in Figure 1, and EGG was recorded for 30 min during a fasting period and again during a postprandial period. We evaluated the percentages of bradygastria (< 2.4 c/min), normogastria (2.4 - 3.6 c/min), and tachygastria (> 3.6 c/min), as well as the dominant frequency (DF) and the postprandial-to-fasting power ratio (PR). We also examined clinical abdominal symptoms, using the questionnaire reported by Svedlund *et al.* (Gastrointestinal Symptom Rating Scale, GSRS)[8] which was translated into Japanese. The translated form was provided by Astra Zeneca (Tokyo, Japan). Measured values were expressed as mean ± SE. Comparisons before and after therapy were performed by the paired Student's *t* test, and $P < 0.05$ was accepted as a significant difference.

The results are summarized in Table 1. Because the similar EGG patterns were obtained from all the channels, the data of channel 1 are shown in Table 1. After percutaneous local therapies for HCC, the percentages of bradygastria in the fasting period were significantly increased, while the percentages of normogastria in the same period were significantly decreased. The PR of normogastria and bradygastria was significantly decreased after therapy. Conversely, no significant differences were found in the calculated GSRS scores obtained from the questionnaire before and after therapy. This study is the first report to estimate the effect of percutaneous local therapies for HCC on the gastric myenteric activity. The present results indicate that percutaneous therapies for HCC may impair gastric function even when clinical symptoms are not apparent.

The first EGG measurement in humans was performed by Alvarez [9]. This method can be used to noninvasively assess the electrical activity generated by gastric smooth

Table 1 Comparisons between before and after therapy (mean ± SE)

	Before	After
Bradygastria (%)	54.0 ± 7.4	77.4 ± 5.6[a]
Normogastria (%)	42.4 ± 7.5	18.6 ± 5.2[a]
Tachygastria (%)	3.2 ± 1.8	4.0 ± 4.0
PR of badygastria (%)	1.3 ± 0.1	0.8 ± 0.1[a]
PR of normogastria (%)	2.5 ± 0.5	1.8 ± 0.5[a]
PR of tachygastria (%)	2.1 ± 0.5	1.7 ± 0.5
DF (c/min)	2.3 ± 0.1	1.9 ± 0.1
Gastrointestinal symptoms Total score	1.8 ± 0.2	2.2 ± 0.5

[a]$p < 0.05$ *vs* before therapy

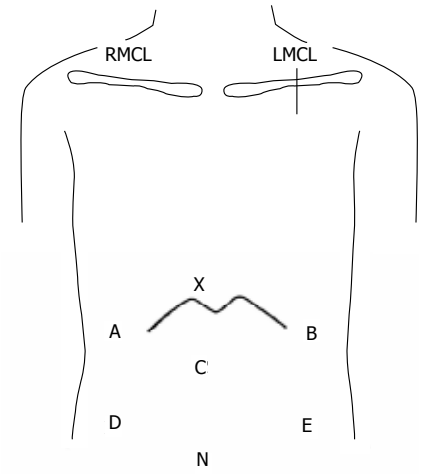


Figure 1 The positions of electrodes for EGG recording. X, xyphoid process; N, navel; RMCL, right mid-clavicular line; LMCL, left mid-clavicular line; C, central terminal electrode placed on the patient's ventral midline about halfway between the umbilicus and the xyphoid process; A, channel 1 placed on an intersecting point between RMCL and a vertical bisectrix of the line XC; B, channel 2 placed on intersecting point between LMCL and a vertical bisectrix of the line XC; D, channel 3 placed on intersecting point between RMCL and a vertical bisectrix of the line NC; E, channel 4 placed on intersecting point between LMCL and a vertical bisectrix of the line NC.

muscles. EGG is believed to reflect the electrical control activity and gastric motility regulated by pacemakers. In humans, these EGG waves originated from the pacemaker area along the major curvature of the stomach and propagated aborally with increasing velocity, at intervals of approximately 20 s[10]. EGG has been shown to provide useful information for clinical diagnoses[10]. EGG abnormalities have been observed in disorders of gastric emptying, nausea and vomiting[10].

Percutaneous local therapies is of great significance in the treatment of HCC and metastatic liver tumors. PELI, PEI-RFA and PELI-RFA are new therapeutic methods for HCC, which we have developed. We have confirmed the usefulness of these novel percutaneous local therapies in the treatment of HCC [1-7]. It has been shown that transcatheter arterial chemoembolization affected the gastric myenteric activity and that overproduction of endogenous prostaglandin was related to dysrhythmia of the gastric myenteric activity [11]. In this pilot study, we demonstrated that the gastric myenteric activity was affected by percutaneous local therapies for HCC, although abdominal symptoms were not apparent and GSRS scores obtained from the questionnaire did not change significantly after therapy. It is a significant clinical matter that delayed gastric transit may occur after percutaneous therapy for HCC. Because it has been reported that patients with HCC tend to have gastrointestinal dysfunction [12], we have to pay attention to gastric dysfunction after percutaneous local therapies for HCC even when there are no clinical symptoms. The mechanisms underlying the effect of percutaneous local therapies for HCC on extrahepatic abdominal organs need further exploration.

REFERENCES

1 **Kurokohchi K**, Watanabe S, Masaki T, Hosomi N, Funaki T, Arima K, Yoshida S, Nakai S, Murota M, Miyauchi Y, Kuriyama S. Combination therapy of percutaneous ethanol injection and radiofrequency ablation against hepatocellular carcinomas difficult to treat. *Int J Oncol* 2002; **21**: 611-615
2 **Kurokohchi K**, Watanabe S, Masaki T, Hosomi N, Funaki T, Arima K, Yoshida S, Miyauchi Y, Kuriyama S. Combined use of percutaneous ethanol injection and radiofrequency ablation for the effective treatment of hepatocelluar carcinoma. *Int J Oncol* 2002; **21**: 841-846
3 **Watanabe S**, Kurokohchi K, Masaki T, Miyauchi Y, Funaki T, Inoue H, Himoto T, Kita Y, Uchida N, Touge T, Tatsukawa T, Kuriyama S. Enlargement of thermal ablation zone by the combination of ethanol injection and radiofrequency ablation in excised bovine liver. *Int J Oncol* 2004; **24**: 279-284
4 **Kurokohchi K**, Masaki T, Miyauchi Y, Funaki T, Yoneyama H, Miyoshi H, Yoshida S, Himoto T, Morishita A, Uchida N, Watanabe S, Kuriyama S. Percutaneous ethanol and lipiodol injection therapy for hepatocellular carcinoma. *Int J Oncol* 2004; **24**: 381-387
5 **Kurokohchi K**, Masaki T, Miyauchi Y, Hosomi N, Yoneyama H, Yoshida S, Himoto T, Deguchi A, Nakai S, Inoue H, Watanabe S, Kuriyama S. Efficacy of combination therapies of percutaneous or laparoscopic ethanol-lipiodol injection and radiofrequency ablation. *Int J Oncol* 2004; **25**: 1737-1743
6 **Kurokohchi K**, Masaki T, Himoto T, Deguchi A, Nakai S, Yoneyama H, Yoshida S, Kimura Y, Inoue H, Kinekawa F, Yoshitake A, Izuishi K, Watanabe S, Kuriyama S. Successful laparoscopic radiofrequency ablation of hepatocellular carcinoma adhered to the mesentery after transcatheter arterial embolization. *Oncol Rep* 2005; **13**: 65-68
7 **Kurokohchi K**, Watanabe S, Masaki T, Hosomi N, Miyauchi Y, Himoto T, Kimura Y, Nakai S, Deguchi A, Yoneyama H, Yoshida S, Kuriyama S. Comparison between combination therapy of percutaneous ethanol injection and radiofrequency ablation and radiofrequency ablation alone for patients with hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 1426-1432
8 **Svedlund J**, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988; **33**: 129-134
9 **Alvarez WC**. The electrogastrogram and what it shows. *JAMA* 1922; **78**: 1116-1119
10 **Verhagen MA**, Van Schelven LJ, Samsom M, Smout AJ. Pitfalls in the analysis of electrogastrographic recordings. *Gastroenterology* 1999; **117**: 453-460
11 **Chang CS**, Yang SS, Yeh HZ, Ko CW, Lien HC, Chen GH. Mediation of transcatheter arterial chemoembolization induced gastric slow-wave dysrhythmia by endogenous prostaglandin. *J Gastroenterol Hepatol* 2002; **17**: 46-51
12 **Chen CY**, Lu CL, Chang FY, Lih-Jiun K, Luo JC, Lu RH, Lee SD. Delayed gastrointestinal transit in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2002; **17**: 1254-1259

S- Editor Wang XL and Guo SY **L- Editor** Elsevier HK **E- Editor** Wu M

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 159
World Journal of Gastroenterology ISSN 1007-9327

Acknowledgments to Reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those were published and those were rejected in this issue) during the last editing period of time.

Kyoichi Adachi, MD
Department of Gastroenterology and Hepatology, Shimane University, School of Medicine Shimane, 89-1 Enya-cho, Izumo-shi Shimane 693-8501, Japan

Masahiro Arai, MD, PhD
Department of Gastroenterology, Toshiba General Hospital, 6-3-22 Higashi-ooi, Shinagawa-ku,Tokyo 140-8522, Japan

Yusuf Bayraktar, Professor
Department of Gastroenterology, School of Medicine, Hacettepe University, Ankara 06100, Turkey

Luigi Bonavina, Professor
Department of Surgery, Policlinico San Donato, University of Milano, via Morandi 30, Milano 20097, Italy

Josep M Bordas, MD
Department of Gastroenterology IMD, Hospital Clinic", Llusanes 11-13 at, Barcelona 08022, Spain

Wang-Xue Chen, Dr
Institute for Biological Sciences, National Research Concil Canada, 100 Sussex Drive, Room 3100, Ottawa, Ontario K1A 0R6, Canada

Franck Carbonnel, Professor
Department of Gastroenterology, Chu de Besancon, Boulevard Fleming,Besancon 25000, France

Mitsuhiro Fujishiro, Dr
Department of Gastroenterology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan

Masayoshi Ito, MD
Department of Endoscopy, Yotsuya Medical Cube, 5-5-27-701 Kitashinagawa, Shinagawa-ku, Tokyo 1410001, Japan

Kei Ito, MD
Department of Gastroenterology, Sendai City Medical Center, 5-22-1,Tsurugaya, Miyagino-ku, Sendai City 983-0824, Japan

Zahariy Krastev, Professor
Department of Gastroneterology, Universiti Hospital "St. Ivan Rilski",#15, blvd "Acad. Ivan Geshov", Sofia 1431, Bulgaria

Edward Loftus, V Jr, Associate Professor
Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, United States

María Isabel Torres López,Professor
Experimental Biology, University of Jaen, araje de las Lagunillas s/n, Jaén 23071, Spain

Yasushi Matsuzaki, Associated Professor
Division of Gastroenterology and Hepatology, Graduate School of Comprehensive Human Sciences and University Hospital, 1-1-1 Tennodai, Tsukuba 305-8575, Japan

Yoshiharu Motoo, Professor
Department of Medical Oncology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

Hisato Nakajima, MD
Department of Gastroenterology and Hepatology, The Jikei University School of Medicine, 3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan

Raffaele Pezzilli, MD
Department of Internal Medicine and Gastroenterology, Sant' Orsola-Malpighi Hospital, Via Massarenti, 9, Bologna 40138, Italy

CS Pitchumoni, Professor
Robert Wood Johnson School of Medicine, Robert Wood Johnson School of Medicine, New Brunswiuc NJ D8903,
United States

Adrian Reuben, Professor of Medicine
Department of Medicine, Medical University of South Carolina, Division of Gastroenterology and Hepatology, 210 Clinical Sciences Building, PO Box 250327, 96 Jonathan Lucas Street, Charleston SC 29425, United States

Manuel Romero-Gómez, MD, Professor
Hepatology Unit, Hospital Universitario de Valme, Ctra de Cádiz s/n, Sevilla 41014, Spain

Wolfgang Stremmel, R Professor
Department of Gastroenterology, University Hospital Heidelberg, Medizinische Universitafsklinik, Heidelberg 49120,
Germany

Tadatoshi Takayama, Professor
Department of Digestive Surgery, Nihon University School of Medicine, 30-1 Oyaguchikami-machi, Itabashi-ku, Tokyo 173-8610, Japan

Wei Tang, MD, EngD, Assistant Professor
H-B-P Surgery Division, Artificial Organ and Transplantation Division, Department of surgery, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan

Simon D Taylor-Robinson, MD
Department of Medicine A, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0HS, United KingdomTennodai, Tsukuba 305-8575, JapanTsurugaya, Miyagino-ku, Sendai City 983-0824, Japan

Yvan Vandenplas, Professor
Department of Pediatrics, AZ-VUB, Laarbeeklaan 101, Brussels 1090, Belgium

Steven David Wexner, MD, Professor
The Cleveland Clinic Foundation Health Sciences Center of the Ohio State University, and Clinical Professor, Department of Surgery, Division of General Surgery, University of South Florida College of Medicine, 21st Century Oncology Chair in Colorectal Surgery, Chairman Department of Colorectal Surgery, Chief of Staff, Cleveland Clinic Florida, 2950 Cleveland Clinic Boulevard, Weston, Florida 33331, United States

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 160
World Journal of Gastroenterology ISSN 1007-9327

Meetings

MAJOR MEETINGS COMING UP

Digestive Disease Week
107th Annual Meeting of AGA, The American Gastroenterology Association
May 20-25, 2006
Loas Angeles Converntion Center, California
www.ddw.org

EVENTS AND MEETINGS IN THE UPCOMING 6 MONTHS

10 th World Congress of the International Society for Diseases of the Esophagus (ISDE 2006)
February 22-25, 2006
Adelaide
isde@sapmea.asn.au
www.isde.net

EASL 2006 - The 41st Annual Meeting
April 26-30, 2006
Vienna, Austria

International Gastrointestinal Fellows Initiative
February 22-24, 2006
Banff, Alberta
CAGOffice@cag-acg.org
www.cag-acg.org

Canadian Digestive Disease Week
February 24-27, 2006
Banff, Alberta
CAGOffice@cag-acg.org
www.cag-acg.org

European Multidisciplinary Colorectal Cancer Congress 2006
February 12-14, 2006
Berlin
info@congresscare.com
www.colorectal2006.org

ILTS 12th Annual International Congress
May 3-6, 2006
Milan
www.ilts.org

World Congress on Gastrointestinal Cancer
June 14-17, 2006
Barcelona, Spain
c.chase@imedex.com

5th International Congress of The African Middle East Association of Gastroenterology
February 24-26, 2006
Sharjah
infoevent@infomedweb.com
www.infomedweb.com

Digestive Disease Week 2006
May 20-25, 2006
Los Angeles
www.ddw.org

Annual Postgraduate Course
May 25-26, 2006
Los Angeles, CA
www.asge.org/education

EVENTS AND MEETINGS IN 2006

10th World Congress of the International Society for Diseases of the Esophagus (ISDE 2006)
February 22-25, 2006
Adelaide
isde@sapmea.asn.au
www.isde.net

10th International Congress of Obesity
September 3-8, 2006
Sydney
enquiries@ico2006.com
www.ico2006.com

EASL 2006 - The 41st Annual Meeting
April 26-30, 2006
Vienna, Austria

International Gastrointestinal Fellows Initiative
February 22-24, 2006
Banff, Alberta
CAGOffice@cag-acg.org
www.cag-acg.org

Canadian Digestive Disease Week
February 24-27, 2006
Banff, Alberta
CAGOffice@cag-acg.org
www.cag-acg.org

Prague Hepatology Meeting 2006
September 14-16, 2006
Prague
veronika.revicka@congressprague.cz
www.czech-hepatology.cz/phm2006

European Multidisciplinary Colorectal Cancer Congress 2006
February 12-14, 2006
Berlin
info@congresscare.com
www.colorectal2006.org

World Congress on Controversies in Obesity, Diabetes and Hypertension (CODHy)
October 25-28, 2006
Berlin
codhy@codhy.com
www.codhy.com

ILTS 12th Annual International Congress
May 3-6, 2006
Milan
www.ilts.org

XXX pan-american congress of digestive diseases
November 25-December 1, 2006
Cancun
amg@gastro.org.mx
www.gastro.org.mx

World Congress on Gastrointestinal Cancer
June 14-17, 2006
Barcelona, Spain
c.chase@imedex.com

5th International Congress of the African Middle East Association of Gastroenterology
February 24-26, 2006
Sharjah
infoevent@infomedweb.com
www.infomedweb.com

7th World Congress of the International Hepato-Pancreato-Biliary Association
September 3-7, 2006
Edinburgh
convention@edinburgh.org
www.edinburgh.org/conference

Digestive Disease Week 2006
May 20-25, 2006
Los Angeles
www.ddw.org

Annual Postgraduate Course
May 25-26, 2006
Los Angeles, CA
www.asge.org/education

71st ACG Annual Scientific Meeting and Postgraduate Course
October 20-25, 2006
Venetian Hotel, Las Vegas, Neveda

AASLD 57th Annual Meeting - The Liver Meeting™
October 27-31, 2006
Boston, MA

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 161-162
World Journal of Gastroenterology ISSN 1007-9327

Instructions to authors

GENERAL INFORMATION

World Journal of Gastroenterology (*WJG, World J gastroenterology* ISSN 1007-9327 CN 14-1219/R) is a weekly journal of more than 48 000 circulation, published on the 7th, 14th, 21st and 28th of every month.

Original Research, Clinical Trials, Reviews, Comments, and Case Reports in esophageal cancer, gastric cancer, colon cancer, liver cancer, viral liver diseases, *etc*., from all over the world are welcome on the condition that they have not been published previously and have not been submitted simultaneously elsewhere.

Published by
The WJG Press

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed double-spaced on A4 (297mm×210 mm) white paper with outer margins of 2.5 cm. Number all pages consecutively, and start each of the following sections on a new page: Title Page , Abstract, Introduction, Materials and Methods, Results, Discussion, acknowledgements, References, Tables, Figures and Figure Legends. Neither the editors nor the Publisher is responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of The WJG Press and may not be reproduced by any means, in whole or in part without the written permission of both the authors and the Publisher. We reserve the right to put onto our website and copy-edit accepted manuscripts. Authors should also follow the guidelines for the care and use of laboratory animals of their institution or national animal welfare committee.

Authors should retain one copy of the text, tables, photographs and illustrations, as rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for the loss or damage to photographs and illustrations.

Online submission
Online submission is strongly advised. Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/index.jsp. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/wjg/help/instructions.jsp) before attempting to submit online. Authors encountering problems with the Online Submission System may send an email you describing the problem to wjg@wjgnet.com for assistance. If you submit your manuscript online, do not make a postal contribution. A repeated online submission for the same manuscript is strictly prohibited.

Postal submission
Send 3 duplicate hard copies of the full-text manuscript typed double-spaced on A4(297 mm×210 mm) white paper together with any original photographs or illustrations and a 3.5 inch computer diskette or CD-ROM containing an electronic copy of the manuscript including all the figures, graphs and tables in native Microsoft Word format or *.rtf format to:

Editorial Office

World Journal of Gastroenterology
Editorial Department: Apartment 1066, Yishou Garden,
58 North Langxinzhuang Road,
PO Box 2345, Beijing 100023, China
E-mail: wjg@wjgnet.com
http: //www.wjgnet.com

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using a word-processing software. All submissions must be typed in 1.5 line spacing and in word size 12 with ample margins. The letter font is Tahoma. For authors from China, one copy of the Chinese translation of the manuscript is also required (excluding references). Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page
Full manuscript title, running title, all author(s) name(s), affiliations, institution(s) and/or department(s) where the work was accomplished, disclosure of any financial support for the research, and the name, full address, telephone and fax numbers and email address of the corresponding author should be included. Titles should be concise and informative (removing all unnecessary words), emphasize what is new, and avoid abbreviations. A short running title of less than 40 letters should be provided. List the author(s)' name(s) as follows: initial and/or first name, middle name or initial(s) and full family name.

Abstract
An informative, structured abstract of no more than 250 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections: AIM: Only the purpose should be included. METHODS: The materials, techniques, instruments and equipments, and the experimental procedures should be included. RESULTS: The observatory and experimental results, including data, effects, outcome, *etc*. should be included. Authors should present *P* value where necessary, and the significant data should accompany. CONCLUSION: Accurate view and the value of the results should be included.

The format of structured abstracts is at: http://www.wjgnet.com/wjg/help/11.doc

Key words
Please list 3-10 key words that could reflect content of the study.

Text
For most article types, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include in appropriate Figures and Tables. Data should be presented in the body text or in Figures and Tables, but not in both.

Illustrations
Figures should be numbered as 1, 2, 3 and so on, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. No detailed legend should be involved under the figures. This part should be added into the text where the figures are applicable. Digital images: black and white photographs should be scanned and saved in TIFF format at a resolution of 300 dpi; color images should be saved as CMYK (print files) but not as RGB (screen-viewing files). Place each photograph in a separate file. Print images: supply images of size no smaller than 126×76 mm printed on smooth surface paper; label the image by writing the Figure number and orientation using an arrow. Photomicrographs: indicate the original magnification and stain in the legend. Digital Drawings: supply files in EPS if created by freehand and illustrator, or TIFF from photoshops. EPS files must be accompanied by a version in native file format for editing purposes. Existing line drawings should be scanned at a resolution of 1200 dpi and as close as possible to the size where they will appear when printed. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...

Tables
Three-line tables should be numbered as 1, 2, 3 and so on, and mentioned clearly in the main text. Provide a brief title for each table. No detailed legend should be included under the tables. This part should be added into the text where the tables are applicable. The information should complement but not duplicate that contained in the text. Use one horizontal line under the title, a second under the column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations
Data that are not statistically significant should not be noted. $^{a}P<0.05$, $^{b}P<0.01$ should be noted ($P>0.05$ should not be noted). If there are other series of *P* values, $^{c}P<0.05$ and $^{d}P<0.01$ are used. Third series of *P* values can be expressed as $^{e}P<0.05$ and $^{f}P<0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or some other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc*. in a certain sequence.

Acknowledgments
Brief acknowledgments of persons who have made genuine contributions to the manuscripts and who endorse the data and conclusions are included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should code the references according the citation order in text in Arabic numerals, put references codes in square brackets, superscript it at the end of citation content or the author name of the citation. For those citation content as the narrate part, the coding number and square brackets should be typeset normally. For example, Crohn's disease (CD) is associated with increased intestinal permeability[1,2]. If references are directly cited in the text, they would be put together with the text, for example, from references [19,22-24], we know that...

When the authors code the references, please ensure that the order in text is the same as in reference part and also insure the spelling accuracy of the first author's name. Do not code the same citation twice.

PMID requirement

PMID roots in the abstract serial number indexed by PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). The author should supply the PMID for journal citation. For those references that have not been indexed by PubMed, a printed copy of the first page of the full reference should be submitted.

The accuracy of the information of the journal citations is very important. Through reference testing system (http://www.aushome.cn/cgi-bin/index.pl), the authors and editor could check the authors name, title, journal title, publication date, volume number, start page, and end page. We will interlink all references with PubMed in ASP file so that the readers can read the abstract of the citations online immediately.

Style for journal references

Authors: the first author should be typed in bold-faced letter. The surname of all authors should be typed with the initial letter capitalized and followed by their name in abbreviation (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). Title of the cited article and italicized journal title (Journal title should be in its abbreviation form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634]

Note: The author should test the references through reference testing system (http://www.aushome.cn/cgi-bin/index.pl)

Style for book references

Authors: the first author should be typed in bold-faced letter. The surname of all authors should be typed with the initial letter capitalized and followed by their name in abbreviation (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Standard jounal article (list all authors and include the PubMed ID [PMID] where applicable)

1 **Das KM,** Farag SA. Current medical therapy of inflammatory bowel disease. *World J Gastroenterol* 2000; 6: 483-489 [PMID: 11819634]

2 **Pan BR**, Hodgson HJF, Kalsi J. Hyperglobulinemia in chronic liver disease: Relationships between *in vitro* immunoglobulin synthesis, short lived suppressor cell activity and serum immunoglobulin levels. *Clin Exp Immunol* 1984; 55: 546-551 [PMID: 6231144]

3 **Lin GZ,** Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; 7: 285-287

Books and other monographs (list all authors)

4 **Sherlock S,** Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

5 **Lam SK.** Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Electronic journal (list all authors)

6 **Morse SS.** Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1):24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Inappropriate references

Authors should always cite references that are relevant to their article, and avoid any inappropriate references. Inappropriate references include those that are linked with a hyphen and the difference between the two numbers at two sides of the hyphen is more than 5. For example, [1-6], [2-14] and [1, 3, 4-10, 22] are all considered as inappropriate references. Authors should not cite their own unrelated published articles.

Statistical data

Present as mean ± SD and mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as γ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p(B)= 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2 not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format about how to accurately write common units and quantum is at: http://www.wjgnet.com/wjg/help/15.doc

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further mention.

Italicis

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyr*A, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *Helicobacter pylori, H pylori, E coli, etc.*

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJG*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. Author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, the final check list for authors, and responses to reviewers by a courier (such as EMS) (submission of revised manuscript by e-mail or on the *WJG* Editorial Office Online System is NOT available at present).

Language evaluation

The language of a manuscript will be graded before sending for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing; (4) Grade D: rejected. The revised articles should be in grade B or grade A.

Copyright assignment form

It is the policy of *WJG* to acquire copyright in all contributions. Papers accepted for publication become the copyright of *WJG* and authors will be asked to sign a transfer of copyright form. All authors must read and agree to the conditions outlined in the Copyright Assignment Form (which can be downloaded from http://www.wjgnet.com/wjg/help/9.doc).

Final check list for authors

The format is at: http://www.wjgnet.com/wjg/help/13.doc

Responses to reviewers

Please revise your article according to the comments/suggestions of reviewers. The format for responses to the reviewers' comments is at: http://www.wjgnet.com/wjg/help/10.doc

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Publication fee

Authors of accepted articles must pay publication fee.

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 163
World Journal of Gastroenterology ISSN 1007-9327

World Journal of Gastroenterology standard of quantities and units

Number	Nonstandard	Standard	Notice
1	4 days	4 d	In figures, tables and numerical narration
2	4 days	four days	In text narration
3	day	d	After Arabic numerals
4	Four d	Four days	At the beginning of a sentence
5	2 hours	2 h	After Arabic numerals
6	2 hs	2 h	After Arabic numerals
7	hr, hrs,	h	After Arabic numerals
8	10 seconds	10 s	After Arabic numerals
9	10 year	10 years	In text narration
10	Ten yr	Ten years	At the beginning of a sentence
11	0,1,2 years	0,1,2 yr	In figures and tables
12	0,1,2 year	0,1,2 yr	In figures and tables
13	4 weeks	4 wk	
14	Four wk	Four weeks	At the beginning of a sentence
15	2 months	2 mo	In figures and tables
16	Two mo	Two months	At the beginning of a sentence
17	10 minutes	10 min	
18	Ten min	Ten minutes	At the beginning of a sentence
19	50% (*V/V*)	500 mL/L	
20	50% (m/*V*)	500 g/L	
21	1 M	1 mol/L	
22	10 μM	10 μmol/L	
23	1N HCl	1 mol/L HCl	
24	1N H_2SO_4	0.5 mol/L H_2SO_4	
25	4rd edition	4^{th} edition	
26	15 year experience	15- year experience	
27	18.5 kDa	18.5 ku,18 500u or M_r18 500	
28	25 $g.kg^{-1}/d^{-1}$	25 g/(kg·d) or 25 g/kg per day	
29	6900	6 900	
30	1000 rpm	1 000 r/min	
31	sec	s	After Arabic numerals
32	1 pg $\cdot L^{-1}$	1 pg/L	
33	10 kilograms	10 kg	
34	13 000 rpm	13 000 *g*	High speed; *g* should be in italic and suitable conversion.
35	1000 *g*	1 000 r/min	Low speed. *g* cannot be used.
36	Gene bank	GenBank	International classified genetic materials collection bank
37	Ten L	Ten liters	At the beginning of a sentence
38	Ten mL	Ten milliliters	At the beginning of a sentence
39	umol	μmol	
40	30 sec	30 s	
41	1 g/dl	10 g/L	10-fold conversion
42	OD_{260}	A_{260}	"OD" has been abandoned.
43	One g/L	One microgram per liter	At the beginning of a sentence
44	*A*260 nm	A_{260} nm	*A* should be in italic.
	$^{b}P<0.05$	$^{a}P<0.05$	In Table, no note is needed if there is no significance instatistics: $^{a}P<0.05$, $^{b}P<0.01$ (no note if $P>0.05$). If ther is a second set of *P* value in the same table, $^{c}P<0.05$ and $^{d}P<0.01$ are used for a third set: $^{e}P<0.05$, $^{f}P<0.01$.
45	$^{*}F=9.87$, $^{\S}F=25.9$, $^{\#}F=67.4$	$^{1}F=9.87$,$^{2}F=25.9$, $^{3}F=67.4$	Notices in or under a table
46	KM	km	kilometer
47	CM	cm	centimeter
48	MM	mm	millimeter
49	Kg, KG	kg	kilogram
50	Gm, gr	g	gram
51	nt	N	newton
52	l	L	liter
53	db	dB	decibel
54	rpm	r/min	rotation per minute
55	bq	Bq	becquerel, a unit symbol
56	amp	A	ampere
57	coul	C	coulomb
58	HZ	Hz	
59	w	W	watt
60	KPa	kPa	kilo-pascal
61	p	Pa	pascal
62	ev	EV	volt (electronic unit)
63	Jonle	J	joule
64	J/mmol	kJ/mol	kilojoule per mole
65	$10\times10\times10cm^3$	10 cm×10 cm×10 cm	
66	N·km	KN·m	moment
67	$x\pm s$	mean±SD	In figures, tables or text narration
68	Mean±SEM	mean±SE	In figures, tables or text narration
69	*im*	im	intramuscular injection
70	– *iv*	iv	intravenous injection
71	Wang et al	Wang *et al.*	
72	EcoRI	*Eco*RI	*Eco* in italic and RI in positive. Restriction endonuclease has its prescript form of writing.
73	Ecoli	*E.coli*	Bacteria and other biologic terms have their specific expression.
74	Hp	*H pylori*	
75	Iga	*Iga*	writing form of genes
76	*igA*	IgA	writing form of proteins
77	-70 kDa	~70 ku	

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 January 7; 12(1): 164-180
World Journal of Gastroenterology ISSN 1007-9327

World Journal of Gastroenterology®

Brief Introduction of Editorial Board

2004-2006

Published by The WJG Press, PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893 E-mail: wjg@wjgnet.com http://www.wjgnet.com

The *World Journal of Gastroenterology* Editorial Board consists of 685 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 52 countries including: Albania (1), Argentina (1), Australia (16), Austria (7), Belarus (1), Belgium (11), Brazil (1), Bulgaria (1), Canada (10), China (58), Croatia (2), Czech(1), Denmark (5), Egypt (3), Finland (1), France (27), Germany (71), Greece (1), Hungary(1), Iceland (1), India (6), Iran (2), Ireland (2), Israel (5), Italy (51), Japan (137), Lebanon(2), Lithuania (1), Macedonia(1), Malaysia (3), Mexico (2), Monaco (1), Netherlands (15), New Zealand (1), Nigeria(1), Norway(2), Pakistan(1), Poland (6), Portugal (1), Russia (3), Singapore (3), Slovakia (1), South Africa (1), South Korea (9), Spain (27), Sweden (6), Switzerland (8), Turkey (7), United Arab Emirates(1), United Kingdom (37), United States (121), Yugoslavia (1).

HONORARY EDITORS-IN-CHIEF

Ke-Ji Chen, Professor, Xiyuan Hospital, Chinese Tranditional Medicine University, Beijing 100091, China

Li-Fang Chou, Professor, Department of Public Finance, National Chengchi University, Chih-Nan Rd., Sec. 2, Zhi-Nan Road, Taipei 11623, Taiwan, China

Dai-Ming Fan, Professor, Director of Department of Gastroenterology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province,China

Zhi-Qiang Huang, Professor, Abdominal Surgery Institute of General Hospital of PLA, Fuxing Road, Beijing 100853, China

Shinn-Jang Hwang, Professor, Department of Family Medicine, Taipei Veterans General Hospital, VGH, 201, Shih-Pai Road, Section 2, 11217, Taiwan, China

Min-Liang Kuo, PhD, Laboratory of Molecular and Cellular Toxicology, Institute of Toxicology, College of Medicine, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei, Taiwan, China

Nicholas F LaRusso, MD, Center for Basic Research in Digestive Diseases, Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, United States

Jie-Shou Li, Professor, Academician of Chinese Academy of Engineering, Department of General Surgery, General Hospital of Nanjing Command Area, 305 East Zhongshan Road, Nanjing 210002, Jiangsu Province, China

Geng-Tao Liu, Professor, Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Lein-Ray Mo, MD, Department of Internal Medicine, Tainan Municipal Hospital, 670, Chung-Te Rd., Tainan 701, Taiwan, China

Fa-Zu Qiu, MD, Hepatic Surgery Center of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China

Eamonn M Quigley, Professor, Department of Medicine National University of Ireland, Cork, Cork University Hospital Clinical Sciences Building Wilton, Cork, Ireland

David S Rampton, Professor, Centre for Gastroenterology, Institute of Cell and Molecular Science, Queen Mary School of Medicine and Dentistry, London E1 2AD, United Kingdom

Rudi Schmid, MD, 211 Woodland Road, Kentfield, California 94904, United States

Nicholas J Talley, MD, PhD, Professor of Medicine, Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine, 200 First Street S.W., PL-6-56, Rochester, MN 55905, United States

PRESIDENT AND EDITOR-IN-CHIEF

EDITOR-IN-CHIEF

ASSOCIATE EDITORS-IN-CHIEF

MEMBERS OF THE EDITORIAL BOARD

Albania

Argentina

Australia

Austria

NO-7006, Norway

Pakistan

Muhammad Saeed Khokhar, Professor, Department of Medicine and Gastroenterology, Services Institute of Medical Sciences, Services Hospital Jail Road Lahore, Project Director SIMS Services Hospital Lahore, Lahore 54000, Pakistan

Poland

Tomasz Brzozowski, Professor, Department of Physiology, Jagiellonian University Medical College, 16 Grzegorzecka Str, Cracow 31-531, Poland

Robert Flisiak, PhD, Department of Infectious Diseases, Medical University of Bialystok, 15-540 Bialystok, Zurawia str., 14, Poland

Hanna Gregorek, Assistant Professor, PhD, Department of Microbiology and Clinical Immunology, The Children's Memorial Health Institute, Al. Dzieci Polskich 20, Warsaw 04-730, Poland

Hanna Gregorek, PhD, Assistant Professor, Department of Microbiology and Clinical Immunology, The Children's Memorial Health Institute, Warsaw 04 730, Poland

Dariusz Marek Lebensztejn, Associate Professor, IIIrd Department of Pediatrics, Medical University of Bialystok, 17 Waszyngtona Str, Bialystok 15-274, Poland

Wojciech G Polak, Dr, Department of Vascular, General and Transplant Surgery University Hospital in Wroclaw ul. Poniatowskiego 2 PL-50-326 Wroclaw, Poland

Portugal

Miguel Carneiro De Moura, Professor, Department of Gastroenterology, Medical School of Lisbon, Av Prof Egas Moniz,1649-028 Lisboa, Portugal

Russia

Vladimir T Ivashkin, MD, Director of The Clinic of Internal Diseases, Gastroenterology and Hepatology Named After V.Vasilenko of The Moscow Sechenov Medical Academy, 119992 Pogodinskaya 1, Moscow, Russian

Leonid Lazebnik, Professor, Moscow Health Department, CRI of Gastroenterology, Shosse Entusiastov 86, Moscow 111123, Russian

Vasiliy Ivanovich Reshetnyak, Professor, Institute of General Reanimatology, 25-2, Petrovka Str., Moscow 107031, Russian

Singapore

Bow Ho, Professor, Department of Microbiology, National University of Singapore, 5 Science Drive 2, Block MD4a, Room 03-12, Kent Ridge 117597, Singapore

Khek-Yu Ho, Professor, Department of Medicine, National University Hospital, 119074, Singapore

Francis Seow-Choen, Professor, Seow-Choen Colorectal Centre, Mt Elizabeth Medical Centre, Singapore, 3 Mt Elizabeth Medical Centre #09-10 , 228510, Singapore

Slovakia

Anton Vavrecka, MD, Clinic of Gastroenterology, SZU, NSP SV.CAM, Antolska 11, Bratislava 85107, Slovakia

South Africa

Michael Charles Kew, Professor, Department of Medicine, University of the Witwatersrand Medical School, 7 York Road, Parktown 2193, Johannesburg, South Africa

South Korea

Byung Ihn Choi, Professor, Department of Radiology, Seoul National University Hospital, 28, Yeongeon-dong, Jongno-gu, Seoul 110-744, South Korea

Ho Soon Choi, Professor, Gastroenterology, department of Internal Medicine, Hanyang University, 17 aengdang-dong, Sungdong-gu, Seoul 133-792, South Korea

Jae J Kim, MD, PhD, Associate Professor, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50, Irwon-dong, Gangnam-gu, Seoul 135-710, South Korea

Jin-Hong Kim, Professor, Department of Gastroenterology, Ajou University Hospital, San 5, Wonchon-dong, Yeongtong-gu, Suwon 442-721, South Korea

Myung-Hwan Kim, Professor, Department of Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-dong, Songpa-gu, Seoul 138-736, South Korea

Jong Kyun Lee, Associate Professor, Department of Gastroenterology, Sungkyunkwan University School of Medicine, Ilwom-Dong 50,Gangnam-GU, Seoul 135-710, South Korea

Eun-Yi Moon, Dr, Laboratory of Human Genomics, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, South Korea

Jae-Gahb Park, Professor, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744, South Korea

Dong-Wan Seo, Professor, Department of Internal Medicine, Division of Gastroenterology, Asan Medical Center, Univeristy of Ulsan College of Medicine, 388-1 Pungnapdong, Songpagu, Seoul 138-736, South Korea

Spain

Juan Gonzalez Abraldes, MD, Hepatic Hemodynamic Laboratory, Liver Unit. Hospital Clinic. University of Barcelona, Hepatic Hemodynamic Lab. Liver Unit, Hospital Clinic, Villarroel 170, Barcelona 08036, Spain

Agustin Albillos, Associate Professor, Departamento de Medicina, Facultad de Medicina-Campus Universitario, Universidad de Alcalá, Carretera Madrid-Barcelona km.33.600, Alcalá de Henares, Madrid 28871, Spain

Raul J Andrade, Associate Professor, Department of Medicine, University of Malaga, Boulevar louis pasteur 32, málaga 29010, Spain

Luis Aparisi, PhD, Unit of Heptatology, University Clinic Hospital, Valencia 46010, Spain

Fernando Azpiroz, MD, Digestive System Research Unit, University Hospital Vall d'Hebron, Paseo Vall d'Hebron, 119-129, Barcelona 08035, Spain

Ramon Bataller, MD, Liver Unit, Hospital Clinic,Villarroel 170, Barcelona 08036, Spain

Josep M Bordas, MD, Department of Gastroenterology IMD, Hospital Clinic", Llusanes 11-13 at, Barcelona 08022, Spain

Xavier Calvet, Professor, Department of Digestive Diseases, Hospital de Sabadell, Parc taulí s/n, Sabadell 08221, Spain

Vicente Carreño, Dr, Fundacion Estudio Hepatitis Virales, C/ Guzman el Bueno 72, Semisotano, Madrid 28015, Spain

Antoni Castells, MD, Gastroenterology Department, Hospital Clínic, University of Barcelona, Villarroel 170, Barcelona 08036, Spain

Vicente Felipo, Dr, Laboratory of Neurobiology, Centro de Investigación Príncipe, Avda del Saler, 16, 46013 Valencia. Spain

Juan Carlos Garcia-Pagán, MD, Liver Unit Hospital Clinic, Villaroel 170, Barcelona 08036, Spain

Jaime Bosch Genover, Professor, Department of Hepatology, Hospital Clínic i Provincial, c/Villarroel 170, Escalera 7, 3 Planta, Barcelona 08036, Spain

Jaime Guardia, Professor, Internal Medicine and Liver Unit, Hospital Universitari 'Vall d'Hebron'. Universitat Autonoma de Barcelona, Hospital Universitari Vall d'Hebron, Pg Vall d'Hebron, 119, Barcelona 08035, Spain

Angel Lanas, Professor, Service of Gastroenterology, Hospital Clinico Universitario, Service of GastroenterologyUniversity HospitalC/ San Juan Bosco 15, Zaragoza 50009, Spain

María Isabel Torres López, Professor, Experimental Biology, University of Jaen, araje de las Lagunillas s/n, Jaén 23071, Spain

Gastroenterology and Hepatology, Mayo Clinic College of Medicine, 200 First Street, SW, Rochester, MN 55905, United States

Robin G Lorenz, Associate Professor, Department of Pathology, University of Alabama at Birmingham, 845 19th Street South BBRB 730, Birmingham, AL 35294-2170, United States

James David Luketich, MD, Professor and Chief, Division of Thoracic and Foregut Surgery University of Pittsburgh Medical Center Pittsburgh, PA 15213 , United States

Henry Thomson Lynch, MD, Department of Preventive Medicine, Creighton University School of Medicine, 2500 California Plaza, Omaha NE 68178, United States

John Frank Di Mari, Assistant Professor, Internal Medicine, Gastroenterology ,9.138 MRB 301 University Blvd. Galveston, Texas 77555-1064, United States

John M Mariadason, PhD, Assistant Professor, Department of Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, Hofheimer Bldg. 413, 111 East 210th Street, Bronx, NY 10467, United States

Wendy Michelle Mars, PhD, Department of Pathology , University of Pittsburgh , S-411B South Biomedical Science Tower Pittsburgh , PA 15261, United States

George W Meyer, MD, Gastroenterology Section Kaiser Permanente Medical Center UC Davis Medical School University of California at Davis Sacramento, California, 025 Morse Ave, Station 2A, Sacramento, CA 95628, United States

George Michalopoulos, MD, PhD, Department of Pathology, University of Pittsburgh, School of Medicine, S-410 Biomedical Science Tower, Pittsburgh, PA 15261,United States

Satdarshan P Singh Monga, Dr, Pathology and Medicine, University of Pittsburgh, SOM, S421-BST, 200 Lothrop Street, Pittsburgh PA 15261, United States

Timothy H Moran, Professer, Department of Psychiatry, Johns Hopkins University School of Medicine, Ross 618, 720 Rutland Ave, Baltimore, Maryland 21205, United States

Hiroshi Nakagawa, Assistant Professor, Gastroenterology Division, University of Pennsylvania, 415 Curie Blvd. 638BCRB, Philadelphia 19104, United States

Douglas B Neison, Dr, Department of Gastroenterology, Minneapalis VA Medical Cencer, One Veterons Dive(111-D), Minneapalis 55417, United States

Curtis T Okamoto, Associate Professor, Department of Pharmaceutical Sciences, University of Southern California, 1985 Zonal Ave. PSC 404A, Los Angeles CA 90089-9121, United States

Stephen J Pandol, MD, Department of Veterans Affairs, University of California, Los Angeles, Building 258, Room 340, 11301 Wilshire Boulevard, Los Angeles CA 90073, United States

Pankaj Jay Pasricha, MD, Department of Internal Medicine, The University of Texas Medical Branch, 301 University Boulevard, Rt. 0764, Galveston, TX 77555-0764, United States

Zhiheng Pei, Assistant Professor, Department of Pathology and Medicine, New York University School of Medicine, Department of Veterans Affairs, New York Harbor Healthcare System, 6001W, 423 East 23rd street, New York NY 10010, United States

Michael A Pezzone, MD, PhD, Assistant Professor, Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh, 200 Lothrop Street, Pittsburgh, PA 15213, United States

CS Pitchumoni, Professor, Robert Wood Johnson School of Medicine, Robert Wood Johnson School of Medicine, New Brunswiuc NJ D8903, United States

Jay Pravda, MD, Inflammatory Disease Research Center, Gainesville, Florida, 32614-2181, United States

Massimo Raimondo, Dr, Division of Gastroenterology and Hepatology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, United States

Adrian Reuben, Professor of Medicine, Department of Medicine, Medical University of South Carolina, Division of Gastroenterology and Hepatology, 210 Clinical Sciences Building, PO Box 250327, 96 Jonathan Lucas Street, Charleston SC 29425, United States

Victor E Reyes, PhD, Professor, Departments of Pediatrics and Microbiology & Immunology, University of Texas Medical Branch, 301 University Blvd., Route 0366, Galveston, TX 77555, United States

Richard A Rippe, Dr, Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7038, United States

Marcos Rojkind, MD, PhD, Professor, Department of Biochemistry and Molecular Biology, The George Washington University Medical Center, 2300 Eye street, NW Washington DC 20037, United States

Hemant Kumar Roy, Dr, Department of Internal Medicine, Institute of Evanston-Northwestern Healthcare, 2650 Ridge Avenue,Walgreen's Building SB 521, Evanston 60201, United States

Shawn David Safford, Dr, Department of Surgery, Duke University Medical Center, 994 West Ocean View Avenue, Norfolk VA23503, United States

Nicholas James Shaheen, Dr, Department of Medicine, Carolina Campus, University of North Carolina, 130 Mason Farm Road, CB#7080, Chapel Hill 27599-7080, United States

Stuart Sherman, Professor of Medicine and Radiology, Department of Gastroenterology and Hepatology, Indiana University Medical Center, 550 N. University Blvd, Suite 4100, Indianapolis 46202, United States

Shivendra Shukla, Professor, Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, 1 Hospital Drive, M530 Medical Sciences Bldg., Columbia MO 65212, United States

Alphonse E. Sirica, MS, PhD,Professor and Chair, Division of Cellular and Molecular Pathogenesis, Department of Pathology, Medical College of Virginia Campus, Virginia Commonwealth University,Richmond, Virginia 23298-0297, United States

Michael Steer, Professor, Department of Surgery, Tufts-Nemc, 860 Washington St, Boston, Ma 02111, United States

Gary D Stoner, PhD, Department of Internal Medicine, Ohio State University, CHRIRm 1148, 300 W. 10th Avenue, Columbus 43210, United States

Yvette Taché, PhD, Digestive Diseases Research Center and Center for Neurovisceral Sciences and Women's Health, Division of Digestive Diseases, Department of Medicine, David Geffen School of Medicine at UCLA, University of California, Los Angeles and VA Greater Los Angeles Healthcare System; 11301 Wilshire Boulevard, CURE Building 115, Room 117, Los Angeles, CA, 90073, United States

Jayant A Talwalkar, MD, Division of Gastroenterology and HepatologyMayo Clinic and Foundation,200 First Street SW, Rochester, MN 55901, United States

Kam-Meng Tchou-Wong, Assistant Professor, Departments of Environmental Medicine and Medicine, NYU School ofMedicine, 57 Old Forge Road, Tuxedo, New York 10987, United States

Paul Joseph Thuluvath, Professor, Department of Gastroenterology and Hepatology, The Johns Hopkins Hospital, 1830 E. Monument St, Baltimore MD 21205, United States

Swan Nio Thung, Professor, Department of Pathology, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York 10029, United States

R Alberto Travagli, Professor, Department of Neuroscience, Pennington Biomedical Research Center-LSU 6400 Perkins Road, Baton Rouge, LA 70808, United States

George Triadafilopoulos, Clinical Professor of Medicine, Division of Gastroenterology and Hepatology,Alway Building, Room M 211, 300 Pasteur Drive, MC: 5187, Stanford, CA 94305-5187, United States

Chung-Jyi Tsai, MD, Division of Digestive Diseases and Nutrition, University of Kentucky Medical Center, 800 Rose Street, Lexington 40536-0298, Kentucky, United States

Hugo E Vargas, Associate Professor of Medicine, Division of