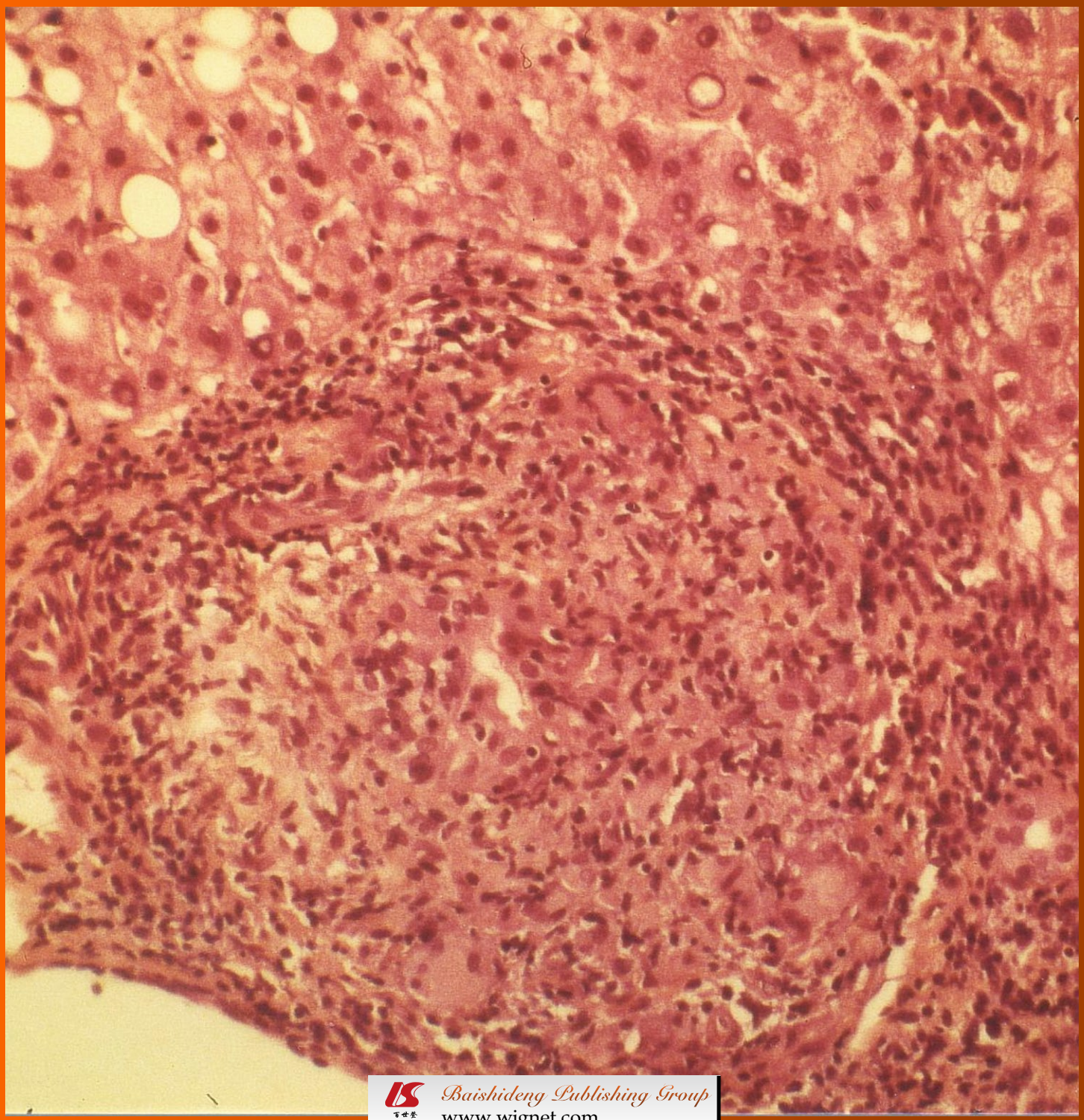
ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2011 June 27; 3(6): 137-174



Baishideng Publishing Group
www.wjgnet.com

WJH World Journal of Hepatology

A peer-reviewed, online, open-access journal of hepatology

Editorial Board

2009-2013

The *World Journal of Hepatology* Editorial Board consists of 573 members, representing a team of worldwide experts in hepatology. They are from 46 countries, including Argentina (4), Australia (7), Austria (2), Bangladesh (1), Belgium (3), Botswana (2), Brazil (8), Brunei Darussalam (1), Bulgaria (1), Canada (10), Chile (1), China (89), Denmark (1), Egypt (3), Finland (1), France (15), Gambia (1), Germany (28), Greece (8), Hungary (3), India (20), Ireland (1), Israel (7), Italy (65), Japan (45), Malaysia (1), Mexico (4), Netherlands (4), Pakistan (2), Poland (1), Portugal (1), Philippines (1), Romania (1), Saudi Arabia (1), Singapore (4), South Korea (17), Spain (22), Sri Lanka (1), Sudan (1), Switzerland (2), Thailand (6), Tunisia (2), Turkey (13), United Kingdom (17), United States (144), and Venezuela (1).

Chiranjib Chakraborty, *Beijing*
Stephen Lam Chan, *Hong Kong*
George G Chen, *Hong Kong*
Min-Shan Chen, *Guangzhou*
Yang Cheng, *Shanghai*
Siu Tim Cheung, *Hong Kong*
Thomas YC Cheung, *Hong Kong*
Yick-Pang Ching, *Hong Kang*
William Chi-shing Cho, *Hong Kong*
Chui Chung-hin, *Hong Kong*
Shuang-Suo Dang, *Xi'an*
Yi-Tao Ding, *Nanjing*
Jian-Gao Fan, *Shanghai*
Yuen Man Fung, *Hong Kang*
Zuo-Jiong Gong, *Wuhan*
Tian-Quan Han, *Shanghai*
Jin-Yang He, *Guangzhou*
Garrett CL Ho, *Hong Kong*
Ji-Ming Hu, *Wuhan*
Can-Hua Huang, *Chengdu*
Zhi-Yong Huang, *Wuhan*
Jian-Hui Jiang, *Changsha*
Dong-Yan Jin, *Hong Kong*
Hsiang-Fu Kung, *Hong Kong*
Lai PBS Lai, *Hong Kong*
Wan YJ Lau, *Hong Kong*
Nancy WY Leung, *Hong Kong*
Jin-Qing Li, *Guangzhou*
Li-Ying Li, *Beijing*
Shu-Chen Li, *Harbin*
Xin-Wei Li, *Shanghai*
Yu-Yuan Li, *Guangzhou*
En-Qi Liu, *Xi'an*
Yin-Kun Liu, *Shanghai*
Chung-Mau Lo, *Hong Kong*
Lun-Gen Lu, *Shanghai*
Ming-De Lu, *Guangzhou*
John M Luk, *Hong Kong*
Guang-Hua Luo, *Changzhou*
Shuang Mei, *Shanghai*
Kelvin KC Ng, *Hong Kong*
Qin Ning, *Wuhan*
Qin Pan, *Shanghai*
Qi-Jun Qian, *Shanghai*
Jian-Min Qin, *Shanghai*
Xian-Jun Qu, *Jinan*
Xue-Ying Sun, *Harbin*
Qin Su, *Beijing*
Wu-Yi Sun, *Hefei*
Hui-Ru Tang, *Wuhan*
Peng Tao, *Nanning*
Eric WC Tse, *Hong Kong*
Bin Wang, Weifang
Xiao-Zhong Wang, *Fuzhou*
Xiu-Jie Wang, *Chengdu*
Zhen-Xia Wang, *Huhhot*
Grace LH Wong, *Hong Kong*
Nathalie Wong, *Hong Kong*
Xiong-Zhi Wu, *Tianjin*
De-Xiang Xu, *Hefei*
Rui-An Xu, *Quanzhou*
Xun-Di Xu, *Changsha*
Xiao Yang, *Beijing*
Zhen-Fan Yang, *Hong Kong*
Boon Hun Yong, *Hong Kong*
Ting-He Yu, *Chengdu*
Benny CY Zee, *Hong Kong*
Jia-Ning Zhang, *Dalian*
Xiao-Dong Zhang, *Tianjin*
Xiao-Lan Zhang, *Shijiazhuang*
Xiao-Yan Zhang, *Shanghai*
Hong-Chuan Zhao, *Hefei*
Xiao-Ping Zhao, *Beijing*
Jiang-Fan Zhu, *Shanghai*
Yi-Ping Zou, *Beijing*

Denmark

Henning Grønbæk, *Aarhus*

Egypt

Nabil Mohie Abdel-Hamid, *Minia*
Laila AF Eissa, *Mansoura*
Mona Mostafa Fahmy Nosseir, *Giza*

Finland

Thomas Kietzmann, *Oulu*

France

Aramando Abergel, *Clenmont -Ferrant*
Henri Bismuth, *Villejuif Cedex*
Ana CFN Cardoso, *Paris*
Nicolas Chignard, *Paris*
Claude C de Fromentel, *Lyon*
Zdenko Herceg, *Lyon*
Nathalie Janel, *Paris*
Victor de Ledinghen, *Pessac cedex*
Antoinette Lemoine, *Villejuif*
Marcellin Patrick, *Clichy*
Raoul Poupon, *Paris*
Rodrigue Rossignol, *Bordeaux cedex*
Christian Trépo, *Lyon*
Dominique A Vuitton, *Besancon*
Virginie Wautot, *Pierre Benite*

Gambia

Maimuna Ebirunkeh Mendy, *Banjul*

Germany

Thomas Bock, *Tuebingen*
Ali Canbay, *Essen*
Enrico Narciso De Toni, *München*
Joachim Drevs, *Freiburg*
Volker Fendrich, *Marburg*
Peter R Galle, *Mainz*
Erich Gulbins, *Essen*
Roland Kaufmann, *Jena*
Sebastian Hinz, *Kiel*
Philipp Kobbe, *Aachen*
Michael Kremer, *Heidelberg*
Christian Liedtke, *Aachen*
Martin Loss, *Regensburg*
Arun Kumar Mankan, *Munich*
Lars Müller, *MD, Kiel*
Michael D Menger, *Saarbrucken*
Andreas K Nussler, *Munich*
Margarete Odenthal, *Koeln*
Claus Petersen, *Hannover*
Andrej Potthoff, *Hannover*
Thomas Pusl, *München*
Elke Roeb, *Giessen*
Frank Tacke, *Aachen*
Stefan Rose-John, *Kiel*
Andreas Teufel, *Mainz*
Lothar Thomas, *Frankfurt*
Jens JW Tischendorf, *Aachen*
Arndt Vogel, *Hannover*

Greece

Alex P Betrosian, *Athens*
Spiros G Delis, *Athens*
Ioannis Diamantis, *Athens*
Papandreou Dimitrios, *Mela*
Elias A Kouroumalis, *Crete*
George Papatheodoridis, *Athens*
Stamatios E. Theocharis, *Athens*

Hungary

Gábor Bánhegyi, *Budapest*
Subhamay Ghosh, *Pécs*
Peter Nagy, *Budapest*

India

Anjali Deepak Amarapurkar, *Mumbai*
DN Amararpurkar, *Mumbai*
Runu Chakravarty, *Kolkata*
Pronobesh Chattopadhyay, *Moradabad*
Puneet Chopra, *Gurgaon Haryana*
Tanya Das, *Kolkata*
Radha Krishan Dhiman, *Chandigarh*
Ajay Duseja, *Chandigarh*
Devendra K Gupta, *New Delhi*
P Kar, *New Delhi*
Sudhir Kumar, *Lucknow*
Vijay Kumar, *New Delhi*
Anoop Misra, *New Delhi*
Devendra Parmar, *Lucknow*
Rajendra Prasad, *Chandigarh*
K Rajeshwari, *New Delhi*
Pallu Reddanna, *Hyderabad*
Barjesh Chander Sharma, *New Delhi*
Sarman Singh, *New Delhi*
Ajith TA, *Thrissur*

Ireland

Matthew William Lawless, *Dublin*

Israel

Yaron Ilan, *Jerusalem*

Yaakov Maor Kendler, *Tel Hashomer*
Ran Oren, MD, *Tel Aviv*
Amir Shlomai, *Modiin*
Rifaat Safadi, *Jerusalem*
Shira Zelber Sagi, *Tel Aviv*
Yehuda Julius Shoenfeld, *Tel Hahsomer*

Italy

Luca Aasaloni, *Bologna*
Giovanni Addolorato, *Rome*
Luigi E Adinolfi, *Naples*
Pietro Andreone, *Bologna*
M Appetecchia, *Rome*
Antonio Ascione, *Napoli*
Ferruccio Bonino, *Milano*
Bruno D Bruno, *Benevento*
Savino Bruno, *Milano*
Melchiorre Cervello, *Palermo*
Claudio Chiesa, *Rome*
Stefano Colagrande, *Firenze*
Massimo G Colombo, *Milan*
Samuele De Minicis, *Montegranaro*
Alessandro Vitale, *alessandro*
Fabio Farinati, *Padova*
Paolo Feltracco, *Padova*
Domenico Ferri, *Bari*
Amalia Gastaldelli, *Pisa*
Domenico Girelli, *Verona*
Fernando Goglia, *Benevento*
Alessandro Grasso, *Savona*
Ignazio Grattagliano, *Bari*
Pietro Invernizzi, *Milan*
Francesco Izzo, *Naples*
Amedeo Lonardo, *Modena*
Malaguarnera Lucia, *Trecastagni*
Massimo Di Maio, *Rossano*
Melania Manco, *Rome*
Andrea Mancuso, *Palermo*
F Marotta, *Milano*
Fabio Marra, *Florence*
Roberto Mazzanti, *Florence*
Giulia Morsica, *Milan*
Antonio Moschetta, *Bari*
Massimo Negrini, *Ferrara*
Andrea Nicolini, *Pisa*
Giuseppe R Nigri, *Rome*
Valerio Nobili, *Rome*
Valentina Pallottini, *Rome*
Adriano M Pellicelli, *Rome*
Marcello Persico, *Naples*
Massimo Pinzani, *Firenze*
Giovanni Polimeni, *Messina*
Camillo Porta, *Pavia*
Piero Portincasa, *Bari*
Emilio Quaia, *Trieste*
Giuseppe Remuzzi, *Bergamo*
Domenico Ribatti, *Bari*
Massimo Roncalli, *Rozzano*
Carlo Sabbà, *Bari*
Orazio Schillaci, *Rome*
Gaetano Serviddio, *Foggia*
Aurelio Sonzogni, *Bergamo*
Paolo Sorrentino, *Salerno*
Enea Spada, *Roma*
Giovanni Tarantino, *Naples*
Luciano Tarantino, *Naples*
Claudio Tiribelli, *Trieste*
Pierluigi Toniutto, *Udine*
Pietro Vajro, *Naples*
Luca Vigano, *Torino*

Japan

Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Jiro Fujimoto, *Osaka*
Atsushi Hosui, *Osaka*
Kazuo Ikeda, *Nagoya*
Toru Ishikawa, *Niigata*
Yoshiaki Iwasaki, *Okayama*
Satoru Kakizaki, *Gunma*
Naoya Kato, *Tokyo*
Takumi Kawaguchi, *Kurume*
Kiminori Kimura, *Tokyo*
Tsuneo Kitamura, *Chiba*
Keiichi Kubota, *Tochigi*
Sabina Mahmood, *Okayama*
Hitoshi Maruyama, *Chiba*
Sachiko Matsuhashi, *Saga*
Toshihiro Mitaka, *Sapporo*
Eiji Miyoshi, *Yamada-oka Suita*
Zenichi Morise, *Toyoake Aichi*
Ryuichi Morisihita, *Osaka*
Yoshiki Murakami, *Kyoto*
Satoru Murata, *Tokyo*
Atsushi Nakajima, *Kanagawa*
Yasuni Nakanuma, *Kanazawa*
Waka Ohishi, *Hiroshima*
Morikazu Onji, *Matsuyama*
Toshiji Saibara, *Nankoku*
Hiroaki Shiba, *Tokyo*
Ikuo Shoji, *Hyogo*
Ryo Sudo, *Yokohama*
Yoshio Sumida, *Nara*
Shinji Tanaka, *Tokyo*
Takuji Tanaka, *Gifu*
Akihiko Tsuchida, *Tokyo*
Takato Ueno, *Kurume*
Shinichi Ueno, *Kagoshima*
Kiyohito Yagi, *Osaka*
Yo-ichi Yamashita, *Hiroshima*
Teruyoshi Yanagita, *Saga*
Shuang-Qin Yi, *Kanazawa*
Hiroshi Yoshida, *Tokyo*
Hitoshi Yoshiji, *Nara*

Malaysia

Kamsiah Jaarin, *Kuala Lumpur*

Mexico

Norberto C Chavez-Tapia, *Tlalpan*
Javier Lizardi Cervera, *Tlalpan CP*
Saúl Villa-Treviño, *México DF*
Florencia V Vorackova, *México DF*

Netherlands

Robert Jacobus de Knegt, *Rotterdam*
TU Hoogenraad, *Heidelberglaan*
Maarten E Tushuizen, *MB Amsterdam*
Robert C Verdonk, *RB Groningen*

Pakistan

Syed Hamid Ali, *Karachi*
Huma IQ TI, *Islamabad*

Poland

Maria ES Lotowska, *Bialystok*

Portugal

Felix Dias Carvalho, *Porto*

Philippines

Janus P Ong, *Manila*

Romania

Eugen Georgescu, *Craiova*

Saudi Arabia

Ahmed Helmy, *Riyadh*

Singapore

Wei Ning Chen, *Singapore*
Si-Shen Feng, *Singapore*
Lang Zhuo, *Singapore*
Chun-Tao Wai, *Singapore*

South Korea

Sang Hoon Ahn, *Seoul*
Sun Pyo Hong, *Yongin*
Byung Ihn Choi, *Seoul*
Seok Joo Han, *Seoul*
Kyung Lib Jang, *Busan*
Bum-Joon Kim, *Seoul*
Dong Goo Kim, *Seoul*
Kyung Sik Kim, *Seoul*
Meehyein Kim, *Yongin*
Young Chul Kim, *Seoul*
Mi-Kyung Lee, *Jeonnam*
Young-Ik Lee, *Taejon*
Kwan-Kyu Park, *Daegu*
Hyunchul Rhim, *Seoul*
In Kyoung Lim, *Gyunggi-do*
Dae-Yeul Yu, *Daejeon*
Jong Won Yun, *Kyungbuk*

Spain

Jose AG Agundez, *Badajoz*
Maria Angeles, *Madrid*
Agustin Castiella, *Mendaro*
Ruben Ciria, *Cordoba*
Joan Clari, *Barcelona*
Maria Buti Ferret, *Barcelona*
Puri Fortes, *Pamplona*
Joan Genescà, *Barcelona*
María J Gómez-Lechón, *Valencia*
Arias Jaime, *Madrid*
Ángeles Pajares María, *Madrid*
Jordi Muntane, *Cordoba*
Jose JG Marin, *Salamanca*
Julia P Onsurbe, *Barcelona*
Albert Parés, *Barcelona*
Sonia Ramos, *Madrid*
Cristina Ripoll, *Madrid*
Isabel F Romero, *Barcelona*
Marta R Romero, *Salamanca*
Juan Macias Sanchez, *Sevilla*
Juan Sastre, *Valencia*
Manuel Vázquez-Carrera, *Barcelona*

Sri Lanka

EGD Shaman Rajindrajith, *Ragama*

Sudan

Hatim MY Mudawi, *Khartoum*

Switzerland

Beat Mullhaupt, *Zurich*
Maurer A Christoph, *Liestal*

Thailand

Nattiya Hirankarn, *Bangkok*
Somchai Pinlaor, *Khon Kaen*
Yong Poovorawan, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Chanitra Thuwajit, *Bangkok*
Sopit Wongkham, *Khon Kaen*

Tunisia

Olfa Bahri, *Tunis-Belvedere*
Chadli Dziri, *Tunis*

Turkey

Inci Alican, *Istanbul*
Ahmet Atessahin, *Elazig*
Yasemin Hatice Balaban, *Ankara*
Hayrullah Derici, MD, *Izmir*
Cigdem Ulukaya Durakbasa, *Istanbul*
Muhsin MM Harputluoglu, *Malatya*
Abdurrahman Kadayifci, *Gaziantep*
Adnan Kadayifci, *Antalya*
Ali Sazci, *Kocaeli*
Ilker Tasci, *Ankara*
Mehmet Yalniz, *Elazig*
Serkan Yener, *Izmir*
Yusuf Yilmaz, *Istanbul*

United Kingdom

Alastair David Burt, *Newcastle*
David O Cosgrove, *London*
Anil Dhawan, *London*
Indra Neil Guha, *Nottingham*
Phillip M Harrison, *London*
Hübscher SG Hübscher, *Birmingham*
Long R Jiao, *London*
AT Koulaouzidis, *Edinburgh*
Patricia Lalor, *Birmingham*
David A Lomas, *Cambridge*
Rajeshwar P Mookerjee, *London*
Gareth J Morris-Stiff, *Wales*
Kathryn L Nash, *Southampton*
Derek Anthony O'Reilly,
Christian P Selinge, *Bolton*
Konstantinos Tziomalos, *London*
Feng Wu, *Oxford*

United States

Gary A Abrams, *Montgomery*
Hassan H A-Kader, *Tucson*
Hans-Olov Adami, *Massachusetts*
Joseph Ahn, *Maywood*
Shannon Marie Bailey, *Alabama*
Numan Cem Balci, *St Louis MO*
Edmund J Bini, *New York*
Victor E Buckwold, *Frederick*
Roniel Cabrera, *Gainesville*
Guoqing Cao, *Indiana*
Disaya Chavalitdhamrong, *New York*
Chien-Shing Chen, *Loma Linda*
Fei Chen, *Morgantown*
Su Chen, *San Antonio*
Youhai H Chen, *Philadelphia*
Anne M Covey, *New York*
Mark J Czaja, *New York*
Srikanta Dash, *New Orleans*
Anthony JB Demetris, *Pittsburgh*
Sridevi Devaraj, *California*
Lisa Ross Dixon, *Gainesville*
Terrence M Donohue, *Omaha*
Q Ping Dou, *Detroit*
Murray N Ehrinpreis, *Detroit*
Marwan Ghazi Fakih, *Buffalo*
Shengyun Fang, *Maryland*
Claus J Fimmel, *Illinois*
Robert Anthony Fisher, *Virginia*
Samuel W French, *Torrance*
Phillip A Furman, *Princeton*
M Eric Gershwin, *California*
Jalal K Ghali, *Michigan*
Grace Liejun Guo, *Kansas City*
Dieter Haemmerich, *Charleston*
Young S Hahn, *Charlottesville*
Stephen A Harrison, *Texas*
Dee Harrison-Findik, *Nebraska*
Sidhartha Hazari, *Louisiana*
Thomas S Helling, *Jackson*
Alan W Hemming, *Florida*
Iryna S Hepburn, *Evans*
Ai-Xuan L Holterman, *Chicago*
Ke-Qin Hu, *California*
Guangcun Huang, *Ohio*
Wendong Huang, *California*
Rachel M Hudacko, *New Brunswick*
Michael John Jacobs, *Michigan*
Hartmut W Jaeschke, *Kansas City*
Ravi Jhaveri, *North Carolina*
Lynt B Johnson, *Washington*
Neil Louis Julie, *Bethesda*
Sanjay Kakar, *San Francisco*
Sanjeeva P Kalva, *Boston*
Jing X Kang, *Massachusetts*
Hetal Karsan, *Georgia*
Emmet B Keeffe, *California*
Nancy Ellen Kemeny, *New York*
Andrew Scott Kennedy, *Cary*
Kusum K Kharbanda, *Omaha*
David H Kirn, *California*
Hyam Lerner Leffert, *La Jolla*
Stacee Marie Lerret, *Milwaukee*
Fengzhi Li, *New York*
Wei Li, *Houston*
Shuang Liu, *Indiana*
Su Hao Lo, *Davis*
Daniel G Maluf, *Richmond*
Jose E Manautou, *Storrs*
Richard S Mangus, *Indiana*
Mary Ko Manibusan, *Virginia*
Paul Martin, *Miami,*
Jochen Mattner, *Ohio*
James A McCubrey, *North Carolina*
Valentina Medici, *Sacramento*
George Michalopoulos, *Pittsburgh*
Smruti R Mohanty, *Illinois*
John T Moore, *GlaxoSmithKline*
Ravi Murthy, *Texas*
Laura E Nagy, *Cleveland*
Sagar U Nigwekar, *Rochester*
Eileen M O'Reilly, *New York*
Kevin FS O'Carroll, *Hershey*
Melissa Kay Osborn, *Atlanta*
Helieh Saatara Oz, *Kentucky*
Igor P Pogribny, *Arkansas*
Nicholas C Popescu, *Bethesda Maryland*
Daniel S Pratt, *Boston*
Ratna B Ray, *Louis*
Nancy Reau, *Chicago*
Janardan K Reddy, *Chicago*
Martin J Ronis, *Little Rock*
Phillip Ruiz, *Florida*
Tanios B Saab, *Columbus*
Adnan Said, *Madison*
Neeraj Saxena, *Georgia*
Raymund R Saxena, *Minnesota*
Ann Scheimann, *Baltimore*
Timothy M Schmitt, *Charlottesville*
Bernd Schnabl, *La Jolla*
Kunwar Shailubhai, *Pennsylvania*
Muhammad Y Sheikh, *California*
Perry Shen, *Winston-Salem*
Viji Shridhar, *Rochester*
Shivendra D Shukla, *Missouri*
Ashwani K Singal, *Galveston*
Keshav K Singh, *New York*

WJH

World Journal of Hepatology

Contents

Monthly Volume 3 Number 6 June 27, 2011

World Journal of Hepatology
Volume 3 Number 6 June 27, 2011

Contents

NAME OF JOURNAL
World Journal of Hepatology

LAUNCH DATE
October 31, 2009

SPONSOR
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

EDITING
Editorial Board of *World Journal of Hepatology*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Baishideng Publishing Group Co., Limited,
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai,
Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
June 27, 2011

ISSN
ISSN 1948-5182 (online)

EDITORIAL OFFICE
Hai-Ning Zhang, Director
World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/1948-5182office

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i6.137

World J Hepatol 2011 June 27; 3(6): 137-141
ISSN 1948-5182 (online)

EDITORIAL

Management of primary sclerosing cholangitis

Holger H Lutz, Jens JW Tischendorf

Holger H Lutz, Jens JW Tischendorf, Medical Department Ⅲ (Gastroenterology, Hepatology and Metabolic Diseases), University Hospital Aachen (RWTH), Aachen, Germany
Author contributions: Lutz HH drafted the article; Tischendorf JJW provided critical revision and final approval of the version to be published.
Correspondence to: Jens JW Tischendorf, MD, Medical Department Ⅲ (Gastroenterology, Hepatology and Metabolic Diseases), University Hospital Aachen (RWTH), Pauwelsstr. 30, Aachen 52074, Germany. jtischendorf@ukaachen.de
Telephone: +49-241-80-80860 Fax: +49-241-80-82455
Received: December 24, 2010 Revised: May 10, 2011
Accepted: May 17, 2011
Published online: June 27, 2011

Abstract

Primary sclerosing cholangitis (PSC) is a rare cholestatic liver disease with major morbidity and mortality. Therapeutic management is difficult, due to lack of conclusive data and individual disease progression. High-dose UDCA was used for years as a pharmacotherapeutic agent to prevent disease progression, based on a positive trend in pilot studies, but has recently been proven to have a negative effect in advanced disease. Immunosuppressants might be useful in patients with overlap syndromes. Dominant bile duct stenoses should be treated endoscopically, and cholangiocellular carcinoma (CCC) still remains a therapeutic challenge in PSC patients. Early diagnosis of CCC must be improved and new strategies such as neoadjuvant radiochemotherapy with subsequent liver transplantation in selected patients are further options to be considered.

Key words: Primary sclerosing cholangitis; Ursodeoxycholic acid; NorUDCA; Cholangiocellular carcinoma; Cholestatic liver disease; Endoscopy; Dominant stenoses

Peer reviewer: Pietro Invernizzi, MD, PhD, Division of Internal Medicine and Hepatobiliary Immunopathology Unit, IRCCS Istituto Clinico Humanitas, via A. Manzoni 113, Rozzano 20089, Milan, Italy

Lutz HH, Tischendorf JJW. Management of primary sclerosing cholangitis. *World J Hepatol* 2011; 3(6): 137-141 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i6/137.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i6.137

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease, characterized by intra- and extrahepatic bile duct inflammation and consecutive fibrosis[1]. PSC is generally a rare disease typically affecting middle aged men with ulcerative colitis. It presents with an increased risk of disease in relatives[2], showing a genetic susceptibility, combined with an autoimmune effect and toxicity of bile acids. Despite its low incidence, it is one of the main reasons for liver transplantation in northern European countries. The risk of developing cholangiocellular carcinoma (CCC) is high and associated with a dismal outcome. Optimizing treatment is difficult, and conflicting data on pharmacotherapy prevail in the literature. Endoscopic therapy of dominant stenoses, and liver transplantation in advanced disease, are effective treatment options. Small-duct PSC rarely progresses to large-duct PSC and has a more benign course, with slower progression and lower rates of cholangiocarcinoma[3,4]. In this editorial, therapeutic management of PSC to date is presented.

PHARMACOLOGICAL THERAPY

Although many drugs have been tested, effective medical treatment of PSC remains a problem. Promising results from pilot studies have raised hopes of slowing down or even reversing disease progression, but these results could not be proven in larger prospective randomized studies. In the following, the most important pharmacotherapeutic therapies evaluated in PSC are discussed.

UDCA

Since the 1990s, Ursodeoxycholic acid (UDCA), a hydrophilic bile acid, has been used for patients with PSC,

initially in a dosage of 10-15 mg/kg bodyweight per day. Multiple possible effects of UDCA have been discussed since then: increased bile flow, direct and indirect cytoprotective mechanisms and immunomodulation. In 2005, Olsson *et al*. published a prospective randomized trial, which showed a trend towards increased survival for patients treated with high-dose UDCA, though statistical significance was not reached due to lack of power[5]. Although UDCA used to be the standard therapy relating to this and two further promising pilot studies, it has recently come back into the limelight[6]: having been proven to be effective in other cholestatic liver diseases, UDCA does not seem to improve the outcome of patients with advanced PSC. On the contrary, high dosages could be toxic. It is clear that further studies are needed to assess the risk of UDCA in patients with advanced PSC, and to reevaluate the use of UDCA in the prevention of disease progression. Based on the available data to date, routine use of high-dose ursodeoxycholic acid in patients with advanced PSC can no longer be recommended[7,8].

NorUDCA

With UDCA having lost its pivotal role in PSC medication, 24-*nor*ursodeoxycholic acid (*Nor*UDCA) has recently been further evaluated as a future therapy, since it is possible that it does not have the same toxic effect recently discovered for UDCA. As this hydrophobic C_{23}-homolog of UDCA is poorly conjugated, different physiological and therapeutical mechanisms (presumably cholehepatic shunting) in comparison to UDCA have been proposed[9]. So far, promising results could be shown primarily in MDR2 (-/-) mice, which are an established model for sclerosing cholangitis[10,11]: *Nor*UDCA treatment in mice reduced periductal fibrosis, hydroxyproline content, proliferating hepato- and cholangiocytes, infiltrating immune cells and improved biochemical markers of cholestatic hepatopathy.

In addition, in a NEMO/NF-κB knockout mouse model for NASH, *Nor*UDCA has shown anti-inflammatory effects through downregulation of TNF, IL-12, IFN-γ and CCL5 expression as well as anticholestatic and strikingly antifibrotic potency through normalization of expression of key bile transporters and genes involved in hepatic fibrosis (e.g. FXR)[12]. Despite all these results, reliable data for *Nor*UDCA-treatment in PSC patients is still missing. Positive effects of *Nor*UDCA have to be proven in prospective studies with a large number of patients and an adequate period of observation, long enough to reach primary endpoints, and to finally provide statistical significance.

Antibiotics

The role of antibiotics today is limited to the treatment of cholangitis in the case of significant stenosis with cholestasis[13]. Trying to prevent recurrent cholangitis by continuous antibiotic therapy, a small prospective study could show lower levels of alkaline phosphatase, however, liver histology was not significantly improved[14]. Evidence favoring a continuous antibiotic therapy is not available.

Immunosuppressive and immunomodulating substances

Multiple immunosuppressants, such as methotrexate, steroids, cyclosporin A, azathioprine or tacrolimus, have been evaluated, with rather disappointing results. When addressing steroid therapy, it is important to consider possible IgG4-associated cholangitis or an overlap with features of autoimmune hepatitis, especially in younger patients. Current AASLD guidelines favor the use of steroids and other immunosuppressants in these cases. Anti-inflammatory substances like infliximab - or etanercept have been tested, but have failed to show any beneficial effect[15]. Penicillamine, with copper-chelating and immunomodulatory functions, had no positive effects in one prospective study[16], neither did cholestyramine, which failed to change prognosis through reduction of the enterohepatic circulation of bile acids.

Antifibrotic agents

Various antifibrotic substances have been evaluated in the past for all sorts of chronic liver disease, in which treatment of the underlying disease is difficult or impossible. The results so far have been disappointing. Interferon gamma, as one of the most promising substances, failed to reverse fibrosis in patients with advanced liver disease caused by chronic hepatitis C[17]. Furthermore, pathomechanisms of progressive fibrosis in cholestatic liver diseases are not well understood, and cannot easily be adopted from other chronic liver diseases. Blocking activation of Kupffer cells and consecutive cytokine release with pentoxifylline could not improve liver tests or symptoms in PSC patients[18]. Colchicine, though promising in various studies of chronic liver diseases with improvement of biochemical parameters, has not been shown to have positive effects on fibrosis progression in PSC patients.

On the other hand, as already mentioned earlier, *Nor*UDCA might have a significant anti-fibrotic potency itself (see above). Bezafibrate is not only known to reduce alkaline phosphatase levels[19], but can also prevent fibrogenesis through prevention of stellate cell activation in a murine model[20]. As silymarin is able to reduce fibrosis in bile duct ligated rats[21], a first clinical trial raised hope for a positive effect in humans[22]. In summary though, a significant clinical effect still has to be proven for all of these substances in prospective trials.

ENDOSCOPIC TREATMENT IN OBSTRUCTIVE DISEASE

During the natural course of the disease, worsening of symptoms such as pruritus, abdominal pain, fever with chills, and jaundice due to insufficient biliary drainage can be observed. Cholestasis with consecutive cholangitis is often caused by dominant bile duct stenoses, which develop in up to 50% of PSC patients (see also Figure 1). Endoscopic treatment of these stenoses is recommended, especially because of bad outcomes in patients with these stenoses[13,23]. Repeated endoscopic balloon dilatation has

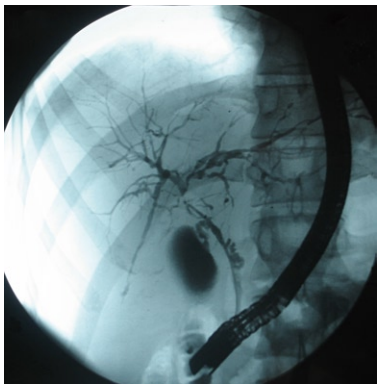


Figure 1 Primary sclerosing cholangitis and a benign, high-grade stenosis in a 22-year-old man. Cholangiography shows partially bilateral intrahepatic ductal dilatations and irregularity consistent with primary sclerosing cholangitis, and a short, high-grade stenotic segment in the proximal portion of the bile duct.

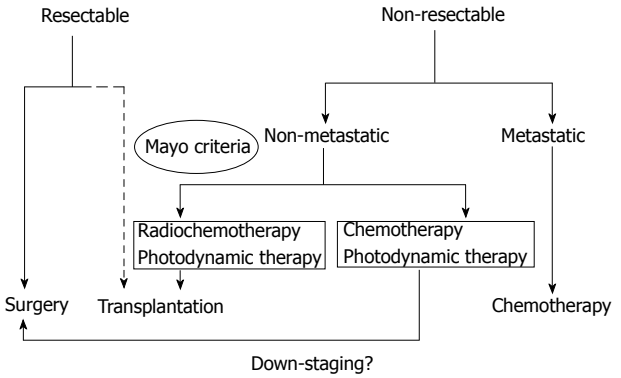


Figure 2 Therapeutic algorithm in PSC and CCC[34]. Patients with resectable carcinoma should preferably undergo surgical therapy as a potentially curative approach. In advanced stages, endoscopic treatment is the first step in the management of the disease in order to reconstitute biliary drainage. Patients without metastases can undergo local treatment such as photodynamic therapy or radiochemotherapy. Cholangiocarcinoma is considered a contraindication for transplantation, but may offer a possibility for selected patients after extensive preoperative treatment within controlled studies. Systemic chemotherapy remains the only treatment option in metastatic patients.

been shown to be a useful technique to preserve common bile duct function[24] and has been established as a standard therapy over the past years.

FOLLOW-UP

A regular follow-up using ultrasound and measurement of blood parameters is recommended at least annually, due to the risk of cholangiocellular carcinoma and because of the higher risk of malignant gall polyps in patients with PSC[25]. Cholecystectomy should be performed even when polyps do not reach 1 cm in diameter.

CCC

Cholangiocellular carcinoma is the most lethal complication in patients with PSC, and has a frequency of up to 20% in end-stage PSC. Diagnosis of CCC can be difficult, due to negative or inconclusive sampling. This is especially problematic in PSC, because of a five times higher risk of developing a desmoplastic reaction in PSC-associated CCC, compared to idiopathic CCC. Polysomy shown in a FISH(fluorescent in situ hybridization)-Test could be a diagnostic sign of CCC in suspected malignancy, but can´t be used as a screening parameter in more highly progressed PSC[26]. Furthermore, lack of highly sensitive imaging methods or blood parameters make reliable early CCC diagnosis extremely difficult. Intraductal ultrasound is a promising method to distinguish benign from malignant stenoses[27]. Due to frequent multifocal tumor growth in PSC, local R0 resection is usually not possible. Photodynamic therapy, with or without surgery, is capable of improving outcome[28], as well as a multimodal approach including chemoembolization[29] or a combination of local and systemic chemotherapy[30]. In selected patients, transplantation might be a therapeutic option (see below).

TRANSPLANTATION

Transplanted end-stage PSC patients show an excellent outcome, based on the Mayo Score[31,32]. However, choosing the optimal time for transplantation remains a major problem. Listing for transplantation is based on the MELD-Score and the appearance of refractory bacterial cholangitis. In addition to that, sufficient screening for malignancy (cholangiocellular carcinoma and colonic cancer) must have taken place. In the case of non-resectable CCC, newer therapeutic options, with neoadjuvant radiochemotherapy and liver transplantation can be considered, though only a small number of patients qualify for this regimen (see also Figure 2). These patients, following a special protocol with explorative laparotomy and lymphadenectomy to exclude lymphatic metastasis, have a one year survival of 91 % and a five year survival of 76%[33].

CONCLUSION

Preventing disease progression remains the major problem in PSC patients. Based on new studies, the therapeutic use of UDCA might not be beneficial for all patients. Especially in advanced stages of the disease, UDCA therapy at high doses could possibly bring with it substantial risk - the positive effect at lower doses has not been proven, and still needs to be validated. *Nor*UDCA, with fewer toxic effects could be a possible option for the future, but further prospective studies still have to prove a positive effect in humans. The existence of overlap syndromes should be evaluated in each patient to discover possible immunosuppressive therapeutic options. In case of advanced disease, liver transplantation is the best therapeutic option, and is known to have an excellent outcome.

REFERENCES

1 **Tischendorf JJ**, Hecker H, Krüger M, Manns MP, Meier PN. Characterization, outcome, and prognosis in 273 patients with primary sclerosing cholangitis: A single center study. *Am J Gastroenterol* 2007; **102**: 107-114

2 **Bergquist A**, Montgomery SM, Bahmanyar S, Olsson R, Danielsson A, Lindgren S, Prytz H, Hultcrantz R, Lööf LA, Sandberg-Gertzén H, Almer S, Askling J, Ehlin A, Ekbom A. Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2008; **6**: 939-943
3 **Björnsson E**, Olsson R, Bergquist A, Lindgren S, Braden B, Chapman RW, Boberg KM, Angulo P. The natural history of small-duct primary sclerosing cholangitis. *Gastroenterology* 2008; **134**: 975-980
4 **Broomé U**, Glaumann H, Lindstöm E, Lööf L, Almer S, Prytz H, Sandberg-Gertzén H, Lindgren S, Fork FT, Järnerot G, Olsson R. Natural history and outcome in 32 Swedish patients with small duct primary sclerosing cholangitis (PSC). *J Hepatol* 2002; **36**: 586-589
5 **Olsson R**, Boberg KM, de Muckadell OS, Lindgren S, Hultcrantz R, Folvik G, Bell H, Gangsøy-Kristiansen M, Matre J, Rydning A, Wikman O, Danielsson A, Sandberg-Gertzén H, Ung KA, Eriksson A, Lööf L, Prytz H, Marschall HU, Broomé U. High-dose ursodeoxycholic acid in primary sclerosing cholangitis: a 5-year multicenter, randomized, controlled study. *Gastroenterology* 2005; **129**: 1464-1472
6 **Lindor KD**, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, Harnois D, Jorgensen R, Petz J, Keach J, Mooney J, Sargeant C, Braaten J, Bernard T, King D, Miceli E, Schmoll J, Hoskin T, Thapa P, Enders F. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009; **50**: 808-814
7 **Chapman R**, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, Gores GJ. Diagnosis and management of primary sclerosing cholangitis. *Hepatology* 2010; **51**: 660-678
8 **Chapman RW**. High-dose ursodeoxycholic acid in the treatment of primary sclerosing cholangitis: throwing the urso out with the bathwater? *Hepatology* 2009; **50**: 671-673
9 **Denk GU**, Maitz S, Wimmer R, Rust C, Invernizzi P, Ferdinandusse S, Kulik W, Fuchsbichler A, Fickert P, Trauner M, Hofmann AF, Beuers U. Conjugation is essential for the anticholestatic effect of NorUrsodeoxycholic acid in taurolithocholic acid-induced cholestasis in rat liver. *Hepatology* 2010; **52**: 1758-1768
10 **Halilbasic E**, Fiorotto R, Fickert P, Marschall HU, Moustafa T, Spirli C, Fuchsbichler A, Gumhold J, Silbert D, Zatloukal K, Langner C, Maitra U, Denk H, Hofmann AF, Strazzabosco M, Trauner M. Side chain structure determines unique physiologic and therapeutic properties of norursodeoxycholic acid in Mdr2-/- mice. *Hepatology* 2009; **49**: 1972-1981
11 **Fickert P**, Wagner M, Marschall HU, Fuchsbichler A, Zollner G, Tsybrovskyy O, Zatloukal K, Liu J, Waalkes MP, Cover C, Denk H, Hofmann AF, Jaeschke H, Trauner M. 24-norUrsodeoxycholic acid is superior to ursodeoxycholic acid in the treatment of sclerosing cholangitis in Mdr2 (Abcb4) knockout mice. *Gastroenterology* 2006; **130**: 465-481
12 **Beraza N**, Ofner-Ziegenfuss L, Ehedego H, Boekschoten M, Bischoff SC, Mueller M, Trauner M, Trautwein C. Nor-ursodeoxycholic acid reverses hepatocyte-specific nemo-dependent steatohepatitis. *Gut* 2011; **60**: 387-396
13 **Tischendorf JJ**, Schirin-Sokhan R. Primary sclerosing cholangitis: The importance of treating stenoses and infections. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 691-692
14 **Färkkilä M**, Karvonen AL, Nurmi H, Nuutinen H, Taavitsainen M, Pikkarainen P, Kärkkäinen P. Metronidazole and ursodeoxycholic acid for primary sclerosing cholangitis: a randomized placebo-controlled trial. *Hepatology* 2004; **40**: 1379-1386
15 **Hommes DW**, Erkelens W, Ponsioen C, Stokkers P, Rauws E, van der Spek M, ten Kate F, van Deventer SJ. A double-blind, placebo-controlled, randomized study of infliximab in primary sclerosing cholangitis. *J Clin Gastroenterol* 2008; **42**: 522-526
16 **LaRusso NF**, Wiesner RH, Ludwig J, MacCarty RL, Beaver SJ, Zinsmeister AR. Prospective trial of penicillamine in primary sclerosing cholangitis. *Gastroenterology* 1988; **95**: 1036-1042
17 **Pockros PJ**, Jeffers L, Afdhal N, Goodman ZD, Nelson D, Gish RG, Reddy KR, Reindollar R, Rodriguez-Torres M, Sullivan S, Blatt LM, Faris-Young S. Final results of a double-blind, placebo-controlled trial of the antifibrotic efficacy of interferon-gamma1b in chronic hepatitis C patients with advanced fibrosis or cirrhosis. *Hepatology* 2007; **45**: 569-578
18 **Bharucha AE**, Jorgensen R, Lichtman SN, LaRusso NF, Lindor KD. A pilot study of pentoxifylline for the treatment of primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 2338-2342
19 **Mizuno S**, Hirano K, Tada M, Yamamoto K, Yashima Y, Yagioka H, Kawakubo K, Ito Y, Kogure H, Sasaki T, Arizumi T, Togawa O, Matsubara S, Nakai Y, Sasahira N, Tsujino T, Isayama H, Kawabe T, Omata M, Koike K. Bezafibrate for the treatment of primary sclerosing cholangitis. *J Gastroenterol* 2010; **45**: 758-762
20 **Nakano S**, Nagasawa T, Ijiro T, Inada Y, Tamura T, Maruyama K, Kuroda J, Yamazaki Y, Kusama H, Shibata N. Bezafibrate prevents hepatic stellate cell activation and fibrogenesis in a murine steatohepatitis model, and suppresses fibrogenic response induced by transforming growth factor-beta1 in a cultured stellate cell line. *Hepatol Res* 2008; **38**: 1026-1039
21 **Boigk G**, Stroedter L, Herbst H, Waldschmidt J, Riecken EO, Schuppan D. Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. *Hepatology* 1997; **26**: 643-649
22 **Angulo P**, Jorgensen RA, Kowdley KV, Lindor KD. Silymarin in the treatment of patients with primary sclerosing cholangitis: an open-label pilot study. *Dig Dis Sci* 2008; **53**: 1716-1720
23 **Rudolph G**, Gotthardt D, Klöters-Plachky P, Kulaksiz H, Rost D, Stiehl A. Influence of dominant bile duct stenoses and biliary infections on outcome in primary sclerosing cholangitis. *J Hepatol* 2009; **51**: 149-155
24 **Gotthardt DN**, Rudolph G, Klöters-Plachky P, Kulaksiz H, Stiehl A. Endoscopic dilation of dominant stenoses in primary sclerosing cholangitis: outcome after long-term treatment. *Gastrointest Endosc* 2010; **71**: 527-534
25 **Buckles DC**, Lindor KD, Larusso NF, Petrovic LM, Gores GJ. In primary sclerosing cholangitis, gallbladder polyps are frequently malignant. *Am J Gastroenterol* 2002; **97**: 1138-1142
26 **Bangarulingam SY**, Bjornsson E, Enders F, Barr Fritcher EG, Gores G, Halling KC, Lindor KD. Long-term outcomes of positive fluorescence in situ hybridization tests in primary sclerosing cholangitis. *Hepatology* 2010; **51**: 174-180
27 **Tischendorf JJ**, Meier PN, Schneider A, Manns MP, Krüger M. Transpapillary intraductal ultrasound in the evaluation of dominant bile duct stenoses in patients with primary sclerosing cholangitis. *Scand J Gastroenterol* 2007; **42**: 1011-1017
28 **Quyn AJ**, Ziyaie D, Polignano FM, Tait IS. Photodynamic therapy is associated with an improvement in survival in patients with irresectable hilar cholangiocarcinoma. *HPB (Oxford)* 2009; **11**: 570-577
29 **Kiefer MV**, Albert M, McNally M, Robertson M, Sun W, Fraker D, Olthoff K, Christians K, Pappas S, Rilling W, Soulen MC. Chemoembolization of intrahepatic cholangiocarcinoma with cisplatinum, doxorubicin, mitomycin C, ethiodol, and polyvinyl alcohol: a 2-center study. *Cancer* 2010; **117**: 1498-1505
30 **Andrašina T**, Válek V, Pánek J, Kala Z, Kiss I, Tuček S, Slampa P. Multimodal oncological therapy comprising stents, brachytherapy, and regional chemotherapy for cholangiocarcinoma. *Gut Liver* 2010; **4 Suppl 1**: S82-S88
31 **Brandsaeter B**, Friman S, Broomé U, Isoniemi H, Olausson M, Bäckman L, Hansen B, Schrumpf E, Oksanen A, Ericzon BG, Höckerstedt K, Mäkisalo H, Kirkegaard P, Bjøro K. Outcome following liver transplantation for primary sclerosing cholangitis in the Nordic countries. *Scand J Gastroenterol* 2003; **38**: 1176-1183
32 **Kornasiewicz O**, Lewandowski Z, Dudek K, Stankiewicz R,

Nyckowski P, Krawczyk M. Prediction of graft loss and death in patients with primary sclerosing cholangitis. *Transplant Proc* 2009; **41**: 3110-3113

33 **Petrowsky H**, Hong JC. Current surgical management of hilar and intrahepatic cholangiocarcinoma: the role of resection and orthotopic liver transplantation. *Transplant Proc* 2009; **41**: 4023-4035

34 **Tischendorf JJ**, Geier A, Trautwein C. Current diagnosis and management of primary sclerosing cholangitis. *Liver Transpl* 2008; **14**: 735-746

S- Editor Zhang HN **L- Editor** Herholdt A **E- Editor** Zhang L

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i6.142

World J Hepatol 2011 June 27; 3(6): 142-146
ISSN 1948-5182 (online)

DREAM 2020

A key problem and challenge for hepatology: Obesity-related metabolic liver diseases

Yasemin Hatice Balaban

Yasemin Hatice Balaban, Hacettepe University, Medical Faculty Internal Medicine Department, Gastroenterology Unit, Ankara 06100, Turkey
Author contributions: Balaban YH contributed solely to this work.
Correspondence to: Yasemin Hatice Balaban, MD, Associate Professor, Hacettepe University, Medical Faculty Internal Medicine Department, Gastroenterology Unit, Ankara 06100, Turkey. ybalaban@hacettepe.edu.tr
Telephone: +90-533-234-4232 Fax: +90-312-442-2161
Received: December 24, 2010 Revised: May 9, 2011
Accepted: May 16, 2011
Published online: June 27, 2011

Abstract

With the arrival of the new millennium, gastroenterologists have been faced with the problem of metabolic liver diseases associated with obesity. The active role of the liver in metabolism and inflammation make it a key organ in the war against the rapidly-spreading worldwide epidemic of obesity. Many lives and much money could be saved if the work of hepatologists led to the development of effective diagnostic and therapeutic strategies against this growing leader of cirrhosis.

Key words: Metabolic liver diseases; Steatohepatitis; Obesity; Insuline resistance; Lipotoxicity; Adiposopaty.

Peer reviewers: Joel Faintuch, MD, PhD, Department of Gastroenterology, Hospital das Clinicas, ICHC, 9th Floor, Room 9077, Sao Paulo 05403-900, Brazil; Amedeo Lonardo, MD, Internal Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio, Emilia 41100 Modena, Italy

Balaban YH. A key problem and challenge for Hepatology: Obesity-related Metabolic Liver Diseases. *World J Hepatol* 2011; 3(6): 142-146 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i6/142.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i6.142

INTRODUCTION

The term "metabolic liver disease" classically implies rare disorders of metabolism causing liver diseases; such as hereditary hemachromatosis, Wilson's disease or alpha-1 anti-trypsin deficiency. However, knowledge of lipid and glucose metabolism has increased considerably since obesity and its associated complications have been recognized as a world-wide epidemic. This necessitates a redefinitionof the concept of metabolic liver disease, which now covers common liver diseases accompanying metabolic syndrome[1,2]. The main component of metabolic syndrome is obesity (particularly visceral); others are glucose intolerance (impaired glucose tolerance test, impaired fasting glucose, and type 2 diabetes mellitus), hypertension, dyslipidaemia (hipertriglyceridemia, low HDL, increased LDL), and atherosclerotic cardiovascular disease. Since each of these components is characterized by insulin resistance, metabolic syndrome is also referred to as "insulin resistance syndrome"[3].

Fatty liver disease occurs if fat content exceeds 5% of the total weight of liver. It can develop either secondary to various factors (HCV, Wilson disease, drugs, alcohol, hipotiroidism, etc) or as a primary metabolic disorder, similar to diabetes and hypertension. Non-alcoholic fatty liver disease (NAFLD) associates the primary fatty liver diseases with a wide spectrum of histopathologic changes, ranging from simple steatosis to steatohepatitis. Non-alcoholic steatohepatitis (NASH) represents the most

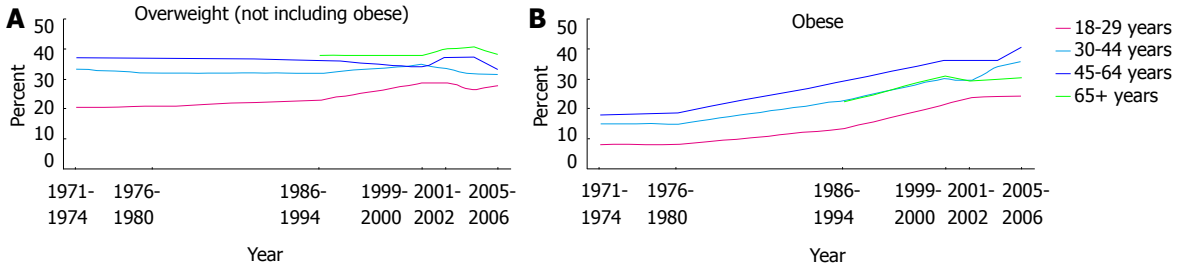


Figure 1 Trends of overweight and obesity in the United States. A: Overweight (not including obese); B: Obese.

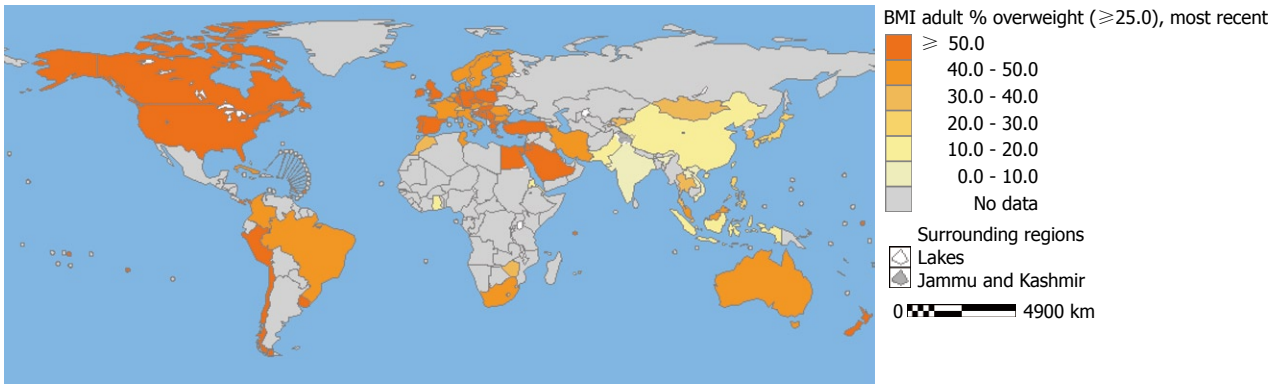


Figure 2 Global data of overweight population. BMI: Body mass index.

severe part of the NAFLD spectrum, in which hepatic steatosis is associated with hepatic inflammation. Since NAFLD is strongly associated with obesity and insulin resistance, it is classically identified as the hepatic component of metabolic syndrome. Recently Brunt EM *et al,* have strongly argued for a change in the nomenclature to "metabolic fatty liver disease" or "metabolic steatohepatitis", in order to drop the negative definition of "non-alcoholic" and to recognize the likely causal role of insulin resistance in NAFLD[1].

ECONOMICAL BURDEN OF OBESITY AND FATTY LIVER DISEASE

Obesity seems to be the major factor that moves the world away from the target of increased general health in the world population by 2020. Since weight-gain has historically been associated with wealth and prosperity in many cultures, in the past, obesity has been neglected as a public health problem. Moreover, during the last century, global eating habits have shifted from fiber-rich foods to an obesity prone Western diet[4]. Figure 1 presents a critical example of the steady and significant increase rates of obesity in the United States since 1970s[4]. Obesity has affected about one third of US adults aged 20 older in 2010. Similarly, the age-adjusted prevalence of clinically diagnosed diabetes has increased to 59 cases per 1000 population in 2008, from the 40 cases per 1000 in 1997. Because of these accelerating rates, one of the aims of the high-priority areas for motivating societal action in connection with health in the US, entitled "10 Leading Health Indicators and Focus Areas for Healthy People 2010", is to decrease the incidence of diabetes to 25 cases per 1000 in 2020[5]. Estimates for the UK show that the rate of obesity in adults will rise from 23% in 2007 to 50% in 2050[7]. Although the epidemiological data is lacking for most parts of the world (Figure 2), available data warn that obesity is a global epidemic "disease" threatening the future health of the world[8].

The cost of obesity increases as more people in a population become obese. The total direct costs attributable to overweight and obesity have been calculated to reach $6.0 billion in Canada, a 15% increase from 2004 to 2006. The attributed ratios for overweight and obesity are approximately 35% and 65% of this $6.0 billion. The cost of obesity in 2006 corresponded to 4.1% of the total health expenditure in Canada[9].

The commonest chronic liver disease in the Western world and also in the Asia-Pacific Region in the new millennium is going to be NAFLD[10,11]. The sizes of world populations infected with HBV and HCV are respectively 350 and 180 million, whereas the current number of obese adults in the world is approximately 312 million. Furthermore, at least 2.5 billion people, which is one third of the estimated world population, are predicted to be obese in 2020[12]. The frequencies of NAFLD and NASH can be as high as 80% and 30% among the obese, so the expected number of patients with NAFLD and NASH in the world will be 2 billion and 800 million, respectively. Advanced fibrosis develops in 10%-30% of NAFLD patients, so 200-600 million people will face the risks of cirrhosis and hepatocellular carcinoma, both of which are well established complications of NAFLD.

These numbers clearly identify metabolic liver disease as the leading cause of cirrhosis and its complications in the new millennium. Although the definition of NAFLD requires the exclusion of other etiologies of chronic liver disease, the high prevalence of clinical and histopathologic features of NAFLD causes problems in the diagnosis and management of liver disease. The metabolic effects underlying NAFLD are shown to exacerbate ongoing liver damage, particular in chronic hepatitis C, alcoholic liver disease and hemochromatosis[13,14,15]. From all these, it can be concluded that the hepatologists of 2020 will mainly be struggling with metabolic liver diseases and their complications.

PATHOPHYSIOLOGY OF NAFLD

NAFLD results from a series of liver insults, commonly referred to as the "multi-hit" hypothesis. Bearing in mind that this is a hypothesis that needs to be proven and is thus prone to changes, the main components this hypothesis are insulin resistance, lipotoxicity and adiposopathy. The underlying metabolic, genetic and/or environmental factors determine the degree of steatosis, inflammation and fibrosis in the liver, and, consequently, the severity of NAFLD in an individual. The NAFD is the underwater portion of the iceberg of obesity, due to high regeneration capacity of liver.

Obesity results from the metabolic need to deposit unused calories coming into the body. Excessive food intake and lack of physical exercise cause overload of the body with fat, which is accompanied by a specific etiopathologic condition, insulin resistance. The adipose tissue in the body is redistributed from the subcutaneous area to the intra-abdominal/omental side. An ectopic fat accumulation occurs in muscle, liver, and heart, as well as "fat infiltration" in other tissues. The end results are cellular dysfunction, apoptosis and consequent pathologic stress on organs, such as heart failure, β-cell dysfunction in the pancreas, peripheral insulin resistance in both the liver and muscle tissue[16,17,18].

"Lipotoxicity" is the term that describes the deleterious effect of tissue fat accumulation on glucose metabolism. The insulin resistance accompanying obesity results in increased lipolysis in adipocytes, leading to an increase in plasma non-esterified fatty acids (NEFA). The high levels of plasma NEFA shift the substrate preference of mitochondrial oxidation from glucose to NEFA, and this diminishes the insulin secretor response of islet β-cells to glucose, leading to relative insulin insufficiency. On the other hand, the enhanced influx of NEFA into muscle and liver decreases fat oxidation at these sites. The increased intracellular fat causes peripheral insulin resistance through over-expression of lipoprotein lipase in the liver and muscle tissue. The over-expressed lipoprotein lipase increases hepatic fat content, induces severe hepatic insulin resistance and impairs hepatic insulin signaling. As a result, steatosis of the liver is established by means of this vicious cycle between lipotoxicity and insulin resistance[3,19].

Adipokines are the cyokines secreted by adipocytes and mononuclear cells infiltrating adipose tissue. Adipocytokines travel to distant sites such as, muscular, liver, and arterial tissue; and affect metabolism and vascular functions negatively. These distant effects of adipokines are another form of lipotoxicity, called adiposopathy[3]. Visceral adipose tissue is particularly prone to inflammation; and it can contribute to hepatic fibro-inflammation by releasing inflammatory adipokines direct into the portal vein. Additionally, a systemic state of chronic inflammation is created by macrophages of visceral adipose tissue in obese and type 2 diabetic patients.

TLR4 on adipocytes and macrophages initiates insulin resistance and inflammatory response by functioning as a sensor for elevated NEFA concentrations. The detrimental complications of obesity-associated insulin resistance develop when the balance between adipokines of pro-inflammatory M1 macrophages (TNF-α, IL-6, and IL-1 β) and those of the anti-inflammatory M2 macrophages (IL-10, adiponectin and the IL-1 receptor antagonist), shift towards M1 macrophages. Pro-inflammatory adipokines exert insulin-resistance, in contrast to the insulin-sensitizing effect of anti-inflammatory ones. Similar to adipose tissue, activation of Kupffer cells promotes a pro-inflammatory Th1 immune response in a liver affected by NAFLD[18,20,21].

The peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs) are subgroups of the nuclear receptor superfamilies that regulate pro-inflammatory cytokine production by macrophages. Differentiation into M1 phenotype macrophages is inhibited by PPARγ signaling. The activation of PPARγ or PPARδ *via* IL-4, promotes polarization of macrophages toward M2 phenotype and thus avoids metabolic inflammation associated with insulin resistance. Another potential basis for the initiation of inflammation in obesity is endoplasmic reticulum (ER) stress. ER stress induces inflammatory signaling and in so doing, directly contributes to insulin resistance at insulin target cells. Therefore, dysregulation of macrophage-mediated inflammation by PPARs, LXRs and ER stress underlies the development of insulin resistance and inflammation in obesity[5,18,19].

TREATMENT STRATEGIES FOR NAFLD

NAFLD is a neglected component of metabolic syndrome due to the high regenerative capacity of the liver. However, the liver is the pathogenetic as well as the targeted organ of obesity-induced insulin resistance and lipotoxicity, which form two components of the vicious cycle ending in metabolic syndrome. Furthermore, NAFLD has been shown to precede other components of metabolic syndrome, such as diabetes, or atherosclerotic heart disease, by years[22]. Significant, but clinically silent liver disease can be detected during initial diagnosis of diabetes and is called "diabetic hepatopathy"[23,24]. All these facts indicate that diagnosis and successful treatment of NAFLD can have an impact not only on the liver but on the whole body.

The obesity epidemic poses enormous medical challenges for the near future. The patients have to be protected from deleterious psychological and social problems, as

well as its systemic complications, which include atherosclerosis, diabetes and metabolic liver diseases. Treatment should mainly be based on prevention of obesity, since significant and sustained weight loss is very hard to achieve in real-life. As one of the systemic complications of obesity, treatment of NAFLD should be primarily directed at improving insulin resistance, and complemented by the treatment of other accompanying components of metabolic syndrome, such as diabetes, dyslipidemia or hypertension.

When the obesity has been brought under control, treatment options are dietary changes with exercise, medication, endoscopic or surgical bariatric approaches. The goal of the treatment is the loss of 8%-10% of body weight, by reducing total body fat content, which can bring about significant improvements in insulin sensitivity, adiponectin level, and liver histopathology, including steatosis, ballooning, inflammation and NAFLD score (NAS). Current medication such as ortistat or sibutramine, are most appropriate in patients who are highly motivated and have mild obesity. Because of the difficulties in achieving a "sustained" weight loss only by medical treatment, the weight loss by bariatric surgery, together with dietary changes and exercise is preferred to medication-assisted weight loss in patients with moderate to severe obesity [11].

The role of insulin-sensitizing drugs is another hot topic in the prevention and/or treatment of metabolic complications associated with obesity. The major insulin sensitizers are metformin and thiazolidinediones (TZDs). While metformin treatment may or may not be improving histopathology in NAFLD, it has utility due to its ability to improve insulin resistance and the cardiometabolic risk factors often accompanying NAFLD[15]. TZDs are PPARγ agonists, such as rosiglitazone and pioglitazone. TZDs have unique effects, such as decreasing plasma NEFA by inhibition of lipolysis, reduction of muscle long-chain fatty acyl CoA levels, and redistribution of fat within the body from the visceral to the subcutaneous side. Even though they cause significant weight gain, TZDs can ameliorate lipotoxicity and adiposopathy overall[3]. As a treatment option in NAFLD, TZDs improve steatosis, transaminase levels, adiponectin, and insulin sensitivity, but have no significant effect on fibrosis or NAS. A recent study has shown that pioglitazone has more limited benefit than vitamin E for the treatment of NASH in adults without diabetes. Long-term treatment with TZDs may be necessary in NAFLD patients, but concerns about cardiotoxicity, congestive heart failure, edema, osteoporosis, weight gain and also hepatotoxicity exist[11].

There is also a long list of agents with some shown efficacy in treatment of NAFLD, such as ursodeoxycholic acid (UDCA), omega-3 fatty acids, pentoxifylline, betaine, S-adenosylmethionine (SAM), N-acetyl-cysteine (NAC), probiotics, and angiotensin-receptor blockers. Fibrates activate PPAR-α, leading to increased HDL and decreased triglycerides, LDL, and VLDL. Similarly, statins also improve liver enzymes, but not insulin resistance or histopathology.

CONCLUSION

NAFLD has become the most common cause of liver disease in the world of the new millennium. It affects approximately 20%-30% of adults and as high a percentage as 70%-90% of patients with obesity or diabetes. The liver is not only a central organ of metabolism, but also has active role in immunity and inflammation. The liver should therefore be the pathogenic as well as targeted organ of insulin resistance and inflammation associated with obesity. One of the corner stones of the current approach to obesity should be to recognize and to treat NAFLD as an early indicator complication of metabolic syndrome. Today, there is no single treatment modality or pharmacological agent that has established efficacy in the treatment of NAFLD. Since policies on awareness of metabolic liver diseases could potentially save lives and millions of dollars, metabolic liver diseases have become a hot topic of hepatologists.

REFRENCES

1 **Brunt EM**. What's in a NAme? *Hepatology* 2009; **50**: 663-667
2 **Loria P**, Lonardo A, Carulli N. Should nonalcoholic fatty liver disease be renamed? *Dig Dis* 2005; **23**: 72-82
3 **DeFronzo RA**. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. *Diabetologia* 2010; **53**: 1270-1287
4 Obesity: preventing and managing the global epidemic. Report of a WHO Consultation (WHO Technical Report Series 894). Geneva: World Health Organization; 2000. 252p
5 CDC/NCHS, Health, United States, 2008, Figure 7. Data from the National Health and Nutrition Examination Survey. Accessed on December 1, 2010. Available at: http: //www.cdc.gov/nchs/data/hus/hus08.pdf.
6 **Koh HK**. A 2020 vision for healthy people. *N Engl J Med* 2010; **362**: 1653-1656
7 **Zeyda M**, Stulnig TM. Obesity, inflammation, and insulin resistance--a mini-review. *Gerontology* 2009; **55**: 379-386
8 http: //apps.who.int/bmi/index.jsp. Accessed December 1, 2010.
9 **Anis AH**, Zhang W, Bansback N, Guh DP, Amarsi Z, Birmingham CL. Obesity and overweight in Canada: an updated cost-of-illness study. *Obes Rev* 2010; **11**: 31-40
10 **Haslam DW**, James WP. Obesity. *Lancet* 2005; **366**: 1197-1209
11 **Lam B**, Younossi ZM. Treatment options for nonalcoholic fatty liver disease. *Therap Adv Gastroenterol* 2010; **3**: 121-137
12 World population to 2300. Department of Economic and Social Affairs. United Nations, 2004; 1-254. Accessed December 1, 2010. Available at: http: //www.un.org/esa/population/publications/longrange2/WorldPop2300final.pdf.
13 **Jacobson IM**, Davis GL, El-Serag H, Negro F, Trépo C. Prevalence and challenges of liver diseases in patients with chronic hepatitis C virus infection. *Clin Gastroenterol Hepatol* 2010; **8**: 924-933; quiz e117
14 **Williams R**. Global challenges in liver disease. *Hepatology* 2006; **44**: 521-526
15 **Balsano C**, Alisi A, Nobili V. Liver fibrosis and therapeutic strategies: the goal for improving metabolism. *Curr Drug Targets* 2009; **10**: 505-512
16 **Hill MJ**, Metcalfe D, McTernan PG. Obesity and diabetes: lipids, 'nowhere to run to'. *Clin Sci (Lond)* 2009; **116**: 113-123
17 **Capeau J**. Insulin resistance and steatosis in humans. *Diabetes Metab* 2008; **34**: 649-657
18 **Balistreri CR**, Caruso C, Candore G. The role of adipose tis-

sue and adipokines in obesity-related inflammatory diseases. *Mediators Inflamm* 2010; **2010**: 802078
19 **Fuentes L**, Roszer T, Ricote M. Inflammatory mediators and insulin resistance in obesity: role of nuclear receptor signaling in macrophages. *Mediators Inflamm* 2010; **2010**: 219583
20 **Baffy G**. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. *J Hepatol* 2009; **51**: 212-223
21 **Tordjman J**, Guerre-Millo M, Clément K. Adipose tissue inflammation and liver pathology in human obesity. *Diabetes Metab* 2008; **34**: 658-663
22 **Targher G**, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**: 1341-1350
23 **Porepa L**, Ray JG, Sanchez-Romeu P, Booth GL. Newly diagnosed diabetes mellitus as a risk factor for serious liver disease. *CMAJ* 2010; **182**: E526-E531
24 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685

S- Editor Zhang HN **L- Editor** Herholdt A **E- Editor** Zhang L

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i6.147

World J Hepatol 2011 June 27; 3(6): 147-156
ISSN 1948-5182 (online)

BRIEF ARTICLE

HBV vaccine efficacy and detection and genotyping of vaccineé asymptomatic breakthrough HBV infection in Egypt

Eman AE Abushady, Magda MA Gameel, John D Klena, Salwa F Ahmed, Kouka SE Abdel-Wahab, Sanya M Fahmy

Eman AE Abushady, Microbiology department, Faculty of Medicine Nourthern Border University, Arar 1321, Saudi Arabia
Magda MA Gameel, Kouka Abdelwahab, Microbiology department, Faculty of Medicine, AL-Azhar University, Cairo R695-11651, Egypt
John D Klena, Salwa F Ahmed, Molecular Epidemiology unit, Clinical Trials Program, NAMRU-3, Cairo 11157, Egypt
Sanya M Fahmy, Pediatric department, Faculty of Medicine, AL-Azhar University, Cairo R695-11651, Egypt
Author contributions: Abushady EAE was the principal investigator responsible for the design of study, supervision, manuscript writing, acquisition of funding; Gameel MMA did the PCR and multiplex PCR practical work, shared in paper revision and correction; Klena JD provided us with technology and materials needed for confirmation of the multiplex PCR results and sequencing of the obtained genome, interpretated and analyzed the data, and shared in paper revision and correction; Ahmed SF confirmed the multiplex PCR results and sequenced the obtained genome and shared in interpretation of the sequencing data; Fahmy SM collected the serum samples done under her supervision; Abdel-Wahab KSE participated in paper revision and correction.
Correspondence to: Eman AE Abushady, Professor, Microbiology Department, Faculty of Medicine, Nourthern Border University, Arar-1321, Saudi Arabia. eman_abushady@yahoo.ie
Telephone: +966-540489688 Fax: +966-46640705
Received: October 20, 2010 Revised: May 15, 2011
Accepted: May 22, 2011
Published online: June 27, 2011

Abstract

AIM: To evaluate the impact of mass vaccination against the hepatitis B virus (HBV) in Egypt, and to search for vaccinee asymptomatic breakthrough HBV infection and its genotype.

METHODS: Seven hundred serum samples from vaccinated children and adults (aged 2-47 years) were used for quantitative and qualitative detection of HBsAb by ELISA. Three hundred and sixty serum samples representing undetectable or low or high HBsAb were screened for markers of active HBV infection (HBsAg, HBcAb (IgG) and HBeAb by ELISA, plus HBsAg by AxSYM) and HBV-DNA genotyping by nested multiplex PCR and by DNA sequencing.

RESULTS: It was found that 65% of children aged 2-4 years, and 20.5% aged 4-13 years, as well as 45% adults were good responders to HBV vaccination mounting protective level HBsAb. Poor responders were 28%, 59.5% and 34%, and non-responders were 7%, 20% and 21% respectively, in the three studied groups. Markers of asymptomatic HBV infections were HBsAg detected by ELISA in 2.5% *vs* 11.39% by AxSYM. Other markers were HBcAb (IgG) in 1.38%, HBeAb in 0.83%, and HBV-DNA in 7.8%. All had HBV genotype E infection.

CONCLUSION: It is concluded that HBV vaccine is efficient in controlling HBV infection among children and adults. The vaccine breakthrough infection was by HBV genotype E. A booster dose of vaccine is recommended, probably four years after initial vaccination.

Key words: HBV vaccine evaluation; Egyptain children; Adults; Genotype E vaccine escape HBV

Peer reviewers: Hatim Mohamed Yousif Mudawi, Associate Professor of Medicine, department of Internal medicine, Faculty of medicine, University of Khartoum, Sudan, PO BOX 2245, Khartoum, Sudan; Sang Hoon Ahn, MD, PhD, Professor, Department of Internal Medicine, Yonsei University College of Medicine, 250 Sungsanno, Seodaemun-gu, Seoul 120-752, South Korea

Abushady EAE, Gameel MMA, Klena JD, Ahmed SF, Abdel-Wahab KSE, Fahmy SM. HBV vaccine efficacy and detection and genotyping of vaccineé asymptomatic breakthrough HBV infection in Egypt. *World J Hepatol* 2011; 3(6): 147-156 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i6/147.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i6.147

INTRODUCTION

The Hepatitis B virus (HBV) is endemic in many developing countries, including Egypt[1-3]. As the majority of chronic carriers of HBV in the worldbecome soas a result of infections which occur prior to the age of 6, the Technical Advisory Group (TAG) of the World Health Organization has recommended that the HB vaccine must be added as a component of the Expanded Program of Immunization (EPI) in countries with a moderate to high prevalence[4]. The recombinant DNA-based vaccine, targeting the HBV major surface protein (r-HBsAg)has been incorporated in the national childhood vaccination program in Egypt since October 1992. Three doses of 10 μg r-HBsAg are given at 2, 4 and 6 mo of age[5,6]. The vaccination of infants, children and adolescents have produced high rates of seroconversion (95%) and induced adequate levels of HBsAb[7]. The accepted level of seroconversion is 10 or more mIU/L, which provides protection against HBV infection. While the success of the vaccine cannot be denied, it was noticed that vaccine efficiency was improved by the addition of preS$_1$ and preS$_2$ components. Universal vaccination of infants with the HBV vaccine has dramatically reduced infection, as well as the hepatitis-B surface antigen (HBsAg) carrier rate of chronic HBV, in addition to a significant decrease in the incidence of childhood hepatocellular carcinoma[1,8,9]. Protective immunity has been demonstrated in persons and populations up to 5 to 10 years post-vaccination, with associated decrease of asymptomatic breakthrough HBV infection[10-12]. In HBV endemic areas, postnatal or prenatal at the time of delivery mother-to-infant transmission of infection occurs frequently, especially if the maternal serum is hepatitis-Be antigen positive, which is the stage at which 90% of babies acquire HBV infection. Despite r-HBsAg vaccination, some of these infants become persistently infected and increase the worldwide HBV reservoir[13]. Furthermore, 5%-10% of the vaccinees display an inadequate antibody response following primary vaccination with triple doses of either plasma-derived or r-HBsAg vaccine, in addition to 3%-20% of non-responders, and may not be protected from subsequent exposure to HBV infection[6,13]. The introduction of a safe, effective, hepatitis B vaccine has led to universal infant vaccination, resulting in a reduced rate of perinatal HBV infection from infected mothers. Because of appropriate hepatitis B vaccination and passive immunoprophylaxis with hepatitis B immune globulin (HBIG) in infants of mothers with HBV infection, perinatal transmission has been reduced to less than 5% to 10%[14]. The configuration of the HBsAg used in current vaccine formulations contains a determinantwhich is located between amino acids (aa) 121-149 of the HBsAg immunogenic epitope, that trigger the production of polyclonal antibodies against the HBV major surface protein (HBsAb). The emergence of HBsAg variants, with mutations within aa 121-149 with altered antigencity, and binding to the HBsAb, has been reported in HBV from vaccinees in several areas of the world[15,16]. Using the currently available diagnostic assays, these variants may go undetected, and could potentially cause breakthrough infections in a vaccinated population, posing a potential threat to the long term success of HBV vaccination programmes[17-19]. Booster doses of Hepatitis B vaccine are recommended only in certain circumstances, for example, for hemodialysis patients, and for those with an ongoing risk of exposure. Annual anti-HBs testing and a booster dose should be administered when anti-HBs levels decline to < 10 mIU/mL. For other immuno-compromised persons, the need for booster doses has not been determined[20]. It has been appreciated that long-term immunity derives from immunological memory, which outlasts the loss of antibody levels.Hence subsequent testing and administration of booster doses is not required in successfully vaccinated immunocompetent individuals. With the passage of time and longer experience, protection has been shown to last for at least 25 years in those who showed an adequate initial response to the primary course of vaccinations[21].

In 1988, genotypes of HBV were proposed by a sequence divergence in the entire genome exceeding 8%, based on a comparison of 18 HBV isolates[22]. Four genotypes were recognized and they were given capital letters of the alphabet from A to D. In 1994, Norder *et al*[23] found an additional two HBV genotypes by means of the same criteria, and named them E and F; genotype G was reported in 2000[24], and genotype H was reported in 2002[25]. The vaccine "a" component of HBsAg is stable, due to conserved gene sequences encoding it in all HBV genotypes. On the other hand, the preS$_1$, preS$_2$ containing vaccines, which are more immunogenic, have high specific motifs. Thus there is a need to tailor the vaccine to the HBV genotype prevalent in the geographic areas of vaccination.

The objective of the current study was to evaluate the impact of mass childhood HBV vaccination in Egypt on asymptomatic HBV breakthrough infection in vaccinated individuals, and to determine the causative genotype.

MATERIALS AND METHODS

This study was done in 2004, 12 years after the start of the vaccination programme. Laboratory testing was conducted from October 2004 to January 2007 at the Virology Laboratory, Microbiology Department, Faculty of Medicine, Al-Azhar University, the Virology Laboratory in the Military Central Laboratory and the Molecular Epidemiology Department NAMRU#3 Cairo, Egypt.

Subjects of study

Six hundred serum samples from vaccinated children were collected (after obtaining the legal guardian consent). The six hundred children had received HBV vaccine [Engerix-B (Simksline "Sigma" licensed at 1989) from October 1992 until 1996. They received Euvax vaccine (Korea)] at 2, 4, and 6 mo of age, according to the vaccine schedule of the Egyptian Ministry of Health Population (MOHP). Serum samples from children aged 2- < 4 years of age were obtained from the Pediatric out-patient clinic

at Al- Zahraa University Hospital, Cairo Governorate, Egypt. Serum samples of children aged from 4-13 years were obtained from the Maternal and Child Health Care Center in Qusena City, Menofya Governorate, or from Al-Zahraa University Hospital, Cairo Governorate, Egypt.

A hundred serum samples from vaccinated adults were collected (after obtaining individual consent) from Benha Teaching Hospital, the Motor Rehabilitation Institute and the Hearing and Speaking Institute, Qualyubia Governorate. All serum samples were collected between October 2004 and August 2005. Sera were divided according to the age of vaccinees into three groups:

Group Ⅰ: two hundred children aged 2-<4 years. These children were considered healthy, with no history of medical or surgical problems, or risk factors for HBV infection, except for males who had beencircumcised.

Group Ⅱ: four hundred children aged 4-13 years, some of whom had past surgical history (circumcision, tonsillectomy, para-umblical & inguinal herniorraphy).

Group Ⅲ: one hundred sera from healthy adults, whose age ranged from 20-47 years. This group was without any history of risk for HBV infection, except that some females had a history of previous operations, such as caesarean section, tube dilatation and cervical curettage. All adults had received the 20 μgm r- HBsAg at a 0, 1, 6 mo interval schedule. The last dose of vaccine in the 90 adult volunteers was administered within theyear before inclusion in the study, six adults had had their last dose between one and four years previously, and four volunteers had had their last dose six years or more prior to the study. This study did not include another age and gender matched non-vaccinated subject, as almost all children had been vaccinated within the MOHP vaccine schedule.

HBV serological testing

BIO ELISA (BioKit, Barcelona, Spain) HBsAb kits were used to both quantitatively and qualitatively assess HBsAb in the 700 post-vaccination serum samples. A random selection of 360/585 serum samples that were either negative 82/360 or demonstrated low antibody titer 287/360 (< 10 mIU/L) together with 41/360 serum samples with high antibody titer (> 10 mIU/L), were subsequently screened for HBsAg, using two commercial kits; one to detect both wild and mutant strains (AxSYM from ABBOTT), and the other the ELISA Bioelisa for HBsAg (Bio-Kit, Barcelona, Spain). All serum samples positive for HBsAg were screened for the detection of HBcAb (IgG) (Bio-Kit, Barcelona, Spain) and HBeAb using (Diasorin, S.P.E Italy).

HBV-DNA detection and genotyping by nested multiplex PCR

Serum DNA was extracted using Kaucner and Stinear 1998 heat shock method[26]. In some cases, DNA was extracted using the Qiagen DNA Blood mini kit, according to the manufacturer's protocol (Qiagen, Valencia, CA, USA). Extracted DNA was subjected to HBV-DNA detection and genotyping using the nested multiplex PCR (nm PCR) method[27]. Briefly, for the first round PCR, the primer pair P1 sense (5' TCACCATATTCTTGGGAACAAGA 3') and S 1-2 antisense (5' CGAACCACTGAACAAATGGC 3') were used to amplify the conserved regions of the pre-S1 and S-gene (1063 bases). The reaction mixture contained 5 μL of extracted DNA in 25 μL 1 × PCR buffer containing 1.5MgCl2, 5 pmol of each primer completed 200 μmol/L of each of the four deoxynucleotides, 1U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Norwalk, Conn.) and completed to 50 μl with DEPEC treated sterile water. The samples were incubated at 95ºC for 10 min, followed by 40 ampilification cycles of 94ºC for 20 sec (denaturation), 55ºC for 20 sec (annealing), 72ºC for 1 min (extension), and then followed by further extension at 72ºC for 10 min. After that the product was kept at 4ºC. The second-round PCR was performed for each specimen using inner pair primers in two different combinations, Mix-A: *B2*:5' GGCTCMAGTTCMGGAACAGT 3' (nt 67-86, types A to E specific, sense), *BA1R*: 5' CTCGCGGAGATTGACGAGATGT 3' (nt 113-134, type A specific, anti sense), *BB1R*: 5' CAGGTTGGTGAGTGACTGGAGA 3' (nt 324-345, type B specific, antisense), *BC1R*: 5' GGTCCTAGGAATCCTGATGTTG 3' (nt 165-186, type C specific, antisense) for genotypes A, B and C using universal common primer B2 (sense) and specific primers A4, B5 and C6. *Mix B: BD1*: 5' GCCAACAAGGTAGGAGCT 3' (nt 2979-2996, type D specific, sense), *BE1*: 5' CACCAGAAATCCAGATTGGGACCA 3' (nt 2955-2978, type E specific, sense), *BF1*: 5' GYTACGGTCCAGGGTTACCA 3' (nt 3032-3051, type F specific, sense), *B2R*: 5' GGAGGCGGATYTGCTGGCAA 3' (nt 3078-3097, type D specific, antisense), for genotype D, E, and F using universal primer R10 (antisense) and specific primers D7, E8 and F9. In the second round PCR 5 μL of the first PCR product was added to each mix with the same components of the first round PCR. These were amplified for 40 cycles, with the following parameters: hot start at 95°C for 10 min, followed by 25 cycles of 94°C for 20 sec (denaturation), 58°C for 20 sec (annealing), 72°C for 30 sec (extension), and an additional 20 cycles of 94°C for 20 sec, 60°C for 20 se, 72°C for 30 sec, which was then followed by further extension at 72°C for 10 min, then the product was kept at 4°C. The products of this second round PCR were visualized by electrophoreses on 3% agarose gel, and are differentiated by the size of genotype-specific DNA bands, compared to a 50 base-pair DNA marker (Amersco).

To assess changes in the HBV S gene, the following primers were designed to ampilify the whole S gene, and used in a semi-nested PCR, HBsP1f (forward): 5' GGAGYKGGAGCATTCGGS 3', HBsP2f (forward) : 5' GTTACAGGCGGGGTTTTTCTTG 3' and HBsP4r (reverse): 5' TCACACATCATCCATATARCTGAAAGC. The first round PCR was done using HBsP1f and HBsP4r, the reaction

Table 1 Percent of positive cases, for HBs antibodies (IgG) by ELISA in the three studied population

	Group Ⅰ Children (2- < 4 years) *N* = 200	Group Ⅱ Children (4-13 years) *N* = 400	Group Ⅲ Adults (20-47 years) *N* = 100	Total No *N* = 700
Anti-HBs IgG	*n* (%)	*n* (%)	*n* (%)	*n* (%)
High +ve samples (10-99.9 mIU/mL)	130 (65)	82 (20.5)	45 (45)	257(36.7)
Low +ve samples (<10 mIU/mL)	56 (28)	238 (59.5)	34 (34)	328(46.9)
Negative samples (Non-responders)	14 (7)	80 (20)	21 (21)	115 (16.4)
Total positive samples	186 (93)	320 (80)	79 (79)	585 (83.6)

mixture consists of 5 μL of extracted DNA in 50 μL of a reaction buffer made of 30 pmol of each primer, 10 μmol/L of each of the four deoxynucleotides, 1 U of AmpliTaq Gold DNA polymerase, and 5 × PCR buffer containing 25 mol/L $MgCl_2$. The samples were incubated at 95ºC for 10 min, followed by 35 cycles of 94ºC for 30 sec (denaturation), 60ºC for 1 min (annealing), 72ºC for 1 min (extension), followed by further extension at 72ºC for 10 min. The second round PCR was done in the same way as the first round, using HBsP2f and HBsP4r primers. To confirm the obtained genotype, and to determine the existence of any mutations, DNA sequencing of the S gene product was conducted using BigDye terminator technology and an ABI 377 fluorescent automated sequencer. DNA sequence was manually edited, using the software program Bioedit v7.0.5[28]. Sequence comparisons of the obtained Egyptian strain (a positive control chronic hepatitis patient EGYAZC P1P2P4 bankit 1229997 GQ253-10e) were made using the program Clustal X[29] and HBV gene sequences retrieved from the Gene Bank: HBV genotypeE (AB183AB274977, LAGOS558AJ604967, CAR19-4AM494753, X75657, EU239226 PW6, 235-01DQ-060830, MAL136AJ604992, CMR936 AB194948)[30] and HBV genotype A X75669, HBV genotype B X75660, HBV genotype C X75656, HBV genotype F X75658[23]. In addition, alignment of 87 nucleotide sequence obtained from preS1 genes from Egyptian HBV isolate (a sample from a vaccinated child EGYAZV B2 265R) and Gene Bank strains as indicated: HBV genotype E PW6 EU239226[31], HBV genotype A DQ788725[32], HBV genotype B FM211366[33], HBV genotype Cx75656 and HBV genotype D x75658[23] was done, and Phylogenetic analysis and distance calculations for molecular evolutionary analyses were conducted using MEGA version 3.1[34]. Phylogenies were constructed using the Neighbor-joining method and substitutions were modeled using the Kimura 2-parameter model, and phylogenetic analysis of selected sequences based on$PreS_1$ and S fragments was done according to Felsetien(1988)[35].

RESULTS

Egyptian children and adults' immune response to r-HBsAg vaccination

The r-HBsAg vaccine used in Egypt appears to be efficient in inducing HBsAb immune response, which may, in turn, be efficient in controlling HBV infection in children and adults (⩾ 10 mIU/mL), as 93% and 80% of children aged 2- < 4 years, 4-13 years respectively, and 79% adults acquired protective HBsAb. Having low HBsAb titre (less than 10 mIU/mL) were 28%, 59.5% and 34% in the three studied groups respectively (Table 1).

Breakthrough HBV infection in vaccinated children and adults

The infection determined by detection of HBsAg by Bio ELISA test was 2.5% *vs* 11.39% by AxSYM. Active HBV replication by DNA amplification procedures was 6.11%, and other serological markers of infectivity were HBcAb (IgG) 1.38% and HBeAb 0.83% (Tables 2, 3) respectively.

Among serum samples from children aged 2- < 4 years old, 65% had a high HBsAb titer**,** 28% had low titer, and 7 % did not have detectable HBsAb. The majority of these children had no markers of HBV infection by BioELISA test for HBsAg. Only 5/55 (9.09%) were infected as determined by detection of the HBsAg by AxSYM (Tables 1-3).

In contrast, only 20.5% of children aged 4-13 years had a high HBsAb titer, 59.5% had a low HBsAb titer, and 20% did not have detectable HBsAb. Screening for HBV infection in this age group revealed that 2.04% and 12.24% were positive for HBsAg by BioELISA and AxSYM respectively. It was noticed that 7.14%, 9.55% and 20.75% of high, low and undetectable HBsAb were positive for HBsAg, 7.34% (2.25% in low HBsAb and 26.4% in undetectable HBsAb) for HBV-DNA, 0.81% for HBcAb and 0.4% for HBeAb (Tables 1-3).

Finally, within the adult age group 45% of the serum samples had a high antibody titer, 34% of samples had a low antibody titer and 21% were negative. In this age group, 6.66% and 11.66% were positive for HBsAg by ELISA and AxSYM, respectively, 5% were positive for HBcAb, and 3.33% were positive for HBeAb; HBV-DNA was detected in 6.66% of the serum samples (Tables 1-3).

On examining 41 high HBsAb positive samples, regardless of the assay, they were negative for all markers tested, except for one sample which was positive for HBsAg by AxSYM assay (Table 2)

Out of 22 cases positive for HBV DNA; 4 cases of group Ⅲ who were also HBsAg positive by both methods,

Table 2 Frequency of HBs antigen by ELISA and AxSYM among r-HBsAg vaccine responders and non responders

	Anti-HBs IgG antibody level	Samples tested	HBs Ag	
			(ELISA)	(AxSYM)
		n	Positive *n* (%)	Positive *n* (%)
Group Ⅰ Children	Low	27	0	2 (7.4)
(2-<4 years)	Negative	8	0	3 (37.5)
	High	20	0	0
Group Ⅱ Children	Low	178	4 (2.25)	17 (9.55)
(4-13 years)	Negative	53	1 (1.88)	11 (20.8)
	High	14	0	1[a] (7.14)
Group Ⅲ Adults	Low	32	2 (6.25)	2 (6.25)
(20-47 years)	Negative	21	2 (9.52)	5 (23.8)
	High	7	0	0
Total		360	9 (2.5)	41[b] (11.39)

[a]Examination of 41 high anti-HBs IgG antibodies serum samples demonstrated that all markers tested were negative, except for one sample that was positive for HBsAg by AxSYM; [b]The number of positive samples for HBs antigen were 41 samples by AxSYM in comparison with 9 samples.

Table 3 Frequency of HBV-DNA by nested Multiplex PCR, HBc and HBe anti bodies among r-HBsAg vaccine responders and non responders

Age group	anti-HBs IgG level	Number of serum samples Tested	HBV-DNA by nested Multiplex PCR *n* (%)	HBc antibody IgG *n* (%)	HBe IgG antibody *n* (%)
Group I Children	Low	27	0	0	0
(2-<4 years)	Negative	8	0	0	0
	High	20	0	0	0
Group II Children	Low	178	4 (2.5)	2 (1.2)	0
(4-13 years)	Negative	53	14 (26.4)	1 (1.9)	1 (1.9)
	High	14	0	0	0
Group III Adults	Low	32	2[a] (6.25)	1 (3.1)	1 (3.1)
(20-47 years)	Negative	21	2[a] (9.52)	1 (4.8)	1 (4.8)
	High	7	0	0	0
Total		360	22 (6.11)	5 (1.38)	3 (0.83)

[a]These four +ve sera are also +ve for HBs antigen &HBcAb detected by ELISA.

and 18 cases of group Ⅱ (5 of them were HBsAg positive by BioELISA 11 by AxYM and two were HBcAb positive), while 12 participants had HBV-DNA as the only marker for HBV infection. Considering HBV-DNA detection by nmPCR as a reference test, it was found that BioELISA specificity (100%), BioELISA sensitivity (96.29%), AxYM specificity (50%), AxYM sensitivity (96.65%).

Genotyping and sequencing of the S gene

In all the HBV-DNA positive samples, genotype E positive control and samples were detected at 167bp specific for type E. The result was confirmed by DNA sequencing in the available PCR products (Figure 1 and 2). A correlation between HBsAg detection by BioELISA and HBV genotyping by nm PCR revealed that all samples positive by BioELISA (9 samples) and the 11 samples positive by AxYM were also positive by nm PCR. HBV DNA was detected in 12 samples that were HBsAg negative by both techniques.

It was also found that tested subjects, either HBsAb positive or negative, were infected with the same genotype. Sequence comparisons of 565 nucleotide (genome position: ~155-720) obtained from HBV S- gene from Egyptian strain (a positive control chronic hepatitis patient) EGYAZC P1P2P4 and HBV/E gene sequences retrieved from the Gene Bank revealed only five nucleotide sequence differences (No 186, 201, 473, 515, 521) between them. The closest sequence was from CMR936 AB194948 (Figure 1). Furthermore, alignments of 87 nucleotide sequence (genome position: ~ 3048- 3135) obtained from preS1 genes from Egyptian HBV isolate (a sample from a vaccinated child) EGYAZV B2 265R and the Gene Bank strains indicated that there is 100 % similarity with HBV genotype E PW6 EU239226 (Figure 2).

DISCUSSION

Evaluation of r- HBsAg vaccination in Egypt

Studies on vaccinated children during infancy and early childhood in countries with a high endemicity of chronic HBV infectionhave shown that more than 50% of participants had measurable anti-HBs levels of at least 10 mIU/L from 4 to 10 years after vaccination[36,37].

The main finding in this study was that 93% of children aged 2- < 4 years had responded to the vaccine, compared with 80% of children aged 4-13 years, and 79% of adults. In the present study, adults had a higher rate of high-titre antibodies (45%) than children (age 4-13 years) (20.5%), because the last vaccine dose in 90

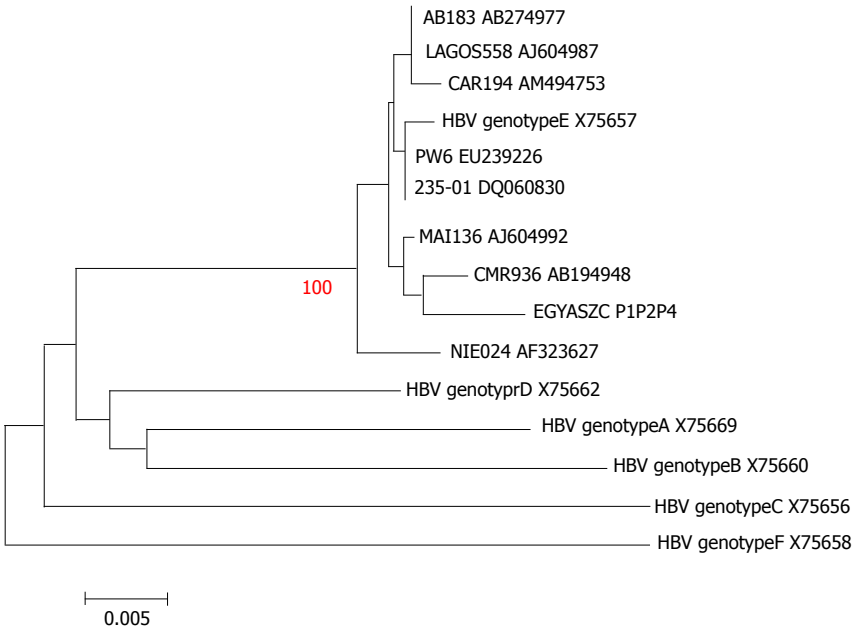


Figure 1 Phylogenetic analysis between 560 nucleotide sequence of an Egyptian strain (a positive control chronic hepatitis patient) EGYAZC P1P2P4 (bankit1229997 GQ253108) and HBV/E gene sequences and HBV genotype A, HBV genotype, HBV genotype C, HBV genotype F retrieved from gene bank revealed that the closest sequence was from HBV/E gene sequences CMR936 AB194948.

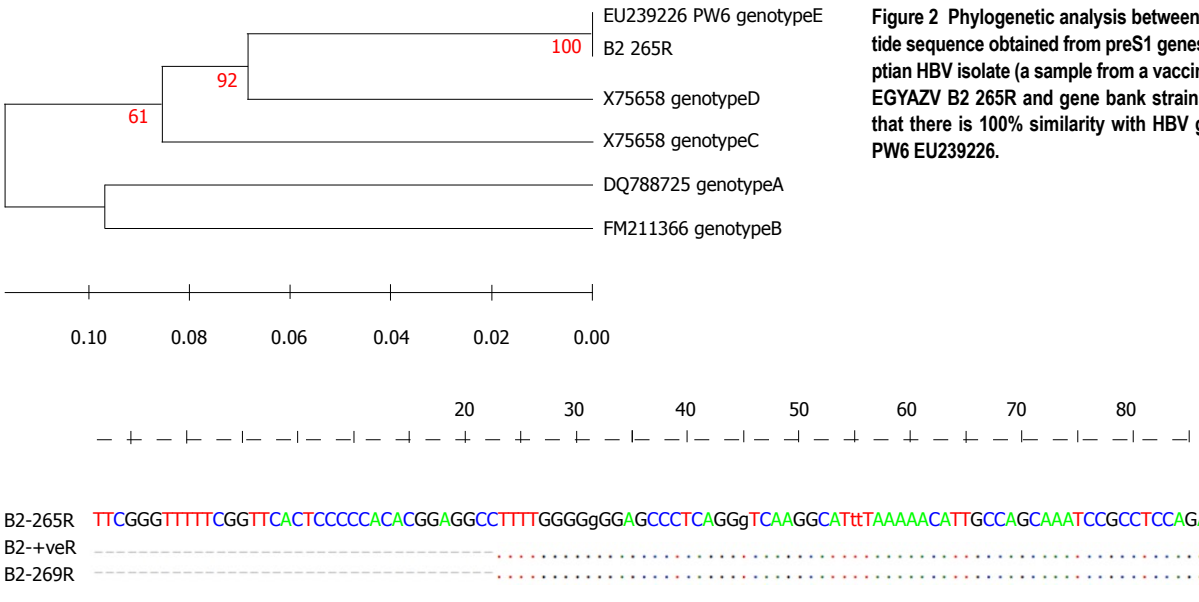


FIgure 3 Alignment between the three of our samples (265, 269, +ve control) amplification PCR product there were 100% alignment they were all of the same type.

adult volunteers was administered within a year before their inclusion in the study, six adults had their last dose between one and four years previously, and four volunteers had had their last dose six years or greater prior to the study. However, the children had received their doses since their birth, consequently they had had the vaccine administered between the ages of 4 to 13 years. It is well known that HBsAb level declines by time. The percentage of undetectable HBsAb level was lowest (7%) in 2- < 4 years old vaccinees, and rose to 20% and 21% as the age of the vacinee increased by the time of vaccination. This is comparable to McMahon *et al*, 2005[38] who found HBsAb response in 89% of the study population with 19% between 2 and 9.9 mIU/L and 70% had levels greater than 10 mIU/L. In the current study, 83.6% had detectable HBsAb, but only 36.7% of them had levels of ⩾ 10 mIU/L. In the study of Zanetti *et al*, 2005[39], more than 60% of children and nearly 90% of recruits maintained protective HBsAb, and recorded undetectable concentrations in about 9% of children and 4% of recruits and detectable amounts lower than 10 mIU/L in 27% and 7% respectively, more than 10 years after vaccination. In Egypt, El-Sawy and Mohamed (1999)[40] tested the post-vaccination seroprotection rate in sera collected during one month (93.3%) and 5 years (53.3%). In Taiwan, the HBsAb was detected post- vaccination in 100% of 2 year-olds, and in 75% of 6 year old children[41]. In a study from Taiwan, they noted that a single dose of vaccine boosted the immune response in almost all individuals. The results led to the suggestion that booster doses may be necessary

in seronegative subjects for at least 15 years after neonatal immunization. They believed that this applies to both hyper-endemic and low endemic areas of the world[42].

Breakthrough HBV infection

In the present study, detection of markers of HBV infection in the form of HBsAg was 0% among children aged 2- < 4 years, 2% among children aged 4 -13 years and 6.66% among adults, as established by the ELISA method. Similarly Alam *et al* (2007)[43] found in their study that the frequency of HBV infection in the Pakistani population was higher in individuals aged from 20-40 years. In the present study, none of the high HBsAb- positive individuals were positive for HBsAg, supporting a previous observation that an HBsAb titer greater than 10 mIU/mL can be considered protective[44,45].

Population-based studies of HB immunization after 10-15 years follow-up showed a reduction in chronic HBsAg carrier prevalence from high (8% or greater) to low (< 2%) endemicity in immunized cohorts of infants[46]. The current results are comparable to the Shatat *et al* (2000)[47] study in Alexandria, Egypt, in which only one child out of 184 vaccinated 5 year old children who had received the full course of EPI r- vaccine was HBsAg positive, while El-Sawy and Mohamed (1999)[40] did not find HBsAg positive sera among 180 children with a one month to 5 year time lapse since their last dose of vaccination.Moreover, in a serosurvey in Alexandria, Reda *et al* (2003)[48] revealed that the number of HBsAg carriers is significantly lower among the vaccinated (0.8%), compared to the unvaccinated in 6 year old children (2.2%). All these findings, as well as the present study reflect the impact of HB vaccination in lowering the HBsAg carriage rate in Egypt, but it also raises several questions. Is the time schedule for the 2nd and 3rd dose of r- HB vaccine appropriate? How frequent is post- vaccination breakthrough HBV infection, and which genotype is associated with it?

The same observations have been recorded all over the world, in Gambia[49], in Taiwan[50], in Indonesia[51], in Senegal[52], in a hyper-endemic area in Southern Italy[53], in Chinese children[54] and in Saudi Arabia[55]. However, the current results have revealed a decrease in the titer of HBsAb as subjects grow older, associated with an increased probability of becoming infected over time. A higher rate of HBV-positive cases was observed among the non-responders, when compared to subjects who mounted an elevated level of anti-HBs IgG antibodies.

By comparison, the current results showed that the AxSYM system yielded significantly more positive results than the ELISA test with respect to the detection of HBsAg 11.39% by AxSYM in comparison to 2.5% by ELISA. This finding may be due to the emergence of mutant HBV that could not be detected by ELISA or false positive AxSYM results as noted previously[56].

It was found that 1.38% of the studied groups had HBcAb, and 0.83% had HBeAb. Since serological data were not obtained either before or after vaccination, it is impossible to conclude whether these individuals were already infected at the time of vaccination or whether they had been subsequently infected with the hepatitis B virus.

Considering viraemia, in the present study, 22 (6.11%) of all participants were positive for HBV DNA; 4 cases of group Ⅲ were HBsAg positive by both methods, and 18 cases of group Ⅱ (5 of them were HBsAg positive by BioELISA 11 by AxYM and two were HBcAb positive), so 12 participants had occult HBV infection. Similarly, McMahon *et al* (2005)[38] found that all detected cases of HBV DNA were HBsAb negative. In China, a higher result of HBV viraemia (36% of the vaccinated one year old children) was reported[57].

In the current study, HBV-DNA/HBsAg positive children may be either born to an HBV-positive mother or infected with an HBV mutant. It was noticed in study of Karthigesu *et al* (1999)[58] that vaccinated children may show serological evidence of breakthrough infections, particularly if they had a low HBsAb titer. They recorded that single-point mutation at nucleotide 421 of the S gene is associated with such breakthrough infections. It was recorded also by Coleman *et al* (2006)[59] that a child remained both DNA/HBsAg positive for > 12 years, despite having a protective HBsAb titer against the wild type virus that had a substitution mutation of glycine to arginine at HBsAg aa position 145.

The HBV genotype E recorded in this study has not previously been reported in Egypt; the most prevalent genotype in Mediterranean, Middle East and Egypt is the genotype D[60,61]. Sequence comparisons of the obtained Egyptian strain EGYAZC P1P2P4 and HBV/E gene sequences retrieved from the Gene Bank revealed that there were only five nucleotide sequence differences between them, the closest sequence was from CMR936 AB194948. Also alignments of 87 nucleotide sequence obtained from preS1 genes from the Egyptian HBV isolate EGYAZV B2 265R and the Gene Bankstrains indicated that there is a 100% similarity with HBV genotype E PW6 EU239226. The same conclusion was arrived at by Mulders *et al* (2003)[30], who reported that HBV genotype/E has low sequence diversity throughout the expanses of the HBV/E crescent, which covers almost 6000 km from Senegal to Angola. this suggest that it has a short evolutionary history in humans, and is incompatible with the evolution from the closest human virus genotype D. Transmission during childhood is supposed to be the most common mode of infection in Africa, and most children infected before the age of 6 mo become chronic carriers[62]. Early age of infection and high probability of chronic carrier status results in a high rate of transmission[63]. It was speculated from this study that the presence of this genotype in Egypt for the first time may be due to virus mutation in the "a" determinant that causes this vaccine escape mutant infection. Similarly, in Argentine they found that HBV genotype E was detected in two Argentinean sisters; one of them had been vaccinated against HBV[64].

Conclusions and recommendations

HBV breakthrough infection was induced by a novel HBV genotype (E) with respect to that reported in Egypt (geno-

type D). The Hepatitis-B vaccine appears to be efficient in controlling HBV infection in children and adults. It was noticed that the HBsAb level decreases by age, with increased liability to get infected, and that those with undetectable HBsAb also had a higher rate of infection. Further studies are needed to evaluate the spread of this genotype in Egypt. Furthermore, the need for and timing of a booster dose should be studied (by whom and when?).

ACKNOWLEDGEMENTS

The research team expresses their gratitude and deep thanks to: All those who supported this work, special thanks are to Biotechnology and Genetic Engineering National Strategy- Academy for Science, Research and Biotechnology for their financial support to this project. Also, Dr. Wael Abd-El Hamied, lecturer in the Military Academy and head of the Virology Laboratory, in the Military Central Laboratories for technology support in HBsAg detection by AxSYM.

COMMENTS

Background

The apparent prevalence of hepatitis B virus (HBV) infection in Egypt has decreased after the Expanded Program of Immunization (EPI) vaccination program; however, the frequency of asymptomatic HBV carriers in response to the vaccination program needs to be determined.

Research frontier

Studies on vaccinated children during infancy and early childhood in countries having high endemicity of chronic HBV infection have shown that more than 50% of participants had measurable anti-HBs levels of at least 10 mIU/L 4 to 10 years after vaccination. Population-based studies in Alexandria, Egypt, of HB immunization after 10-15 years follow-up, showed a reduction in chronic HBsAg carrier prevalence from high (8% or greater) to low (< 2%) endemicity in immunized cohorts of infants. Thesame observations were recorded all over the world, in Gambia, in Taiwan, in Indonesia, in Senegal, in a hyper endemic area in Southern Italy, in Chinese children and in Saudi Arabia.

Innovations and breakthroughs

Considering viraemia and breakthrough infections, it was observed that there is a decrease in the titer of HBsAb as age progresses, with an increased probability to become infected over time. Similar results were recorded by McMahon *et al*, in China. Kato *et al* and Karthigesu *et al* all detected cases of positive HBV DNA in those who were HBsAb negative than in those who had HBsAb. The genotype of HBV in this study was genotype E, which has not previously been reported in Egypt; genotype D is the most prevalent in Mediterranean and Middle East and Egypt. Similarly in Argentina they found the HBV genotype E in two Argentinean sisters; one of them had been vaccinated against HBV.

Applications

Further studies are needed to evaluate the spread of the HBV genotype E in Egypt. The Hepatitis -B vaccine appears to be efficient in controlling HBV infection in both children and adults, so it is recommended that it should be given to the high risk groups all over the country. It was noticed that HBsAb level decreases with age leading to increased liability to get infected, and that those with undetectable HBsAb have is a higher rate of infection, so the need for a booster dose should be studied (to whom and when?). The number of HBsAg positive samples by AxSYM was higher than that of the BioELIS test. This raises the question of whether they were true or false positives? Further studies using different kinds of ELISA tests are needed to confirm or deny this observation.

Peer review

This is an interesting study that investigated HBV vaccine efficacy in Egypt. It is readable and publishable.

REFERENCES

1 **Bassily S**, Strickland GT, Abdel-Wahab MF, Esmat GE, Narooz S, el-Masry NA, Constantine NT, Struewing JP. Efficacy of hepatitis B vaccination in primary school children from a village endemic for Schistosoma mansoni. *J Infect Dis* 1992; **166**: 265-268

2 **el-Sayed HF**, Abaza SM, Mehanna S, Winch PJ. The prevalence of hepatitis B and C infections among immigrants to a newly reclaimed area endemic for Schistosoma mansoni in Sinai, Egypt. *Acta Trop* 1997; **68**: 229-237

3 **Heijtink RA**, van Bergen P, van Roosmalen MH, Sünnen CM, Paulij WP, Schalm SW, Osterhaus AD. Anti-HBs after hepatitis B immunization with plasma-derived and recombinant DNA-derived vaccines: binding to mutant HBsAg. *Vaccine* 2001; **19**: 3671-3680

4 **West DJ**, Calandra GB, Hesley TM, Ioli V, Miller WJ. Control of hepatitis B through routine immunization of infants: the need for flexible schedules and new combination vaccine formulations. *Vaccine* 1993; **11 Suppl 1**: S21-S27

5 **Mansour E**, Baron ER. The expanded programme on immunization in Egypt 1984–1995. Cairo: Ministry of Health, Child Survival Project 1995; XXVIII.

6 **El Ghandoure S**, El Sayed H, Abdel Hamid A, Gad S. Effectiveness of hepatitis- B vaccination in Egyptian Infant in Ismailia Governorate. *Suez Canal Univ Med J* 1998; **1**: 123-130

7 **Mansour E**, Abdul-Rahim S, Batouty G, Zaghloul I, Abdel-Hadi S. Integration of hepatitis B immunization in the Expanded Program on Immunization of the Child Survival Project. *J Egypt Public Health Assoc* 1993; **68**: 487-494

8 **André F**. Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine* 2000; **18 Suppl 1**: S20-S22

9 **Banerjee A**, Banerjee S, Chowdhury A, Santra A, Chowdhury S, Roychowdhury S, Panda CK, Bhattacharya SK, Chakravarty R. Nucleic acid sequence analysis of basal core promoter/precore/core region of hepatitis B virus isolated from chronic carriers of the virus from Kolkata, eastern India: low frequency of mutation in the precore region. *Intervirology* 2005; **48**: 389-399

10 **Ding L**, Zhang M, Wang Y, Zhou S, Kong W, Smego RA. A 9-year follow-up study of the immunogenicity and long-term efficacy of plasma-derived hepatitis B vaccine in high-risk Chinese neonates. *Clin Infect Dis* 1993; **17**: 475-479

11 **Xu ZY**, Duan SC, Margolis HS, Purcell RH, Ou-Yang PY, Coleman PJ, Zhuang YL, Xu HF, Qian SG, Zhu QR. Long-term efficacy of active postexposure immunization of infants for prevention of hepatitis B virus infection. United States-People's Republic of China Study Group on Hepatitis B. *J Infect Dis* 1995; **171**: 54-60

12 **Shokrgozar MA**, Shokri F. Enumeration of hepatitis B surface antigen-specific B lymphocytes in responder and non-responder normal individuals vaccinated with recombinant hepatitis B surface antigen. *Immunology* 2001; **104**: 75-79

13 **Jenson HB**. Nonresponders to hepatitis B vaccine. *Postgrad Med* 2000; **107**: 97-98

14 **Tran T**, Keeffe E. Management of the Pregnant Hepatitis B Patient. *Current Hepatitis Reports* 2008, **7**: 12–17

15 **Fortuin M**, Karthigesu V, Allison L, Howard C, Hoare S, Mendy M, Whittle HC. Breakthrough infections and identification of a viral variant in Gambian children immunized with hepatitis B vaccine. *J Infect Dis* 1994; **169**: 1374-1376

16 **Hsu HY**, Chang MH, Ni YH, Lin HH, Wang SM, Chen DS. Surface gene mutants of hepatitis B virus in infants who develop acute or chronic infections despite immunoprophylaxis. *Hepatology* 1997; **26**: 786-791

17 **Hsu HY**, Chang MH, Ni YH, Chen HL. Survey of hepatitis B surface variant infection in children 15 years after a nationwide vaccination programme in Taiwan. *Gut* 2004; **53**: 1499-1503

18 **Carman WF**, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336**: 325-329
19 **Singh H**, Aggarwal R, Singh RL, Naik SR, Naik S. Frequency of infection by hepatitis B virus and its surface mutants in a northern Indian population. *Indian J Gastroenterol* 2003; **22**: 132-137
20 **Gabbuti A**, Romanò L, Blanc P, Meacci F, Amendola A, Mele A, Mazzotta F, Zanetti AR. Long-term immunogenicity of hepatitis B vaccination in a cohort of Italian healthy adolescents. *Vaccine* 2007; **25**: 3129-3132
21 **Van Damme P**, Van Herck K. A review of the long-term protection after hepatitis A and B vaccination. *Travel Med Infect Dis* 2007; **5**: 79-84
22 **Okamoto H**, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 1988; **69 (Pt 10)**: 2575-2583
23 **Norder H**, Couroucé AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 1994; **198**: 489-503
24 **Stuyver L**, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; **81**: 67-74
25 **Arauz-Ruiz P**, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; **83**: 2059-2073
26 **Kaucner C**, Stinear T. Sensitive and rapid detection of viable Giardia cysts and Cryptosporidium parvum oocysts in large-volume water samples with wound fiberglass cartridge filters and reverse transcription-PCR. *Appl Environ Microbiol* 1998; **64**: 1743-1749
27 **Naito H**, Hayashi S, Abe K. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. *J Clin Microbiol* 2001; **39**: 362-364
28 Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 1999; **41**: 95-98
29 **Thompson JD**, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; **25**: 4876-4882
30 **Mulders MN**, Venard V, Njayou M, Edorh AP, Bola Oyefolu AO, Kehinde MO, Muyembe Tamfum JJ, Nebie YK, Maiga I, Ammerlaan W, Fack F, Omilabu SA, Le Faou A, Muller CP. Low genetic diversity despite hyperendemicity of hepatitis B virus genotype E throughout West Africa. *J Infect Dis* 2004; **190**: 400-408
31 **Zahn A**, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, Allain JP. Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. *J Gen Virol* 2008; **89**: 409-418
32 **Märschenz S**, Endres AS, Brinckmann A, Heise T, Kristiansen G, Nürnberg P, Krüger DH, Günther S, Meisel H. Functional analysis of complex hepatitis B virus variants associated with development of liver cirrhosis. *Gastroenterology* 2006; **131**: 765-780
33 **Fang ZL**, Sabin CA, Dong BQ, Wei SC, Chen QY, Fang KX, Yang JY, Huang J, Wang XY, Harrison TJ. Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma: a matched nested case-control study. *J Gen Virol* 2008; **89**: 2882-2890
34 **Kumar S**, Tamura K, Nei M. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* 2004; **5**: 150-163
35 **Felsenstein J**. Phylogenies from molecular sequences: inference and reliability. *Annu Rev Genet* 1988; **22**: 521-565
36 **Huang LM**, Chiang BL, Lee CY, Lee PI, Chi WK, Chang MH. Long-term response to hepatitis B vaccination and response to booster in children born to mothers with hepatitis B e antigen. *Hepatology* 1999; **29**: 954-959
37 **Ni YH**, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, Tsai KS, Chen DS. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med* 2001; **135**: 796-800
38 **McMahon BJ**, Bruden DL, Petersen KM, Bulkow LR, Parkinson AJ, Nainan O, Khristova M, Zanis C, Peters H, Margolis HS. Antibody levels and protection after hepatitis B vaccination: results of a 15-year follow-up. *Ann Intern Med* 2005; **142**: 333-341
39 **Zanetti AR**, Mariano A, Romanò L, D'Amelio R, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti C, Stroffolini T, Mele A. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* ; **366**: 1379-1384
40 **el-Sawy IH**, Mohamed ON. Long-term immunogenicity and efficacy of a recombinant hepatitis B vaccine in Egyptian children. *East Mediterr Health J* 1999; **5**: 922-932
41 **Lin YC**, Chang MH, Ni YH, Hsu HY, Chen DS. Long-term immunogenicity and efficacy of universal hepatitis B virus vaccination in Taiwan. *J Infect Dis* 2003; **187**: 134-138
42 **Lu CY**, Chiang BL, Chi WK, Chang MH, Ni YH, Hsu HM, Twu SJ, Su IJ, Huang LM, Lee CY. Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. *Hepatology* 2004; **40**: 1415-1420
43 **Alam MM**, Zaidi SZ, Malik SA, Naeem A, Shaukat S, Sharif S, Angez M, Khan A, Butt JA. Serology based disease status of Pakistani population infected with hepatitis B virus. *BMC Infect Dis* 2007; **7**: 64
44 **Poland GA**, Jacobson RM. Clinical practice: prevention of hepatitis B with the hepatitis B vaccine. *N Engl J Med* 2004; **351**: 2832-2838
45 Centers for Disease Control and Prevention. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part 1: Immunization of Infants, Children, and Adolescents. *MMWR* 2005; **54**: 1-23
46 **Kane MA**. Status of hepatitis B immunization programmes in 1998. *Vaccine* 1998; **16 Suppl**: S104-S108
47 **Shatat HZ**, Kotkat AM, Imam ZI. Follow-up study of the immunogenecity and efficiency of hepatitis B vaccine in Egyptian children. *J Med Res Inst* 2000; **21**: 126-136
48 **Reda AA**, Arafa MA, Youssry AA, Wandan EH, Ab de Ati M, Daebees H. Epidemiologic evaluation of the immunity against hepatitis B in Alexandria, Egypt. *Eur J Epidemiol* 2003; **18**: 1007-1011
49 **Viviani S**, Jack A, Hall AJ, Maine N, Mendy M, Montesano R, Whittle HC. Hepatitis B vaccination in infancy in The Gambia: protection against carriage at 9 years of age. *Vaccine* 1999; **17**: 2946-2950
50 **Hsu HM**, Lu CF, Lee SC, Lin SR, Chen DS. Seroepidemiologic survey for hepatitis B virus infection in Taiwan: the effect of hepatitis B mass immunization. *J Infect Dis* 1999; **179**: 367-370
51 **Ruff TA**, Gertig DM, Otto BF, Gust ID, Sutanto A, Soewarso TI, Kandun N, Marschner IC, Maynard JE. Lombok Hepatitis B Model Immunization Project: toward universal infant hepatitis B immunization in Indonesia. *J Infect Dis* 1995; **171**: 290-296
52 **Maupas P**, Chiron JP, Barin F, Coursaget P, Goudeau A, Perrin J, Denis F, Mar ID. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Controlled trial in an endemic area (Senegal). *Lancet* 1981; **1**: 289-292
53 **Da Villa G**, Piccinino F, Scolastico C, Fusco M, Piccinino R, Sepe A. Long-term epidemiological survey of hepatitis B virus infection in a hyperendemic area (Afragola, southern Italy): results of a pilot vaccination project. *Res Virol 1998*; **149**: 263-270

54 **Liao SS**, Li RC, Li H, Yang JY, Zeng XJ, Gong J, Wang SS, Li YP, Zhang KL. Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. *Vaccine* 1999; **17**: 2661-2666

55 **Al-Faleh FZ**, Al-Jeffri M, Ramia S, Al-Rashed R, Arif M, Rezeig M, Al-Toraif I, Bakhsh M, Mishkkhas A, Makki O, Al-Freihi H, Mirdad S, AlJuma A, Yasin T, Al-Swailem A, Ayoola A. Seroepidemiology of hepatitis B virus infection in Saudi children 8 years after a mass hepatitis B vaccination programme. *J Infect* 1999; **38**: 167-170

56 **Diepersloot RJ**, van Zantvliet-van Oostrom Y, Gleaves CA. Comparison of a chemiluminescent immunoassay with two microparticle enzyme immunoassays for detection of hepatitis B virus surface antigen. *Clin Diagn Lab Immunol* 2000; **7**: 865-866

57 **Kato H**, Nakata K, Hamasaki K, Hida D, Ishikawa H, Aritomi T, Nakao K, Kato Y, Yano M, Eguchi K. Long-term efficacy of immunization against hepatitis B virus in infants at high-risk analyzed by polymerase chain reaction. *Vaccine* 1999; **18**: 581-587

58 **Karthigesu VD**, Allison LM, Ferguson M, Howard CR. A hepatitis B virus variant found in the sera of immunised children induces a conformational change in the HBsAg "a" determinant. *J Med Virol* 1999; **58**: 346-352

59 Coleman PF**.** Surveillance for hepatitis B surface antigen mutants. *J Med Virol* 2006; **78** Suppl 1: S56-S58

60 **Norder H**, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289-309

61 **Zekri AR**, Hafez MM, Mohamed NI, Hassan ZK, El-Sayed MH, Khaled MM, Mansour T. Hepatitis B virus (HBV) genotypes in Egyptian pediatric cancer patients with acute and chronic active HBV infection. *Virol J* 2007; **4**: 74

62 **Edmunds WJ**, Medley GF, Nokes DJ, Hall AJ, Whittle HC. The influence of age on the development of the hepatitis B carrier state. *Proc Biol Sci* 1993; **253**: 197-201

63 Medley GF, Lindop NA, Edmunds WJ, Nokes DJ. Hepatitis-B virus endemicity: heterogeneity, catastrophic dynamics and control. *Nat Med* 2001; **7**: 619-624

64 **Mathet VL**, Cuestas ML, Ruiz V, Minassian ML, Rivero C, Trinks J, Daleoso G, León LM, Sala A, Libellara B, Corach D, Oubiña JR. Detection of hepatitis B virus (HBV) genotype E carried--even in the presence of high titers of anti-HBs antibodies--by an Argentinean patient of African descent who had received vaccination against HBV. *J Clin Microbiol* 2006; **44**: 3435-3439

S- Editor Zhang HN **L- Editor** Herholdt A **E- Editor** Zhang L

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i6.157

World J Hepatol 2011 June 27; 3(6): 157-163
ISSN 1948-5182 (online)

BRIEF ARTICLE

MELD score, insulin-like growth factor 1 and cytokines on bone density in end-stage liver disease

Rebecca Mitchell, Jill McDermid, Mang M Ma, Constance L Chik

Rebecca Mitchell, Constance L Chik, Divisions of Endocrinology and Metabolism, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2S2, Alberta, Canada
Jill McDermid, Mang M Ma, Division of Gastroenterology, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB T6G 2S2, Alberta, Canada
Author contributions: Mitchell M was involved in data collection, data interpretation, drafting of the manuscript and revision of the manuscript; McDermid J was involved in data collection, study conception, design and oversight, critical review and revision of the manuscript. Ma MM and Chik CL were involved in study conception, design and oversight, data interpretation, critical review and revision of the manuscript.
Supported by the Transplant Program of the University of Alberta Hospital
Correspondence to: Constance L Chik, MD, PhD, 362 HMRC, University of Alberta, Edmonton, AB T6G 2S2, Canada. cchik@ualberta.ca
Telephone: +1-780-4073636 Fax: +1-780-4076702
Received: November 30, 2010 Revised: May 20, 2011
Accepted: May 27, 2011
Published online: June 27, 2011

Abstract

AIM: To determine the contributions of insulin-like growth factor 1 (IGF-1), cytokines and liver disease severity to bone mineral density in patients pre-transplantation.

METHODS: Serum IGF-1, tumor necrosis factor-α (TNFα) and interleukin 6 (IL-6) were measured and the Model for End-Stage Liver Disease (MELD) score calculated in 121 adult patients referred to a single centre for liver transplantation. Bone mineral density (BMD) of the lumbar spine and femoral neck were assessed via dual energy X-ray absorptiometry. Demographics, liver disease etiology, medication use and relevant biochemistry were recorded.

RESULTS: A total of 117 subjects were included, with low BMD seen in 68.6%, irrespective of disease etiology. In multivariable analysis, low body mass index (BMI), increased bone turnover and low IGF-1 were independent predictors of low spinal bone density. At the hip, BMI, IGF-1 and vitamin D status were predictive. Despite prevalent elevations of TNFα and IL-6, levels did not correlate with degree of bone loss. The MELD score failed to predict low BMD in this pre-transplant population.

CONCLUSION: Osteopenia/osteoporosis is common in advanced liver disease. Low serum IGF-1 is weakly predictive but serum cytokine and MELD score fail to predict the severity of bone disease.

Key words: Hepatic osteodystrophy; Insulin-like growth factor-1; Cytokines; Bone mineral density; MELD score

Peer reviewers: Julia Peinado Onsurbe, Assistant Professor, Department of Biochemistry and Molecular Biiology, Faculty of Biology, University of Barcelona, Avda. Diagonal, 645. Barcelona 08028, Spain; Branko Stefanovic, PhD, Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL 32312, United States

Mitchell R, McDermid J, Ma MM, Chik CL. MELD score, insulin-like growth factor 1 and cytokines on bone density in end-stage liver disease. *World J Hepatol* 2011; 3(6): 157-163 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i6/157.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i6.157

INTRODUCTION

Low bone mineral density (BMD) is a known complication of chronic liver disease[1,2], demonstrated in diverse etiologies, including cholestasis[3,4], alcohol[5-7] and viral hepatitis[8,9]. However, the mechanism by which chronic liver disease mediates bone loss is not clearly defined and is likely to be multifactorial.

Declining serum insulin-like growth factor-1 (IGF-1) has been shown to play a role in the pathogenesis of bone loss in elderly normal subjects[10,11] and in males with idiopathic osteoporosis[12,13]. While known to decline in hepatic disease, correlating with the degree of dysfunction[14,15], the contribution of reduced IGF-1 to bone loss is controversial in this population. A positive correlation between IGF-1 and BMD has been reported in males with viral cirrhosis[8,9] but refuted in subjects with early hepatic disease[16]. Its role in the pathogenesis of hepatic osteopenia in advanced liver failure has not been ascertained.

Elevated levels of serum cytokines, including tumor necrosis factor-α (TNFα), interleukin-6 (IL-6) and interleukin-1, are seen in liver disease from both alcohol[17,18] and viral hepatitis[9,19], with levels shown to correlate with disease severity[19,20]. In the general population, TNF is known to stimulate formation and activity of osteoclasts, with a resultant increase in bone resorption[21,22]. A recent study supports the role of soluble TNF receptor p55 (sTNFR-55), a marker of TNF function, in mediating bone loss in males with viral cirrhosis[9]. However, little is known about the contribution of TNFα to hepatic osteopenia in other groups, including females and non-viral disease. IL-6 is also involved in bone remodeling through stimulation of osteoclast formation and, in conjunction with IL-1, stimulation of bone resorption[23,24]. Its contribution to bone loss in end-stage liver disease has not been determined.

Finally, the Model for End-Stage Liver Disease (MELD) score has been prospectively developed and validated to predict the severity of chronic liver disease[25] and has been adopted by United Network for Organ Sharing (UNOS) as the basis for cadaveric liver allocation. It is being considered for adoption in many other transplant programs worldwide. Despite its increasing utility, to our knowledge, only one study has examined the relationship between the MELD score and bone density and it was limited to primary sclerosing cholangitis[26]. The ability of the MELD score to predict low bone density in advanced liver failure warrants further study.

The aim of the present study was to investigate the relationship between advanced chronic liver disease and low bone density in a pre-transplant population, examining specifically the roles of serum IGF-1, TNF, IL-6 and the predictive ability of disease severity reflected by the MELD score. Other factors known to affect bone mass, including serum 25-hydroxyvitamin D (25OHD), parathyroid hormone (PTH), testosterone and body mass index (BMI), were included in the analysis.

MATERIALS AND METHODS

Patients

The study was conducted according to the Declaration of Helsinki and approved by the University of Alberta Health Research Ethics Board. A total of 121 adult patients were evaluated for orthotopic liver transplantation at the University of Alberta, Edmonton, Canada, between 1998 and 2000. 117 subjects were included in the analysis; 4 subjects were excluded because of acute fulminant hepatic failure.

Methods

The etiology of liver failure was determined by biochemical, serological and liver biopsy data (where available), and patients were classified according to disease etiology, including viral (hepatitis B or C), alcohol, cholestasis [primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)] and other (hemochromatosis, Wilson's disease, alpha-1 anti-trypsin deficiency).

The main explanatory variables of interest included serum IGF-1, measured *via* radioimmunoassay (Nichols Institute Diagnostics Inc., San Juan Capistrano, CA), serum IL-6 and TNFα, measured via chemiluminesence (Diagnostic Products Corp., Los Angeles, CA) and MELD score, calculated using an internet-based calculator (http://www.mayoclinic.org/gi-rst/mayomodel7.html). Serum bilirubin, INR and creatinine were used in the calculation.

The covariates included in the analysis were those that may influence BMD, including age, gender, body mass index (BMI)(based upon measured height and weight), smoking status (current *vs* former or never smoker) and self-reported fracture history. Data on family history of osteoporosis or maternal hip fracture was not available. Medication use prior to bone densitometry was recorded, including use of osteoporosis therapy (bisphosphonates, raloxifene, calcitonin and hormone replacement therapy), nutritional measures (calcium and/or vitamin D supplementation) and corticosteroids, due to its known deleterious effects on bone. Additional biochemical data included indices of calcium and vitamin D metabolism (serum calcium, albumin, 25OHD, PTH), thyroid stimulating hormone (TSH) and gonadal function (total testosterone in males only). 24-hour urine for N-telopeptides (NTx) was measured *via* enzyme-linked immunosorbent assay (Diagnostic Products Corp., Los Angeles, CA), serving as a marker of bone turnover. Biochemical and clinical data measured closest to the time of bone densitometry were used in the analysis.

The outcome variable was BMD of the lumbar spine (L1-L4) and femoral neck, measured via dual energy X-ray absorptiometry (DXA) (Hologic QDR1000, Hologic Inc, Waltham, MA) using standard protocols. Vertebral BMD were standardized using the Hologic reference database; hip BMD values were standardized using the National Health and Nutrition Examination Study Ⅲ (NHANES Ⅲ) database. BMD values were expressed as the T-score, the number of standard deviations of the patients' BMD from the mean peak value for a reference population of the same race and gender. According to World Health Organization criteria, osteopenia was defined as a T-score between -1.0 and -2.5 and osteoporosis as a T-score less than -2.5[27]. Abnormal BMD was defined as a T-score less than -1.0 at either site.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL). All results were expressed as the mean ± SD unless indicated

Table 1 Patient demographics and biochemical data

Variable	*n* = 117
Age in years	50.4 (10.5)
Male gender - no. (%)	74 (63%)
Etiology of liver disease - no. (%)	
Viral	46 (39%)
Alcohol	20 (17%)
Cholestasis	25 (21%)
Other	26 (22%)
BMI (kg/m^2)	24.8 (5.0)
Current smoking - no. (%)	43 (37%)
History of fracture - no. (%)	10 (9%)
MELD score	15.2 (6.0)
Calcium[a] (N 2.1 - 2.6 nmol/L)	2.41 (0.22)
PTH - mean (SD)(N 1.1 - 6.8 ng/L)	2.86 (2.44)
25 OHD (N 40 - 200 ng/mL)	32.2 (19.5)
Total testosterone (N 10 - 29.5 nmol/L)	12.0 (9.9)
IGF-1 (ng/mL) (N, age and sex dependent)	41.3 (54.0)
TNFα (N < 8.1 pg/mL)	14.8 (8.7)
IL-6 (N < 9.7 pg/mL)	17.1 (46.2)
UNTx (N < 81 nmol BCE/ mmol creatinine)	57.3 (36.4)

[a]Corrected calcium presented; Values are presented as mean (SD) unless indicated otherwise, N: normal range. BCE: bone collagen equivalents; PTH: Parathyroid hormone; BMI: Body mass index; 25OHD: 25-hydroxyvitamin D; IGF-1: Insulin-like growth factor-1; TNFα: Tumor necrosis factor-α; IL-6: Interleukin-6

otherwise. Subjects were grouped by etiology of liver disease, with continuous variables compared *via* one-way ANOVA with post-hoc testing, and dichotomous variables *via* χ^2 testing. Where variables were non-normally distributed, data were transformed (IGF-1, IL-6). Subjects were then grouped by severity of bone disease (normal, osteopenia and osteoporosis), comparing groups by one-way ANOVA. Univariate regression (correlation) was performed with Pearson standard linear regression analysis (normal distribution) or the Spearman test (non-normal distribution) to assess the association and prediction of BMD by continuous variables. Multivariable linear regression was then completed to adjust for the influence of covariates on the relationship between IGF-1, serum cytokines and disease severity on bone density. Covariates of clinical and statistical significance were included, including age, gender, BMI, 25OHD, testosterone, IGF-1, TNFα, IL-6 and MELD score. A *P*-value less than 0.05 was considered significant.

RESULTS

Clinical and biochemical data are summarized in Table 1 (all subjects) and Table 2, where subjects are grouped by etiology of liver disease. The mean age was 50.4 years (range 18-73) and 63.2% of subjects were male. None were receiving treatment for osteoporosis (bisphosphonates, raloxifene, calcitonin or hormone replacement therapy) at the time of bone densitometry. No significant differences were seen between disease groups with respect to age or gender, while a significantly higher number of subjects

Table 2 Clinical and Biochemical Data by Etiology of Cirrhosis. Mean Value (SD)

Variable	Viral	Alcohol	Cholestasis	Other
	n = 46	*n* = 20	*n* = 25	*n* = 26
BMI (kg/m^2)	25.4 (5.1)	24.4 (5.3)	23.3 (3.2)	25.8 (5.9)
MELD score	15.1 (6.14)	16.2 (6.14)	14.2 (5.06)	15.6 (6.51)
Corrected calcium (nmol/L)	2.39 (0.28)	2.44 (0.21)	2.37 (0.11)	2.41 (0.22)
PTH (ng/L)	2.14 (1.2)[a]	3.73 (2.7)	2.83 (1.5)	3.48 (3.9)
25OHD (μg/L)	30.6 (16.1)	34.4 (20.1)	37.0 (20.2)	28.7 (17.0)
Testosterone (nmol/L)	12.7 (11.2)	7.0 (4.5)[b]	15.7 (7.4)	12.2 (11.5)
IGF-1 (μg/L)	34.5 (47.3)	28.3 (27.1)	45.9 (47.7)	59.5 (79.2)
TNFα (ng/L)	16.1 (10.9)	13.7 (6.7)	15.0 (8.4)	13.0 (5.0)
IL-6 (ng/L)	26.7 (72.4)	19.3 (20.7)	7.1 (5.3)	9.9 (12.2)
UNTx (nmol BCE/ mmol creatinine)	54.9 (38.0)	69.3 (25.2)	56.5 (41.6)	53.7 (36.2)

[a]PTH significantly lower in viral group than in alcohol and other ($P < 0.05$); [b]Testosterone significantly lower in alcohol group than in cholestasis ($P < 0.05$). BCE: bone collagen equivalents; PTH: Parathyroid hormone; BMI: Body mass index; 25OHD: 25-hydroxyvitamin D; IGF-1: Insulin-like growth factor-1; TNFα: Tumor necrosis factor-α; IL-6: Interleukin-6.

with viral disease were current smokers ($P < 0.001$) (data not shown). PTH was significantly lower in the viral group ($P = 0.015$), while total testosterone was significantly lower in males with alcohol-induced disease than in those with cholestasis ($P = 0.027$)(Table 2). No other significant differences were seen between disease groups.

Prevalence of abnormal bone mineral density

Low BMD was seen in 80 (68.4%) patients, including 55 (47.0%) with osteopenia and 25 (21.4%) with osteoporosis. Low bone density was common at both the lumbar spine (56.4%) and femoral neck (57.8%). Table 3 summarizes T-scores and prevalence of low BMD by disease etiology. No differences were seen with respect to T-scores and prevalence of low bone density between liver disease groups.

Relationship between BMD, bone turnover, and variables of interest serum IGF-1

Serum IGF-1 was below that expected for age and gender in 96/109 (88%) of subjects, including 58/109 (53%) of subjects in whom the level was below the detection limit of 15 μg/L and assigned a value of 15 μg/L (Figure 1). A significant positive correlation was seen between IGF-1 and bone density at both sites (Table 4). However, when grouped by severity of bone disease (normal *vs* osteopenia *vs* osteoporosis), IGF-1 did not significantly differ (Table 5).

Serum cytokines

TNFα values exceeded the reference range (< 8.1 pg/L) in 89/110 (80.9%) subjects in whom values were available (Figure 1). However, there was no correlation between TNFα and BMD or bone turnover (Table 4). When only those with viral cirrhosis were included (data not shown), the lack of correlation persisted at both BMD sites (Lumbar

Table 3 T-scores and prevalence of abnormal BMD between disease groups

	Viral n = 46	Alcohol n = 20	Cholestasis n = 25	Other n = 26	P value
T-score lumbar spine mean (SD)	-1.08 (1.35)	-1.51 (1.35)	-1.43 (1.23)	-1.30 (1.43)	0.552
T-score femoral neck mean (SD)	-1.11 (1.07)	-1.31 (1.02)	-1.30 (0.81)	-1.03 (1.05)	0.706
Low BMD at 1 or both sites number (%)	31 (67.4%)	13 (65.0%)	18 (72.0%)	18 (69.2%)	0.963
Low BMD lumbar spine number (%)	22 (47.8%)	11 (55.0%)	15 (60.0%)	18 (69.2%)	0.556
Low BMD femoral neck number (%)	26 (56.5%)	12 (60.0%)	15 (60.0%)	14 (53.8%)	0.993

T-score values represent the mean (SD); BMD: Bone marrow density.

Table 4 Correlation between BMD, bone turnover and variables of interest

Variable	T-score (lumbar spine)		T-score (femoral neck)		Urine NTx	
	r Value	P Value	r Value	P Value	r Value	P Value
BMI	0.324[a]	< 0.001	0.360[a]	< 0.001	-0.249[a]	0.013
MELD	-0.255[a]	0.016	-0.038	0.692	0.311[a]	0.002
25OHD	0.278[a]	0.016	0.227[a]	0.003	-0.426[a]	< 0.001
Testosterone (males only)	0.13	0.273	0.003	0.978	-0.377[a]	0.003
IGF-1	0.215[a]	0.024	0.209[a]	0.029	-0.07	0.5
TNFα	-0.139	0.15	-0.084	0.39	0.07	0.51
IL-6	-0.137	0.19	-0.073	0.49	0.276[a]	0.015
Urine NTx	-0.346[a]	< 0.001	-0.102	0.319		

[a]Significant at P < 0.05. BMI: Body mass index; 25OHD: 25-hydroxyvitamin D; IGF-1: Insulin-like growth factor-1; TNFα: Tumor necrosis factor-α; IL-6: Interleukin-6.

Table 5 Clinical and biochemical parameters ranked by severity of bone loss

	Normal	Osteopenia	Osteoporosis	P value
BMI	26.8 (5.1)	24.4 (5.1)[a]	22.9 (3.6)[a]	0.006
MELD	15.5 (5.9)	14.2 (5.7)	16.9 (6.5)	0.18
25OHD	33.9 (15.7)	33.4 (20.2)	27.9 (15.3)	0.25
Testosterone	11.1 (9.8)	13.7 (10.8)	10.1 (8.3)	0.41
IGF-1	38.3 (35.5)	44.4 (67.5)	39.2 (46.1)	0.86
TNFα	14.3 (10.7)	15.2 (7.6)	14.7 (8.0)	0.89
IL-6	11.8 (12.9)	12.4 (14.0)	15.6 (17.4)	0.64
UNTx	47.2 (27.3)	59.4 (38.7)	70.2 (41.6)[b]	0.08

Values are presented as mean (SD); [a]Significantly lower than in subjects with normal BMD; [b]Significantly higher than UNTx in subjects with normal BMD (P=0.027) (Using post-hoc test of ANOVA). BMI: Body mass index; 25OHD: 25-hydroxyvitamin D; IGF-1: Insulin-like growth factor-1; TNFα: Tumor necrosis factor-α; IL-6: Interleukin-6.

spine: r = 0.249, P = 0.102; Femoral neck: r = 0.098, P = 0.526). Additionally, the mean value of TNFα did not differ between subjects with normal *vs* low BMD (Table 5). IL-6 values were abnormally elevated above the lab reference range (< 9.7 ng/L) in 27/92 (29.3%) of subjects (Figure 1). Again, there was no significant association between IL-6 and BMD, although IL-6 did correlate with bone turnover (Table 4). The mean value of IL-6 did not differ between subjects with normal and low BMD (Table 5).

MELD Score

A significant inverse correlation was seen between the MELD score and spinal bone density. There was no association at the hip (Table 4). However, when grouped by severity of bone disease, the MELD score did not significantly differ between groups (Table 5) but a significant positive correlation was noted between MELD score and bone turnover (Table 4).

Other covariates

In bivariate analysis, a significant positive correlation was seen between lumbar spine bone density, BMI and vitamin D status. At the femoral neck, BMI and vitamin D status again correlated significantly with bone density (Table 4). Low total testosterone was seen in 43.8% of our male subjects, the prevalence of which did not differ by disease group. There was no association between testosterone and BMD, although levels did inversely correlate with bone turnover (Table 4). Bone turnover was inversely correlated with spinal bone density, while there was no association at the hip (Table 4). As expected, bone turnover was significantly higher in those with osteoporosis compared to those with normal BMD (Table 4). BMI was significantly lower in those with low BMD compared to those with normal BMD (Table 5).

Multivariable linear regression

Variables approaching statistical significance (P < 0.2) in univariate regression or that are clinically relevant in affecting bone density were included in a multivariate model attempting to predict changes in BMD. Model inclusion variables included age, BMI, MELD score, 25OHD, testosterone, IGF-1, TNF, IL-6 and UNTx. At the lumbar spine, the model of best fit included BMI, UNTx and IGF-1 as independent predictors of spinal T-score (r = 0.523, adjusted r^2 = 0.249, P < 0.01). TNF, IL-6 and MELD score were not independent predictors. At the hip, BMI, age and 25OHD remained as independent predictors of bone density (r = 0.502, adjusted r^2 = 0.232, P < 0.001). Again, TNF, IL-6 and MELD score were not predictive and at this site, there was no effect from IGF-1.

DISCUSSION

Patients with end stage liver disease have complex hormonal and cytokine profiles. These changes can affect bone mineral density. Low BMD was seen in nearly 70% of subjects in a heterogeneous population referred for evalua-

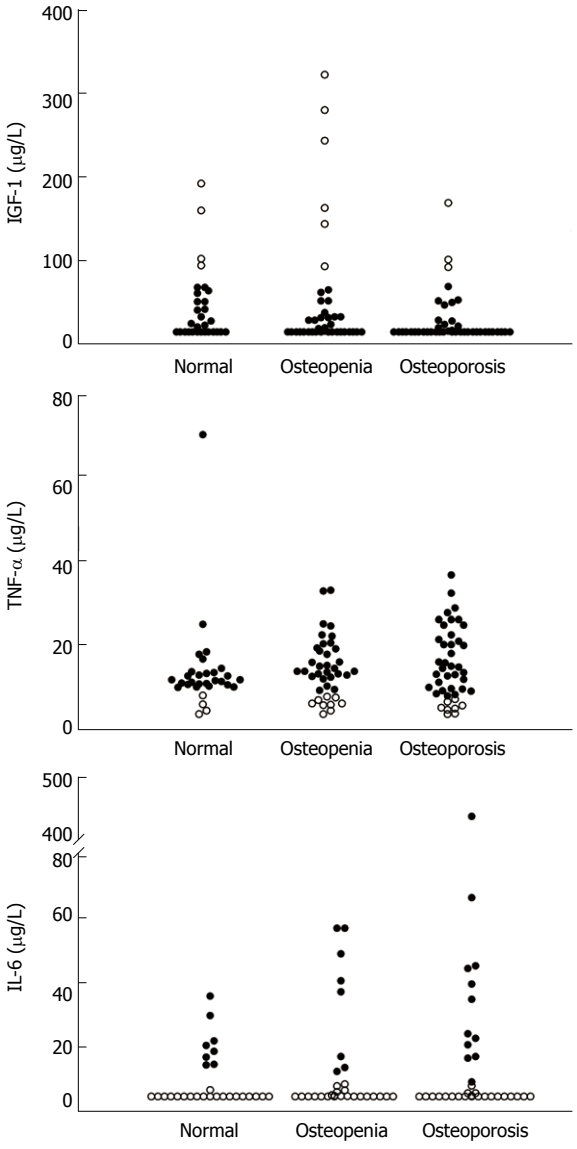


Figure 1 Individual IGF-1, TNFα and IL-6 values grouped according to severity of bone loss. ○ depicts individuals with values within the normal range and ● depicts individuals with values below (IGF-1) or above (TNFα and IL-6) the normal range. IGF-1: Insulin-like growth factor-1; TNFα: Tumor necrosis factor-α; IL-6: Interleukin-6

tion of liver transplantation. Prevalence did not differ by disease etiology and was common in sites of both trabecular and cortical bone. Serum IGF-1 was subnormal in nearly 90% of subjects and levels correlated directly with bone density. When adjusted for potential confounders, IGF-1 remained predictive of low spinal BMD. Despite elevated levels of the osteoclastogenic cytokines TNFα and IL-6, levels failed to correlate with bone density and were not independently predictive of bone loss. The MELD score was inversely associated with spinal bone density but lost predictive value when adjusted for potential confounders.

The prevalence of abnormal BMD in our population with end-stage liver disease is similar to that which has been previously reported[8,9,28]. Our patients had extremely low levels of serum IGF-1 which did not differ by disease etiology. Given the advanced stage of disease, this is not surprising and is in accordance with previous findings[14,15]. IGF-1 is known to play an important role in bone formation and in mineralization of bone surface through stimulation of bone cell proliferation and collagen synthesis[29,30]. Declining levels in hepatic disease would be expected to contribute to reduced bone density. Gallejo-Rojo *et al*[8,9] reported a significant association between serum IGF-1 and spinal bone density in a smaller group of male subjects with viral cirrhosis. Conversely, Ormarsdottir *et al*[16] showed no association in male and female subjects with non-viral disease. However, 78% of these patients were Child-Pugh A and subjects with viral disease were excluded. Our study expands upon existing literature as the first to report a significant association and independently predictive, albeit small, role for serum IGF-1 in hepatic osteopenia among a diverse population with advanced liver disease. Low serum IGF-1 may play an increasingly prominent role in the pathogenesis of low bone mass with advancing liver failure. A longitudinal study design which incorporates the role of a declining IGF-1 and that of its more stable IGF binding proteins on its relationship to bone loss may provide further insight.

Hypogonadism is a risk factor for reduced BMD[31] and is a known complication of advanced cirrhosis, irrespective of etiology[32,33]. While common in our male subjects, there was no significant association between total testosterone and BMD, with no difference in levels of testosterone between subjects with and without reduced BMD. While supporting previous results[9], interpretation is limited by the lack of measurement of free testosterone. Sex hormone binding globulin is known to increase in advanced cirrhosis[33], elevating total testosterone, whereas it is unbound testosterone that is available for tissue binding, including at the bone. We likely under report true hypogonadism and may under represent the importance of bioavailable testosterone levels on bone mass in this population.

To our knowledge, our study is the first to expand upon the contribution of TNFα to bone loss beyond males with viral cirrhosis. In this limited population, an inverse correlation was recently reported between bone density and soluble TNF receptor p55 (sTNFRp55), a modulator of the biological function of TNF[9]. In our expanded population, we failed to detect an association between TNFα and BMD or bone turnover and levels of TNF did not differ between those with and without reduced bone density. TNFα is rapidly cleared from circulation and, while elevated in liver disease, its levels are known to fluctuate widely[34]. The lack of an apparent role for TNFα elevation in low BMD in our population may relate to limitations in its direct quantification. sTNFRp55 is more stable, correlates well with TNFα[35] and may serve as a more accurate marker in this population. The measurement of this soluble receptor and its ability to predict reduced bone density in this population warrants further study.

IL-6 has been shown to mediate osteoclastogenesis through stimulation of osteoclast formation and, in conjunction with IL-1, stimulation of bone resorption[23,24]. In

post-menopausal women, serum levels of IL-6 have been found to predict bone loss[36]. The biological effects of IL-6 are dependent on soluble IL-6 receptors (sIL-6r) and both IL-6 and sIL-6r are increased in chronic liver disease, correlating with disease severity[37]. However, the contribution of IL-6 to hepatic osteopenia has not been previously reported. To our knowledge, our study is the first to report a lack of significant association between serum IL-6 and BMD. Numerous studies have noted an association between IL-6 polymorphisms and lower BMD in postmenopausal women[38], healthy males[39] and in inflammatory bowel disease[40]. The lack of significant association in our study may reflect the importance of gene polymorphisms and the role of more stable IL-6 receptors in affecting bone loss.

To date, only one study has previously explored the association and predictive ability of the MELD score on metabolic bone disease. Restricted to primary sclerosing cholangitis and limited by a small sample size and lack of biochemical measures of vitamin D and gonadal function, this study revealed no association between MELD score and spinal bone density[26]. We expand upon the literature to include larger patient numbers and diverse disease, and describe a significant inverse association between MELD score and spinal bone density. However, in multivariate analysis, this lacks predictive ability. This relationship may be better examined with a longitudinal study design whereby progression of hepatic dysfunction is followed in parallel with changes in bone density. However, it is important to note that as part of a pre-transplant work-up, the MELD score does not appear to be a useful marker for the presence or absence of metabolic bone disease.

Limitations to our study include the cross-sectional study design, where examination of causation is not possible. The absence of accurate data on the gonadal status of women limits examination of its contribution in female subjects. Finally, the lack of measurement of more stable serum cytokine receptors may lead to an under representation of their effects on mediating bone loss.

In conclusion, this study confirms the high prevalence of low bone density in advanced liver disease, regardless of underlying disease etiology. The pathogenesis of low BMD is multifactorial. Low serum IGF-1 is common in all disease groups, is associated with low BMD in both trabecular and cortical bone, and is a weak but significant predictor of low spinal bone density. Serum cytokine elevation is common, reflecting advanced hepatic disease, but levels do not predict the presence or absence of bone disease. While the MELD score is a useful predictor of survival in end-stage liver disease, it does not appear to serve a predictive function for the presence of low bone density.

COMMENTS

Background

Low bone mineral density (BMD) is a known complication of chronic liver disease of diverse etiologies. Little is known about the contributions of serum insulin growth factor 1 (IGF-1) and pro-inflammatory cytokines to low bone density or the ability of the Model for End Stage Liver Disease (MELD) score to predict bone loss in end-stage liver disease.

Research frontiers

Reduced serum IGF-1 and elevation of interleukin 6 (IL-6) and tumor necrosis factor α (TNF α) are markers of osteopenia in some hepatic disorders. The MELD score can predict the severity of chronic liver disease and may be a good predictor of hepatic osteopenia.

Innovations and breakthroughs

Low serum IGF-1 which is common in end-stage liver disease of various etiologies can predict low spinal bone density. Neither serum cytokine elevation nor MELD score can predict low bone density.

Applications

As part of a pre-transplant work-up, the MELD score does not appear to be a useful marker for the presence or absence of low BMD. Low serum IGF-1 may play a prominent role in the pathogenesis of low BMD in end-stage liver disease. A longitudinal study design may provide further insight.

Terminology

MELD score has been validated to predict the severity of chronic liver disease and adopted by United Network for Organ Sharing as the basis for cadaveric liver allocation.

Peer review

The study examines an issue important to the management of pre-transplant end stage liver disease (ESLD) patients. Post-liver transplant fracture is a very concerning problem in this patient population and screening patients for hepatic osteopenia is crucial. The authors have selected pathologically relevant potential predictors: the MELD score. The study revealed, interestingly and contrary to presumptions about the MELD score, that the score is not a good predictor of severity of hepatic osteopenia.

REFERENCES

1 **Diamond TH**, Stiel D, Lunzer M, McDowall D, Eckstein RP, Posen S. Hepatic osteodystrophy. Static and dynamic bone histomorphometry and serum bone Gla-protein in 80 patients with chronic liver disease. *Gastroenterology* 1989; **96**: 213-221

2 **Compston JE**. Hepatic osteodystrophy: vitamin D metabolism in patients with liver disease. *Gut* 1986; **27**: 1073-1090

3 **Matloff DS**, Kaplan MM, Neer RM, Goldberg MJ, Bitman W, Wolfe HJ. Osteoporosis in primary biliary cirrhosis: effects of 25-hydroxyvitamin D3 treatment. *Gastroenterology* 1982; **83**: 97-102

4 **Hodgson SF**, Dickson ER, Wahner HW, Johnson KA, Mann KG, Riggs BL. Bone loss and reduced osteoblast function in primary biliary cirrhosis. *Ann Intern Med* 1985; **103**: 855-860

5 **Bikle DD**, Genant HK, Cann C, Recker RR, Halloran BP, Strewler GJ. Bone disease in alcohol abuse. *Ann Intern Med* 1985; **103**: 42-48

6 **Conte D**, Caraceni MP, Duriez J, Mandelli C, Corghi E, Cesana M, Ortolani S, Bianchi PA. Bone involvement in primary hemochromatosis and alcoholic cirrhosis. *Am J Gastroenterol* 1989; **84**: 1231-1234

7 **Diamond T**, Stiel D, Lunzer M, Wilkinson M, Posen S. Ethanol reduces bone formation and may cause osteoporosis. *Am J Med* 1989; **86**: 282-288

8 **Gallego-Rojo FJ**, Gonzalez-Calvin JL, Muñoz-Torres M, Mundi JL, Fernandez-Perez R, Rodrigo-Moreno D. Bone mineral density, serum insulin-like growth factor I, and bone turnover markers in viral cirrhosis. *Hepatology* 1998; **28**: 695-699

9 **Gonzalez-Calvin JL**, Gallego-Rojo F, Fernandez-Perez R, Casado-Caballero F, Ruiz-Escolano E, Olivares EG. Osteoporosis, mineral metabolism, and serum soluble tumor necrosis factor receptor p55 in viral cirrhosis. *J Clin Endocrinol Metab* 2004; **89**: 4325-4330

10 **Janssen JA**, Burger H, Stolk RP, Grobbee DE, de Jong FH, Lamberts SW, Pols HA. Gender-specific relationship between serum free and total IGF-I and bone mineral density in elderly men and women. *Eur J Endocrinol* 1998; **138**: 627-632

11 **Barrett-Connor E**, Goodman-Gruen D. Gender differences in insulin-like growth factor and bone mineral density associa-

tion in old age: the Rancho Bernardo Study. *J Bone Miner Res* 1998; **13**: 1343-1349
12 **Kurland ES**, Rosen CJ, Cosman F, McMahon D, Chan F, Shane E, Lindsay R, Dempster D, Bilezikian JP. Insulin-like growth factor-I in men with idiopathic osteoporosis. *J Clin Endocrinol Metab* 1997; **82**: 2799-2805
13 **Ljunghall S**, Johansson AG, Burman P, Kämpe O, Lindh E, Karlsson FA. Low plasma levels of insulin-like growth factor 1 (IGF-1) in male patients with idiopathic osteoporosis. *J Intern Med* 1992; **232**: 59-64
14 **Møller S**, Becker U. Insulin-like growth factor 1 and growth hormone in chronic liver disease. *Dig Dis* 1992; **10**: 239-248
15 **Caregaro L**, Alberino F, Amodio P, Merkel C, Angeli P, Plebani M, Bolognesi M, Gatta A. Nutritional and prognostic significance of insulin-like growth factor 1 in patients with liver cirrhosis. *Nutrition* 1997; **13**: 185-190
16 **Ormarsdóttir S**, Ljunggren O H, Olofsson H, Blum WF, Lööf L. Circulating levels of insulin-like growth factors and their binding proteins in patients with chronic liver disease: lack of correlation with bone mineral density. *Liver* 2001; **21**: 123-128
17 **Khoruts A**, Stahnke L, McClain CJ, Logan G, Allen JI. Circulating tumor necrosis factor, interleukin-1 and interleukin-6 concentrations in chronic alcoholic patients. *Hepatology* 1991; **13**: 267-276
18 **Diez Ruiz A**, Santos Perez JL, Lopez Martinez G, Gonzalez Calvín J, Gil Extremera B, Gutierrez Gea F. Tumour necrosis factor, interleukin-1 and interleukin-6 in alcoholic cirrhosis. *Alcohol Alcohol* 1993; **28**: 319-323
19 **Zylberberg H**, Rimaniol AC, Pol S, Masson A, De Groote D, Berthelot P, Bach JF, Bréchot C, Zavala F. Soluble tumor necrosis factor receptors in chronic hepatitis C: a correlation with histological fibrosis and activity. *J Hepatol* 1999; **30**: 185-191
20 **Genesca J**, Gonzalez A, Segura R, Catalan R, Marti R, Varela E, Cadelina G, Martinez M, Lopez-Talavera JC, Esteban R, Groszmann RJ, Guardia J. Interleukin-6, nitric oxide, and the clinical and hemodynamic alterations of patients with liver cirrhosis. *Am J Gastroenterol* 1999; **94**: 169-177
21 **Pfeilschifter J**, Chenu C, Bird A, Mundy GR, Roodman GD. Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclastlike cells in vitro. *J Bone Miner Res* 1989; **4**: 113-118
22 **Bertolini DR**, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature* 1986; **319**: 516-518
23 **Manolagas SC**, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N Engl J Med* 1995; **332**: 305-311
24 **Manolagas SC**. The role of IL-6 type cytokines and their receptors in bone. *Ann N Y Acad Sci* 1998; **840**: 194-204
25 **Kamath PS**, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, D'Amico G, Dickson ER, Kim WR. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001; **33**: 464-470
26 **Campbell MS**, Lichtenstein GR, Rhim AD, Pazianas M, Faust T. Severity of liver disease does not predict osteopenia or low bone mineral density in primary sclerosing cholangitis. *Liver Int* 2005; **25**: 311-316
27 Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. *World Health Organ Tech Rep Ser* 1994; **843**: 1-129
28 **Sokhi RP**, Anantharaju A, Kondaveeti R, Creech SD, Islam KK, Van Thiel DH. Bone mineral density among cirrhotic patients awaiting liver transplantation. *Liver Transpl* 2004; **10**: 648-653
29 **Hock JM**, Centrella M, Canalis E. Insulin-like growth factor I has independent effects on bone matrix formation and cell replication. *Endocrinology* 1988; **122**: 254-260
30 **Reed BY**, Zerwekh JE, Sakhaee K, Breslau NA, Gottschalk F, Pak CY. Serum IGF 1 is low and correlated with osteoblastic surface in idiopathic osteoporosis. *J Bone Miner Res* 1995; **10**: 1218-1224
31 **Jackson JA**, Kleerekoper M, Parfitt AM, Rao DS, Villanueva AR, Frame B. Bone histomorphometry in hypogonadal and eugonadal men with spinal osteoporosis. *J Clin Endocrinol Metab* 1987; **65**: 53-58
32 **Pignata S**, Daniele B, Galati MG, Esposito G, Vallone P, Fiore F, Ricchi P, Pergola M. Oestradiol and testosterone blood levels in patients with viral cirrhosis and hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 1997; **9**: 283-286
33 **Kaymakoğlu S**, Okten A, Cakaloğlu Y, Boztaş G, Beşişik F, Taşçioğlu C, Yalçin S. Hypogonadism is not related to the etiology of liver cirrhosis. *J Gastroenterol* 1995; **30**: 745-750
34 **Engelberts I**, Möller A, Schoen GJ, van der Linden CJ, Buurman WA. Evaluation of measurement of human TNF in plasma by ELISA. *Lymphokine Cytokine Res* 1991; **10**: 69-76
35 **Aukrust P**, Liabakk NB, Müller F, Lien E, Espevik T, Frøland SS. Serum levels of tumor necrosis factor-alpha (TNF alpha) and soluble TNF receptors in human immunodeficiency virus type 1 infection--correlations to clinical, immunologic, and virologic parameters. *J Infect Dis* 1994; **169**: 420-424
36 **Scheidt-Nave C**, Bismar H, Leidig-Bruckner G, Woitge H, Seibel MJ, Ziegler R, Pfeilschifter J. Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. *J Clin Endocrinol Metab* 2001; **86**: 2032-2042
37 **Migita K**, Abiru S, Maeda Y, Daikoku M, Ohata K, Nakamura M, Komori A, Yano K, Yatsuhashi H, Eguchi K, Ishibashi H. Serum levels of interleukin-6 and its soluble receptors in patients with hepatitis C virus infection. *Hum Immunol* 2006; **67**: 27-32
38 **Ferrari SL**, Garnero P, Emond S, Montgomery H, Humphries SE, Greenspan SL. A functional polymorphic variant in the interleukin-6 gene promoter associated with low bone resorption in postmenopausal women. *Arthritis Rheum* 2001; **44**: 196-201
39 **Lorentzon M**, Lorentzon R, Nordström P. Interleukin-6 gene polymorphism is related to bone mineral density during and after puberty in healthy white males: a cross-sectional and longitudinal study. *J Bone Miner Res* 2000; **15**: 1944-1949
40 **Schulte CM**, Dignass AU, Goebell H, Röher HD, Schulte KM. Genetic factors determine extent of bone loss in inflammatory bowel disease. *Gastroenterology* 2000; **119**: 909-920

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Zhang L

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i6.164

World J Hepatol 2011 June 27; 3(6): 164-169
ISSN 1948-5182 (online)

CASE REPORT

Budd-Chiari syndrome in a patient with ulcerative colitis and no inherited coagulopathy

Sunil Dacha, Manjari Devidi, Evan Osmundson

Sunil Dacha, Manjari Devidi, Evan Osmundson, Department of Internal Medicine, Saint Joseph Hospital, 2900 N. Lake Shore Drive, Chicago, IL 60657, United States
Author contributions: Dacha S and Osmundson E contributed equally to this work; Dacha S, Devidi M and Osmundson EC established concepts, gathered data and performed the literature review; and Dacha S and Osmundson E wrote the paper.
Correspondence to: Evan Osmundson, MD, PhD, Department of Internal Medicine, Saint Joseph Hospital, 2900 N. Lake Shore Drive, Chicago, IL 60657,
United States. evan.osmundson@gmail.com
Telephone: +1-773-665-3017 Fax: +1-773-665-3384
Received: December 14, 2010 Revised: May 17, 2011
Accepted: May 24, 2011
Published online: June 27, 2011

Abstract

We report a case of 27 year old female patient who was admitted to the hospital with an acute flare up of ulcerative colitis. The patient presented with complaints of persistent abdominal pain and bloody diarrhea despite aggressive therapy for her ulcerative colitis. A CT scan of the abdomen on admission revealed hepatic vein thrombosis, suggesting a diagnosis of Budd-Chiari syndrome. Significantly, an associated thrombosis of the inferior mesenteric vein was also detected. Based on imaging data and clinical assessment, the patient was started on anticoagulation therapy and an extensive work-up for hypercoagulability was initiated. Up to the time of publication, no significant findings suggesting this patient has an underlying coagulation disorder have been found. Based on our search of PUBMED, this report is one of only five reported adult cases of Budd-Chiari Syndrome associated with ulcerative colitis in the English literature in living patients without evidence of a co-existing coagulation disorder. This case highlights the potential for thrombosis at unusual sites in ulcerative colitis patients even in the absence of classical coagulation abnormalities. In addition to the case presented, we provide a brief review of previously reported cases of Budd-Chiari Syndrome occurring in patients with inflammatory bowel disease.

Key words: Ulcerative colitis; Inflammatory bowel disease; Budd-Chiari syndrome; Thrombosis; Coagulopathy

Peer reviewers: Hongzhi Xu, Dr., Massachusetts General Hospital, 51 Blossom Street, Room 435, Boston, MA 02148, United States

Dacha S, Devidi M, Osmundson E. Budd-Chiari syndrome in a patient with ulcerative colitis and no inherited coagulopathy. *World J Hepatol* 2011; 3(6): 164-169 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i6/164.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i6.164

INTRODUCTION

Patients with inflammatory bowel disease are at increased risk for thromboembolic complications. We present the case of a patient with ulcerative colitis (UC) complicated by the development of Budd-Chiari Syndrome (BCS) likely precipitated by an acute flare up of her UC. The incidence of venous thrombosis in UC was found to be 39% in one necropsy study[1], but hepatic vein thrombosis and BCS have been reported only as a rare extra intestinal complication of UC. Very few cases occurring in patients without an underlying coagulation disorder have been reported in the literature. In addition to the presentation of our patient, we review the literature describing the other reported cases and provide a brief clinical overview and outcome of all reported adult cases.

CASE REPORT

The patient was a 27 year old woman with a history of UC (pancolitis) diagnosed six months prior to admission. Her

disease was not well controlled despite aggressive management including treatment with certolizumab. The patient presented to the hospital after she had three episodes of bloody stools on the day of admission. She complained of diarrhea 12-15 times a day along with some abdominal pain for two days before admission. In addition, the patient stated she had decreased appetite and had lost six pounds in two months. She underwent a colonoscopy with biopsy six months prior to admission at the time of diagnosis of UC and no evidence of malignancy was detected. On admission, she denied any other symptoms, including fevers, chills, abdominal distention or mental status changes.

Physical exam on admission revealed an ill appearing woman with moderate pain but not in distress. She had a temperature of 98.7 F, a blood pressure of 96/60 mmHg (baseline for this patient), a pulse of 80 beats per minute (regular) and a respiratory rate of 16/min with an oxygen saturation of 99% on room air. Auscultation of the heart and lungs revealed no detectable abnormalities. Her abdomen was moderately tender to deep palpation but was not distended. Bowel sounds were present and there was an absence of rebound tenderness, rigidity or guarding. Her neurological examination was normal.

Pertinent labs on admission included hemoglobin of 9.2 gm/dL, a serum albumin of 2.3 gm/dL and liver enzymes within normal limits. On admission, mesalamine and Ⅳ steroids were started as initial therapy for the exacerbation of UC. A CT scan of abdomen was performed for intractable abdominal pain, which revealed hepatic vein thrombosis, supporting the diagnosis of BCS (Figure 1A); these findings were not present on a CT scan of the abdomen performed two months prior to admission (Figure 1B). She was therapeutically anticoagulated with enoxaparin as a bridge to warfarin therapy. The patient's stool collected on admission tested positive for clostridium difficile toxin on hospital day 3, which further complicated her inpatient management and she was started on oral vancomycin therapy. Fortunately, over the course of her stay in the hospital, the patient's clinical condition improved and she was discharged in good health.

Given this unusual presentation, a thorough investigation into other potential causes of this patient's hypercoagulability was performed which included a bone marrow biopsy to evaluate for myeloproliferative diseases, as well as laboratory studies to assess for the presence of paroxysmal nocturnal hemoglobulinuria, antiphospholipid antibody syndromes, deficiencies of natural anticoagulants (antithrombin Ⅲ, protein C and protein S), Factor Ⅴ Leiden mutation, prothrombin Ⅱ mutation, hyperhomocysteinemia, and a leukemia and lymphoma screen. No alternate etiology for the patient's apparent hypercoagulable state was identified. Of note, the patient was not on oral contraceptive medications on admission and for at least 7 mo prior to admission.

A follow up CT scan of the patient's abdomen two months later demonstrated a partial resolution of the hepatic vein thrombosis and her hepatic function remains normal (Figure 1C). She is currently in remission for her UC on Certolizumab and will continue on coumadin therapy indefinitely.

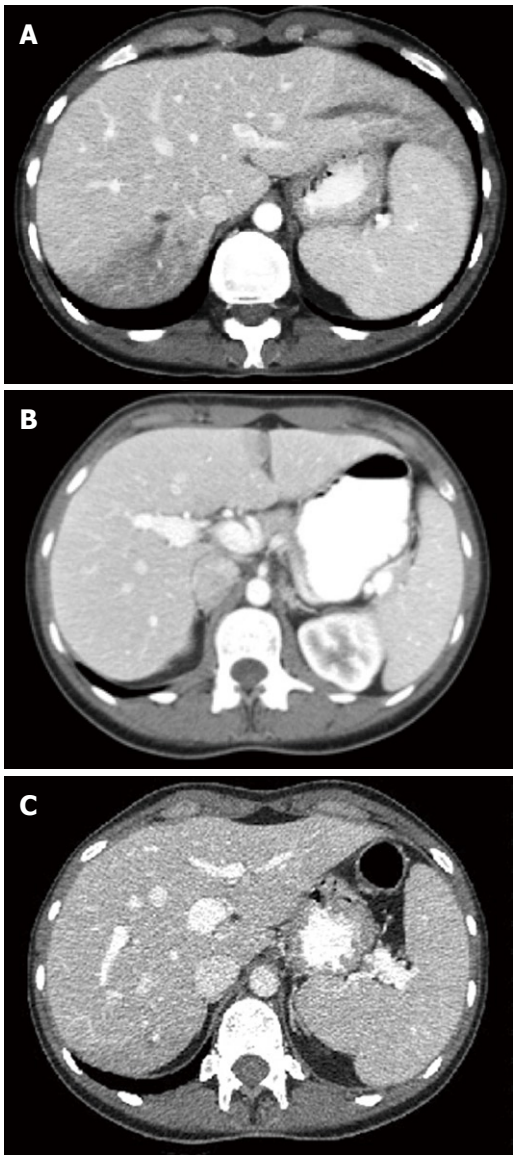


Figure 1 Abdominal computed tomography scans of patient at time points indicated. A: Abdominal computed tomography (CT) scan of the patient on admission demonstrates clear evidence of hepatic vein thrombosis; B: CT scan of the abdomen performed two months prior to admission revealed no evidence of hepatic vein thrombosis; C: Partial resolution of hepatic vein thrombosis two months after admission and initiation of anticoagulation.

DISCUSSION

BCS is characterized by venous congestion secondary to processes that interrupt or diminish the normal blood flow out of the liver[2]. Fatty changes, pericholangitis, sclerosing cholangitis and cirrhosis are well-recognized hepatic complications of UC but UC as a precipitant of BCS in the absence of other heritable causes has been only rarely reported. Patients with UC are at increased risk for venous thromboembolism at baseline but the risk is eight times higher during a flare up[3]. UC is also associated with an increased

risk of arterial thromboembolic events[4]. The incidence of venous thrombosis in UC was found to be 39% in necropsy studies[1], but hepatic vein thrombosis is a rare extra intestinal complication of UC. This case report is only one of five published reports of BCS diagnosed in living adult patients with IBD. In addition to our findings, we briefly summarize all 14 published BCS cases associated with UC and highlight features of the 12 reported cases published in English, with a focus on adult patients and those with co-exisiting inherited hypercoagulability.

The relative contribution of inherited thrombophilia to the development of VTE in patients with IBD remains unclear. Solem *et al* have found the rates of thrombophilic abnormalities among IBD patients in their cohort to be collectively similar to patients without IBD; however, the incidence of specific inherited coagulopathies was found to be individually uncommon[5]. Moreover, data from a case-control analysis published by Spina *et al*[3] suggest that classical inherited coagulation disorders are less frequent among IBD patients than age matched and VTE site-matched controls without IBD. Both of these studies have shown that the risk of VTE development among IBD patients is positively associated with both disease extent and activity (temporal association). Furthermore, as a whole, VTE in IBD patients occurs earlier in life than in those without IBD[6,7]. These and other findings support the classification of IBD as an independent risk factor for the development of VTE. However, as noted by previous authors, the acquisition of non-heritable risk factors for thromboembolic disease among IBD patients, particularly during acute flare ups is likely contributory. Although limited by its size, the study by Solem *et al* also found that UC patients remain at higher risk of venous thromboembolism even after a proctocolectomy, suggesting the systemic as opposed to the local origin of thrombophilia[5]. Taken together with other data, this suggests that VTE is a true extraintestinal manifestation of IBD.

More recent analyses have examined in greater depth whether consistent abnormalities in either the levels or functionality of components of the coagulation cascade can be detected in patients with IBD (reviewed in[8]). Various reports have demonstrated abnormalities in select haemostatic pathways in IBD patients including abnormalities of coagulation[9-13], platelet function[14-17], fibrinolysis[18-20] and endothelial function[21-23]. However, difficulties with intercomparison between studies and discordant results have made it challenging to draw firm conclusions. Furthermore, work over the past decade has elucidated functional links between both innate and adaptive immune function and coagulation cascades[24-26]. Indeed, IBD-associated alterations in the interaction between the immune system and coagulation pathways have been explored as a mechanism of VTE in these patients, as has the effect of nutritional deficiencies[8], which can be common in IBD patients. However, no single underlying abnormality in these interrelationships has been consistently linked to the development of VTE in IBD patients.

Although the precise mechanism responsible for increased risk of thromboembolism in patients with IBD remains obscure, several possible mechanisms have been hypothesized to contribute. While patients with IBD are predisposed to VTE even during remission, they appear to be particularly at risk for VTE during acute flare ups[27]. During an acute flare up of UC, increased systemic levels of various cytokines and other inflammatory mediators (e.g. IL-1, 1L-6 and TNF-alpha) can activate pro-inflammatory signaling in endothelial and immune cells and can modulate coagulation cascades predisposing patients to thrombosis[8]. Furthermore, Vassiliadis *et al* have proposed that increased intestinal epithelial permeability during an acute flare up facilitates bacterial translocation resulting in systemic endotoxinemia, which leads to a lowered threshold for activation of the coagulation cascade[28]. Notably, several groups have demonstrated that pro-inflammatory cytokines can counteract natural anticoagulant activity leading to a hypercoagulable state[29]. Rosenberg *et al* and Socha *et al* have suggested that loco-regional imbalances of endothelial cell-dependent procoagulant and anticoagulant activity underlies the thrombotic selectivity for some vessels such as the hepatic veins in cases of BCS[30,31]. However, given the low number of reported cases of BCS associated with IBD patients, the relevance of these putative mechanisms to cases such as that presented here is difficult to estimate. Our patient was found to have a concomitant *Clostridium difficile*-associated enterocolitis at presentation that we suggest could have caused additional regional proinflammatory signaling and coagulation dysfunction, possibly explaining the VTE in such a unique site leading to BCS.

We searched PUBMED and found 14 published cases of BCS coexisting with UC in the English literature (Table 1). One case was reported in German and another in Korean. Four of these reported patients were pediatric patients. Of these published reports, eight describe adult cases including four patients that were diagnosed based on necropsy studies. To our knowledge, our patient represents only the fifth reported case of coexisting BCS and UC reported in living adult patients. Excluding those diagnosed at necropsy, it is notable that three out of four of the previously reported patients had evidence of a coexisting coagulation disorder; two had polycythemia vera and one had antiphospholipid antibody syndrome, while the presence of a coexisiting coagulation disorder in the remaining patient was unknown at publication. Interestingly, among the 4 reported pediatric patients, only one was found to have a coexisting coagulation disorder upon work-up. A brief analysis of these patients demonstrates them to be predominantly female with BCS presenting or diagnosed during an acute exacerbation of UC. Importantly, those successfully diagnosed with BCS when alive survived the acute event in all cases.

Among reported adult patients, Kelsey *et al* first described a case of coexisting BCS and UC in 1945 during a necropsy study[32]. The next three reported cases were also from necropsy studies; one case by Jorgensen in 1958[33] and two cases by Chesner IM *et al* in 1986[34]. The first case of BCS and UC diagnosed in a living patient was reported

Table 1 Reported cases of Budd-Chiari Syndrome occurring in patients with Inflammatory Bowel Disease

Age (year)	Gender	Clinical scenario	Liver function tests	Secondary causes	Outcome	Ref.
33	F	Necropsy study (Active Ulcerative Colitis)	-	Unknown	Deceased	[32]
22	F	Necropsy study	-	Unknown	Deceased	[33]
35	M	Necropsy study (Bloody diarrhea)	Elevated AlkP	Unknown	Deceased	[34]
54	F	Necropsy study (vomiting, diarrhea)	Elevated AlkP	Unknown	Deceased	[34]
22	F	Active Ulcerative Colitis	Elevated AST, ALT	Unknown	Survived	[35]
16	M	Abdominal pain	Elevated AST, ALT, AlkP	None	Survived	[43]
40	F	Restorative proctocolectomy	Elevated AlkP	Polycythemia vera	Survived	[36]
32	M	Fever asthenia	Elevated AST, ALT	Antiphospholipid antibody	Survived	[37]
11	F	Jaundice Hepatosplenomegaly	Normal	Polycythemia vera	Survived	[44]
? (Gn)	F	Thrombotic complications, needed liver transplant	-	Antiphospholipid antibody, Protein C deficiency	Survived	[45]
27 (Kn)	F	Fever, Vomiting, Hematochezia	Elevated AST; Normal ALT	Probable Protein C deficiency	Survived	[46]
14	F	Bloody diarrhea	Normal	None	Survived	[47]
12	F	Bloody diarrhea	Normal	None	Survived	[30]
33	F	RUQ Abdominal Pain	Normal	Polycythemia vera	Survived	[28]

Cases are organized by publication date with references (Ref.) listed at far right. RUQ: Right Upper Quadrant; AST: Aspartate Amino Transferase; ALT: Alanine Amino Transferase; AlkP: Alkaline Phosphatase; Gn: Article written in German; Kn: Article written in Korean.

by Brinson *et al* in 1988[35]. This patient was a 22 year old female who had an active flare up of UC and elevated liver enzymes. Whiteford *et al* 1999[36] reported the second case to be diagnosed in a living patient, believed to be secondary to polycythemia that was unmasked after restorative proctocolectomy. Apparently, this patient's polycythemia went initially undetected due IBD-associated chronic blood loss from her gastrointestinal tract. In 2000, Praderio *et al* reported a third adult case in a patient who was found to have coexisting antiphospholipid antibody syndrome[37]. In 2009, a fourth adult case was reported by Vassiliadis T *et al* in a 33 year old female secondary to polycythemia[28]. This patient required a transjugular intrahepatic portosystemic shunt (TIPS) and thereafter was treated with anticoagulants for BCS and corticosteroids for IBD. Our patient will be the fifth reported adult case, but the first adult patient presenting without any evidence of classical/inherited coagulation disorder after extensive work-up. Since most of the cases were female patients it is possible that female gender is an independent risk factor; however, it is difficult to draw such conclusions based on the limited number of cases available for analysis.

We postulate that our patient developed a hepatic vein thrombus secondary to an IBD-associated hypercoagulable state exacerbated during an acute flare up of UC. The absence of evidence of a detectable thrombus in an abdominal CT scan administered 2 mo prior to admission supports the temporal relationship between thrombus development and acute flare up of her IBD. Although our patient's serum transaminase levels were not severely elevated on admission, it is possible that her abdominal pain was, at least partially, related to early venous congestion associated with the hepatic vein thrombus we discovered when evaluating for causes of intractable abdominal pain. It is noteable that our patient responded well to anticoagulation and that her UC was brought to remission, with both factors likely contributing to the resolution of her hepatic vein thrombus.

It is plausible that other unique factors associated with our patient's disease predisposed her to VTE and BCS. While our patient was found to have *Clostridium difficile* toxin positive stool on admission, the precise contribution of this finding to her development of BCS is difficult to assess. Many experts believe that *Clostridium difficile*-associated colitis can precipitate an IBD-flare up; however, whether this was causative in our patient is not clear. However, our search of the literature revealed no reported relationship between *Clostridium difficile*-associated colitis and BCS in patients with or without IBD. One could hypothesize the development of *Clostridium difficile*-associated colitis in our patient exacerbated (or even caused) her IBD flare leading to an even greater degree of local and systemic inflammation in our patient, thus putting her at greater risk for VTE and, more specifically BCS, for reasons discussed above.

The diagnosis of BCS can be made in patients who sometimes present with abdominal pain, ascites and hepatomegaly or with other findings raising a high level of suspicion in the clinician. The diagnostic modalities that have been found to be most helpful are Doppler ultrasound[38] and Computed tomography[39]. Magnetic Resonance Angiography has been shown in a few studies to be more accurate in delineating the hepatic vasculature to more precisely define the location of the obstruction[40]. Nevertheless, clear cut indications for MRI over CT have not been established. The gold standard for diagnosis is hepatic venography but it is more invasive and is typically performed when less invasive methods of evaluation are equivocal or negative. Liver biopsy can be diagnostic in some acute and subacute cases. One study by Tang TJ *et al* suggested that there was no evidence for a relationship between early liver pathology and survival[41]. However, the Child-Pugh score, serum ALT levels and evidence of porto-systemic shun-

ting appear to be prognostic indicators for patients with BCS[42]. Treatment guidelines for BCS were established in 2009 by the American Association for the Study of Liver Diseases (AASLD) (www.aasld.org). In summary, anticoagulation should be initiated immediately and continued for life unless contraindicated. An extensive workup for secondary causes of hypercoagulability should be performed. In symptomatic patients, percutaneous angiography may be helpful to look for venous obstruction and stents may be placed if necessary. TIPS is reserved for those not improving with anticoagulation and who have failed other management strategies. Liver transplantation should be considered for fulminant liver failure or failure to respond to TIPS. Medical therapy alone is recommended in patients without evidence of ongoing hepatic necrosis[42].

This case, along with the previous reports outlined above, recapitulates the need for a high level of suspicion for VTE in patients presenting with IBD.

REFERENCES

1 **Sloan WP**, Bargen JA, Gage RB. Life histories of patients with chronic ulcerative colitis: a review of 2,000 cases. *Gastroenterology* 1968; **54**: Suppl: 819-822
2 **Valla DC**. Budd-Chiari syndrome and veno-occlusive disease/sinusoidal obstruction syndrome. *Gut* 2008; **57**: 1469-1478
3 **Spina L**, Saibeni S, Battaglioli T, Peyvandi F, de Franchis R, Vecchi M. Thrombosis in inflammatory bowel diseases: role of inherited thrombophilia. *Am J Gastroenterol* 2005; **100**: 2036-2041
4 **Ha C**, Magowan S, Accortt NA, Chen J, Stone CD. Risk of arterial thrombotic events in inflammatory bowel disease. *Am J Gastroenterol* 2009; **104**: 1445-1451
5 **Solem CA**, Loftus EV, Tremaine WJ, Sandborn WJ. Venous thromboembolism in inflammatory bowel disease. *Am J Gastroenterol* 2004; **99**: 97-101
6 **Jackson LM**, O'Gorman PJ, O'Connell J, Cronin CC, Cotter KP, Shanahan F. Thrombosis in inflammatory bowel disease: clinical setting, procoagulant profile and factor V Leiden. *QJM* 1997; **90**: 183-188
7 **Grip O**, Svensson PJ, Lindgren S. Inflammatory bowel disease promotes venous thrombosis earlier in life. *Scand J Gastroenterol* 2000; **35**: 619-623
8 **Danese S**, Papa A, Saibeni S, Repici A, Malesci A, Vecchi M. Inflammation and coagulation in inflammatory bowel disease: The clot thickens. *Am J Gastroenterol* 2007; **102**: 174-186
9 **Saibeni S**, Vecchi M, Valsecchi C, Faioni EM, Razzari C, de Franchis R. Reduced free protein S levels in patients with inflammatory bowel disease: prevalence, clinical relevance, and role of anti-protein S antibodies. *Dig Dis Sci* 2001; **46**: 637-643
10 **Heneghan MA**, Cleary B, Murray M, O'Gorman TA, McCarthy CF. Activated protein C resistance, thrombophilia, and inflammatory bowel disease. *Dig Dis Sci* 1998; **43**: 1356-1361
11 **Hudson M**, Chitolie A, Hutton RA, Smith MS, Pounder RE, Wakefield AJ. Thrombotic vascular risk factors in inflammatory bowel disease. *Gut* 1996; **38**: 733-737
12 **Lee LC**, Spittell JA, Sauer WG, Owen CA, Thompson JH. Hypercoagulability associated with chronic ulcerative colitis: changes in blood coagulation factors. *Gastroenterology* 1968; **54**: 76-85
13 **Aadland E**, Odegaard OR, Røseth A, Try K. Free protein S deficiency in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol* 1992; **27**: 957-960
14 **Danese S**, Motte Cd Cde L, Fiocchi C. Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. *Am J Gastroenterol* 2004; **99**: 938-945
15 **Collins CE**, Cahill MR, Rampton DS. Clinical significance of platelet size in inflammatory bowel disease? *Thromb Haemost* 1997; **77**: 218-219
16 **Danese S**, Katz JA, Saibeni S, Papa A, Gasbarrini A, Vecchi M, Fiocchi C. Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. *Gut* 2003; **52**: 1435-1441
17 **Danese S**, de la Motte C, Sturm A, Vogel JD, West GA, Strong SA, Katz JA, Fiocchi C. Platelets trigger a CD40-dependent inflammatory response in the microvasculature of inflammatory bowel disease patients. *Gastroenterology* 2003; **124**: 1249-1264
18 **Van Bodegraven AA**, Tuynman HA, Schoorl M, Kruishoop AM, Bartels PC. Fibrinolytic split products, fibrinolysis, and factor XIII activity in inflammatory bowel disease. *Scand J Gastroenterol* 1995; **30**: 580-585
19 **Vrij AA**, Rijken J, van Wersch JW, Stockbrügger RW. Coagulation and fibrinolysis in inflammatory bowel disease and in giant cell arteritis. *Pathophysiol Haemost Thromb* 2003; **33**: 75-83
20 **Sonoda K**, Ikeda S, Mizuta Y, Miyahara Y, Kohno S. Evaluation of venous thromboembolism and coagulation-fibrinolysis markers in Japanese patients with inflammatory bowel disease. *J Gastroenterol* 2004; **39**: 948-954
21 **Stevens TR**, James JP, Simmonds NJ, McCarthy DA, Laurenson IF, Maddison PJ, Rampton DS. Circulating von Willebrand factor in inflammatory bowel disease. *Gut* 1992; **33**: 502-506
22 **Meucci G**, Pareti F, Vecchi M, Saibeni S, Bressi C, de Franchis R. Serum von Willebrand factor levels in patients with inflammatory bowel disease are related to systemic inflammation. *Scand J Gastroenterol* 1999; **34**: 287-290
23 **Binion DG**, West GA, Volk EE, Drazba JA, Ziats NP, Petras RE, Fiocchi C. Acquired increase in leucocyte binding by intestinal microvascular endothelium in inflammatory bowel disease. *Lancet* 1998; **352**: 1742-1746
24 **Massberg S**, Grahl L, von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, Brinkmann V, Lorenz M, Bidzhekov K, Khandagale AB, Konrad I, Kennerknecht E, Reges K, Holdenrieder S, Braun S, Reinhardt C, Spannagl M, Preissner KT, Engelmann B. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med* 2010; **16**: 887-896
25 **Danese S**, Vetrano S, Zhang L, Poplis VA, Castellino FJ. The protein C pathway in tissue inflammation and injury: pathogenic role and therapeutic implications. *Blood* 2010; **115**: 1121-1130
26 **Esmon CT**. Interactions between the innate immune and blood coagulation systems. *Trends Immunol* 2004; **25**: 536-542
27 **Grainge MJ**, West J, Card TR. Venous thromboembolism during active disease and remission in inflammatory bowel disease: a cohort study. *Lancet* 2010; **375**: 657-663
28 **Vassiliadis T**, Mpoumponaris A, Giouleme O, Hatzidakis A, Patsiaoura K, Zezos P, Vakalopoulou S, Kargiotis K, Gkisakis D, Katsinelos P, Evgenidis N. Late onset ulcerative colitis complicating a patient with Budd-Chiari syndrome: a case report and review of the literature. *Eur J Gastroenterol Hepatol* 2009; **21**: 109-113
29 **Esmon CT**. The impact of the inflammatory response on coagulation. *Thromb Res* 2004; **114**: 321-327
30 **Socha P**, Ryzko J, Janczyk W, Dzik E, Iwanczak B, Krzesiek E. Hepatic vein thrombosis as a complication of ulcerative colitis in a 12-year-old patient. *Dig Dis Sci* 2007; **52**: 1293-1298
31 **Rosenberg RD**, Aird WC. Vascular-bed--specific hemostasis and hypercoagulable states. *N Engl J Med* 1999; **340**: 1555-1564
32 **PALMER ED**. Budd-Chiari syndrome (occlusion of the hepatic veins); seven cases. *Ann Intern Med* 1954; **41**: 261-271
33 **JORGENSEN L**. Occlusion of the hepatic veins; a report of two cases. *Acta Pathol Microbiol Scand* 1958; **44**: 68-79
34 **Chesner IM**, Muller S, Newman J. Ulcerative colitis complicated by Budd-Chiari syndrome. *Gut* 1986; **27**: 1096-1100

35 **Brinson RR**, Curtis WD, Schuman BM, Mills LR. Recovery from hepatic vein thrombosis (Budd-Chiari syndrome) complicating ulcerative colitis. *Dig Dis Sci* 1988; **33**: 1615-1620

36 **Whiteford MH**, Moritz MJ, Ferber A, Fry RD. Budd-Chiari syndrome complicating restorative proctocolectomy for ulcerative colitis: report of a case. *Dis Colon Rectum* 1999; **42**: 1220-1224

37 **Praderio L**, Dagna L, Longhi P, Rubin G, Sabbadini MG. Budd-Chiari syndrome in a patient with ulcerative colitis: association with anticardiolipin antibodies. *J Clin Gastroenterol* 2000; **30**: 203-204

38 **Bolondi L**, Gaiani S, Li Bassi S, Zironi G, Bonino F, Brunetto M, Barbara L. Diagnosis of Budd-Chiari syndrome by pulsed Doppler ultrasound. *Gastroenterology* 1991; **100**: 1324-1331

39 **Lupescu IG**, Dobromir C, Popa GA, Gheorghe L, Georgescu SA. Spiral computed tomography and magnetic resonance angiography evaluation in Budd-Chiari syndrome. *J Gastrointestin Liver Dis* 2008; **17**: 223-226

40 **Noone TC**, Semelka RC, Siegelman ES, Balci NC, Hussain SM, Kim PN, Mitchell DG. Budd-Chiari syndrome: spectrum of appearances of acute, subacute, and chronic disease with magnetic resonance imaging. *J Magn Reson Imaging* 2000; **11**: 44-50

41 **Tang TJ**, Batts KP, de Groen PC, van Hoek B, Haagsma EB, Hop WC, Janssen HL. The prognostic value of histology in the assessment of patients with Budd-Chiari syndrome. *J Hepatol* 2001; **35**: 338-343

42 **Menon KV**, Shah V, Kamath PS. The Budd-Chiari syndrome. *N Engl J Med* 2004; **350**: 578-585

43 **Kraut J**, Berman JH, Gunasekaran TS, Allen R, McFadden J, Messersmith R, Pellettiere E. Hepatic vein thrombosis (Budd-Chiari syndrome) in an adolescent with ulcerative colitis. *J Pediatr Gastroenterol Nutr* 1997; **25**: 417-420

44 **Sood A**, Midha V, Sood N, Kaushal V. Hepatic vein thrombosis with ulcerative colitis. *Indian J Gastroenterol* 2000; **19**: 145-146

45 **Junge U**, Wienke J, Schüler A. Acute Budd-Chiari syndrome, portal and splenic vein thrombosis in a patient with ulcerative colitis associated with antiphospholipid antibodies and protein C deficiency. *Z Gastroenterol* 2001; **39**: 845-852

46 **Park SY**, Kim YJ, Park IH, Moon CM, Choi CW, Lee SK, Kim H, Park YN, Kim TI, Kim WH. [A case of Budd-Chiari syndrome and superior mesenteric vein thrombosis in ulcerative colitis]. *Korean J Gastroenterol* 2005; **45**: 201-205

47 **Rahhal RM**, Pashankar DS, Bishop WP. Ulcerative colitis complicated by ischemic colitis and Budd Chiari syndrome. *J Pediatr Gastroenterol Nutr* 2005; **40**: 94-97

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Zhang L

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v3.i6.170

World J Hepatol 2011 June 27; 3(6): 170-174
ISSN 1948-5182 (online)

CASE REPORT

Ductopenia related liver sarcoidosis

Nourr-Eddine Farouj, Jean-François D Cadranel, Ali Mofredj, Vincent Jouannaud, Maria Lahmiri, Pierre Le Lann, Alain Cazier

Nourr-Eddine Farouj, Ali Mofredj, Intensive Care Department, Centre Hospitalier Laënnec BP 72 60100 Creil, France
Jean-François D Cadranel, Vincent Jouannaud, Maria Lahmiri, Liver, Gastroenteroly and Nutrition Department, Centre Hospitalier Laënnec BP 72 60100 Creil, France
Pierre Le Lann, Chest and Lung Department, Centre Hospitalier Laënnec BP 72 60100 Creil, France
Alain Cazier, Pathological, Centre Hospitalier Laënnec BP 72 60100 Creil, France
Author contributions: Farouj NE wrote the manuscript; Cadranel JF was the patient's main physician, made the diagnosis and wrote the manuscript. Mofredj A participated in manuscript writing; Lahmiri M and Jouannaud V and Le Lann P collected data; Cazier A made all pathological examinations and wrote pathological comments; all authors approved the submitted manuscript.
Correspondence to: Jean François Cadranel, MD, Professor, CMHP, Liver, Gastroenteroly and Nutrition Department, Centre Hospitalier Laënnec BP 72 60100 Creil, France. jfrançois.cadranel@ch-creil.fr
Telephone: +33-3-44-61-64-46 Fax: +33-3-44-61-64-40
Received: January 8, 2011 Revised: May 16, 2011
Accepted: May 23, 2011
Published online: June 27, 2011

Abstract

Sarcoidosis is a systemic granulomatous disease which may involve many organs. In approximately 95% of patients there is liver involvement, with noncaseating hepatic granulomas occurring in 21 to 99% of patients with sarcoidosis. Liver involvement is usually asymptomatic and limited to mild to moderate abnormalities in liver biochemistry. The occurrence of jaundice in sarcoidosis is rare; extensive imaging procedures and the examination of liver biopsies permit a precise diagnostic. Ductopenia associated with sarcoidosis has been reported in less than 20 cases and can lead to biliary cirrhosis and liver- related death. We report here on a case of ductopenia-related sarcoidosis in which primary biliary cirrhosis and extrahepatic cholestasis have been carefully excluded. The patient follow up was 8 years. Although ursodesoxycholic acid appears to improve liver biochemistry it does not preclude the rapid occurrence of extensive fibrosis. A review of the literature of reported cases of ductopenia related to sarcoidosis is provided.

Key words: Sarcoidosis; Cholestasis ductopenia; Ursodeoxycholic acid

Peer reviewers: Wing-Kin Syn, MD, Hepatologist, 1GI Division, Duke University, Suite 1073, 595 LaSalle Street, Durham, NC 27710, United States

Farouj NE, Cadranel JFD, Mofredj A, Jouannaud V, Lahmiri M, Le Lann P, Cazier A. Ductopenia related liver sarcoidosis. *World J Hepatol* 2011; 3(6): 170-174 Available from: URL: http://www.wjgnet.com/1948-5182/full/v3/i6/170.htm DOI: http://dx.doi.org/10.4254/wjh.v3.i6.170

INTRODUCTION

Sarcoidosis is a systemic granulomatous disease which may involve many organs, although the lungs remain the most frequent and specific localization[1]. An incidence rate of 71/100 000 and prevalence of 2.0% was reported in a large recent study performed in black American women[2]. Asymptomatic liver involvement may be present in up to 95% of the patients and is usually limited to liver test abnormalities The most common histological lesions are noncaseating hepatic granulomas[3]. The occurrence of jaundice in sarcoidosis is rare. Imaging procedures and liver biopsy are clues to the pathogenesis of jaundice. Ductopenia associated with sarcoidosis has been reported in less than 15 cases and can progress to biliary cirrhosis and liver-related death.

CASE REPORT

A 54-year-old Caucasian woman was admitted in our hospital on June 2nd 1996 with abdominal pain, fever, jaundice,

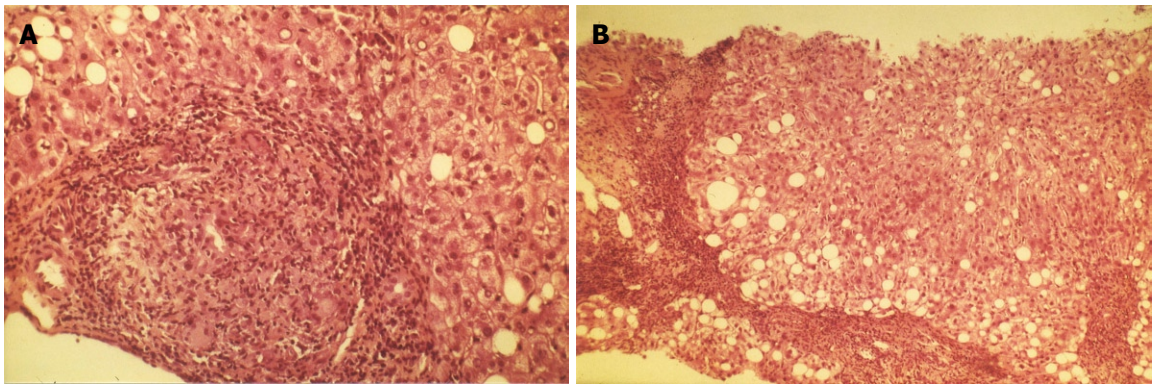


Figure 1 Hematoxilin Eosin Safran staining. A: × 40, non caseating granulomas with giant and epithelioid cells associated with periportal fibrosis and lobular chronic inflammatory cells are seen, as well as moderate steatosis (< 20%) without signs of steatohepatitis. There is no evidence of cirrhosis; B: × 20, distortion of the normal liver architecture, with bridging and annular fibrosis containing noncaseating granulomas including giant and epithelioid cells with significantly diminution in number of interlobular bile ducts are seen.

night sweats and weight loss that began in April. She was born in France and had never left that country. Her medical history was remarkable for obesity (body index: BMI 35kg/m^2), arterial hypertension and type 2 diabetes mellitus treated for several years with metformin(850 mg bid). In 1988, she underwent a cholecystectomy for (histologically proven) calculus cholecystitis. Liver biopsy was not performed at that time. There was no history of alcoholism, viral hepatitis exposure or blood transfusion. She has no risk factors for tuberculosis. Physical examination on admission showed a body temperature of 40°C and moderate scleral icterus. The abdomen was soft, without tenderness, there was no cutaneous rash, or flitting arthalgia. There was no hepatomegaly, splenomegaly, ascites or collateral venous circulation. Laboratory values on admission showed normal complete blood count and C-reactive protein 28, 9 mg/L [N < 10]. Bacteriological examination of blood, urine and stools was negative. Prothrombin index was 98%. Results of liver tests were as follows: total bilirubin, 63 μmol/L [N = 5-20](conjugated 50); gamma-glutamyl-transpeptidases, 52 times upper limit of normal (ULN) [N = 5-45]; alkaline phosphatases, 5 ULN L [N = 30-100 UI/L]; aspartate aminotransferases, 1.1 ULN [N = 6-40]; and alanine aminotransferases, 3 ULN [N = 6-45]. Protidogram showed a mild decrease in albumin levels (36 g/L; 37-42) and increased beta-globulins (16 g/L; 5-8); IgM was below 2 g/L. Stools were beige and the urine dark. Serologic tests for hepatitis A, B and C were negative. Screening for antinuclear antibodies, anti-smooth muscle antibodies and antimitochondrial antibodies was negative. The tuberculin test was mildly positive. Serum angiotensin converting enzyme was elevated, 60 U/mL [N = 20-40]. Abdominal ultrasonography showed homogeneous hepatomegaly without splenomegaly, ascites, or biliary tract dilatation.

Chest x-ray studies showed no pneumonia, and revealed bilateral hilar adenopathies. Thoracic computed tomography showed bilateral hilar adenopathies without lobar or interstitial pneumonia. Abdominal tomodensitometry showed moderate hepatomegaly without splenomegaly, no biliary tract dilatation, and no pancretic abnormality. Biliary tract endosonography did not show biliary tract abnormalities but revealed calcified mediastinal adenopathies. Biliary MRI scans were normal as were upper gastrointestinal endoscopy and colonoscopy. There was no improvement in liver biochemistry or physical condition in spite of parenteral administration of antibiotics. The transbronchial biopsy (June 10th 1996) showed multiple epithelioid granulomas in the bronchial mucosa with normal pulmonary parenchyma. A large transparietal liver biopsy with 10 to 12 portal tracts (June 15th 1996) revealed diffuse noncaseating granulomas including giant and epithelioid cells within the portal tracts and within the lobules, associated with periportal fibrosis without bridging, as well as lobular chronic inflammatory cells. These granulomas were not aggressive against the bile ducts. There was moderate pure macrovacuolar steatosis (< 20%) without signs of steatohepatitis or cholangitis. There was no cirrhosis (Figure 1A). The number of bile ducts was significantly reduced, suggesting ductopenia (between 30 to 50 % of the portal tracts did not contain bile ducts). This liver histological pattern, associated with the presence of multiple epithelioid granulomas in the bronchial mucosa, was compatible with the diagnosis of hepatic sarcoidosis rather than of primary biliary cirrhosis.

Therapy with an oral steroid (prednisolone), the standard treatment for sarcoidosis, was started at a dose of 0.5 mg/kg per day on June 17th. After one month of treatment, the fever had disappeared and the patient's condition had markedly improved. However, the initial abnormalities in liver biochemistry persisted. Ursodesoxycholic acid was added to the treatment on July 15th at a daily dose of 13 mg/kg, and induced a progressive improvement in liver function tests (Table 1). Prednisolone treatment was maintained a 7 mg per day. A subsequent liver biopsy performed after 16 mo (January 1998) of ursodiol treatment revealed noncaseating granulomas including giant and epithelioid cells, and progressive diminution in the number of interlobular bile ducts. Marked steatosis (> 50%) was noted, in part related to a 10 kg increase in body weight. Fibrosis in portal and periportal areas was present, but there was no pericellular fibrosis destroying bile ducts, through bridging and loss of lobular architecture. There was

Table 1 Clinical course and laboratory Findings after treatment with ursodeoxycholic acid in our patient

Treatment with UDCA	ALP UI/L (N+ 30 - 100)	GGT UI/L (N + 5 - 45)	ALT UI/L (N + 6 - 45)	AST UI/L (N + 6 - 40)	Jaundice[a]
1996-07-15	520	1210	150	48	+++
1996-09-24	370	966	89	26	+++
1996-12-23	226	249	29	21	++
1997-02-21	123	215	31	22	0
1997-04-22	113	210	27	43	0
1997-07-08	134	235	26	17	0
1999-04-16	143	258	47	34	0
2000-05-05	103	152	40	29	0
2001-01-10	113	167	35	31	0
2002-12-05	88	170	30	36	0
2003-06-19	92	181	23	29	0

[a]Degree of change: 0: Absent; ++: Moderate; +++: Marked; N: Value of laboratory; Abbreviations: ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transpeptidase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; UDCA: Ursodeoxycholic acid.

no balloon degeneration or Mallory bodies present. This process may eventually lead to secondary biliary cirrhosis (Figure 1B). At final follow up in November 2004, liver tests were normal, no GP 210 antibodieswere found, and abdominal ultrasonography showed moderate hepatomegaly.

DISCUSSION

In this report, the diagnosis of sarcoidosis was supported by abnormalities found in chest x-ray studies and the presence of multiple noncaseating granulomas in the liver. Diagnosis of hepatic sarcoidosis with ductopenia was based on liver histology and normal findingd on biliary tract endosonography and biliary MRI; thereby excluding common bile duct stone or primary sclerosing cholangitis.

Sepsis was unlikely to be the cause of cholestasis since there was no improvement in liver biochemistry or physical condition despite intravenous antibiotic treatment. The presence of a systemic disease was confirmed, with bilateral hilar adenopathies and bronchial involvement, and subsequent liver histology showing numerous and well-defined granulomas, suggesting sarcoidosis rather than primary biliary cirrhosis. The immunological features and the results of liver biopsy allowed us to exclude primary biliary cirrhosis,the main possible differential diagnosis.

Sarcoidosis is a multisystem disease of unknown etiology. The disease, more common in blacks than whites, is characterized pathologically by diffuse noncaseating granulomatous lesions[1,5]. Usual sites of disease are the lungs and the mediastinum, although the liver seems to be frequently involved[5]. Clinical signs of hepatic involvement may range from asymptomatic forms to chronic cholestasis portal hypertension[6,7,8] and signs of hepatic vein obstruction (Budd-Chiari syndrome[8]). Jaundice, however is very rare[9,10].

Hepatic sarcoidosis may resemble primary biliary cirrhosis, as both diseases can lead to chronic cholestasis and to biliary cirrhosis. Thus, it may be very difficult to distinguish between these two entities. Primary biliary cirrhosis characteristically affects women of middle age, but sarcoidosis particularly affects black males under 40 years[6]. Hepatomegaly has been reported in 2%-21% of patients with sarcoidosis[11,12]. These are often incidental findings, but abdominal pain, dizziness may suggest hepatic involvement[13]. The frequency of abnormal findings in liver function tests in patients with sarcoidosis is highly variable, ranging from 2%-60%[14]. Elevation in alkaline phosphatase activities is the most common laboratory indicator, being detected in up to one third of patients[10, 14]. Anti-mitochondrial antibodies are detected in primary biliary cirrhosis, but are absent in sarcoidosis[15].

In seronegative primary biliary cirrhosis, anti GP 210 antibodies are found in 47% of patients without anti-mitochondrial antibodies[16] but were absent from the serum of our patient. Absence of anti GP 210 antibodies was an additional surrogate marker against PBC diagnosis. Elevated serum angiotensin-converting enzyme levels (observed in our patient) favoured of the diagnosis of sarcoidosis[17], although elevated levels have been observed in patients with hepato-cellular diseases of varied aetiologies[17,18].

Despite a mild increase in ACE, tuberculosis was excluded by clinical, radiological, pathological data as well as by the outcome.

Histological features of liver sarcoidosis usually consist of well defined and abundant confluent noncaseating granulomas, predominantly in the portal and periportal areas, as well as mononuclear cell infiltration, hepatocyte injury, and foci of fibrosis or cirrhosis[19]. Severe intrahepatic cholestasis and bile ductopenia only occur in a subset of patients with advanced disease[4,14]. In a study of 100 patients with sarcoidosis and abnormal liver function tests, liver biopsies showed cholestasis in 58%, a necroticoinflammatory process in 41%, and vascular changes in 20% of the patients[3]. In the cholestatic group, 19% of the patients had bile lesions similar to those of primary biliary cirrhosis. An additional 13% of the patients had a dense periductal fibrosis without accompanying inflammation, similar to that usually seen in primary sclerosing

Table 2 Epidemiological, clinical features and outcome of reported cases with hepatic sarcoidosis and ductopenia

Ref.	Age (Year)/Sex/Race	Jaundice	Maximum ALP	Maximun AST	Clinical outcome
[4]	29/M/Black	Yes	10 X	1 X	Died 9 years after diagnosis
	31/M/Black	Yes	14 X	5 X	Died 11 years after diagnosis
	23/M/Black	Yes	30 X	13 X	Alive, portal hypertension
	20/M/Black	Yes	35 x	9 X	Died 10 years after first seen
	33/M/Black	Yes	30 X	6 X	Died 18 years after first seen
[20]	56/M/White	Yes	3 X	4 X	Alive, no symptoms
[21]	50/F/Black	Yes	6 X	2 X	Alive, portal hypertension
	46/M/Black	Yes	3 X	2 X	Alive, portal hypertension
[15]	-/M/	Yes	9 X	4 X	Alive, no symptoms
[22]	62/F/-	No	10 X	2 X	Alive, cirrhosis
[23]	38/F/White	Yes	12 X	3 X	Died 26 years after diagnosis
	40/M/-	Yes	10 X	4 X	Died 10 years after diagnosis
[5]	27/M/Black	Yes	10 X	3 X	Alive, portal hypertension
	28/M/Black	No	9 X	2 X	alive, disabled
	44/M/Black	No	7 X	2 X	Alive, well
	24/F/Black	Yes	7 X	1 X	Alive, well
[24]	52/M/Black	No	8 X	1 X	Died, 4 years after diagnosis
[25]	44/M/-	No	4 X	1, 5 X	Alive, cirrhosis
[26]	62/M/-	No	1, 8 X	ND	Died, 3 years after diagnosis
Present case	54/F/White	Yes	5 X	1 X	Alive, cirrhosis

X: Upper limit of normal; ND: Not done; Ref: Reference; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

cholangitis. A decreased number of interlobular bile ducts were noted in 37%[3] of liver specimens. The female gender of our patient, the absence of inflammatory bowel disease , the course of the disease , the normality of Biliary MRI and of liver the histological specimens, ruled out a primary or secondary biliary sclerosing cholangitis in our patient. The liver biopsy did not show florid lesions of bile ducts or disruption of the biliary epithelium although parenchyma granulomas, paucity of bile ducts, and intense fibrosis did resemble primary sclerosing chlolangitis. The syndrome of disappearing intrahepatic bile ducts or "ductopenia" associated with sarcoidosis has, to our knowledge, previously been reported in 19 patients[4,5,21-26].

Epidemiological and clinical features of theses cases are summarized in Table 2. The majority of patients were black men and their ages ranged from 20 to 62 years. Jaundice was present in 13 of 19 the patients (70%). Liver biopsies showed noncaseating granulomas, bile duct depletion and cholestasis in all of these patients.

The clinical outcome was poor with death occurring in 9 patients, chronic sequelae consisting of severe liver disease in 6 others, and four patients who were free of symptoms. In our case, the second liver histological examination showed portal and periportal fibrosis destroying bile ducts and causing ductopenia, which may lead to secondary biliary cirrhosis. The intrahepatic bile ducts are open to several form of attack. The mechanisms of destruction may be immunological, vascular, infective or chemical[27]. Among possible immunological causes of of destruction are PBC, graft-versus-host disease and sarcoidosis. PSC is usually associated with immunological features, but the hepatic histology is not that of an immunological disease[27]. Among possible chemical casues, a variety of therapeutic drugs have occasionally been associated with bile duct destruction and loss. These may include chlorpromazine, prochlorperazin, organic arsenicals and tolbutamid[28, 29].

Diseases with disappearing bile ducts have a long natural history and hepatocellular failure usually occurs late in the process. At this stage, hepatic transplantation may give good results[27,30,31]. Treatment of patients with hepatic sarcoidosis is difficult to evaluate due to the variable clinical course of the disease, the variable timing of therapy and the possibility of sampling error in flow-up liver biopsies[23]. Steroids could improve clinical symptoms, but Valla *et al* showed that they were ineffective in improving liver function tests[6]. In addition, they did not prevent the development of portal hypertension[6]. Murphy *et al*, reported on 2 patients with symptoms of small-duct sarcoid biliary disease who showed biochemical- but not histological- improvement under steroid therapy[5]. In our patient, the clinical symptoms improved, but biochemical changes persisted despite steroid treatment. When ursodiol was added, a decrease in jaundice and cholestasis were observed. However, the liver histological findings did not improve. In end-stage liver sarcoidosis, there orthotopic liver transplantation is indicated[32], although recurrent sarcoidosis has been reported in such cases[33].

REFERENCES

1 **Cozier YC**, Berman JS, Palmer JR, Boggs DA, Serlin DM, Rosenberg L. Sarcoidosis in black women in the United States: data from the Black Women's Health Study. *Chest* 2011; **139**: 144-150

2 **Chesnutt AN**. Enigmas in sarcoidosis. *West J Med* 1995; **162**: 519-526

3 **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

4 **Albu E**, Saraiya RJ, Carvajal SJ, Sehonanda A, Balar N, Gerst PH. Sarcoidosis presenting as obstructive jaundice. *Am Surg* 1995; **61**: 516-517

5 **Rudzki C**, Ishak KG, Zimmerman HJ. Chronic intrahepatic cholestasis of sarcoidosis. *Am J Med* 1975; **59**: 373-387
6 **Murphy JR**, Sjogren MH, Kikendall JW, Peura DA, Goodman Z. Small bile duct abnormalities in sarcoidosis. *J Clin Gastroenterol* 1990; **12**: 555-561
7 **Valla D**, Pessegueiro-Miranda H, Degott C, Lebrec D, Rueff B, Benhamou JP. Hepatic sarcoidosis with portal hypertension. A report of seven cases with a review of the literature. *Q J Med* 1987; **63**: 531-544
8 **Russi EW**, Bansky G, Pfaltz M, Spinas G, Hammer B, Senning A. Budd-Chiari syndrome in sarcoidosis. *Am J Gastroenterol* 1986; **81**: 71-75
9 **Bloom R**, Sybert A, Mascatello VJ. Granulomatous biliary tract obstruction due to sarcoidosis. Report of a case and review of the literature. *Am Rev Respir Dis* 1978; **117**: 783-787
10 **Baughman RP**. Sarcoidosis. Usual and unusual manifestations. *Chest* 1988; **94**: 165-170
11 **Kochar R**, Fallon MB. Pulmonary diseases and the liver. *Clin Liver Dis* 2011; **15**: 21-37
12 **Maddrey WC**, Johns CJ, Boitnott JK, Iber FL. Sarcoidosis and chronic hepatic disease: a clinical and pathologic study of 20 patients. *Medicine (Baltimore)* 1970; **49**: 375-395
13 **James DG**, Sherlock S. Sarcoidosis of the liver. *Sarcoidosis* 1994; **11**: 2-6
14 **Mayock RL**, Bertrand P, Morrison CE, Scott JH. Manifestations of sarcoidosis. Analysis of 145 patients, with a review of nine series selected from the literature. *Am J Med* 1963; **35**: 67-89
15 **Hercules HD**, Bethlem NM. Value of liver biopsy in sarcoidosis. *Arch Pathol Lab Med* 1984; **108**: 831-834
16 **Bandin O**, Courvalin JC, Poupon R, Dubel L, Homberg JC, Johanet C. Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. *Hepatology* 1996; **23**: 1020-1024
17 **Studdy P**, Bird R, James DG. Serum angiotensin-converting enzyme (SACE) in sarcoidosis and other granulomatous disorders. *Lancet* 1978; 2: 1331-1334
18 **Schweisfurth H**, Wernze H. Changes of serum angiotensin I converting enzyme in patients with viral hepatitis and liver cirrhosis. *Acta Hepatogastroenterol (Stuttg)* 1979; **26**: 207-210
19 **Johnson DA**, Diehl AM, Sjogren MH, Lazar J, Cattau EL, Smallridge RC. Serum angiotensin converting enzyme activity in evaluation of patients with liver disease. *Am J Med* 1987; **83**: 256-260
20 **Nakanuma Y**, Ohta G, Yamazaki Y, Doishita K. Intrahepatic bile duct destruction in a patient with sarcoidosis and chronic intrahepatic cholestasis. *Acta Pathol Jpn* 1979; **29**: 211-219
21 **Hughes GS**, Kataria YP, O'Brien TF. Sarcoidosis presenting as biliary cirrhosis: treatment with chlorambucil. *South Med J* 1983; **76**: 1440-1442
22 **Bass NM**, Burroughs AK, Scheuer PJ, James DG, Sherlock S. Chronic intrahepatic cholestasis due to sarcoidosis. *Gut* 1982; **23**: 417-421
23 **Thomas E**, Micci D. Chronic intrahepatic cholestasis with granulomas and biliary cirrhosis. Enigmatic disease and therapeutic dilemma. *JAMA* 1977; **238**: 337-338
24 **Pereira-Lima J**, Schaffner F. Chronic cholestasis in hepatic sarcoidosis with clinical features resembling primary biliary cirrhosis. Report of two cases. *Am J Med* 1987; **83**: 144-148
25 **Moreno-Merlo F**, Wanless IR, Shimamatsu K, Sherman M, Greig P, Chiasson D. The role of granulomatous phlebitis and thrombosis in the pathogenesis of cirrhosis and portal hypertension in sarcoidosis. *Hepatology* 1997; **26**: 554-560
26 **Romero-Gómez M**, Suárez-García E, Otero MA, Rufo MC, Castilla L, Guerrero P, López-Garrido J, Larraona JL, Fernández MC. Sarcoidosis, sclerosing cholangitis, and chronic atrophic autoimmune gastritis: a case of infiltrative sclerosing cholangitis. *J Clin Gastroenterol* 1998; **27**: 162-165
27 **Nakanuma Y**, Kouda W, Harada K, Hiramatsu K. Hepatic sarcoidosis with vanishing bile duct syndrome, cirrhosis, and portal phlebosclerosis. Report of an autopsy case. *J Clin Gastroenterol* 2001; **32**: 181-184
28 **Sherlock S**. The syndrome of disappearing intrahepatic bile ducts. *Lancet* 1987; **2**: 493-496
29 **Zimmerman HJ**. Drug-induced liver disease. In: Schiff ER, Sorrell M, Maddrey WC, eds. Schiff's Diseases of the liver. 8 th ed. Philadelphia: Lippincott-Raven Publishers, 1999: 973-1064
30 **Scheuer PJ**. Drugs and toxins. In: Scheuer PJ, ed. Liver biopsy interpretation. London: Bailliere Tindall, 1988: 99-112
31 **Zafrani ES**, Degott C. [Non-neoplastic diseases of the intrahepatic biliary tracts in adults]. *Ann Pathol* 1995; **15**: 348-356
32 **Poupon R**, Chazouillières O, Poupon RE. Chronic cholestatic diseases. *J Hepatol* 2000; **32 suppl 1**: 129-140
33 **Casavilla FA**, Gordon R, Wright HI, Gavaler JS, Starzl TE, Van Thiel DH. Clinical course after liver transplantation in patients with sarcoidosis. *Ann Intern Med* 1993; **118**: 865-866
34 **Pescovitz MD**, Jones HM, Cummings OW, Lumeng L, Leapman SB, Filo RS. Diffuse retroperitoneal lymphadenopathy following liver transplantation--a case of recurrent sarcoidosis. *Transplantation* 1995; **60**: 393-396

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Zhang L

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2011 June 27; 3(6): I
ISSN 1948-5182 (online)

Acknowledgments to reviewers of *World Journal of Hepatology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Hepatology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Sang Hoon Ahn, MD, PhD, Professor, Department of Internal Medicine, Yonsei University College of Medicine, 250 Sungsanno, Seodaemun-gu, Seoul 120-752, South Korea

Joel Faintuch, MD, PhD, Department of Gastroenterology, Hospital das Clinicas, ICHC, 9th Floor, Room 9077, Sao Paulo 05403-900, Brazil

Pietro Invernizzi, MD, PhD, Division of Internal Medicine and Hepatobiliary Immunopathology Unit, IRCCS Istituto Clinico Humanitas, via A. Manzoni 113, Rozzano 20089, Milan, Italy

Amedeo Lonardo, MD, Internal Medicine, Endocrinology, Metabolism and Geriatrics, University of Modena and Reggio Emilia, Modena 41100, Italy

Hatim Mohamed Yousif Mudawi, Associate Professor of Medicine, department of Internal medicine, Faculty of medicine, University of Khartoum, Sudan, PO BOX 2245, Khartoum, Sudan

Julia Peinado Onsurbe, Assistant Professor, Department of Biochemistry and Molecular Biiology, Faculty of Biology, University of Barcelona, Avda. Diagonal, 645, Barcelona 08028, Spain

Branko Stefanovic, PhD, Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL 32312, United States

Wing-Kin Syn, MD, Hepatologist, 1GI Division, Duke University, Suite 1073, 595 LaSalle Street, Durham, NC 27710, United States

Hongzhi Xu, Dr., Massachusetts General Hospital, 51 Blossom Street, Room 435, Boston, MA 02148, United States

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2011 June 27; 3(6): I
ISSN 1948-5182 (online)

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011
Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011
San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11
Regensburg 93053, Germany

January 28-29, 2011
9. Gastro Forum München
Munich, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference
Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011
Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation
Dublin, Ireland

March 3-5, 2011
42nd Annual Topics in Internal Medicine
Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings
Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011
Birmingham, England, United Kingdom

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011
Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform
Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011
Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease
San Diego, CA 94143, United States

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition
Riyadh, Saudi Arabia

May 7-10, 2011
Digestive Disease Week
Chicago, IL 60446, United States

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671
Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course
Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn,
Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011
Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV
SPIGC, II ESYS
Napoli, Italy

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer
Barcelona, Spain

October 19-29, 2011
Cardiology & Gastroenterology Tahiti 10 night CME Cruise
Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week
Stockholm, Sweden

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course
Washington, DC 20001, United States

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2011 June 27; 3(6): I-V
ISSN 1948-5182 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Hepatology (*World J Hepatol, WJH*, online ISSN 1948-5182, DOI: 10.4254), is a monthly, openaccess, peer-reviewed journal supported by an editorial board of 573 experts in hepatology from 46 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJH* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJH* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJH* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The major task of *WJH* is to rapidly report the most recent results in basic and clinical research on hepatology, specifically including autoimmune, cholestatic and biliary disease, cirrhosis and its complications, liver biology/pathobiology, liver failure, growth , liver failure/cirrhosis/portal hypertension, liver fibrosis, hepatitis B and C virus infection, hepatocellular carcinoma, biliary tract disease, transplantation, genetics, epidemiology, microbiology and inflammatory disorders, molecular and cell biology, nutrition, geriatric hepatology, pediatric hepatology, steatohepatitis and metabolic liver disease, diagnosis and screening, endoscopy, imaging and advanced technology.

Columns

The columns in the issues of *WJH* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in hepatology; (9) Brief Article: To briefly report the novel and innovative findings in hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJH*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of hepatology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in hepatology.

Name of journal

World Journal of Hepatology

ISSN

ISSN 1948-5182 (online)

Indexed and Abstracted in

PubMed Central, PubMed, Digital Object Identifer, and Directory of Open Access Journals.

Published by

Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing

Statisital review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement

In the interests of transparency and to help reviewers assess any potential bias, *WJH* requires authors of all papers to declare any competing commercial, personal, political, in tellectual, or religious interestsin relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent

Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should comform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Bo ok Antiqua with ample margins. 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 are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, 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 copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1948-5182 office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjh@wjgnet.com, or by telephone: +86-10-59080038. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Me-

dical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g., Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJH*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-5182/g_info_list.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under 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. A 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 sometimes as 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 manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, 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 cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in boldfaced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in boldfaced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (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

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **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

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **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)

11 **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

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 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/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or 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 υ (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 for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107115140.htm.

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 explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H. pylori*, *E coli*, *etc*.

Examples for paper writing

Editorial: http://www.wjgnet.com/1948-5182/g_info_20100316080004.htm

Frontier: http://www.wjgnet.com/1948-5182/g_info_20100315103153.htm

Topic highlight: http://www.wjgnet.com/1948-5182/g_info_20100316080006.htm

Observation: http://www.wjgnet.com/1948-5182/g_info_20100107112630.htm

Guidelines for basic research: http://www.wjgnet.com/1948-5182/g_info_20100315103748.htm

Guidelines for clinical practice: http://www.wjgnet.com/1948-5182/g_info_20100315103829.htm

Review: http://www.wjgnet.com/1948-5182/g_info_20100107112834.htm

Original articles: http://www.wjgnet.com/1948-5182/g_info_20100107113351.htm

Brief articles: http://www.wjgnet.com/1948-5182/g_info_20100315104523.htm

Case report: http://www.wjgnet.com/1948-5182/g_info_20100107113649.htm

Letters to the editor: http://www.wjgnet.com/1948-5182/_info_20100107114003.htm

Book reviews: http://www.wjgnet.com/1948-5182/g_info_20100315105017.htm

Guidelines: http://www.wjgnet.com/1948-5182/g_info_20100315105107.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJH*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office

World Journal of Hepatology
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1948-5182/g_info_20100107114726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107114601.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJH will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

WJH is an international, peer-reviewed, Open-Access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, book reviews and letters to the editor are published free of charge.