

# World Journal of *Clinical Cases*

*World J Clin Cases* 2014 July 16; 2(7): 240-315



## Editorial Board

2012-2016

The *World Journal of Clinical Cases* Editorial Board consists of 519 members, representing a team of worldwide experts in clinical medical research. They are from 55 countries, including Albania (1), Australia (8), Bangladesh (3), Belgium (3), Botswana (1), Brazil (10), Bulgaria (1), Canada (11), China (24), Colombia (2), Croatia (4), Cuba (1), Czech (2), Egypt (5), France (5), Germany (14), Greece (15), Hungary (1), India (56), Indonesia (1), Iran (11), Iraq (1), Ireland (1), Israel (5), Italy (56), Japan (33), Lebanon (3), Malaysia (2), Mexico (1), Morocco (2), Netherlands (3), New Zealand (1), Nigeria (1), Oman (1), Pakistan (1), Peru (2), Poland (4), Portugal (3), Qatar (1), Romania (3), Saudi Arabia (4), Serbia (6), Singapore (3), Slovakia (2), Slovenia (1), South Korea (27), Spain (11), Sudan (1), Taiwan (21), Thailand (2), Trinidad and Tobago (1), Tunisia (1), Turkey (28), United Kingdom (26), and United States (82).

### EDITORS-IN-CHIEF

Giuseppe Di Lorenzo, *Naples*  
Jan Jacques Michiels, *Rotterdam*  
Sandro Vento, *Gaborone*  
Shuhei Yoshida, *Boston*

### GUEST EDITORIAL BOARD MEMBERS

Hung-Yang Chang, *Hsinchu*  
Ning-Chia Chang, *Kaohsiung*  
Yao-Lung Chang, *Taoyuan*  
Chang-Han Chen, *Kaohsiung*  
Shao-Tsu Chen, *Hualien*  
Yen-Hsu Chen, *Kaohsiung*  
Kuen-Bao Chen, *Taichung*  
Yi-Ming Chen, *Taipei*  
Chih-Chien Chin, *Taoyuan*  
I-Ching Chou, *Taichung*  
Jun-Te Hsu, *Taoyuan*  
Shu-Pin Huang, *Kaohsiung*  
Chi-Wen Juan, *Taichung*  
Chih-Yuan Lin, *Taipei*  
Chiung-Chyi Shen, *Taichung*  
Jim Jinn-Chyuan Sheu, *Taichung*  
Bing-Wen Soong, *Taipei*  
Hwei-Fang Tien, *Taipei*  
Rong Kung Tsai, *Hualien*  
Han-Ping Wu, *Taichung*  
Hsu-Heng Yen, *Changhua*

### MEMBERS OF THE EDITORIAL BOARD



**Albania**

Ridvan Hamid Alimehmeti, *Tirana*



**Australia**

Roy Gary Beran, *Sydney*  
Jian Cheng, *Melbourne*  
Devang Jitendra Desai, *Brisbane*  
Manuel B Graeber, *Sydney*  
Finlay Alistair Macrae, *Victoria*  
Harrison Scott Weisinger, *Victoria*  
Harunor Rashid, *Sydney*



**Bangladesh**

Forhad Hossain Chowdhury, *Dhaka*  
Md Jafrul Hannan, *Chittagong*  
Aliya Naheed, *Dhaka*



**Belgium**

Guy Cheron, *Brussels*  
Yves Jacquemyn, *Edegem*  
Jean-Yves Luc Reginster, *Angleur*



**Botswana**

Guy Cheron, *Brussels*



**Brazil**

Everson Luiz De Almeida Artifon, *Sao Paulo*  
Juliano Julio Cerci, *Curitiba*

Luciano Pamplona de Góes, *Fortaleza*  
Márcio Ajudarte Lopes, *Piracicaba*  
Jose Mario Franco de Oliveira, *Rio de Janeiro*  
Daniel Cesar de Araujo Santos, *Rio de Janeiro*  
Hélio Afonso Ghizoni Teive, *Curitiba*  
Eduardo Neubarth Trindade, *Porto Alegre*  
Fabio Francesconi do Valle, *Manaus*  
Flavia Mariana Valente, *Sao Jose do Rio Preto*



**Bulgaria**

Plamen Kostov Nedev, *Varna*



**Canada**

Mark Otto Baerlocher, *Barrie*  
Kunihiko Hiraiwa, *Vancouver*  
Ali Izadpanah, *Quebec*  
Gang Li, *Vancouver*  
Habib-Ur-Rehman, *Regina*  
Abdul Qayyum Rana, *Toronto*  
Consolato Sergi, *Alberta*  
Rashmi Singh, *Vancouver*  
Jennifer L Spratlin, *Alberta*  
Ted L Tewfik, *Montreal*  
Sam Wiseman, *Vancouver*



**China**

Shiu-Yin Cho, *Hong Kong*  
Lian Duan, *Beijing*  
Lee Fung Yee Janet, *Hong Kong*  
David Harolo Garfield, *Shanghai*

Yong-Song Guan, *Chengdu*  
 Guo-Rong Han, *Nanjing*  
 Bin Jiang, *Beijing*  
 Alice Pik Shan Kong, *Hong Kong*  
 Jian-Jun Li, *Beijing*  
 De-Zhi Mu, *Chengdu*  
 Simon Siu-Man Ng, *Hong Kong*  
 Shi-Su Sheng, *Beijing*  
 Huai-Yin Shi, *Beijing*  
 Xue-Ying Sun, *Harbin*  
 Xue-Rui Tan, *Shantou*  
 Gang Wang, *Chengdu*  
 Feng Wang, *Shanghai*  
 Nian-Song Wang, *Shanghai*  
 Ge Xiong, *Beijing*  
 Zheng-Feng Yin, *Shanghai*  
 Qing Zhang, *Jingzhou*  
 Ming-Hua Zheng, *Wenzhou*  
 Jun Zhong, *Shanghai*  
 Yan-Ming Zhou, *Xiamen*



#### Colombia

Iván Darío Vélez Bernal, *Medellín*  
 Carlos Alberto Calderón-Ospina, *Bogota*



#### Croatia

Iva Brcic, *Zagreb*  
 Srđana Čulić, *Spinciceva*  
 Tomislav Kulis, *Zagreb*  
 Zvonimir Lovrić, *Zagreb*



#### Cuba

Alain Cruz Portelles, *Holguin*



#### Czech

David Bludovský, *Plzen*  
 Antonin Marik, *Prague*



#### Egypt

Farid Mohammed Sabry El-Askary, *Cairo*  
 Reda Abd Elhady Hemida, *Mansoura*  
 Sherifa Ahmad Hamed, *Assiut*  
 Ahmad Abd-Elgawad Nofal, *Zagazig*  
 Mohamed Ismail Seleem, *Cairo*



#### France

I Alain Braillon, *Amiens*  
 Jean-François Bosset, *Besançon*  
 Isabelle Andrée Chemin, *Lyon*  
 Emile Jean-François, *Boulogne*  
 Christophe Martinaud, *Clamart*



#### Germany

Sebastian Decker, *Hannover*  
 Andreas Martin Fette, *Weissach im Tal*  
 Michael Froehner, *Dresden*  
 Wolf Christoph Mueller, *Leipzig*  
 Andres Hao Ming Neuhaus, *Berlin*  
 Arndt Hartmann, *Erlangen*  
 Dirk M Hermann, *Essen*  
 Karl-Anton Kreuzer, *Berlin*  
 Ingo Stefan Nölte, *Mannheim*  
 Andreas G Schreyer, *Regensburg*  
 Crispin Schneider, *Bristol*  
 Hans-Joachim Schmoll, *Halle*  
 Martin Paul Schencking, *Witten*  
 Mathias Z Strowski, *Berlin*



#### Greece

Andrew P Andonopoulos, *Patras*  
 Dimitrios Daoussis, *Patras*  
 Ioanna Dimopoulou, *Athens*  
 Moses S Elisaf, *Ioannina*  
 Costas Fourtounas, *Rio-Patras*  
 Olga-Elpis Kolokitha, *Thessaloniki*  
 Sophia Lionaki, *Athens*  
 Marilita M Moschos, *Athens*  
 Michail N Varras, *Athens*  
 Nikolaos Papanas, *Alexandroupolis*  
 Athanasios Papatsoris, *Athens*  
 Zervoudis Stephane, *Athens*  
 Konstantinos Tepetes, *Larissa*  
 Apostolos Tsapas, *Thessaloniki*  
 Dimitrios Vavilis, *Thessaloniki*



#### Hungary

Tibor Hortobágyi, *Debrecen*



#### India

Subrat Kumar Achaya, *New Delhi*  
 Amit Arvind Agrawal, *Nasik*  
 Hena A Ansari, *Aligarh*  
 MS Ansari, *Lucknow*  
 Laxminarayan Bhadrani, *Calicut*  
 Ashu Seith Bhalla, *New Delhi*  
 Sachin Anil Borkar, *New Delhi*  
 Bhuvan Chanana, *New Delhi*  
 Kanishka Das, *Bangalore*  
 Reena Das, *Chandigarh*  
 Nilay Kanti Das, *Kolkata*  
 Deep Dutta, *Kolkata*  
 Mimi Gangopadhyay, *Siliguri*  
 Rakesh Garg, *New Delhi*  
 Sandeep Grover, *Chandigarh*  
 Mahendra Singh Hada, *Rajasthan*  
 P Hazarika, *Manipal*  
 Sachin Bhalchandra Ingle, *Latur*  
 Parwez Sajad Khan, *Srinagar*  
 Pradeep Kumar, *Bangalore*  
 Amol Lunkad, *Pune*

Dale A Maharaj, *Trinidad*  
 Nikhil Marwah, *Rajasthan*  
 Meena Gupta, *New Delhi*  
 Amit Kumar Mishra, *Indore*  
 Soma Mukherjee, *Mumbai*  
 Deb Sanjay Nag, *Jamshedpur*  
 Kushal Naha, *Karnataka*  
 Janardhanan C Narayanaswamy, *Bangalore*  
 Soubhagya Ranjan Nayak, *Nadia*  
 Narendra Pamidi, *Karnataka*  
 Murali Prabhakaran Vettath, *Kerala*  
 Samir Kumar Praharaj, *Karnataka*  
 Peralam Yegneswaran Prakash, *Manipal*  
 C S Pramesh, *Mumbai*  
 Kishore Puthezhath, *Kerala*  
 Harbans Singh Randhawa, *Delhi*  
 M Rangarajan, *Coimbatore*  
 Sayantan Ray, *Kolkata*  
 Bharat Rekhi, *Maharashtra*  
 S Sharija, *Thiruvananthapuram*  
 Dhananjaya Sabat, *New Delhi*  
 Sachin Chakradhar Sarode, *Pune*  
 Ashish Sharma, *Coimbatore*  
 Hakim Irfan Showkat, *Srinagar*  
 Rikki Singal, *Mullana*  
 Deepak Kumar Singh, *Lucknow*  
 Yashpal Singh, *Meerut*  
 Naorem Gopendro Singh, *New Delhi*  
 Shyam Sundar, *Varanasi*  
 Naveen S Tahasildar, *Hubli*  
 Devinder Mohan Thappa, *Pondicherry*  
 Pradeep Vaideeswar, *Mumbai*  
 Mukul Vij, *Kanpur*  
 Rajesh Vijayvergiya, *Chandigarh*  
 B Viswanatha, *Bangalore*



#### Indonesia

Coen Pramono, *Surabaya*



#### Iran

Masoud Amiri, *Shahrekord*  
 Mostafa Ghanei, *Tehran*  
 Mahdi Malekpour, *Tehran*  
 Setareh Mamishi, *Tehran*  
 Afshin Mohammadi, *Urmia*  
 Seyyed Amin Ayatollahi Mousavi, *Kerman*  
 Mohammad Taher Rajabi, *Tehran*  
 Amin Saburi, *Tehran*  
 Maryam Sahebari, *Mashhad*  
 Payman Vahedi, *Mashad*  
 Amir Reza Vosoughi, *Shiraz*



#### Iraq

Bassim Irheim Mohammad, *Al-Qadisiya*



#### Ireland

Robbie Seton Rowan Woods, *Dublin*

**Israel**

Nimer Najib Assy, *Safed*  
 Gil Bar-Sela, *Haifa*  
 Itzhak Braverman, *Hadera*  
 Eyal Itshayek, *Jerusalem*  
 Gary Michael Ginsberg, *Jerusalem*

**Italy**

Giovanni Addolorato, *Rome*  
 Piero Luigi Almasio, *Palermo*  
 Francesco Angelico, *Rome*  
 Marialuisa Appetecchia, *Rome*  
 Valeria Barresi, *Messina*  
 Gabrio Bassotti, *San Sisto*  
 Paolo Boffano, *Turin*  
 Maria Luisa Brandi, *Florence*  
 Michelangelo Buonocore, *Pavia*  
 Giovanni Cammarota, *Rome*  
 Isidoro Di Carlo, *Catania*  
 Andrea Ciorba, *Ferrara*  
 Lucio Cocco, *Bologna*  
 Carlo Colosimo, *Rome*  
 Alfredo Conti, *Messina*  
 Giovanni Conzo, *Naples*  
 Gennaro Cormio, *Bari*  
 Alessandro Federico, *Naples*  
 Gabriella Maria Ferrandina, *Rome*  
 Davide Firinu, *Cagliari*  
 Caterina Foti, *Bari*  
 Gennaro Galizia, *Naples*  
 Silvio Garattini, *Milan*  
 Giampietro Gasparini, *Roma*  
 Luigi De Gennaro, *Rome*  
 Giorgio Ghilardi, *Milano*  
 Domenico Girelli, *Verona*  
 Biondi Zoccai Giuseppe, *Latina*  
 Carlo Lajolo, *Rome*  
 Alessandro Landi, *Rome*  
 Salvatore Leonardi, *Catania*  
 Carmela Loguerzio, *Naples*  
 Marianna Luongo, *Potenza*  
 Zippi Maddalena, *Rome*  
 Roberto Manfredini, *Ferrara*  
 Annunziato Mangiola, *Roma*  
 Elia De Maria, *Carpi*  
 Marco Mazzocchi, *Perugia*  
 Roberto Luca Meniconi, *Rome*  
 Marco Milone, *Naples*  
 Paolo Nozza, *Genoa*  
 Pier Paolo Panciani, *Brescia*  
 Desire' Pantalone, *Firenze*  
 Raffale Pezzilli, *Bologna*  
 Giorgina Barbara Piccoli, *Torino*  
 Roberto Pola, *Rome*  
 Marco Romano, *Napoli*  
 Gianantonio Saviola, *Castel Goffredo*  
 Stefania Scala, *Naples*  
 Leonardo A Sechi, *Udine*  
 Matteo Tebaldi, *Ferrara*  
 Riccardina Tesse, *Bari*

Tiziano Testori, *Milano*  
 Gian Vincenzo Zuccotti, *Milan*

**Japan**

Ukei Anazawa, *Ichikwa-shi*  
 Junichi Asaumi, *Okayama*  
 Takashi Asazuma, *Saitama-ken*  
 Norihiro Furusyo, *Fukuoka*  
 Masaru Ishida, *Yokohama*  
 Tatsuaki Ishiguro, *Tokyo*  
 Hajime Isomoto, *Nagasaki*  
 Yokoyama Junkichi, *Sendai*  
 Keita Kai, *Saga*  
 Terumi Kamisawa, *Tokyo*  
 Tatsuo Kanda, *Niigata*  
 Shigeyuki Kawa, *Matsumoto*  
 Kazushi Kishi, *Wakayama-city*  
 Satoru Kyo, *Ishikawa*  
 Nozomi Majima, *Osaka*  
 Kenji Miki, *Tokyo*  
 Atsushi Nakajima, *Tokyo*  
 Rui Niimi, *Tsu city*  
 Masaharu Nomura, *Tokyo*  
 Kenoki Ohuchida, *Fukuoka*  
 Morishita Ryuichi, *Osaka*  
 Yosuke Sato, *Niigata*  
 Mitsushige Sugimoto, *Hamamatsu*  
 Haruhiko Sugimura, *Hamamatsu*  
 Keisuke Uehara, *Nagoya*  
 Manabu Watanabe, *Tokyo*  
 Takayuki Yamamoto, *Yokkaichi*  
 Yoshihito Yokoyama, *Hiroaki*  
 Junkichi Yokoyama, *Tokyo*  
 Han-Seung Yoon, *Nagano*  
 Kiyoshi Yoshino, *Osaka*  
 Yuichi Kasai, *Tsu city*  
 Yuzuru Niibe, *Sagamihara-shi*

**Lebanon**

Maroun Miled Abou-Jaoude, *Beirut*  
 Kassem A Barada, *Beirut*  
 Raja Sawaya, *Beirut*

**Malaysia**

Iman Salahshourifar, *Kubang Kerian*  
 Mohamad Nasir Shafiee, *Kuala Lumpur*

**Mexico**

Ernesto Roldan-Valadez, *Mexico*

**Morocco**

Alae El Koraichi, *Rabat*  
 Faycal Lakhdar, *Rabat*

**Netherlands**

Sijens Paul Eduard, *Groningen*  
 Paul E Sijens, *Groningen*

**New Zealand**

Rita Rita Krishnamurthi, *Auckland*

**Nigeria**

Shamsideen Abayomi Ogun, *Lagos*

**Oman**

Itrat Mehdi, *Muscat*

**Pakistan**

Sabiha Anis, *Karachi*

**Peru**

Eduardo Gotuzzo, *Lima*  
 Eduardo Salazar-Lindo, *Lima*

**Poland**

Łukasz Stanisław Matuszewski, *Lublin*  
 Tadeusz Robak, *Ciolkowskiego*  
 Adam Wysokiński, *Lodz*  
 Witold Antoni Zatoński, *Warsaw*

**Portugal**

Jorge Alves, *Braga*  
 Gustavo Marcondes Rocha, *Porto*  
 Zacharoula Sidiropoulou, *Barreiro*

**Qatar**

Fahmi Yousef Khan, *Doha*

**Romania**

Simona Gurzu, *Targu-Mures*  
 Doina Piciu, *Cluj-Napoca*  
 Mugurel Constantin Rusu, *Bucharest*

**Saudi Arabia**

Ahmed Alkhani, *Riyadh*  
 Iqbal Abdulaziz Bukhari, *Alkhobar*  
 Mohamed Fahmy Ibrahim, *Riyadh*

Jyothi Tadakamadla, *Hyderabad*



#### **Serbia**

Ivona Milorad Djordjevic, *Nis*  
Jelena Lazar Lazic, *Belgrade*  
Djordje Radak, *Beograd*  
Boban Stanojevic, *Belgrade*  
Mihailo Ilija Stjepanovic, *Belgrade*  
Momcilo Pavlovic, *Subotica*



#### **Singapore**

Wei-Sheng Chong, *Singapore*  
Khek-Yu Ho, *Singapore*  
Yong Kuei Lim, *Singapore*



#### **Slovakia**

Michal Mego, *Bratislava*  
Ivan Varga, *Bratislava*



#### **Slovenia**

Pavel Skok, *Maribor*



#### **South Korea**

Young-Seok Cho, *UiJeongbu*  
Tae Hyun Choi, *Seoul*  
Yeun-Jun Chung, *Seoul*  
Ki-Baik Hahm, *Seoul*  
Seung-Jae Hyun, *Seongnam*  
Soo Bin Im, *Bucheon*  
Soung Won Jeong, *Seoul*  
Choun-Ki Joo, *Seoul*  
Chang Moo Kang, *Seoul*  
Seung Taik Kim, *Chungbuk*  
Byung-Wook Kim, *Incheon*  
Myoung Soo Kim, *Seoul*  
Gwi Eon Kim, *Seoul*  
Gyeong-Moon Kim, *Seoul*  
Hahn Young Kim, *Seoul*  
Won Seog Kim, *Seoul*  
Yoon Jun Kim, *Seoul*  
Yun-Hee Kim, *Seoul*  
Sun-Young Lee, *Seoul*  
Sang Chul Lim, *Hwasun-gun*  
Seung Sam Paik, *Seoul*  
Jae Yong Park, *Daegu*  
Jong-Ho Park, *Goyang*  
Jun-Beom Park, *Seoul*  
Songhae Hae Ryong, *Seoul*  
Chan Sup Shim, *Seoul*  
Hwaseung Yoo, *Daejeon*



#### **Spain**

Adrià Arboix, *Barcelona*

FJA Artiles, *Las Palmas de Gran Canaria*

Manuel Benito, *Madrid*

Vicente Carreño, *Madrid*

Rosa Corcoy, *Barcelona*

Exuperio Díez-Tejedor, *Madrid*

Luis Ignacio González Granado, *Madrid*

Carlos Alberto Dussan Luberth, *Torre Vieja*

Juan de Dios Molina Martín, *Madrid*

Sergio Fernández-Pello Montes, *Gijón*

Tomás Sobrino, *Santiago de Compostela*



#### **Sudan**

Samir MH Shaheen, *Khartoum*



#### **Thailand**

Sarunyou Chusri, *Songkhla*

Weekitt Kittisupamongkol, *Bangkok*



#### **Trinidad and Tobago**

Dale Andrew Maharaj, *Port of Spain*



#### **Tunisia**

Makram Koubaa, *Sfax*



#### **Turkey**

Sami Akbulut, *Diyarbakir*

Tamer Akça, *Mersin*

Cengiz Akkaya, *Bursa*

Ahmet Baydin, *Samsun*

Hasan Belli, *Istanbul*

Serbüent Gökhan Beyaz, *Sakarya*

GK Cakmak, *Kozlu Zonguldak*

Turgay Celik, *Ankara*

Yasemin Benderli Cihan, *Kayseri*

Ömür Dereci, *Ankara*

Mehmet Doganay, *Kayseri*

F Neslihan İnal Emiroğlu, *İzmir*

Aylin Türel Ermercan, *Manisa*

Kadir Ertem, *Malatya*

Aydın Gulses, *Canakkale*

Mustafa Koray Gumus, *Kayseri*

Ramazan Kahveci, *Kırıkkale*

Saadettin Kiliçkap, *Ankara*

Fatih Kucukdurmaz, *Istanbul*

Ashihan Küçükler, *Ankara*

Nuray Bayar Muluk, *Ankara*

Orhan Veli Ozkan, *Sakarya*

Zeynep Özkurt-Kayahant, *Istanbul*

Mustafa Sahin, *Ankara*

İbrahim Sakçak, *Ankara*

Feyzi Birol Sarica, *Adana*

Selim Sözen, *Kayseri*

Murat Ugurlucan, *Istanbul*



#### **United Kingdom**

Henry Dushan Atkinson, *London*

Ioannis G Baraboutis, *Cambridgeshire*

I Beegun, *London*

Ricky Harminder Bhogal, *Birmingham*

Kuntal Chakravarty, *Romford*

Deyaa Elsandabese, *Harlow*

Radwan Faraj, *Moorgate Road-Rotherham*

Babatunde Abiodun Gbolade, *Leeds*

Sanju George, *Birmingham*

David Julian Alexander Goldsmith, *London*

Nadey S Hakim, *London*

Koshy Jacob, *Boston*

Anastasios Koulaouzidis, *Edinburgh*

Andrew Richard Lisle Medford, *Bristol*

Panagiotis Peitsidis, *Southend Essex*

Rahul Tony Rao, *London*

Francis Paul Rugman, *Preston*

Khaled Maher Sarraf, *London*

Yousef Shahin, *Hull*

Alexa Shipman, *Birmingham*

Badri Man Shrestha, *Sheffield*

Herrick J Siegel, *Birmingham*

Leonello Tacconi, *London*

Jagdeep Singh Virk, *Harrow*

James Chiun Lon Wong, *Manchester*

Kimia Ziahosseini, *Liverpool*



#### **United States**

Doru Traian Alexandrescu, *San Diego*

Naim Alkhouri, *Cleveland*

Mohammad M Alsolaiman, *Orem Utah*

Bhupinder S Anand, *Houston*

Suresh J Antony, *Oregon*

Normadeane Armstrong, *Rockville Centre*

Wilbert Solomon Aronow, *Valhalla*

Hossam M Ashour, *Detroit*

Rajendra Badgaiyan, *Buffalo*

Joseph Robert Berger, *Lexington*

Dennis A Bloomfield, *New York*

Neil Box, *Denver*

Jeffrey Alan Breall, *Indianapolis*

Susana M Campos, *Boston*

Robert Carter III, *San Antonio*

Kaisorn Lee Chaichana, *Baltimore*

Antonio Joseph Chamoun, *Coatesville*

Vince Clark, *Albuquerque*

C Donald Combs, *Norfolk*

Suzanne Marie Crumley, *Houston*

Parakkal Deepak, *Evanston*

Yuchuan Ding, *Detroit*

Konstantin Hristov Dragnev, *Lebanon*

Cecilia Luminita Dragomir, *New York*

Konstantinos P Economopoulos, *Boston*

James M Ford, *Stanford*

Yun Gong, *Houston*

Zeba Hasan Hafeez, *Novato*

Ardeshtir Hakam, *Tampa*

Jaclyn Frances Hechtman, *New York*

T Patrick Hill, *New Brunswick*

Hitoshi Hirose, *Philadelphia*

Elias Jabbour, *Houston*

Robert Thomas Jensen, *Bethesda*

Huanguang Jia, *Florida*  
Zhong Jiang, *Worcester*  
Theodoros Kelesidis, *Los Angeles*  
Kusum K Kharbanda, *Omaha*  
Praveen Kumar, *Chicago*  
Julius Gene Silva Latorre, *Syracuse*  
Guojun Li, *Houston*  
Yaling Liu, *Rochester*  
Marios-Nikolaos Lykissas, *New York*  
Kenneth Maiese, *Newark*  
Serge Peter Marinkovic, *Lafayette*  
Charles Christian Matouk, *New Haven*  
Kapil Mehta, *Houston*  
Zaher Merhi, *Burlington*  
Ayse Leyla Mindikoglu, *Baltimore*  
Roberto Nicolas Miranda, *Houston*

Majaz Moonis, *Worcester*  
Assad Movahed, *Greenville*  
Mohammad Reza Movahed, *Tucson*  
Saleh A Naser, *Orlando*  
Srinivasan Paramasivam, *New York*  
Edwin Melencio Posadas, *Los Angeles*  
Xiaofa Qin, *Newark*  
Michel Elias Rivlin, *Jackson*  
Jae Y Ro, *Houston*  
Bruce Samuel Rudy, *Hershey*  
Abdulaziz Sachedina, *Charlottesville*  
Ravi Prakash Sahu, *Indiana*  
Michael William Schlund, *Baltimore*  
Eric Lee Scott, *Indianapolis*  
Volney Leo Sheen, *Boston*  
Ilke Sipahi, *Cleveland*

Subbaya Subramanian, *Minneapolis*  
Jessica D Sun, *South San Francisco*  
Ulas Sunar, *Buffalo*  
Scott Tenner, *Brooklyn*  
Diana Olguta Treaba, *Providence*  
Richard Gary Trohman, *Chicago*  
Ming C Tsai, *New York*  
Vassiliy Tsytsarev, *Baltimore*  
Howard J Worman, *New York*  
Jun Yao, *Naperville*  
Shahram Yazdani, *Los Angeles*  
Panitan Yossuck, *Morgantown*  
Stanley Zaslau, *Morgantown*  
Sheng Zhang, *New Haven*  
Xinmin Zhang, *Philadelphia*





## Contents

Monthly Volume 2 Number 7 July 16, 2014

<b>REVIEW</b>	240	Infectious burden and atherosclerosis: A clinical issue <i>Sessa R, Di Pietro M, Filardo S, Turriziani O</i>
<b>MINIREVIEWS</b>	250	Is there a role for fish oil in inflammatory bowel disease? <i>Farrukh A, Mayberry JF</i>
	253	Critical review of topical management of oral hairy leukoplakia <i>Brasileiro CB, Abreu MHNG, Mesquita RA</i>
<b>EVIDENCE-BASED MEDICINE</b>	257	Association between resting energy expenditure, psychopathology and HPA-axis in eating disorders <i>Castellini G, Castellani W, Lelli L, Lo Sauro C, Dini C, Lazzeretti L, Bencini L, Mannucci E, Ricca V</i>
<b>OBSERVATIONAL STUDY</b>	265	Role of ethnicity in social anxiety disorder: A cross-sectional survey among health science students <i>De Jager P, Suliman S, Seedat S</i>
	272	Cut-off of body mass index and waist circumference to predict hypertension in Indian adults <i>Midha T, Krishna V, Nath B, Kumari R, Rao YK, Pandey U, Kaur S</i>
<b>CASE REPORT</b>	279	Perirenal extra-adrenal myelolipoma <i>Hajiran A, Morley C, Jansen R, Kandzari S, Bacaj P, Zaslau S, Cardinal J</i>
	284	Verrucous carcinoma of the esophagus: A case report and literature review <i>Ramani C, Shah N, Nathan RS</i>
	289	360° fusion for realignment of high grade cervical kyphosis by one step surgery: Case report <i>Landi A, Marotta N, Mancarella C, Dugoni DE, Tarantino R, Delfini R</i>
	293	Focal epithelial hyperplasia in a human immuno-deficiency virus patient treated with laser surgery <i>Galanakis A, Palaia G, Tenore G, Del Vecchio A, Romeo U</i>



- 297** Optimal management of a patient with recurrent nasopharyngeal carcinoma  
*Perri F, Dell'Oca I, Muto P, Schiavone C, Aversa C, Fulciniti F, Solla R, Della Vittoria Scarpati G, Buonerba C, Di Lorenzo G, Caponigro F*
- 301** Disseminated infection due to *Mycobacterium bovis* after intravesical BCG instillation  
*Marquez-Batalla S, Fraile-Villarejo E, Belhassen-García M, Gutierrez-Zubiaurre N, Cordero-Sánchez M*
- 304** One-stage revision in two cases of *Salmonella* prosthetic hip infection  
*Jeroense KTV, Kuiper JWP, Colen S, Schade RP, Saouti R*
- 309** Passage of nasogastric tube through tracheo-esophageal fistula into stomach: A rare event  
*Kamble RS, Gupta R, Gupta A, Kothari P, Dikshit KV, Kesan K, Mudkhedkar K*
- 311** Diagnostic pitfall of sebaceous gland metaplasia of the esophagus  
*Chiu KW, Wu CK, Lu LS, Eng HL, Chiou SS*



**APPENDIX** I-V Instructions to authors

**ABOUT COVER** Editorial Board Member of *World Journal of Clinical Cases*, Sachin Chakradhar Sarode, Associate Professor, Department of Oral Pathology and Microbiology, Dr .D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Sant Tukaram nagar, Pimpri, Pune 411018, India

**AIM AND SCOPE** *World Journal of Clinical Cases* (*World J Clin Cases*, *WJCC*, online ISSN 2307-8960, DOI: 10.12998) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

The primary task of *WJCC* is to rapidly publish high-quality Autobiography, Case Report, Clinical Case Conference (Clinicopathological Conference), Clinical Management, Diagnostic Advances, Editorial, Field of Vision, Frontier, Medical Ethics, Original Articles, Clinical Practice, Meta-Analysis, Minireviews, Review, Therapeutics Advances, and Topic Highlight, in the fields of allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, geriatrics and gerontology, hematology, immunology, infectious diseases, internal medicine, obstetrics and gynecology, oncology, ophthalmology, orthopedics, otolaryngology, pathology, pediatrics, peripheral vascular disease, psychiatry, radiology, rehabilitation, respiratory medicine, rheumatology, surgery, toxicology, transplantation, and urology and nephrology.

**INDEXING/ABSTRACTING** *World Journal of Clinical Cases* is now indexed in PubMed Central, PubMed, Digital Object Identifier.

**FLYLEAF** I-V Editorial Board**EDITORS FOR THIS ISSUE**

Responsible Assistant Editor: *Xiang Li*  
Responsible Electronic Editor: *Huan-Liang Wu*  
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*  
Proofing Editorial Office Director: *Xin-Xia Song*

**NAME OF JOURNAL**  
*World Journal of Clinical Cases*

**ISSN**  
ISSN 2307-8960 (online)

**LAUNCH DATE**  
April 16, 2013

**FREQUENCY**  
Monthly

**EDITORS-IN-CHIEF**  
**Giuseppe Di Lorenzo, MD, PhD, Professor**, Genitourinary Cancer Section and Rare-Cancer Center, University Federico II of Napoli, Via Sergio Pansini, 5 Ed. 1, 80131, Naples, Italy

**Jan Jacques Michiels, MD, PhD, Professor**, Primary Care, Medical Diagnostic Center Rijnmond Rotterdam, Bloodcoagulation, Internal and Vascular Medicine, Erasmus University Medical Center, Rotterdam, Goodheart Institute and Foundation, Erasmus Tower, Veenmos 13, 3069 AT, Erasmus City, Rotterdam, The Netherlands

**Sandro Vento, MD**, Department of Internal Medicine, University of Botswana, Private Bag 00713, Gaborone,

Botswana

**Shuhei Yoshida, MD, PhD**, Division of Gastroenterology, Beth Israel Deaconess Medical Center, Dana 509, Harvard Medical School, 330 Brookline Ave, Boston, MA 02215, United States

**EDITORIAL OFFICE**  
Jin-Lei Wang, Director  
Xiu-Xia Song, Vice Director  
*World Journal of Clinical Cases*  
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China  
Telephone: +86-10-85381891  
Fax: +86-10-85381893  
E-mail: editoroffice@wjnet.com  
Help Desk: <http://www.wjnet.com/esp/helpdesk.aspx>  
<http://www.wjnet.com>

**PUBLISHER**  
Baishideng Publishing Group Inc  
8226 Regency Drive,  
Pleasanton, CA 94588, USA  
Telephone: +1-925-223-8242  
Fax: +1-925-223-8243  
E-mail: bpgoffice@wjnet.com  
Help Desk: <http://www.wjnet.com/esp/helpdesk.aspx>

<http://www.wjnet.com>

**PUBLICATION DATE**  
July 16, 2014

**COPYRIGHT**  
© 2014 Baishideng Publishing Group Inc. Articles published by this Open Access 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.

**SPECIAL STATEMENT**  
All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

**INSTRUCTIONS TO AUTHORS**  
Full instructions are available online at [http://www.wjnet.com/2307-8960/g\\_info\\_20100722180909.htm](http://www.wjnet.com/2307-8960/g_info_20100722180909.htm)

**ONLINE SUBMISSION**  
<http://www.wjnet.com/esp/>

## Infectious burden and atherosclerosis: A clinical issue

Rosa Sessa, Marisa Di Pietro, Simone Filardo, Ombretta Turriziani

Rosa Sessa, Marisa Di Pietro, Simone Filardo, Department of Public Health and Infectious Diseases, "Sapienza" University, 00185 Rome, Italy

Ombretta Turriziani, Department of Molecular Medicine, "Sapienza" University, 00185 Rome, Italy

Author contributions: Sessa R, Di Pietro M, Filardo S and Turriziani O contributed to this paper; all authors read and approved the final version of the manuscript before submission.

Supported by Grants to R. Sessa from Center for Social Disease Research, "Sapienza" University, Rome

Correspondence to: Rosa Sessa, PhD, Department of Public Health and Infectious Diseases, "Sapienza" University, P.le Aldo Moro 5, 00185 Rome, Italy. [rosa.sessa@uniroma1.it](mailto:rosa.sessa@uniroma1.it)

Telephone: +39-064-9914102 Fax: +39-064-9914634

Received: December 27, 2013 Revised: May 16, 2014

Accepted: June 10, 2014

Published online: July 16, 2014

### Abstract

Atherosclerotic cardiovascular diseases, chronic inflammatory diseases of multifactorial etiology, are the leading cause of death worldwide. In the last decade, more infectious agents, labeled as "infectious burden", rather than any single pathogen, have been showed to contribute to the development of atherosclerosis through different mechanisms. Some microorganisms, such as *Chlamydia pneumoniae* (*C. pneumoniae*), human cytomegalovirus, *etc.* may act directly on the arterial wall contributing to endothelial dysfunction, foam cell formation, smooth muscle cell proliferation, platelet aggregation as well as cytokine, reactive oxygen specie, growth factor, and cellular adhesion molecule production. Others, such as *Helicobacter pylori* (*H. pylori*), influenza virus, *etc.* may induce a systemic inflammation which in turn may damage the vascular wall (*e.g.*, by cytokines and proteases). Moreover, another indirect mechanism by which some infectious agents (such as *H. pylori*, *C. pneumoniae*, periodontal pathogens, *etc.*) may play a role in the pathogenesis of atherosclerosis is molecular mimicry. Given the complexity of the mechanisms by which each microorganism may contribute to atherosclerosis, defining the interplay of more

infectious agents is far more difficult because the pro-atherogenic effect of each pathogen might be amplified. Clearly, continued research and a greater awareness will be helpful to improve our knowledge on the complex interaction between the infectious burden and atherosclerosis.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Infectious burden; Atherosclerosis; Bacteria; Virus; Pathogenetic mechanisms

**Core tip:** Several studies support the hypothesis that the infectious burden (IB) may be more involved in the pathogenesis of atherosclerosis than any single pathogen. However, because of the complexity of the interplay of more infectious agents in the host and the limitations of the methods available for the assessment of IB, the role of IB in the pathogenesis of atherosclerosis may have been underestimated.

Sessa R, Di Pietro M, Filardo S, Turriziani O. Infectious burden and atherosclerosis: A clinical issue. *World J Clin Cases* 2014; 2(7): 240-249 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/240.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.240>

### INTRODUCTION

Atherosclerosis, a chronic inflammatory disease of multifactorial etiology, may be considered as a multistage process, starting from the endothelial injury to the fibrous cap and thrombus formation in the advanced plaque. Key process in the development of atherosclerosis is low density lipoprotein (LDL) oxidation and accumulation in vascular cells, promoting foam cell formation as well as increased secretion of mediators of inflammation, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)- $\alpha$ <sup>[1]</sup>. The inflammatory state, in turn, can induce oxidative stress by enhancing the production of reactive

oxygen species (ROS) in the vascular wall<sup>[1]</sup>, contributing to the progression and destabilization of atherosclerotic plaque and consequently to cardiovascular diseases (CVDs). It is well known that CVDs are the leading cause of death worldwide, accounting for approximately 17.3 million deaths per year<sup>[2]</sup>.

Current opinion is that increased incidence of CVDs is probably the result of a high prevalence of both traditional risk factors such as hypertension, dyslipidemia, etc. and nontraditional risk factors including inflammation, oxidative stress, and infectious agents<sup>[3]</sup>. In the last decade, infectious agents have acquired a growing importance, since they are able to induce inflammation and/or oxidative stress<sup>[4]</sup>.

More recently, several studies have provided evidence that more infectious agents, for example, *Chlamydia pneumoniae* (*C. pneumoniae*), *Helicobacter pylori* (*H. pylori*), human cytomegalovirus (HCMV), Herpes simplex virus (HSV), labeled as “infectious burden” (IB), rather than any single pathogen, may be involved in the development of atherosclerosis and the subsequent cardiovascular events.

## EVIDENCE LINKING INFECTIOUS BURDEN WITH ATHEROSCLEROSIS

Zhu *et al.*<sup>[5]</sup> were the first to show the association between increasing risk of coronary artery disease (CAD) and increasing number of infectious agents including *C. pneumoniae*, *H. pylori*, HCMV, HSV-1 and 2, and hepatitis A virus (HAV). Indeed, the prevalence of CAD was 48%, 69% and 85% in individuals with seropositivity to  $\leq 2$  pathogens, to 3 or 4 pathogens and to 5 pathogens respectively. Since then, several serological studies found a prospective relation between increasing number of infectious agents (HSV-1 and 2, HCMV, Epstein Barr virus, EBV, *Haemophilus influenzae*, *C. pneumoniae*, *Mycoplasma pneumoniae*, and *H. pylori*) and CVD outcomes<sup>[6-8]</sup>. At the same time, serological assessments demonstrated the association between increasing number of infectious agents (*C. pneumoniae*, *H. pylori*, *M. pneumoniae*, *H. influenzae*, HCMV, EBV, HSV-1 and 2) and progression of atherosclerosis<sup>[9,10]</sup>. Again, cross-sectional and case-control studies confirmed the relationship between the seropositivity to *C. pneumoniae*, *H. pylori*, HAV, HCMV, HSV-1 and 2, and atherosclerosis<sup>[11,12]</sup>.

The evidence for a direct contribution of IB in the pathogenesis of atherosclerosis is based on the simultaneous detection of two pathogens in the atherosclerotic plaque (*C. pneumoniae* and *H. pylori* or *M. pneumoniae* and *C. pneumoniae* or *C. pneumoniae* and HCMV)<sup>[13-15]</sup>. Better yet is the evidence for a synergistic effect of *C. pneumoniae* and *H. pylori*, *M. pneumoniae* and *C. pneumoniae*, HCMV and *C. pneumoniae* in initiating or aggravating atherosclerosis in several animal models<sup>[16-18]</sup>. Similarly, there are some data showing the synergistic effect of the co-infection with *C. pneumoniae* and HCMV on the expression of atherogenic factors including IL-6, IL-8 and basic fibroblast growth factor in vascular smooth muscle cells (VSMCs) involved

in advanced plaque formation<sup>[19]</sup>. Also seropositivity for both *C. pneumoniae* and HCMV infections was found to be associated with premature myocardial infarction even after adjustment for coronary risk factors and socioeconomic status<sup>[20]</sup>.

Interestingly, the significant association between the increasing number of infectious agents together with elevated IL-6, C-reactive protein (CRP), and fibrinogen levels, and CAD prevalence, supports the hypothesis that inflammation may be one pathway by which more infectious agents and CVDs are linked<sup>[5,8,12]</sup>.

The involvement of IB in the pathogenesis of atherosclerosis is expected since numerous infectious agents have been shown to play a role in the development and progression of atherosclerosis<sup>[4]</sup>.

*C. pneumoniae*, an obligate intracellular bacterium, is responsible for respiratory infections such as sinusitis, pharyngitis and pneumonia. Exposure to *C. pneumoniae* is extremely common and epidemiological studies indicate that anti-*C. pneumoniae* antibody prevalence is 50% by the age of 20 and increases with increasing age<sup>[21]</sup>. *C. pneumoniae* is characterized by the ability to systematically disseminate from the lungs through peripheral blood mononuclear cells and to localize in several extrapulmonary tissues<sup>[22-25]</sup>. In recent years, it has been demonstrated that *C. pneumoniae*, in response to several stress conditions (iron or essential amino acid starvation, interferon (IFN)- $\gamma$  or antibiotic treatment), can generate a persistent form during its developmental cycle<sup>[26-29]</sup>. Chlamydial persistent form may endure for a long time inside host cells since it is more suited to evade the host immune response and is more difficult to eradicate with antibiotics, leading to a chronic inflammatory state<sup>[26]</sup>.

Cumulative evidence on the involvement of *C. pneumoniae* and atherosclerosis has been provided by seroepidemiological studies<sup>[30-32]</sup>, *C. pneumoniae* DNA detection in the atherosclerotic plaque<sup>[31-33]</sup>, the isolation of viable bacteria from the atheroma<sup>[4,32]</sup> and *in vivo* studies, demonstrating that *C. pneumoniae* infection may accelerate the progression of atherosclerotic lesion in animal models<sup>[4,31,34]</sup>. Lastly, *in vitro* studies have evidenced that *C. pneumoniae* is able to multiply within vascular cells, such as macrophages, endothelial cells, SMCs and platelets, and to induce chronic inflammation through the elicitation of inflammatory cytokines (*e.g.*, IL-6, IL-1 $\beta$  and TNF- $\alpha$ )<sup>[31,32,35]</sup>. Furthermore, once inside the vascular tissue, *C. pneumoniae* has been shown to induce the production of ROS leading to oxidative stress, which contributes to LDL oxidation and accumulation within vascular cells and to foam cell formation<sup>[36]</sup>.

Periodontal pathogens, such as *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Tannerella forsythia* (*T. forsythia*), *Prevotella intermedia*, *Fusobacterium nucleatum* (*F. nucleatum*), *Treponema denticola*, *Campylobacter rectus*, *Streptococcus sanguis*, and *Streptococcus mutans*, are responsible of a complex group of chronic oral inflammatory diseases like periodontitis or gingivitis. Over the last years, different lines of evidence have supported the role of periodontal bacteria in cardio-

vascular diseases. First of all, it has been demonstrated that oral bacteria can disseminate in the blood stream causing bacteremia<sup>[37]</sup> and localize in vascular wall. Indeed, DNA, RNA and antigens of a variety of oral bacterial species (*e.g.*, *P. gingivalis*, *A. actinomycetemcomitans*, *T. forsythia* and *F. nucleatum*) have been detected in atherosclerotic plaques<sup>[4]</sup>. More importantly, evidence of live *P. gingivalis* and *A. actinomycetemcomitans* in the atheroma<sup>[38]</sup>, supports the direct involvement of these pathogens in the pathogenesis of atherosclerosis. Moreover, *in vivo* studies have shown the ability of *P. gingivalis* to accelerate atherosclerosis in murine models<sup>[4,39]</sup> and to induce aortic and coronary lesions in both normocholesterolemic and hypercholesterolemic pigs<sup>[40]</sup>. *In vitro* studies have demonstrated that periodontal pathogens are able to infect endothelial cells, SMCs and macrophages, eliciting the production of proinflammatory cytokines and chemokines (*e.g.*, IL-6 and monocyte chemoattractant protein (MCP)-1) and the formation of foam cells, hence contributing to atherosclerosis<sup>[41,42]</sup>.

*H. pylori*, a common cause of chronic gastritis as well as a risk factor for gastric cancer, is widespread in the general population. In the last decade, it has been considered as a possible risk factor for atherosclerosis, since *H. pylori* DNA has been found in the atherosclerotic plaque<sup>[4,14]</sup>. Several seroepidemiological studies have confirmed a relationship between *H. pylori* and atherosclerosis although others have failed to demonstrate such an association<sup>[43-46]</sup>. Controversial are data showing the ability of *H. pylori* to accelerate the atherosclerotic lesion development in mouse models<sup>[4]</sup>. However, *H. pylori* may also contribute to the systemic inflammation underlying atherosclerosis through the elicitation of acute-phase reactants (*e.g.*, CRP) and inflammatory cytokines (*e.g.*, IL-6)<sup>[47]</sup>.

Other bacteria, such as *M. pneumoniae*, have been proposed as possible pathogens in atherosclerosis with controversial results. Several seroepidemiological studies have found the association between CVDs and *M. pneumoniae*<sup>[48,49]</sup>. Furthermore, an *in vivo* study has demonstrated that *M. pneumoniae* infection aggravated atherosclerosis in hypercholesterolemic mice<sup>[18]</sup>. However, pathological studies have not supported the association between this microorganism and atherosclerosis, since *M. pneumoniae* DNA has been detected in atherosclerotic tissues as well as in healthy vessels<sup>[4]</sup>.

Lifelong persistent infection with HCMV has been also associated with atherosclerosis. HCMV was first detected in human atheromatous tissue by Benditt *et al*<sup>[50]</sup> in 1983. Experimental data have shown the ability of HCMV to infect the human vascular wall, resulting in altered function of the endothelium<sup>[51]</sup>. Furthermore, both antigen and nucleic acid sequence of HCMV have been detected in SMCs from carotid artery plaques<sup>[52-54]</sup>.

In addition, HCMV DNA has been more often detected in arterial samples from patients with atherosclerosis than in control subjects<sup>[55]</sup>. Similarly, higher prevalence as well as higher titer of HCMV antibody have been observed in patients undergoing vascular surgery for

atherosclerosis than in control subjects<sup>[56]</sup>. In addition, a meta-analysis study has reported a significant increased coronary heart disease risk for patients infected with HCMV<sup>[57]</sup>.

Recently it has been suggested that HSV-2, but not HSV-1, was associated with premature CVD<sup>[58]</sup>. Consistent with a potential relationship between HSV-2 and CVD, Raza-Ahmad *et al*<sup>[59]</sup> previously examined coronary artery specimens of patients undergoing coronary artery bypass grafting and found 45% of them positive for HSV-2 and only 1% positive for HSV-1. Likewise, a large cross sectional study linked HSV-2 to hypertension, but it did not find any association with HSV-1. The reasons of the association with HSV-2 and not with HSV-1 are unclear.

There is also evidence supporting the role of influenza as a trigger for cardiovascular events<sup>[60]</sup>. However, data are debated. Some authors think that influenza (A and B) seropositivity is not a predictor of risk for CAD. Others propose that influenza virus might play a role in atherogenesis or atherothrombosis and that influenza vaccination might reduce the risk of recurrent myocardial infarction<sup>[60,61]</sup>. Recently, a correlation between influenza B virus infection and acute myocardial infarction has been reported<sup>[62]</sup>.

Although there have been positive associations of antibody titers or viral antigens of the hepatitis viruses with CVD<sup>[63-65]</sup>, many recent studies have reported no association. Zhu *et al*<sup>[63]</sup> has suggested a causal role for HAV infection in atherogenesis, on the basis of a significantly higher prevalence of CAD among subjects living in the Washington, DC, area who had serum IgG antibodies to HAV. The same research group has reported a high relative hazard for myocardial infarction or death among individuals positive for IgG antibodies to HAV. However, some authors believe that epidemiological evidence argues against a significant role for HAV infection in atherogenesis, since in countries where HAV infection is far less frequent, such as northern European countries and Australia, the incidence of cardiovascular diseases is remarkably higher than that detected in countries showing an high HAV infection prevalence<sup>[66]</sup>.

Several studies have also investigated the association of atherosclerosis with hepatitis C virus (HCV) infection, with conflicting results. Some studies have reported that the presence of antibody against HCV was associated with an increased risk of carotid artery plaque in the general population<sup>[65]</sup>. In addition, positive-strand HCV RNA has been detected in carotid plaque tissues from anti-HCV antibody-positive patients but it was not detected in anti-HCV antibody-negative patients<sup>[67,68]</sup>. Furthermore, multivariate logistic regression analysis has showed that HCV core protein positivity was an independent predictor of carotid plaque, supporting the possible link between persistent HCV infection and carotid atherosclerosis in subjects without severe liver dysfunction<sup>[69]</sup>. Patients with chronic HCV infection are known to develop not only hepatitis, but also various metabolic disorders<sup>[70,71]</sup>. Indeed, HCV affects both glucose and



lipid metabolism. Recent population-based studies have demonstrated hypolipidemia in subjects with chronic HCV infection<sup>[72,73]</sup>. Although altered lipid metabolism is linked to atherosclerosis, the effect of HCV on atherosclerosis remains controversial<sup>[73-75]</sup>. A systematic review published by Roed *et al*<sup>[76]</sup> has suggested an increased risk of CAD in HCV infected individuals. Recently, a study has revealed that chronic HCV infection was associated with increased insulin resistance and with mild atherosclerosis, thus underlining the complexity of this association<sup>[77]</sup>.

A growing body of literature reports that human immunodeficiency virus (HIV) infected patients suffer from an elevated risk for both subclinical atherosclerotic disease and CVD events than uninfected individuals<sup>[78-81]</sup>. However, the results of a meta-analysis as well as a number of independent studies have questioned this association<sup>[82,83]</sup>. Furthermore, antiretroviral therapy (ART) has been shown to have independent effects on lesion development in several experimental studies, and some compounds, such as protease inhibitors, are associated with lipodystrophy, central adiposity, hyperlipidaemia, and endothelial dysfunction, all recognized risk factors for CVD<sup>[84-86]</sup>. However, the risk of CVD associated with HIV infection is not fully accounted for by the effects of antiretrovirals in these studies. Indeed, other papers have suggested that direct HIV infection of endothelial cells could contribute to atherosclerosis by causing endothelial dysfunction<sup>[87]</sup>. Furthermore, Hsue *et al*<sup>[88]</sup> have shown that increased atherosclerosis can occur in the absence of ART in HIV-infected patients. Recently, Desvarieux *et al*<sup>[89]</sup> have further emphasized the role of HIV in atherosclerosis, reporting the preponderant association of HIV infection (rather than ART) with increased atherosclerosis in never smokers, thus also determining the validity of the relationship independent of this important confounder.

## POSSIBLE MECHANISMS UNDERLYING INFECTIOUS BURDEN RELATED TO ATHEROSCLEROSIS

A substantial body of evidence supports the hypothesis that more infectious agents rather than a single pathogen may contribute to atherosclerosis through different mechanisms (Figure 1). Some microorganisms, such as *A. actinomycetemcomitans*, may act directly on the arterial wall contributing to endothelial dysfunction, foam cell formation, SMC proliferation, platelet aggregation and cytokine production<sup>[4,41]</sup>. Otherwise, microorganisms, such as *H. pylori*, may induce a systemic inflammation which in turn may damage the vascular wall (*e.g.*, by cytokines and proteases). Indeed, many observational studies have reported the association of the seropositivity to *H. pylori* with a sensitive marker of systemic inflammation and even predictor of acute cardiovascular events such as CRP<sup>[90,91]</sup>.

Furthermore, there are also infectious agents, such as *C. pneumoniae* and *P. gingivalis*, that may contribute to atherosclerosis by both direct and indirect mechanisms. As a direct effect, these microorganisms have been

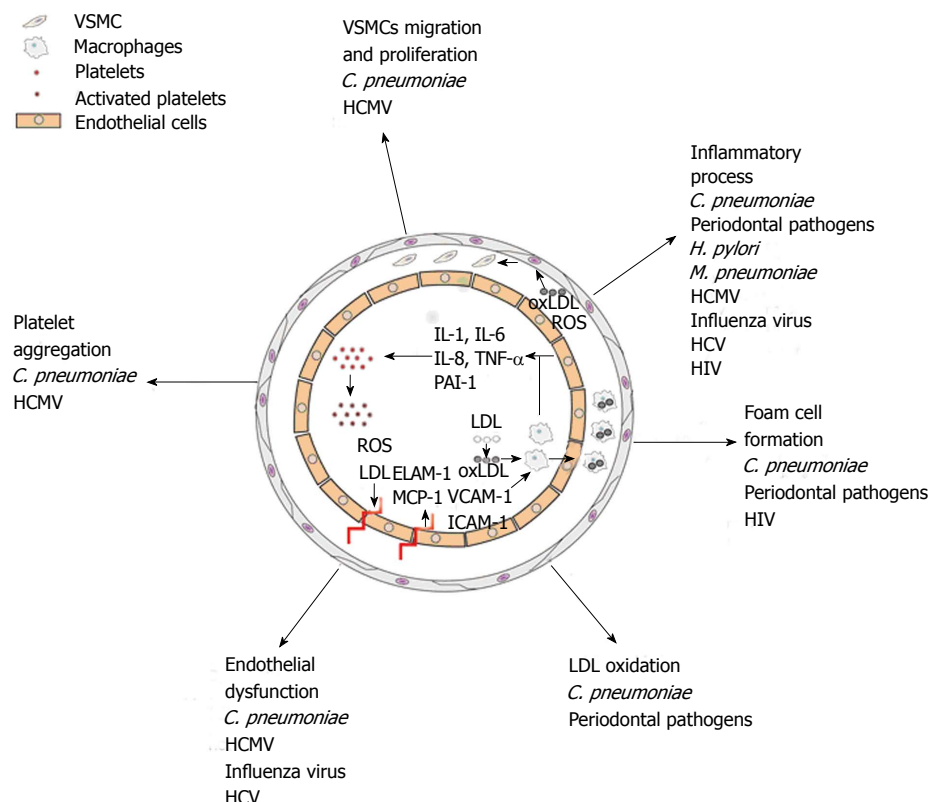
shown to infect macrophages, SMCs and endothelial cells inducing the production of ROS, cytokines (IL-6, IL-1 $\beta$  and TNF- $\alpha$ , *etc.*), growth factors (basic fibroblast growth factor, bFGF, tumor growth factor (TGF)- $\beta$ , *etc.*) and cellular adhesion molecules (vascular cell adhesion molecule-1, VCAM-1, intercellular adhesion molecule-1, ICAM-1, endothelial-leukocyte adhesion molecule-1, ELAM-1, *etc.*), all responsible for the typical pathological changes of the atherosclerotic plaque<sup>[36,41,42]</sup>. On the other hand, *C. pneumoniae* and *P. gingivalis* can contribute to atherosclerosis indirectly by inducing systemic inflammation<sup>[92,93]</sup>. Indeed, circulating cytokines (IL-6) and acute phase proteins (serum amyloid A), produced in response to systemic infection of animal models with *C. pneumoniae* or *P. gingivalis*, have been associated with the progression and destabilization of atherosclerotic lesions<sup>[94,95]</sup>. Also, in human, increases in circulating CRP levels and *P. gingivalis* or *C. pneumoniae* antibody levels have been associated with an increased risk of CAD<sup>[4,96]</sup>.

Another indirect mechanism by which infectious agents play a role in the pathogenesis of atherosclerosis is molecular mimicry. There is evidence that the humoral immune response against the heat shock proteins (HSPs) found in *C. pneumoniae*, *H. pylori* and *P. gingivalis*, may cross-react with human HSPs in vascular cells, initiating an autoimmune process, responsible for vascular endothelial injury<sup>[97-99]</sup>. In fact, antibody levels against HSPs have been associated with early and advanced atherosclerosis<sup>[100]</sup>. In addition, *in vivo* studies have also confirmed that the T-cell immune response against HSP, derived from *H. pylori*, *C. pneumoniae* and *P. gingivalis*, could promote atherogenesis<sup>[101-103]</sup>.

As far as concern viral agents, data supporting a direct effect of these agents on the pathogenesis of atherosclerosis are usually weak; infections with viruses are more likely to have an indirect effect on the initiation and progression of atherosclerosis.

Relative to HCMV, it has been observed that SMCs isolated from atherosclerotic coronary lesions, harbor HCMV DNA sequences and express immediate early proteins, such as IE84, one of the immediate early proteins of the virus that binds and inhibits p53<sup>[104]</sup>. Inhibition of p53 by the virus is held responsible for the enhanced proliferation of SMCs and impaired apoptosis, either of which may contribute to restenosis<sup>[104]</sup>. Furthermore, persistent infection of HCMV in endothelial cells leads to dysfunction of these cells and activates proinflammatory signaling pathways, which promote enhanced proliferation and migration of monocytes and SMCs into *intima* of the vascular wall as well as lipid accumulation and expansion of the atherosclerotic lesion<sup>[105,106]</sup>.

The precise mechanism by which influenza virus infection contributes to atherosclerosis is unclear, however inflammation and coagulopathy seem to be key factors. Specifically, the potential mechanisms may include: (1) antigenic cross-reactivity; (2) an increase in pro-inflammatory and prothrombotic cytokines, such as IL-2, IL-6, IL-10 and IL-18; (3) pronounced expression of inflammatory cytokines by infected monocytes and reduced



**Figure 1 Schematic representation of transversal artery section.** Possible etiopathogenetic mechanisms of the infectious agents in atherosclerotic plaque development. *C. pneumoniae*: *Chlamydia pneumoniae*; HCMV: Human cytomegalovirus; *H. pylori*: *Helicobacter pylori*; *M. pneumoniae*: *Mycoplasma pneumoniae*; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; SMC: Smooth muscle cell; ROS: Reactive oxygen species; LDL: Low-density lipoprotein; Ox-LDL: Oxidized low-density lipoprotein; IL: Interleukin; TNF- $\alpha$ : Tumor necrosis factor; PAI: Plasminogen activator inhibitor-1; ELAM-1: Endothelial-leukocyte adhesion molecule-1; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular adhesion molecule-1; MCP-1: Monocyte chemoattractant protein-1.

clotting time; (4) increased trafficking of macrophages into the arterial wall; and (5) induction of procoagulant activity in infected endothelial cells, reduced clotting time, and increased expression of tissue factor<sup>[60,62]</sup>. Repeated influenza virus infection may injure vascular endothelial cells and initiate the inflammatory response that is required to accelerate and enhance the development of atherosclerosis.

It has been suggested that influenza virus may trigger the destabilization of already present vulnerable plaques. Naghavi *et al*<sup>[107]</sup> have showed that inoculation of influenza virus A in atherosclerotic apolipoprotein E-deficient mice led to a marked increase in inflammation and thrombosis in plaques but not in normal area. Influenza virus infection may cause the production of IL-2, IL-6, IL-10, IL-18, IFN- $\gamma$  and TNF- $\alpha$ , which induces endothelial cells to release endothelin (ET)-1, sICAM-1 and sVCAM-1. These inflammatory cytokines may trigger the destabilization of existing vulnerable plaques and lead to an acute myocardial infarction without being involved in the development or progression of atherosclerosis<sup>[108]</sup>.

As stated before, the role of HCV in atherosclerosis is widely debated. A role of chronic inflammation in atherogenesis has been suggested<sup>[109]</sup> because chronic HCV infection has been associated with vasculitis and mixed cryoglobulinemia, which may cause vascular injury as well as cerebrovascular damage<sup>[110]</sup>. Concentrations of sICAM-1 have been reported to be higher in HCV patients than in control subjects<sup>[111]</sup>, and Cacoub *et al*<sup>[112]</sup> have reported a possible association of anti-endothelial cell auto-antibodies, commonly observed in HCV patients but not in other viral diseases, with vasculitis. However, a recent

paper has demonstrated a favorable effect of HCV on atherosclerosis<sup>[77]</sup> probably due to the alteration in lipid parameters of the subjects with chronic HCV infection caused by the progression of liver disease and partly by a metabolic process associated with HCV replication.

Several papers have reported that atherosclerosis is consistently higher among the HIV positive patients, with or without treatment. Recently Shrestha *et al*<sup>[113]</sup> have postulated three key sequential biological processes that lead to accelerate progression of atherosclerosis: (1) inflammation leads to the recruitment of monocytes; (2) monocytes migrate to the endothelium and differentiate to macrophages and foam cells; and (3) apoptosis of foam cells leads to plaque development through calcium-dependent endoplasmic reticulum stress. The HIV itself, or together with treatment, affects this progression by increasing inflammation, promoting the transformation of monocytes, and increasing apoptosis through ER stress and an imbalance of calcium.

Given the complexity of the mechanisms by which each microorganism may play a role in the pathogenesis of atherosclerosis, defining the interplay of more infectious agents is far more difficult because the pro-atherogenic effect of each pathogen might be amplified.

## ASSESSMENT OF INFECTIOUS BURDEN RELATED TO ATHEROSCLEROSIS

The main unanswered question is the definition of IB. Several infectious agents, such as *C. pneumoniae*, *H. influenzae*, *H. pylori*, *M. pneumoniae*, HCMV, HSV, EBV and HAV, *etc.* have been proposed as constituting the IB related to

atherosclerosis, but, to date, there is no consensus both on the number and on which microorganisms should be considered.

The majority of the infectious agents involved in the IB are widespread, as evidenced from the high prevalence of antibodies in the general population; more than half of the world population is seropositive, for example, to *C. pneumoniae*, *H. pylori*, HCMV and HSV. Again, HSV, HCMV, *C. pneumoniae* and *H. pylori* infections could be acquired early in life, and persist over time. The situation is further complicated by the fact that the infectious agents involved in IB are responsible for persistent infection (*e.g.*, HIV and *C. pneumoniae*), repeated infection (*e.g.*, influenza virus), latent infection followed by life-long reactivation (*e.g.*, HSV and HCMV) or chronic infection (*e.g.*, HBV, HCV and, *H. pylori*).

Nowadays, the assessment of the IB related to atherosclerosis is based mainly on serological methods. The main limitations of serology are to define whether the antibody response reflects a past or chronic infection and to identify the differences in seropositivity between patients and general population, especially if seropositivity is common. In addition, serological diagnostic methods are not appropriate for the detection of novel or rare pathogens. Lastly, most serological assays are designed for diagnostic testing in clinical settings, and not for the assessment of the burden of infections acquired through life. Notably, most of the infectious agents involved in the IB, such as *C. pneumoniae*, *H. pylori*, HSV, and HCMV, can cause asymptomatic infections that are not routinely investigated. As a result, these undiagnosed infections, if left untreated, can contribute to the development of severe complications, including CVDs.

Other technical obstacles in the assessment of the IB related to atherosclerosis include difficulties in obtaining atherosclerotic plaques and in isolating and culturing certain infectious agents. Indeed, atherosclerotic plaques are obtained too late during the course of the disease to be of clinical use.

Another intriguing issue is the interaction of more infectious agents with host factors, such as age, gender, ethnicity, and other concomitant infections or clinical conditions that may impair the host immune system, thus potentially modifying the establishment, progression and outcome of the infection. Moreover, genome-wide association studies have now convincingly shown that the susceptibility to an infection as well as the diverse outcomes (for example the resolution of infection, the clinical deterioration to severe disease, or the progression from acute infection to persistent infection) can be, at least, partly explained by genetic variation<sup>[114]</sup>. In this regard, a recent study has showed that *IL-6* gene polymorphisms appear to influence the susceptibility to the atherogenic effect of more infectious agents including *C. pneumoniae*, CMV, *H. pylori* and HSV-1<sup>[115]</sup>.

## CONCLUSION

Based on the extent of the issues previously described,

the role of the IB in the pathogenesis of atherosclerosis may have been substantially underestimated, so that the true impact of IB is likely to be much greater than it is currently recognized.

Different approaches could be taken to address the problem; one possibility may be to conceive a well-designed protocol that includes the number and the type of infectious agents, the antibody response (IgG and/or IgA) as well as the monitoring of antibody titer, atherosclerotic biological markers and cytokines. The latter are particularly critical in chronic viral infections, such as HIV infection, in which two monocytes surface markers (CD11b and chemokine (C-X-C motif) receptor (CXCR)-1) have been proposed as predictors of CVD<sup>[116]</sup>.

Clearly, continued research and a better awareness of this problem will be helpful to improve our knowledge on the complex interaction between IB and atherosclerosis.

## REFERENCES

- 1 **Hulsmans M**, Holvoet P. The vicious circle between oxidative stress and inflammation in atherosclerosis. *J Cell Mol Med* 2010; **14**: 70-78 [PMID: 19968738 DOI: 10.1096/fj.11-181149]
- 2 **World Health Organization**. Global Atlas on Cardiovascular Disease Prevention and Control. Mendis S, Puska P, Norrving B (Editors). Available from: URL: [http://whqlibdoc.who.int/publications/2011/9789241564373\\_eng.pdf](http://whqlibdoc.who.int/publications/2011/9789241564373_eng.pdf)
- 3 **Balogopal PB**, de Ferranti SD, Cook S, Daniels SR, Gidding SS, Hayman LL, McCrindle BW, Mietus-Snyder ML, Steinberger J. Nontraditional risk factors and biomarkers for cardiovascular disease: mechanistic, research, and clinical considerations for youth: a scientific statement from the American Heart Association. *Circulation* 2011; **123**: 2749-2769 [PMID: 21555711 DOI: 10.1161/CIR.0b013e31821c7c64]
- 4 **Rosenfeld ME**, Campbell LA. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost* 2011; **106**: 858-867 [PMID: 22012133 DOI: 10.1160/TH11-06-0392]
- 5 **Zhu J**, Quyyumi AA, Norman JE, Csako G, Waclawiw MA, Shearer GM, Epstein SE. Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels. *Am J Cardiol* 2000; **85**: 140-146 [PMID: 10955367 DOI: 10.1016/S0002-9149(99)00653-0]
- 6 **Rupprecht HJ**, Blankenberg S, Bickel C, Rippin G, Hafner G, Prellwitz W, Schlumberger W, Meyer J. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. *Circulation* 2001; **104**: 25-31 [PMID: 11435333]
- 7 **Smieja M**, Gnarpe J, Lonn E, Gnarpe H, Olsson G, Yi Q, Dzavik V, McQueen M, Yusuf S. Multiple infections and subsequent cardiovascular events in the Heart Outcomes Prevention Evaluation (HOPE) Study. *Circulation* 2003; **107**: 251-257 [PMID: 12538424 DOI: 10.1161/01.CIR.0000044940.65226.1F]
- 8 **Mundkur LA**, Rao VS, Hebbagudi S, Shanker J, Shivanandan H, Nagaraj RK, Kakkar VV. Pathogen burden, cytomegalovirus infection and inflammatory markers in the risk of premature coronary artery disease in individuals of Indian origin. *Exp Clin Cardiol* 2012; **17**: 63-68 [PMID: 22826649]
- 9 **Espinola-Klein C**, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Victor A, Hafner G, Prellwitz W, Schlumberger W, Meyer J. Impact of infectious burden on progression of carotid atherosclerosis. *Stroke* 2002; **33**: 2581-2586 [PMID: 12411646 DOI: 10.1161/01.STR.0000034789.82859.A4]
- 10 **Espinola-Klein C**, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Rippin G, Victor A, Hafner G, Schlumberger W,



- Meyer J. Impact of infectious burden on extent and long-term prognosis of atherosclerosis. *Circulation* 2002; **105**: 15-21 [PMID: 11772870 DOI: 10.1161/hc0102.101362]
- 11 **Elkind MS**, Ramakrishnan P, Moon YP, Boden-Albala B, Liu KM, Spitalnik SL, Rundek T, Sacco RL, Paik MC. Infectious burden and risk of stroke: the northern Manhattan study. *Arch Neurol* 2010; **67**: 33-38 [PMID: 19901154 DOI: 10.1001/archneurol.2009.271]
  - 12 **Nazmi A**, Diez-Roux AV, Jenny NS, Tsai MY, Szklo M, Aiello AE. The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis. *BMC Public Health* 2010; **10**: 706 [PMID: 21083905 DOI: 10.1186/1471-2458-10-706]
  - 13 **Higuchi Mde L**, Reis MM, Sambiasi NV, Palomino SA, Castelli JB, Gutierrez PS, Aiello VD, Ramires JA. Coinfection with Mycoplasma pneumoniae and Chlamydia pneumoniae in ruptured plaques associated with acute myocardial infarction. *Arq Bras Cardiol* 2003; **81**: 12-22, 1-11 [PMID: 12908069]
  - 14 **Kaplan M**, Yavuz SS, Cinar B, Koksai V, Kut MS, Yapici F, Gercekoglu H, Demirtas MM. Detection of Chlamydia pneumoniae and Helicobacter pylori in atherosclerotic plaques of carotid artery by polymerase chain reaction. *Int J Infect Dis* 2006; **10**: 116-123 [PMID: 16183317 DOI: 10.1016/j.ijid.2004.10.008]
  - 15 **Virok D**, Kis Z, Kari L, Barzo P, Sipka R, Burian K, Nelson DE, Jackel M, Kerenyi T, Bodosi M, Gönczöl E, Endresz V. Chlamydophila pneumoniae and human cytomegalovirus in atherosclerotic carotid plaques--combined presence and possible interactions. *Acta Microbiol Immunol Hung* 2006; **53**: 35-50 [PMID: 16696549 DOI: 10.1556/AMicr.53.2006.1.3]
  - 16 **Burnett MS**, Gaydos CA, Madico GE, Glad SM, Paigen B, Quinn TC, Epstein SE. Atherosclerosis in apoE knockout mice infected with multiple pathogens. *J Infect Dis* 2001; **183**: 226-231 [PMID: 11120928 DOI: 10.1086/317938]
  - 17 **Liuba P**, Pesonen E, Paakkari I, Batra S, Andersen L, Forslid A, Ylä-Herttua S, Persson K, Wadström T, Wang X, Laurini R. Co-infection with Chlamydia pneumoniae and Helicobacter pylori results in vascular endothelial dysfunction and enhanced VCAM-1 expression in apoE-knockout mice. *J Vasc Res* 2003; **40**: 115-122 [PMID: 12808347 DOI: 10.1159/000070708]
  - 18 **Damy SB**, Higuchi ML, Timenetsky J, Reis MM, Palomino SP, Ikegami RN, Santos FP, Osaka JT, Figueiredo LP. Mycoplasma pneumoniae and/or Chlamydophila pneumoniae inoculation causing different aggravations in cholesterol-induced atherosclerosis in apoE KO male mice. *BMC Microbiol* 2009; **9**: 194 [PMID: 19744321 DOI: 10.1186/1471-2180-9-194]
  - 19 **Prochnau D**, Straube E, Figulla HR, Rödel J. Supra-additive expression of interleukin-6, interleukin-8 and basic fibroblast growth factor in vascular smooth muscle cells following coinfection with Chlamydia pneumoniae and cytomegalovirus as a novel link between infection and atherosclerosis. *Can J Infect Dis Med Microbiol* 2012; **23**: e26-e30 [PMID: 23730316]
  - 20 **Gattone M**, Iacoviello L, Colombo M, Castelnovo AD, Soffiantino F, Gramoni A, Picco D, Benedetta M, Giannuzzi P. Chlamydia pneumoniae and cytomegalovirus seropositivity, inflammatory markers, and the risk of myocardial infarction at a young age. *Am Heart J* 2001; **142**: 633-640 [PMID: 11579353 DOI: 10.1067/mhj.2001.118118]
  - 21 **Grayston JT**. Background and current knowledge of Chlamydia pneumoniae and atherosclerosis. *J Infect Dis* 2000; **181** Suppl 3: S402-S410 [PMID: 10839724]
  - 22 **Sessa R**, Di Pietro M, Schiavoni G, Santino I, Cipriani P, Romano S, Penco M, del Piano M. Prevalence of Chlamydia pneumoniae in peripheral blood mononuclear cells in Italian patients with acute ischaemic heart disease. *Atherosclerosis* 2001; **159**: 521-525 [PMID: 11730834 DOI: 10.1016/S0021-9150(01)00537-8]
  - 23 **Wang SS**, Tondella ML, Bajpai A, Mathew AG, Mehranpour P, Li W, Kacharava AG, Fields BS, Austin H, Zafari AM. Circulating Chlamydia pneumoniae DNA and advanced coronary artery disease. *Int J Cardiol* 2007; **118**: 215-219 [PMID: 17023075 DOI: 10.1016/j.ijcard.2006.07.013]
  - 24 **Di Pietro M**, Schiavoni G, Sessa V, Pallotta F, Costanzo G, Sessa R. Chlamydia pneumoniae and osteoporosis-associated bone loss: a new risk factor? *Osteoporos Int* 2013; **24**: 1677-1682 [PMID: 23160916 DOI: 10.1007/s00198-012-2217-1]
  - 25 **Di Pietro M**, Filardo S, Cazzavillan S, Segala C, Bevilacqua P, Bonoldi E, D'Amore ES, Rassu M, Sessa R. Could past Chlamydial vascular infection promote the dissemination of Chlamydia pneumoniae to the brain? *J Biol Regul Homeost Agents* 2013; **27**: 155-164 [PMID: 23489695]
  - 26 **Schoborg RV**. Chlamydia persistence -- a tool to dissect chlamydia--host interactions. *Microbes Infect* 2011; **13**: 649-662 [PMID: 21458583 DOI: 10.1016/j.micinf.2011.03.004]
  - 27 **Di Pietro M**, Tramonti A, De Santis F, De Biase D, Schiavoni G, Filardo S, Zagaglia C, Sessa R. Analysis of gene expression in penicillin G induced persistence of Chlamydia pneumoniae. *J Biol Regul Homeost Agents* 2012; **26**: 277-284 [PMID: 22824742]
  - 28 **Di Pietro M**, De Santis F, De Biase D, Sessa R. The elusive but pathogenic peptidoglycan of Chlamydiae. *Eur J Inflamm* 2013; **11**: 257-260
  - 29 **Di Pietro M**, Filardo S, De Santis F, Sessa R. New insights into Chlamydiae persistence: an energy metabolism strategy? *Int J Immunopathol Pharmacol* 2013; **26**: 525-528 [PMID: 23755769]
  - 30 **Sessa R**, Di Pietro M, Santino I, del Piano M, Varveri A, Dagianti A, Penco M. Chlamydia pneumoniae infection and atherosclerotic coronary disease. *Am Heart J* 1999; **137**: 1116-1119 [PMID: 10347340]
  - 31 **Sessa R**, Nicoletti M, Di Pietro M, Schiavoni G, Santino I, Zagaglia C, Del Piano M, Cipriani P. Chlamydia pneumoniae and atherosclerosis: current state and future perspectives. *Int J Immunopathol Pharmacol* 2009; **22**: 9-14 [PMID: 19309547]
  - 32 **Joshi R**, Khandelwal B, Joshi D, Gupta OP. Chlamydophila pneumoniae infection and cardiovascular disease. *N Am J Med Sci* 2013; **5**: 169-181 [PMID: 23626952 DOI: 10.4103/1947-2714.109178]
  - 33 **Sessa R**, Di Pietro M, Schiavoni G, Santino I, Benedetti-Valentini F, Perna R, Romano S, del Piano M. Chlamydia pneumoniae DNA in patients with symptomatic carotid atherosclerotic disease. *J Vasc Surg* 2003; **37**: 1027-1031 [PMID: 12756349 DOI: 10.1067/mva.2003.200]
  - 34 **Chen S**, Shimada K, Zhang W, Huang G, Crother TR, Arditi M. IL-17A is proatherogenic in high-fat diet-induced and Chlamydia pneumoniae infection-accelerated atherosclerosis in mice. *J Immunol* 2010; **185**: 5619-5627 [PMID: 20935201 DOI: 10.4049/jimmunol.1001879]
  - 35 **Di Pietro M**, Schiavoni G, Del Piano M, Shaik Y, Boscolo P, Caraffa A, Teté S, Conti F, Sessa R. Chlamydia pneumoniae and atherosclerosis: the role of mast cells. *J Biol Regul Homeost Agents* 2009; **23**: 65-69 [PMID: 19589286]
  - 36 **Di Pietro M**, Filardo S, De Santis F, Sessa R. Chlamydia pneumoniae infection in atherosclerotic lesion development through oxidative stress: a brief overview. *Int J Mol Sci* 2013; **14**: 15105-15120 [PMID: 23877837 DOI: 10.3390/ijms140715105]
  - 37 **Castillo DM**, Sánchez-Beltrán MC, Castellanos JE, Sanz I, Mayorga-Fayad I, Sanz M, Lafaurie GI. Detection of specific periodontal microorganisms from bacteraemia samples after periodontal therapy using molecular-based diagnostics. *J Clin Periodontol* 2011; **38**: 418-427 [PMID: 21392048 DOI: 10.1111/j.1600-051X.2011.01717.x]
  - 38 **Kozarov EV**, Dorn BR, Shelburne CE, Dunn WA, Prohulske-Fox A. Human atherosclerotic plaque contains viable invasive Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. *Arterioscler Thromb Vasc Biol* 2005; **25**: e17-e18 [PMID: 15662025]

- 39 **Hayashi C**, Viereck J, Hua N, Phinikaridou A, Madrigal AG, Gibson FC, Hamilton JA, Genco CA. Porphyromonas gingivalis accelerates inflammatory atherosclerosis in the innominate artery of ApoE deficient mice. *Atherosclerosis* 2011; **215**: 52-59 [PMID: 21251656 DOI: 10.1016/j.atherosclerosis.2010]
- 40 **Brodala N**, Merricks EP, Bellinger DA, Damrongsri D, Ofenbacher S, Beck J, Madianos P, Sotres D, Chang YL, Koch G, Nichols TC. Porphyromonas gingivalis bacteremia induces coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1446-1451 [PMID: 15845905 DOI: 10.1161/01.ATV.0000167525.69400.9c]
- 41 **Rodrigues PH**, Reyes L, Chadda AS, Bélanger M, Wallet SM, Akin D, Dunn W, Progulske-Fox A. Porphyromonas gingivalis strain specific interactions with human coronary artery endothelial cells: a comparative study. *PLoS One* 2012; **7**: e52606 [PMID: 23300720 DOI: 10.1371/journal.pone.0052606]
- 42 **Shaik-Dasthagirisahab YB**, Huang N, Baer MT, Gibson FC. Role of MyD88-dependent and MyD88-independent signaling in Porphyromonas gingivalis-elicited macrophage foam cell formation. *Mol Oral Microbiol* 2013; **28**: 28-39 [PMID: 23194377 DOI: 10.1111/omi.12003]
- 43 **Christodoulou DK**, Milonis HJ, Pappa P, Katsanos KH, Sigounas D, Florentin M, Elisaf M, Tsianos EV. Association of Helicobacter pylori infection with cardiovascular disease-is it just a myth? *Eur J Intern Med* 2011; **22**: 191-194 [PMID: 21402252 DOI: 10.1016/j.ejim.2010.11.010]
- 44 **Park MJ**, Choi SH, Kim D, Kang SJ, Chung SJ, Choi SY, Yoon DH, Lim SH, Kim YS, Yim JY, Kim JS, Jung HC. Association between Helicobacter pylori Seropositivity and the Coronary Artery Calcium Score in a Screening Population. *Gut Liver* 2011; **5**: 321-327 [PMID: 21927661 DOI: 10.5009/gnl.2011.5.3.321]
- 45 **Schöttker B**, Adamu MA, Weck MN, Müller H, Brenner H. Helicobacter pylori infection, chronic atrophic gastritis and major cardiovascular events: a population-based cohort study. *Atherosclerosis* 2012; **220**: 569-574 [PMID: 22189198 DOI: 10.1016/j.atherosclerosis.2011.11.029]
- 46 **Chen Y**, Segers S, Blaser MJ. Association between Helicobacter pylori and mortality in the NHANES III study. *Gut* 2013; **62**: 1262-1269 [PMID: 23303440 DOI: 10.1136/gutjnl-2012-303018]
- 47 **Rogha M**, Nikvarz M, Pourmoghaddas Z, Shirneshan K, Dadkhah D, Pourmoghaddas M. Is helicobacter pylori infection a risk factor for coronary heart disease? *ARYA Atheroscler* 2012; **8**: 5-8 [PMID: 23056092]
- 48 **Reunanen A**, Roivainen M, Kleemola M. Increased titer of antibodies to Mycoplasma pneumoniae may be associated with coronary heart disease. *Atherosclerosis* 2005; **180**: 209-210 [PMID: 15823295 DOI: 10.1046/j.1365-2796.2002.01052.x]
- 49 **Daxböck F**, Assadian A, Watkins-Riedel T, Assadian O. Persistently elevated IgA antibodies to Mycoplasma pneumoniae in patients with internal carotid artery stenosis. *GMS Krankenhhyg Interdisziplin* 2011; **6**: Doc04 [PMID: 22242085 DOI: 10.3205/dgkh000161]
- 50 **Benditt EP**, Barrett T, McDougall JK. Viruses in the etiology of atherosclerosis. *Proc Natl Acad Sci USA* 1983; **80**: 6386-6389 [PMID: 6312457]
- 51 **Van Dam-Mieras MC**, Bruggeman CA, Muller AD, Debie WH, Zwaal RF. Induction of endothelial cell procoagulant activity by cytomegalovirus infection. *Thromb Res* 1987; **47**: 69-75 [PMID: 2821649]
- 52 **Melnick JL**, Petrie BL, Dreesman GR, Burek J, McCollum CH, DeBakey ME. Cytomegalovirus antigen within human arterial smooth muscle cells. *Lancet* 1983; **2**: 644-647 [PMID: 6136795 DOI: 10.1016/S0140-6736(83)92529-1]
- 53 **Yi L**, Wang DX, Feng ZJ. Detection of human cytomegalovirus in atherosclerotic carotid arteries in humans. *J Formos Med Assoc* 2008; **107**: 774-781 [PMID: 18926944 DOI: 10.1016/S0929-6646(08)60190-4]
- 54 **Xenaki E**, Hassoulas J, Apostolakis S, Sourvinos G, Spanidos DA. Detection of cytomegalovirus in atherosclerotic plaques and nonatherosclerotic arteries. *Angiology* 2009; **60**: 504-508 [DOI: 10.1177/0003319708322390]
- 55 **Hendrix MG**, Salimans MM, van Boven CP, Bruggeman CA. High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. *Am J Pathol* 1990; **136**: 23-28 [PMID: 2153348]
- 56 **Adam E**, Melnick JL, Probstfield JL, Petrie BL, Burek J, Bailey KR, McCollum CH, DeBakey ME. High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. *Lancet* 1987; **2**: 291-293 [PMID: 2886763 DOI: 10.1016/S0140-6736(87)90888-9]
- 57 **Ji YN**, An L, Zhan P, Chen XH. Cytomegalovirus infection and coronary heart disease risk: a meta-analysis. *Mol Biol Rep* 2012; **39**: 6537-6546 [PMID: 22311014 DOI: 10.1007/s11033-012-1482-6]
- 58 **Mendy A**, Vieira ER, Gasana J. Seropositivity to herpes simplex virus type 2, but not type 1 is associated with premature cardiovascular diseases: a population-based cross-sectional study. *Atherosclerosis* 2013; **231**: 18-21 [PMID: 24125404 DOI: 10.1016/j.atherosclerosis.2013.08.020]
- 59 **Raza-Ahmad A**, Klassen GA, Murphy DA, Sullivan JA, Kinley CE, Landymore RW, Wood JR. Evidence of type 2 herpes simplex infection in human coronary arteries at the time of coronary artery bypass surgery. *Can J Cardiol* 1995; **11**: 1025-1029 [PMID: 8542544]
- 60 **Madjid M**, Naghavi M, Litovsky S, Casscells SW. Influenza and cardiovascular disease: a new opportunity for prevention and the need for further studies. *Circulation* 2003; **108**: 2730-2736 [PMID: 14610013 DOI: 10.1161/01.CIR.0000102380.47012.92]
- 61 **Lavallée P**, Perchaud V, Gautier-Bertrand M, Grabli D, Amarenco P. Association between influenza vaccination and reduced risk of brain infarction. *Stroke* 2002; **33**: 513-518 [PMID: 11823662 DOI: 10.1161/hs0202.102328]
- 62 **Guan X**, Yang W, Sun X, Wang L, Ma B, Li H, Zhou J. Association of influenza virus infection and inflammatory cytokines with acute myocardial infarction. *Inflamm Res* 2012; **61**: 591-598 [PMID: 22373653 DOI: 10.1007/s00011-012-0449-3]
- 63 **Zhu J**, Quyyumi AA, Norman JE, Costello R, Csako G, Epstein SE. The possible role of hepatitis A virus in the pathogenesis of atherosclerosis. *J Infect Dis* 2000; **182**: 1583-1587 [PMID: 11069227 DOI: 10.1086/3171613]
- 64 **Alyan O**, Kacmaz F, Ozdemir O, Deveci B, Astan R, Celebi AS, Ilkay E. Hepatitis C infection is associated with increased coronary artery atherosclerosis defined by modified Reardon severity score system. *Circ J* 2008; **72**: 1960-1965 [PMID: 18957787 DOI: 10.1253/circj.CJ-08-0459]
- 65 **Ishizaka N**, Ishizaka Y, Takahashi E, Toda Ei E, Hashimoto H, Ohno M, Nagai R, Yamakado M. Increased prevalence of carotid atherosclerosis in hepatitis B virus carriers. *Circulation* 2002; **105**: 1028-1030 [PMID: 11877348]
- 66 **Cainelli F**, Concia E, Vento S. Hepatitis A virus infection and atherosclerosis. *J Infect Dis* 2001; **184**: 390-391 [PMID: 11443571]
- 67 **Boddi M**, Abbate R, Chellini B, Giusti B, Giannini C, Pratesi G, Rossi L, Pratesi C, Gensini GF, Paperetti L, Zignego AL. Hepatitis C virus RNA localization in human carotid plaques. *J Clin Virol* 2010; **47**: 72-75 [PMID: 19896417 DOI: 10.1016/j.jcv.2009.10.005]
- 68 **Boddi M**, Abbate R, Chellini B, Giusti B, Solazzo V, Soft F, Pratesi G, Pratesi C, Gensini G, Zignego AL. HCV infection facilitates asymptomatic carotid atherosclerosis: preliminary report of HCV RNA localization in human carotid plaques. *Dig Liver Dis* 2007; **39** Suppl 1: S55-S60 [PMID: 17936225 DOI: 10.1016/S1590-8658(07)80012-0]
- 69 **Ishizaka Y**, Ishizaka N, Takahashi E, Unuma T, Tooda E, Hashimoto H, Nagai R, Yamakado M. Association between hepatitis C virus core protein and carotid atherosclerosis.

- Circ J* 2003; **67**: 26-30 [PMID: 12520147 DOI: 10.1253/circ.67.26]
- 70 **Weinman SA**, Belalcazar LM. Hepatitis C: a metabolic liver disease. *Gastroenterology* 2004; **126**: 917-919 [PMID: 14988846 DOI: 10.1053/j.gastro.2003.01.001]
  - 71 **Kawaguchi T**, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011; **54**: 1063-1070 [PMID: 21563202 DOI: 10.1002/hep.24412]
  - 72 **Miyazaki T**, Honda A, Ikegami T, Saitoh Y, Hirayama T, Hara T, Doy M, Matsuzaki Y. Hepatitis C virus infection causes hypolipidemia regardless of hepatic damage or nutritional state: An epidemiological survey of a large Japanese cohort. *Hepatol Res* 2011; **41**: 530-541 [PMID: 21501354 DOI: 10.1111/j.1872-034X.2011.00803.x]
  - 73 **Mostafa A**, Mohamed MK, Saeed M, Hasan A, Fontanet A, Godslan I, Coady E, Esmat G, El-Hoseiny M, Abdul-Hamid M, Hughes A, Chaturvedi N. Hepatitis C infection and clearance: impact on atherosclerosis and cardiometabolic risk factors. *Gut* 2010; **59**: 1135-1140 [PMID: 20584782 DOI: 10.1136/gut.2009.202317]
  - 74 **Ishizaka N**, Ishizaka Y, Takahashi E, Tooda Ei, Hashimoto H, Nagai R, Yamakado M. Association between hepatitis C virus seropositivity, carotid-artery plaque, and intima-media thickening. *Lancet* 2002; **359**: 133-135 [PMID: 11809259 DOI: 10.1016/S0140-6736(02)07339-7]
  - 75 **Yang KC**, Chen MF, Su TC, Jeng JS, Hwang BS, Lin LY, Liao CS, Lee YT. Hepatitis B virus seropositivity is not associated with increased risk of carotid atherosclerosis in Taiwanese. *Atherosclerosis* 2007; **195**: 392-397 [PMID: 17134707 DOI: 10.1016/j.atherosclerosis.2006.10.018]
  - 76 **Roed T**, Lebech AM, Kjaer A, Weis N. Hepatitis C virus infection and risk of coronary artery disease: a systematic review of the literature. *Clin Physiol Funct Imaging* 2012; **32**: 421-430 [PMID: 23031062 DOI: 10.1111/j.1475-097X.2012.01152.x]
  - 77 **Miyajima I**, Kawaguchi T, Fukami A, Nagao Y, Adachi H, Sasaki S, Imaizumi T, Sata M. Chronic HCV infection was associated with severe insulin resistance and mild atherosclerosis: a population-based study in an HCV hyperendemic area. *J Gastroenterol* 2013; **48**: 93-100 [PMID: 22678465 DOI: 10.1007/s00535-012-0610-3]
  - 78 **Hsue PY**, Lo JC, Franklin A, Bolger AF, Martin JN, Deeks SG, Waters DD. Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection. *Circulation* 2004; **109**: 1603-1608 [PMID: 15023877 DOI: 10.1161/01.CIR.0000124480.32233.8A]
  - 79 **Thiébaud R**, Aurillac-Lavignolle V, Bonnet F, Ibrahim N, Cipriano C, Neau D, Dupon M, Dabis F, Mercié P. Change in atherosclerosis progression in HIV-infected patients: ANRS Aquitaine Cohort, 1999-2004. *AIDS* 2005; **19**: 729-731 [PMID: 15821400]
  - 80 **Jerico C**, Knobel H, Calvo N, Sorli ML, Guelar A, Gimeno-Bayón JL, Saballs P, López-Colomé JL, Pedro-Botet J. Subclinical carotid atherosclerosis in HIV-infected patients: role of combination antiretroviral therapy. *Stroke* 2006; **37**: 812-817 [PMID: 16439699 DOI: 10.1161/01.STR.0000204037.26797.7f]
  - 81 **Lang S**, Mary-Krause M, Simon A, Partisani M, Gilquin J, Cotte L, Boccara F, Costagliola D. HIV replication and immune status are independent predictors of the risk of myocardial infarction in HIV-infected individuals. *Clin Infect Dis* 2012; **55**: 600-607 [PMID: 22610928 DOI: 10.1093/cid/cis489]
  - 82 **Currier JS**, Kendall MA, Zackin R, Henry WK, Alston-Smith B, Torriani FJ, Schouten J, Mickelberg K, Li Y, Hodis HN. Carotid artery intima-media thickness and HIV infection: traditional risk factors overshadow impact of protease inhibitor exposure. *AIDS* 2005; **19**: 927-933 [PMID: 15905673]
  - 83 **Hultén E**, Mitchell J, Scally J, Gibbs B, Villines TC. HIV positivity, protease inhibitor exposure and subclinical atherosclerosis: a systematic review and meta-analysis of observational studies. *Heart* 2009; **95**: 1826-1835 [PMID: 19632982 DOI: 10.1136/hrt.2009.177774]
  - 84 **Stein JH**, Klein MA, Bellehumeur JL, McBride PE, Wiebe DA, Otvos JD, Sosman JM. Use of human immunodeficiency virus-1 protease inhibitors is associated with atherogenic lipoprotein changes and endothelial dysfunction. *Circulation* 2001; **104**: 257-262 [PMID: 11457741]
  - 85 **Badiou S**, Merle De Boever C, Dupuy AM, Baillat V, Cristol JP, Reynes J. Decrease in LDL size in HIV-positive adults before and after lopinavir/ritonavir-containing regimen: an index of atherogenicity? *Atherosclerosis* 2003; **168**: 107-113 [PMID: 12732393 DOI: 10.1016/S0021-9150(03)00058-3]
  - 86 **Asztalos BF**, Schaefer EJ, Horvath KV, Cox CE, Skinner S, Gerrior J, Gorbach SL, Wanke C. Protease inhibitor-based HAART, HDL, and CHD-risk in HIV-infected patients. *Atherosclerosis* 2006; **184**: 72-77 [PMID: 15935358 DOI: 10.1016/j.atherosclerosis.2005.04.013]
  - 87 **Duffy P**, Wang X, Lin PH, Yao Q, Chen C. HIV Nef protein causes endothelial dysfunction in porcine pulmonary arteries and human pulmonary artery endothelial cells. *J Surg Res* 2009; **156**: 257-264 [PMID: 19540523 DOI: 10.1016/j.jss.2009.02.005]
  - 88 **Hsue PY**, Hunt PW, Schnell A, Kalapus SC, Hoh R, Ganz P, Martin JN, Deeks SG. Role of viral replication, antiretroviral therapy, and immunodeficiency in HIV-associated atherosclerosis. *AIDS* 2009; **23**: 1059-1067 [PMID: 19390417 DOI: 10.1097/QAD.0b013e32832b514b]
  - 89 **Desvarieux M**, Boccara F, Meynard JL, Bastard JP, Mallat Z, Charbit B, Demmer RT, Haddour N, Fellahi S, Tedgui A, Cohen A, Capeau J, Boyd A, Girard PM. Infection duration and inflammatory imbalance are associated with atherosclerotic risk in HIV-infected never-smokers independent of antiretroviral therapy. *AIDS* 2013; **27**: 2603-2614 [PMID: 24100713 DOI: 10.1097/QAD.0b013e3283634819]
  - 90 **Jackson L**, Britton J, Lewis SA, McKeever TM, Atherton J, Fullerton D, Fogarty AW. A population-based epidemiologic study of *Helicobacter pylori* infection and its association with systemic inflammation. *Helicobacter* 2009; **14**: 108-113 [PMID: 19751435 DOI: 10.1111/j.1523-5378.2009.00711.x]
  - 91 **Rogha M**, Dadkhah D, Pourmoghaddas Z, Shirneshan K, Nikvarz M, Pourmoghaddas M. Association of *Helicobacter pylori* infection with severity of coronary heart disease. *ARYA Atheroscler* 2012; **7**: 138-141 [PMID: 23205045]
  - 92 **Schiavoni G**, Di Pietro M, Ronco C, De Cal M, Cazzavillan S, Rattu M, Nicoletti M, Del Piano M, Sessa R. Chlamydia pneumoniae infection as a risk factor for accelerated atherosclerosis in hemodialysis patients. *J Biol Regul Homeost Agents* 2010; **24**: 367-375 [PMID: 20846485]
  - 93 **Pejic L**, Kesic LJ, Milasin J. C-reactive protein as a systemic marker of inflammation in periodontitis. *Eur J Clin Microbiol Infect Dis* 2011; **30**: 407-414 [PMID: 21057970 DOI: 10.1007/s10096-010-1101-1]
  - 94 **Campbell LA**, Yaraei K, Van Lenten B, Chait A, Blessing E, Kuo CC, Nosaka T, Ricks J, Rosenfeld ME. The acute phase reactant response to respiratory infection with *Chlamydia pneumoniae*: implications for the pathogenesis of atherosclerosis. *Microbes Infect* 2010; **12**: 598-606 [PMID: 20417302 DOI: 10.1016/j.micinf.2010.04.001]
  - 95 **Kebschull M**, Demmer RT, Papapanou PN. "Gum bug, leave my heart alone!"--epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. *J Dent Res* 2010; **89**: 879-902 [PMID: 20639510 DOI: 10.1177/0022034510375281]
  - 96 **Roivainen M**, Viik-Kajander M, Palosuo T, Toivanen P, Leinonen M, Saikku P, Tenkanen L, Manninen V, Hovi T, Mänttari M. Infections, inflammation, and the risk of coronary heart disease. *Circulation* 2000; **101**: 252-257 [PMID: 10645920]
  - 97 **Okada T**, Ayada K, Usui S, Yokota K, Cui J, Kawahara Y, Inaba T, Hirohata S, Mizuno M, Yamamoto D, Kusachi S, Matsuura E, Oguma K. Antibodies against heat shock protein 60



- derived from *Helicobacter pylori*: diagnostic implications in cardiovascular disease. *J Autoimmun* 2007; **29**: 106-115 [PMID: 17606364 DOI: 10.1016/j.jaut.2007.05.004]
- 98 **Choi J**, Lee SY, Kim K, Choi BK. Identification of immunoreactive epitopes of the *Porphyromonas gingivalis* heat shock protein in periodontitis and atherosclerosis. *J Periodontol Res* 2011; **46**: 240-245 [PMID: 21241301 DOI: 10.1111/j.1600-0765.2010.01339.x]
  - 99 **Kreutmayer S**, Csordas A, Kern J, Maass V, Almanzar G, Offtendering M, Öllinger R, Maass M, Wick G. Chlamydia pneumoniae infection acts as an endothelial stressor with the potential to initiate the earliest heat shock protein 60-dependent inflammatory stage of atherosclerosis. *Cell Stress Chaperones* 2013; **18**: 259-268 [PMID: 23192457 DOI: 10.1007/s12192-012-0378-7]
  - 100 **Andrié RP**, Bauriedel G, Braun P, Höpp HW, Nickenig G, Skowasch D. Prevalence of intimal heat shock protein 60 homologues in unstable angina and correlation with anti-heat shock protein antibody titers. *Basic Res Cardiol* 2011; **106**: 657-665 [PMID: 21416407 DOI: 10.1007/s00395-011-0171-2]
  - 101 **Ausiello CM**, Palazzo R, Spensieri F, Fedele G, Lande R, Ciervo A, Fioroni G, Cassone A. 60-kDa heat shock protein of *Chlamydia pneumoniae* is a target of T-cell immune response. *J Biol Regul Homeost Agents* 2005; **19**: 136-140 [PMID: 16602628]
  - 102 **Ford P**, Gemmell E, Walker P, West M, Cullinan M, Seymour G. Characterization of heat shock protein-specific T cells in atherosclerosis. *Clin Diagn Lab Immunol* 2005; **12**: 259-267 [PMID: 15699420 DOI: 10.1128/CDLI.12.2.259-267.2005]
  - 103 **Ayada K**, Yokota K, Hirai K, Fujimoto K, Kobayashi K, Ogawa H, Hatanaka K, Hirohata S, Yoshino T, Shoenfeld Y, Matsumura E, Oguma K. Regulation of cellular immunity prevents *Helicobacter pylori*-induced atherosclerosis. *Lupus* 2009; **18**: 1154-1168 [PMID: 19880562 DOI: 10.1177/0961203309106600]
  - 104 **Speir E**, Modali R, Huang ES, Leon MB, Shawl F, Finkel T, Epstein SE. Potential role of human cytomegalovirus and p53 interaction in coronary restenosis. *Science* 1994; **265**: 391-394 [PMID: 8023160]
  - 105 **Dengler TJ**, Raftery MJ, Werle M, Zimmermann R, Schönrich G. Cytomegalovirus infection of vascular cells induces expression of pro-inflammatory adhesion molecules by paracrine action of secreted interleukin-1 $\beta$ . *Transplantation* 2000; **69**: 1160-1168 [PMID: 10762222]
  - 106 **Rahbar A**, Söderberg-Nauclér C. Human cytomegalovirus infection of endothelial cells triggers platelet adhesion and aggregation. *J Virol* 2005; **79**: 2211-2220 [PMID: 15681423 DOI: 10.1128/JVI.79.4.2211-2220.2005]
  - 107 **Naghavi M**, Wyde P, Litovsky S, Madjid M, Akhtar A, Naguib S, Siadaty MS, Sanati S, Casscells W. Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice. *Circulation* 2003; **107**: 762-768 [PMID: 12578882 DOI: 10.1161/01.CIR.0000048190.68071.2B]
  - 108 **Naghavi M**, Barlas Z, Siadaty S, Naguib S, Madjid M, Casscells W. Association of influenza vaccination and reduced risk of recurrent myocardial infarction. *Circulation* 2000; **102**: 3039-3045 [PMID: 11120692]
  - 109 **Blake GJ**, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res* 2001; **89**: 763-771 [PMID: 11679405]
  - 110 **Petty GW**, Duffy J, Houston J. Cerebral ischemia in patients with hepatitis C virus infection and mixed cryoglobulinemia. *Mayo Clin Proc* 1996; **71**: 671-678 [PMID: 8656709]
  - 111 **Capra F**, De Maria E, Lunardi C, Marchiori L, Mezzelani P, Beri R, Gabrielli GB. Serum level of soluble intercellular adhesion molecule 1 in patients with chronic liver disease related to hepatitis C virus: A prognostic marker for responses to interferon treatment. *J Infect Dis* 2000; **181**: 425-431 [PMID: 10669322 DOI: 10.1086/315265]
  - 112 **Cacoub P**, Ghillani P, Revelen R, Thibault V, Calvez V, Charlotte F, Musset L, Youinou P, Piette JC. Anti-endothelial cell auto-antibodies in hepatitis C virus mixed cryoglobulinemia. *J Hepatol* 1999; **31**: 598-603 [PMID: 10551381 DOI: 10.1016/S0168-8278(99)80337-7]
  - 113 **Shrestha S**, Irvin MR, Grunfeld C, Arnett DK. HIV, inflammation, and calcium in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2014; **34**: 244-250 [PMID: 24265418 DOI: 10.1161/ATVBAHA.113.302191]
  - 114 **Khor CC**, Hibberd ML. Host-pathogen interactions revealed by human genome-wide surveys. *Trends Genet* 2012; **28**: 233-243 [PMID: 22445588 DOI: 10.1016/j.tig.2012.02.001]
  - 115 **Georges JL**, Rupprecht HJ, Blankenberg S, Poirier O, Bickel C, Hafner G, Nicaud V, Meyer J, Cambien F, Tiret L. Impact of pathogen burden in patients with coronary artery disease in relation to systemic inflammation and variation in genes encoding cytokines. *Am J Cardiol* 2003; **92**: 515-521 [PMID: 12943869 DOI: 10.1016/S0002-9149(03)00717-3]
  - 116 **Westhorpe CL**, Maisa A, Spelman T, Hoy JF, Dewar EM, Karapanagiotidis S, Hearps AC, Cheng WJ, Trevillyan J, Lewin SR, Sviridov D, Elliott JH, Jaworowski A, Dart AM, Crowe SM. Associations between surface markers on blood monocytes and carotid atherosclerosis in HIV-positive individuals. *Immunol Cell Biol* 2014; **92**: 133-138 [PMID: 24296810 DOI: 10.1038/icb.2013.84]

P- Reviewers: He JY, Stover CM S- Editor: Song XX

L- Editor: A E- Editor: Wu HL



## Is there a role for fish oil in inflammatory bowel disease?

Affifa Farrukh, John Francis Mayberry

Affifa Farrukh, John Francis Mayberry, Department of Digestive Diseases, University Hospitals of Leicester NHS Trust, Leicester General Hospital, Leicester LE5 4PW, United Kingdom

**Author contributions:** Both authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; drafted the article and revised it critically for important intellectual content; and final approval of this version to be published.

**Correspondence to:** Dr. Affifa Farrukh, Department of Digestive Diseases, University Hospitals of Leicester NHS Trust, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, United Kingdom. [anupvora@gmail.com](mailto:anupvora@gmail.com)

Telephone: +44-01-162584787 Fax: +44-01-162584787

Received: April 5, 2014 Revised: May 2, 2014

Accepted: June 10, 2014

Published online: July 16, 2014

### Abstract

A number of animal and human studies suggest omega 3-fatty acids are anti-inflammatory. As a result they may have a therapeutic role in inflammatory bowel disease (IBD). The aim of this review is to briefly assess the literature about the utility of poly-unsaturated fatty acids (PUFAs) in the management of IBD. Taken together, almost all studies suggest some beneficial effects of n-3 PUFAs in IBD but the mechanism remains controversial. In addition, clinical benefit seems to be largely confined to ulcerative colitis. However all studies have concluded that these compounds have no potential for a steroid/aminosalicylic acid sparing effect or to maintain remission. Now the question arises as to whether this treatment is of real value to IBD patients? Clearly they have some therapeutic potential but further work is needed.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Fish oil; Ulcerative colitis; Crohn's disease; Treatment; n-3 poly-unsaturated fatty acids

**Core tip:** Fish oil supplements are probably of benefit to

patients with ulcerative colitis. They have a much less certain role in Crohn's disease.

Farrukh A, Mayberry JF. Is there a role for fish oil in inflammatory bowel disease? *World J Clin Cases* 2014; 2(7): 250-252 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/250.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.250>

### INTRODUCTION

Both animal and clinical work suggest that omega 3-fatty acids are anti-inflammatory in their action and so have the potential to be of use in the treatment of inflammatory bowel disease (IBD). In particular, they appeal to patients who see them as both safe and natural. The purpose of this review is to survey the literature and assess the utility of poly-unsaturated fatty acids (PUFAs) in the management of IBD.

### THE UTILITY OF PUFAS IN THE MANAGEMENT OF IBD

The aetiology of IBD remains unclear but local mediators including arachidonic acid metabolites, cytokines and altered cell mediated immunity are likely to contribute to the disease. The rationale for the prescription of n-3 PUFA to promote a healthy gastrointestinal tract has been linked to their suggested anti-inflammatory properties. Different strategies have been adopted in various clinical trials to evaluate n-3 PUFA in patients with IBD. Inhibition of natural cytotoxicity, changes in interleukin 2 (IL-2) and arachidonic acid metabolites, e.g., LTB<sub>4</sub> are the main chemotactic signals seen in the mucosa during a relapse. All are known to mediate the natural killer activity. A second hypothesis is based on the possible deficiency of essential fatty acids (EFAs) in IBD and its effect on cell membranes<sup>[1]</sup>. A further possibility is that fish oil ameliorates oxidative stress in IBD.

In a randomised crossover trial from four units which

involved 18 patients with ulcerative colitis (UC) fish oil supplements reduced LTB<sub>4</sub> levels in a rectal dialysate. Histology improved and patients' weight increased<sup>[2]</sup>.

Conversely, a small Canadian trial of 11 patients found that addition of fish oil was of clinical benefit in UC but did not reduce mucosal LTB<sub>4</sub><sup>[3]</sup>. However, over a six month period serum LTB<sub>4</sub> was insignificant while there was a simultaneous fall in NK cell cytotoxicity<sup>[4]</sup>.

An American study of 47 patients with long-standing bowel problems looked at the frequency of essential fatty acid deficiency. The majority had Crohn's disease. Compared to 56 control subjects, patients' metabolism was comparable to that seen in essential fatty acid deficiency (EFAD). Patients had: (1) 7% lower PUFA levels; (2) Lower concentrations of saturated and monounsaturated fatty acids.

The authors suggested patients with IBD should be assessed for EFAD and receive significant quantities of supplements with a high EFA content<sup>[5]</sup>. In contrast, a Japanese study found that EFAD rarely occurs in Crohn's disease<sup>[6]</sup>. A United Kingdom prospective study based on 26000 recruits living in Norfolk showed that total dietary n-3PUFAs, eicosapentaenoic acid and docosahexaenoic acid were associated with a reduced risk of ulcerative colitis<sup>[7]</sup>. This was not found in a small cross-sectional study of 51 patients with inflammatory bowel disease from Hungary<sup>[8]</sup>.

To gauge whether fish oil can reduce oxidative stress in ulcerative colitis a Brazilian crossover study of nine patients on conventional treatment with sulfasalazine also received omega-3 fatty acids or placebo for two months separated by two months. They were compared with nine healthy people. Disease activity was examined through a range of standard serological measures as well as endoscopy and histology. The results showed that fish oil can act as a free radical scavenger and so protect patients<sup>[9]</sup>. The influence of monounsaturated, n-3, and n-3 + n-6 polyunsaturated fatty acids on histology, mucosal defence, mucosal prostaglandin E<sub>2</sub> and LTB<sub>4</sub> in a rat model was investigated in Spain. It concluded that n-3 PUFAs can prevent inflammation but cause a decrease in the colon's defence system leading to oxidative injury<sup>[10]</sup>. Therefore, although it seems likely that these compounds have anti-inflammatory effects, the mechanism by which they achieve this needs further exploration.

There are some important considerations which should be borne in mind before promoting their use in the clinical care of patients with IBD: (1) Do they decrease disease activity? (2) Do they maintain remission? (3) Can they be used as an alternative to steroids or aminosalicylic acid (ASA) compounds?

There are studies which demonstrated a reduction in disease activity with these compounds, *e.g.*, a pilot study in United Kingdom evaluated their effectiveness both in terms of disease activity and histological scores when compared with pre-treatment measures<sup>[11,12]</sup>. In a German trial 5-ASA compounds were stopped in 64 patients who had ulcerative colitis and had been in remission for three months. They received a fish oil supplement and

their clinical course was followed for 24 mo with colonoscopies at the beginning and end of the study. Freedom from disease activity was only seen in two of the 24 mo and the overall relapse rate was similar for active treatment and placebo groups. It seems that n-3 PUFAs can temporarily retard but not prevent relapse in UC<sup>[12]</sup>. Another randomised blinded control study of 87 patients from United Kingdom found fish oil had some benefit. Corticosteroid requirements were reduced for those 53 patients who had active disease on trial entry. Fish oil induced faster remission, although this trend did not reach significance. In contrast there was no difference in relapse rates if patients were in remission. So, it appears fish oil supplementation has a modest benefit in active disease allowing use of smaller doses of corticosteroids. In contrast they seem to have no role in maintenance<sup>[13]</sup>. A randomised controlled multicentre trial of 204 patients with Crohn's disease in Germany confirmed that omega-3 fatty acids had no role in prolonging remission<sup>[14,15]</sup>. Confirmation of the limited benefit in ulcerative colitis and absence of effect in Crohn's disease was first suggested in 1989 in a study of 39 patients<sup>[16]</sup>. The multicentre EPIC study from North America and Europe which included 738 patients with Crohn's disease again confirmed that omega 3 free fatty acids had no role in maintenance<sup>[17]</sup>. Support for such an interpretation comes from 3 systematic reviews<sup>[18-20]</sup>. However, this might not be the case in children and young people. In a study of 38 patients addition of enteric coated omega-3 fatty acids to 5-ASA treatment appeared of benefit<sup>[21]</sup>. Support for their role in Crohn's disease also came from a rigorous trial in 78 patients<sup>[22]</sup>.

Ten patients with moderately active colitis were assessed in a study which used a randomized cross-over methodology. The treatments were either sulfasalazine or omega-3 fatty acids for 2 mo. Response was measured by endoscopic assessment, histological review and whole-body protein turnover. Treatment with omega-3 fatty acids alone led to a less objective improvement than conventional treatment. This shows that for active ulcerative colitis sulfasalazine is better than omega-3 fatty acids<sup>[23]</sup>.

## CONCLUSION

Taken together, almost all studies suggest some beneficial effects of n-3 PUFAs in IBD but the mechanism remains controversial. In addition, clinical benefit seem largely confined to UC. However all studies have concluded that these compounds have no potential for a Steroid/ASA sparing effect or to maintain remission. Now the question arises as to whether this treatment is of real value to IBD patients? Clearly they have some therapeutic potential but further work is needed with larger numbers and more highly powered trials.

## REFERENCES

- 1 Uchiyama K, Nakamura M, Odahara S, Koido S, Katahira K, Shiraishi H, Ohkusa T, Fujise K, Tajiri H. N-3 polyunsatu-

- rated fatty acid diet therapy for patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 1696-1707 [PMID: 20222122 DOI: 10.1002/ibd.21251]
- 2 **Stenson WE**, Cort D, Rodgers J, Burakoff R, DeSchryver-Kecskemeti K, Gramlich TL, Beeken W. Dietary supplementation with fish oil in ulcerative colitis. *Ann Intern Med* 1992; **116**: 609-614 [PMID: 1312317 DOI: 10.7326/0003-4819-116-8-609]
- 3 **Aslan A**, Triadafilopoulos G. Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, crossover study. *Am J Gastroenterol* 1992; **87**: 432-437 [PMID: 1553930]
- 4 **Almallah YZ**, El-Tahir A, Heys SD, Richardson S, Eremin O. Distal procto-colitis and n-3 polyunsaturated fatty acids: the mechanism(s) of natural cytotoxicity inhibition. *Eur J Clin Invest* 2000; **30**: 58-65 [PMID: 10620003 DOI: 10.1046/j.1365-2362.2000.00581.x]
- 5 **Siguel EN**, Lerman RH. Prevalence of essential fatty acid deficiency in patients with chronic gastrointestinal disorders. *Metabolism* 1996; **45**: 12-23 [PMID: 8544768 DOI: 10.1016/S0026-0495(96)90194-8]
- 6 **Kuroki F**, Iida M, Matsumoto T, Aoyagi K, Kanamoto K, Fujishima M. Serum n3 polyunsaturated fatty acids are depleted in Crohn's disease. *Dig Dis Sci* 1997; **42**: 1137-1141 [PMID: 9201073 DOI: 10.1023/A: 1018873217192]
- 7 **John S**, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. *Eur J Gastroenterol Hepatol* 2010; **22**: 602-606 [PMID: 20216220 DOI: 10.1097/MEG.0b013e3283352d05]
- 8 **Figler M**, Gasztonyi B, Cseh J, Horváth G, Kisbenedek AG, Bokor S, Decsi T. Association of n-3 and n-6 long-chain polyunsaturated fatty acids in plasma lipid classes with inflammatory bowel diseases. *Br J Nutr* 2007; **97**: 1154-1161 [PMID: 17381967 DOI: 10.1017/S0007114507682956]
- 9 **Barbosa DS**, Cecchini R, El Kadri MZ, Rodríguez MA, Burini RC, Dichi I. Decreased oxidative stress in patients with ulcerative colitis supplemented with fish oil omega-3 fatty acids. *Nutrition* 2003; **19**: 837-842 [PMID: 14559317 DOI: 10.1016/S0899-9007(03)00162-X]
- 10 **Nieto N**, Fernandez MI, Torres MI, Ríos A, Suarez MD, Gil A. Dietary monounsaturated n-3 and n-6 long-chain polyunsaturated fatty acids affect cellular antioxidant defense system in rats with experimental ulcerative colitis induced by trinitrobenzene sulfonic acid. *Dig Dis Sci* 1998; **43**: 2676-2687 [PMID: 9881500 DOI: 10.1023/A: 1026655311878]
- 11 **Almallah YZ**, Richardson S, O'Hanrahan T, Mowat NA, Brunt PW, Sinclair TS, Ewen S, Heys SD, Eremin O. Distal procto-colitis, natural cytotoxicity, and essential fatty acids. *Am J Gastroenterol* 1998; **93**: 804-809 [PMID: 9625132 DOI: 10.1111/j.1572-0241.1998.229\_a.x]
- 12 **Loeschke K**, Ueberschaer B, Pietsch A, Gruber E, Ewe K, Wiebecke B, Heldwein W, Lorenz R. n-3 fatty acids only delay early relapse of ulcerative colitis in remission. *Dig Dis Sci* 1996; **41**: 2087-2094 [PMID: 8888725 DOI: 10.1007/BF02093614]
- 13 **Hawthorne AB**, Daneshmend TK, Hawkey CJ, Belluzzi A, Everitt SJ, Holmes GK, Malkinson C, Shaheen MZ, Willars JE. Treatment of ulcerative colitis with fish oil supplementation: a prospective 12 month randomised controlled trial. *Gut* 1992; **33**: 922-928 [PMID: 1353742 DOI: 10.1136/gut.33.7.922]
- 14 **Frey M**. Behavioral correlates of health and illness in youths with chronic illness. *Appl Nurs Res* 1996; **9**: 167-176 [PMID: 8961573 DOI: 10.1016/S0897-1897(96)80029-2]
- 15 **Lorenz-Meyer H**, Bauer P, Nicolay C, Schulz B, Purrmann J, Fleig WE, Scheurlen C, Koop I, Pudel V, Carr L. Omega-3 fatty acids and low carbohydrate diet for maintenance of remission in Crohn's disease. A randomized controlled multicenter trial. Study Group Members (German Crohn's Disease Study Group). *Scand J Gastroenterol* 1996; **31**: 778-785 [PMID: 8858747 DOI: 10.3109/00365529609010352]
- 16 **Lorenz R**, Weber PC, Szimnau P, Heldwein W, Strasser T, Loeschke K. Supplementation with n-3 fatty acids from fish oil in chronic inflammatory bowel disease--a randomized, placebo-controlled, double-blind cross-over trial. *J Intern Med Suppl* 1989; **731**: 225-232 [PMID: 2650694]
- 17 **Feagan BG**, Sandborn WJ, Mittmann U, Bar-Meir S, D'Haens G, Bradette M, Cohen A, Dallaire C, Ponich TP, McDonald JW, Hébuterne X, Paré P, Klvana P, Niv Y, Ardizzone S, Alexeeva O, Rostom A, Kiudelis G, Spleiss J, Gilgen D, Vandervoort MK, Wong CJ, Zou GY, Donner A, Rutgeerts P. Omega-3 free fatty acids for the maintenance of remission in Crohn disease: the EPIC Randomized Controlled Trials. *JAMA* 2008; **299**: 1690-1697 [PMID: 18398081 DOI: 10.1001/jama.299.14.1690]
- 18 **Cabrè E**, Mañosa M, Gassull MA. Omega-3 fatty acids and inflammatory bowel diseases - a systematic review. *Br J Nutr* 2012; **107** Suppl 2: S240-S252 [PMID: 22591898 DOI: 10.1017/S0007114512001626]
- 19 **Marion-Letellier R**, Savoye G, Beck PL, Panaccione R, Ghosh S. Polyunsaturated fatty acids in inflammatory bowel diseases: a reappraisal of effects and therapeutic approaches. *Inflamm Bowel Dis* 2013; **19**: 650-661 [PMID: 23328774 DOI: 10.1097/MIB.0b013e3182810122]
- 20 **Turner D**, Zlotkin SH, Shah PS, Griffiths AM. Omega 3 fatty acids (fish oil) for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2007; **18**: CD006320 [PMID: 17443620]
- 21 **Romano C**, Cucchiara S, Barabino A, Annese V, Sferlazzas C. Usefulness of omega-3 fatty acid supplementation in addition to mesalazine in maintaining remission in pediatric Crohn's disease: a double-blind, randomized, placebo-controlled study. *World J Gastroenterol* 2005; **11**: 7118-7121 [PMID: 16437657]
- 22 **Belluzzi A**, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. *N Engl J Med* 1996; **334**: 1557-1560 [PMID: 8628335]
- 23 **Dichi I**, Frenhane P, Dichi JB, Correa CR, Angeleli AY, Bicu-do MH, Rodrigues MA, Victória CR, Burini RC. Comparison of omega-3 fatty acids and sulfasalazine in ulcerative colitis. *Nutrition* 2000; **16**: 87-90 [PMID: 10696629]

**P- Reviewers:** Esmat S, Guslandi M, Matsumoto S, Naito Y

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Wu HL





## Critical review of topical management of oral hairy leukoplakia

Cláudia B Brasileiro, Mauro Henrique NG Abreu, Ricardo A Mesquita

Cláudia B Brasileiro, Ricardo A Mesquita, Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, Pampulha, 31270-901, Belo Horizonte/MG, Brazil

Mauro Henrique NG Abreu, Department of Community and Preventive Dentistry, School of Dentistry, Universidade Federal de Minas Gerais, Pampulha, 31270-901, Belo Horizonte/MG, Brazil  
Author contributions: Mesquita RA and Abreu MHNG were responsible for the concept and review of the manuscript; Brasileiro CB performed research and wrote the manuscript.

Correspondence to: Ricardo A Mesquita, PhD, Department of Oral Surgery and Pathology, School of Dentistry, Universidade Federal de Minas Gerais, sala 3202-D, Av. Antônio Carlos, 6627, Pampulha, 31270-901, Belo Horizonte/MG, Brazil. [ramesquita@ufmg.br](mailto:ramesquita@ufmg.br)

Telephone: +55-31-34092499 Fax: +55-31-34092430

Received: January 28, 2014 Revised: April 2, 2014

Accepted: May 16, 2014

Published online: July 16, 2014

### Abstract

Oral hairy leukoplakia (OHL) is a disease associated with Epstein-Barr virus and human immunodeficiency virus infections. OHL is usually an asymptomatic lesion, but in some cases treatment is recommended to reestablish the normal characteristics of the tongue, to eliminate pathogenic microorganisms, to improve patient comfort and for cosmetic reasons. Proposed treatments for this condition include surgery, systemic antiviral treatment and topical management. Topical treatment is an inexpensive and safe therapy that is easy to apply, noninvasive, free of systemic adverse effects and effective over a long period of time. The aim of this study was to present a review of the literature for topical therapy for OHL. Gentian violet, retinoids, podophyllin, acyclovir and podophyllin associated with topical antiviral drugs were used to treat OHL. Reports with this focus are limited, and since 2010, no new studies have been published that discuss the efficacy of topical treatments for OHL. Podophyllin with acyclovir

cream was found to be effective, causing regression of lesions with no recurrences. Additional searches are necessary to provide clinical evidence of topical management effectiveness.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Oral hairy leukoplakia; Human immunodeficiency virus infection; Topical treatment; Topical agents; Recurrence rate

**Core tip:** This literature review was performed to assess the evidence for topical treatments for oral hairy leukoplakia (OHL). Although highly active antiretroviral therapy has reduced oral lesions associated with human immunodeficiency virus, prevalence studies revealed that OHL is still observed in patients with HIV infections. Knowledge about appropriate management of this condition is relevant, specifically regarding topical treatments that are less invasive, low-cost, easy to apply and free of systemic adverse effects.

Brasileiro CB, Abreu MHNG, Mesquita RA. Critical review of topical management of oral hairy leukoplakia. *World J Clin Cases* 2014; 2(7): 253-256 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/253.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.253>

### INTRODUCTION

Oral hairy leukoplakia (OHL) was first described in 1984 by Greenspan *et al*<sup>[1]</sup> and is described as a white plaque generally on the lateral borders of the tongue in patients with human immunodeficiency virus (HIV) that later developed acquired immunodeficiency syndrome (AIDS). Later, other studies confirmed OHL as an early indicator of HIV infection and revealed that this disease may be related to the progression to AIDS. However, OHL

**Table 1 Topical agents used in the treatment of oral hairy leukoplakia and study types**

	Case reports	Case series	Randomized clinical trial
Gentian violet	Bhandarkar <i>et al</i> <sup>[3]</sup>		
Retinoids		Schöfer <i>et al</i> <sup>[11]</sup> Alessi <i>et al</i> <sup>[12]</sup>	
Podophyllin		Lozada-Nur <i>et al</i> <sup>[13]</sup> Sanchez <i>et al</i> <sup>[15]</sup> Gowdey <i>et al</i> <sup>[14]</sup> Ficarra <i>et al</i> <sup>[16]</sup>	
Acyclovir			Moura <i>et al</i> <sup>[2]</sup>
Combined topical therapy (Topical antiviral agents and podophyllin)			Moura <i>et al</i> <sup>[4]</sup>

is not exclusive to HIV infection and may be associated with other cases of immunosuppressed patients<sup>[1-3]</sup>. OHL appears clinically as an asymptomatic white lesion on the lateral border of the tongue, unilaterally or bilaterally, with imprecise boundaries, a flat, corrugated or hairy surface, that is not removed by scraping<sup>[4]</sup>. Some patients may present with symptoms including mild pain and alteration of taste<sup>[5]</sup>.

The pathogenesis of OHL is related to the infection of oral squamous epithelial cells with the Epstein-Barr virus (EBV)<sup>[3,4]</sup>. The absence of or high reduction of Langerhans cells in OHL has been demonstrated<sup>[6]</sup>. Langerhans cells are the antigen-presenting immune cells that are required for an immune system response to a viral infection. This deficiency of Langerhans cells may permit EBV to replicate<sup>[5-7]</sup>.

Topical therapy is the most highly recommended treatment for OHL because it has a low cost, is easy to use, has few side effects and is effective for a long period of time<sup>[4]</sup>. However, there are few studies that evaluate the effects of topical treatment in patients with OHL. This can be explained by the significant reduction in the prevalence of the oral lesions in HIV patients after the introduction of highly active antiretroviral therapy<sup>[4,8]</sup>. The purpose of this article is to present a review of topical therapies for OHL. The methodology was a search of the literature, from 1966 through December 2013, related to the topical treatment of OHL and listed on PubMed. The search was conducted in both English and Portuguese, and the keywords used were “oral hairy leukoplakia”, “oral hairy leukoplakia and topical management” and “oral hairy leukoplakia and topical treatment”. Additional studies were found in the reference lists of the selected articles. Randomized clinical trials, case reports and review articles were included in the current paper (Table 1).

## REVIEW

Usually, OHL does not require specific therapy, and when indicated, therapy is intended to restore the patient's comfort, eliminate the hairiness, reestablish the normal appearance of the tongue for aesthetic reasons and remove

niches for bacteria, viruses (EBV) and fungi to prevent the establishment of other oral diseases<sup>[2]</sup>. Proposed treatments include surgery, systemic antiviral therapy and topical management. A search of the literature found 16 articles related to topical treatments for OHL. All forms of topical management of OHL identified in published studies will be presented herein.

### Gentian violet

Gentian violet is a triphenylmethane dye that was synthesized by Charles Lauth, in 1861, under the name of “Violet de Paris”. Churchman, in 1912, demonstrated the bacteriostatic action of gentian violet against Gram-positive microorganisms *in vitro* and in animal models, as well as the antimycotic effects of this agent against multiple species of *Candida*<sup>[9]</sup>. Since then, several studies have evaluated the antibacterial and antifungal actions of gentian violet.

The antiviral properties of gentian violet were investigated based on evidence that EBV viral products induce the generation of reactive oxygen, and gentian violet is a potent inhibitor of reactive oxygen species<sup>[10]</sup>. Given that gentian violet is well-tolerated, approved for human use and is an inexpensive agent, Bhandarkar *et al*<sup>[3]</sup> performed a study using gentian violet (2%) as a topical treatment for OHL in one HIV-infected man. Gentian violet was applied topically to the lesion three times in a one-month period. Complete regression of OHL was observed at a one-month follow-up, and there was no recurrence of the OHL one year after treatment.

### Retinoids

Retin-A is a dekeratinizing agent responsible for the modulation of the presence of Langerhans cells in OHL. Local application of 0.1% vitamin A twice daily was performed in twelve cases of OHL and regression of the lesions was observed after 10 d<sup>[11]</sup>. Daily application of a tretinoin solution (Retin-A) for 15 to 20 d was performed in 22 patients, and 37 patients received no treatment. Lesion healing was observed in 69% of treated patients and spontaneous regression was detected in 10.8% of untreated patients<sup>[12]</sup>. Retin-A is a costly drug and causes a burning sensation after prolonged use<sup>[13,14]</sup>.

### Podophyllin

Podophyllin is a dry, alcoholic extract of rhizomes and roots of *Podophyllum peltatum*. It is a lipid-soluble substance that crosses cell membranes and interferes with cell replication; this substance is commonly used as a topical chemotherapy agent<sup>[14]</sup>. It is inexpensive, simple to apply, and effective over a long period of time. Although podophyllin has a very bitter and unpleasant taste, the palate returns to normal two hours after of application<sup>[2]</sup>.

The results of a 25% alcoholic solution of podophyllin as topical therapy for OHL are significant, especially in the first week after application. In a case series, nine patients were treated with podophyllin resin 25% sol in a benzoin compound tincture. The results showed complete regression of all lesions: five patients within one

week and four after a second application a week later. Those four patients had presented with more extensive lesions<sup>[13]</sup>. In another study, six men with OHL were treated with a once-daily application of podophyllin 25%, and healing of all lesions was verified in three to 5 d<sup>[15]</sup>. Gowdey *et al.*<sup>[14]</sup> assessed ten HIV-infected patients with OHL on the tongue and treated one side with a single application of topical podophyllin resin 25% solution. The other side was used as a control. The patients were evaluated at days two, seven, and thirty of the study. They described a slight change of taste, burning sensation and pain with a short duration. There was regression of lesions, especially on the second day after application.

The dose usually applied in topical therapy for OHL varied from 10 mg to 20 mg of podophyllin<sup>[2,14]</sup>. This dose has not been associated with adverse or systemic effects; these effects are observed after ingestion or when more than 100 mg of podophyllin is topically applied and not removed within 4 to 6 h<sup>[14]</sup>.

### Acyclovir

Acyclovir is a chemotherapeutic antiviral agent that is highly effective against herpes simplex virus types I and II, EBV, Varicella zoster virus, and cytomegalovirus<sup>[2]</sup>. The only previous study performed using acyclovir cream for topical treatment was performed by Ficarra *et al.*<sup>[16]</sup>. The authors observed OHL in 23 out of 120 HIV-positive patients (19%), and found a complete resolution of OHL in two patients and partial regression in one patient after topical application of acyclovir cream.

### Combined topical therapy

Topical antiviral drugs may be used in combination with podophyllin, increasing the efficiency of OHL treatment. After the dekeratinization of superficial epithelial cells by podophyllin, the topical antiviral drug acts on exposed and infected cells located below the surface<sup>[4]</sup>. A clinical trial study, performed randomly, proposed a combined topical therapy of 25% podophyllin and 5% acyclovir cream and compared the results with 25% podophyllin<sup>[2]</sup>. In both protocols, applications were performed weekly. All lesions treated with podophyllin and acyclovir showed total clinical regression, and in the podophyllin group, four lesions did not display total clinical resolution after 25 applications. Furthermore, in the 25% podophyllin group, smaller lesions showed clinical regression with fewer applications than larger lesions. In the 25% podophyllin and 5% acyclovir cream group, there was no significant difference in the number of applications.

Based on their previous study, a new topical treatment for OHL employing 25% podophyllin resin with 1% penciclovir cream was tested and the results were compared to 25% podophyllin resin and 25% podophyllin resin with 5% acyclovir cream applied topically<sup>[14]</sup>. Fourteen patients were treated in each protocol. The authors concluded that about half of the patients (55%) had clinical healing of OHL within 7-8 wk of each topical treatment, but the 25% podophyllin resin with 5% acyclovir cream resulted in a faster clinical healing rate of OHL after the

sixth week; moreover, no recurrent lesions were observed in this treatment group twelve months after clinical healing of OHL.

### Recurrence rate

Some studies evaluated the recurrence rate of OHL after topical treatment. Bhandarkar *et al.*<sup>[3]</sup> and Ficarra *et al.*<sup>[16]</sup> showed no recurrence of the lesion one year after 2% gentian violet treatment and six months after acyclovir cream topical therapy, respectively, in patients with total clinical regression. For topical retinoid, recurrence is observed a few days following discontinuation of treatment<sup>[11]</sup>. Sanchez *et al.*<sup>[15]</sup> observed a recurrence rate of 33.3% of OHL treated with 25% podophyllin. Two of the six cases evaluated presented with regression of the lesion four and nine months after treatment. Moura *et al.*<sup>[2]</sup> showed a recurrence of 11.2% twelve months post-therapy with 25% podophyllin. No recurrence was observed in the 25% podophyllin resin with 5% acyclovir cream group. These data suggest that synergism of podophyllin and acyclovir decreases recurrence of OHL after topical therapy.

Systemic antiviral drugs such as desciclovir, valacyclovir, acyclovir and ganciclovir have been used for OHL treatment with recurrence observed after discontinuation<sup>[17,18]</sup>. The possibility of the occurrence of side effects and drug resistance must be carefully evaluated so that the potential harm of treatment does not exceed the expected benefits<sup>[18]</sup>. Surgical excision as a treatment for OHL has been performed, and no recurrence was observed within three months. However, most patients presented with new foci of OHL after this time<sup>[19]</sup>. Considering this, and comparing it to systemic therapy and surgery, topical treatment is recommended because it does not produce systemic adverse effects, is less invasive and is effective over a long period of time<sup>[4]</sup>.

## CONCLUSION

A combined topical therapy of 25% podophyllin and 5% acyclovir cream is effective, demonstrating fast healing without recurrence. In this case, additional multicenter studies are necessary. As for other agents, gentian violet (2%) was also used successfully in the treatment of OHL, with no recurrences in a year, although only one previous study has evaluated the effectiveness of this therapy. Future double-blind and placebo-controlled trials are needed to provide clinical evidence for the efficacy of topical management of OHL.

## ACKNOWLEDGMENTS

The authors wish to thank National Council for Scientific and Technological Development (CNPq) and Office of the Dean of Research of Universidade Federal de Minas Gerais (PRPq, #01/2014).

## REFERENCES

- 1 Greenspan JS, Greenspan D. Oral hairy leukoplakia: diagno-

- sis and management. *Oral Surg Oral Med Oral Pathol* 1989; **67**: 396-403 [PMID: 2542857 DOI: 10.1016/0030-4220(89)90381-2]
- 2 **Moura MD**, Guimarães TR, Fonseca LM, de Almeida Pordeus I, Mesquita RA. A random clinical trial study to assess the efficiency of topical applications of podophyllin resin (25%) versus podophyllin resin (25%) together with acyclovir cream (5%) in the treatment of oral hairy leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103**: 64-71 [PMID: 17178496 DOI: 10.1016/j.tripleo.2006.02.016]
- 3 **Bhandarkar SS**, MacKelfresh J, Fried L, Arbiser JL. Targeted therapy of oral hairy leukoplakia with gentian violet. *J Am Acad Dermatol* 2008; **58**: 711-712 [PMID: 18342722 DOI: 10.1016/j.jaad.2007.11.017]
- 4 **Moura MD**, Haddad JP, Senna MI, Ferreira e Ferreira E, Mesquita RA. A new topical treatment protocol for oral hairy leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; **110**: 611-617 [PMID: 20813564 DOI: 10.1016/j.tripleo.2010.05.015]
- 5 **Walling DM**. Oral hairy leukoplakia: an Epstein-Barr virus-associated disease of patients with HIV. *Res Initiat Treat Action* 2000; **6**: 10-15 [PMID: 11708168]
- 6 **Daniels TE**, Greenspan D, Greenspan JS, Lennette E, Schiødt M, Petersen V, de Souza Y. Absence of Langerhans cells in oral hairy leukoplakia, an AIDS-associated lesion. *J Invest Dermatol* 1987; **89**: 178-182 [PMID: 3110300 DOI: 10.1111/1523-1747.ep12470556]
- 7 **Dongo M**, Gonçalves LS, Ferreira SM, Noce CW, Dias EP, Júnior AS. Gender differences in oral manifestations among HIV-infected Brazilian adults. *Int Dent J* 2013; **63**: 189-195 [PMID: 23879254 DOI: 10.1111/idj.12029]
- 8 **Patton LL**, Ramirez-Amador V, Anaya-Saavedra G, Nitayananta W, Carrozzo M, Ranganathan K. Urban legends series: oral manifestations of HIV infection. *Oral Dis* 2013; **19**: 533-550 [PMID: 23517181 DOI: 10.1111/odi.12103]
- 9 **Maley AM**, Arbiser JL. Gentian violet: a 19th century drug re-emerges in the 21<sup>st</sup> century. *Exp Dermatol* 2013; **22**: 775-780 [PMID: 24118276 DOI: 10.1111/exd.12257]
- 10 **Perry BN**, Govindarajan B, Bhandarkar SS, Knaus UG, Valo M, Sturk C, Carrillo CO, Sohn A, Cerimele F, Dumont D, Losken A, Williams J, Brown LF, Tan X, Ioffe E, Yancopoulos GD, Arbiser JL. Pharmacologic blockade of angiopoietin-2 is efficacious against model hemangiomas in mice. *J Invest Dermatol* 2006; **126**: 2316-2322 [PMID: 16741507 DOI: 10.1038/sj.jid.5700413]
- 11 **Schöfer H**, Ochsendorf FR, Helm EB, Milbradt R. Treatment of oral 'hairy' leukoplakia in AIDS patients with vitamin A acid (topically) or acyclovir (systemically). *Dermatologica* 1987; **174**: 150-151 [PMID: 3556707 DOI: 10.1159/000249008]
- 12 **Alessi E**, Berti E, Cusini M, Zerboni R, Cavicchini S, Tomasi D, Muratori S. Oral hairy leukoplakia. *J Am Acad Dermatol* 1990; **22**: 79-86 [PMID: 2153716 DOI: 10.1016/0190-9622(90)70012-7]
- 13 **Lozada-Nur F**, Costa C. Retrospective findings of the clinical benefits of podophyllum resin 25% sol on hairy leukoplakia. Clinical results in nine patients. *Oral Surg Oral Med Oral Pathol* 1992; **73**: 555-558 [PMID: 1518642 DOI: 10.1016/0030-4220(92)90097-A]
- 14 **Gowdey G**, Lee RK, Carpenter WM. Treatment of HIV-related hairy leukoplakia with podophyllum resin 25% solution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1995; **79**: 64-67 [PMID: 7614164 DOI: 10.1016/S1079-2104(05)80076-9]
- 15 **Sanchez M**, Spielman T, Epstein W, Moy J. Treatment of oral hairy leukoplakia with podophyllin. *Arch Dermatol* 1992; **128**: 1659 [PMID: 1456770 DOI: 10.1001/archderm.128.12.1659]
- 16 **Ficarra G**, Barone R, Gaglioti D, Milo D, Riccardi R, Romagnoli P, Zorn M. Oral hairy leukoplakia among HIV-positive intravenous drug abusers: a clinicopathologic and ultrastructural study. *Oral Surg Oral Med Oral Pathol* 1988; **65**: 421-426 [PMID: 3283634 DOI: 10.1016/0030-4220(88)90356-8]
- 17 **Scully C**, McCarthy G. Management of oral health in persons with HIV infection. *Oral Surg Oral Med Oral Pathol* 1992; **73**: 215-225 [PMID: 1312692 DOI: 10.1016/0030-4220(92)90197-X]
- 18 **Baccaglini L**, Atkinson JC, Patton LL, Glick M, Ficarra G, Peterson DE. Management of oral lesions in HIV-positive patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103** Suppl: S50.e1-S50.23 [PMID: 17379155 DOI: 10.1016/j.tripleo.2006.11.002]
- 19 **Herbst JS**, Morgan J, Raab-Traub N, Resnick L. Comparison of the efficacy of surgery and acyclovir therapy in oral hairy leukoplakia. *J Am Acad Dermatol* 1989; **21**: 753-756 [PMID: 2808791 DOI: 10.1016/S0190-9622(89)70250-4]

P- Reviewers: Boffano P, Sarode SC S- Editor: Song XX

L- Editor: A E- Editor: Wu HL





## Association between resting energy expenditure, psychopathology and HPA-axis in eating disorders

Giovanni Castellini, Walter Castellani, Lorenzo Lelli, Carolina Lo Sauro, Carla Dini, Lisa Lazzeretti, Lorenza Bencini, Edoardo Mannucci, Valdo Ricca

Giovanni Castellini, Lorenzo Lelli, Lisa Lazzeretti, Lorenza Bencini, Valdo Ricca, Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, 50134 Firenze, Italy

Walter Castellani, Department of Respiratory Physiopathology, Palagi Hospital, 50134 Firenze, Italy

Carolina Lo Sauro, Department of Health Science, University of Florence, 50134 Firenze, Italy

Carla Dini, Nutrition Service, University of Florence, 50134 Firenze, Italy

Edoardo Mannucci, Non Invasive Cardiology, Department of Heart and Vessels, University of Florence, 50134 Firenze, Italy

**Author contributions:** Castellini G, Lelli L and Ricca V contributed equally to this work; Castellini G, Lelli L, Lo Sauro C and Ricca V designed the research; Castellani W, Dini C, Lazzeretti L and Bencini L performed the research; Castellini G and Mannucci E analyzed the data; Castellini G, Lelli L and Ricca V wrote the paper.

**Correspondence to:** Valdo Ricca, MD, Psychiatric Unit, Department of Neuroscience, Psychology, Drug Research and Child Health, University of Florence, Largo Brambilla 3, 50134 Firenze, Italy. [valdo.ricca@unifi.it](mailto:valdo.ricca@unifi.it)

Telephone: +39-055-7947487 Fax: +39-055-7947487

Received: April 14, 2014 Revised: May 30, 2014

Accepted: June 18, 2014

Published online: July 16, 2014

### Abstract

**AIM:** To investigate the complex relationships between resting energy expenditure (REE), eating psychopathology, and Hypothalamus Pituitary Adrenal axis functioning in patients with eating disorders.

**METHODS:** The study was designed as a cross-sectional survey, and it was planned by the Clinic for Eating Disorders of the University of Florence (Italy). The protocol was approved by the Ethics Committee of the Institution. Twenty two anorexia nervosa and twenty one Bulimia Nervosa patients were assessed by means of a clinical interview and the structured clinical

interview for diagnostic and statistical manual of mental disorders, fourth edition. Eating attitudes and behaviour were specifically investigated by means of the eating disorder examination questionnaire (EDE-Q). Patients were also evaluated by means of the symptom checklist (SCL 90-R), REE was measured by means of indirect calorimetry, and blood cortisol morning levels were evaluated.

**RESULTS:** Both anorexia nervosa and bulimia nervosa patients showed a reduced REE as compared with predicted REE. Body mass index (BMI) was positively associated with resting energy expenditure in Bulimics, whereas a strong, negative association between BMI and REE was observed in Anorectics. The pattern of associations between variables supported a mediation model, where shape concern accounted for variations in REE and cortisol levels (mediator), and variations in the mediator significantly accounted for variations in REE. When these associations were taken into account together, the relationship between shape concern and REE was no longer significant, whereas the association between cortisol levels and REE retained its significance, showing strong evidence for a single, dominant mediator. Anorectics and Bulimics showed an opposite pattern of association between BMI and REE. In Anorectics only, a higher REE was associated with a more severe eating disorder specific psychopathology, and cortisol levels represent a possible mediating factor for this relationship.

**CONCLUSION:** The data supported a mediation model where cortisol levels mediated the relationship between eating psychopathology (concern about body shape) and REE.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Anorexia nervosa; Bulimia nervosa; Cortisol; Psychopathology; Resting energy expenditure

**Core tip:** We have investigated the relationship between resting energy expenditure (REE), eating psychopathology, and hypothalamus adrenal axis in EDs. Twenty two anorexia nervosa (AN) and 21 bulimia nervosa (BN) patients were assessed. Both AN and BN showed a reduced REE as compared with predicted REE. AN and BN showed an opposite pattern of association between REE and Body Mass Index (BMI) which was positively associated with REE in BN, whereas a strong, negative association between BMI and REE was observed in AN. In AN only, a higher REE was associated with a more severe eating disorder psychopathology, and higher cortisol levels. The data supported a mediation model where cortisol levels mediated the relationship between eating psychopathology and REE.

Castellini G, Castellani W, Lelli L, Lo Sauro C, Dini C, Lazzeretti L, Bencini L, Mannucci E, Ricca V. Association between resting energy expenditure, psychopathology and HPA-axis in eating disorders. *World J Clin Cases* 2014; 2(7): 257-264 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/257.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.257>

## INTRODUCTION

Chronic underfeeding and binge-purging behaviours can lead to alterations in metabolism and body composition. Several studies investigated energy metabolism in patients with anorexia nervosa (AN)<sup>[1-6]</sup>, and the observed reduction of resting energy expenditure (REE) has been associated to the smaller volume of metabolically active tissues and to an adaptation to underfeeding<sup>[6-8]</sup>. Measured REE is significantly lower than predicted REE in AN subjects<sup>[9]</sup>. As far as bulimic normal weight patients are concerned, most of the published studies found that the resting metabolic rate of these patients was significantly lower than that of controls<sup>[10,11]</sup>, whereas Detzer *et al*<sup>[12]</sup> did not find significant differences between patients with bulimia nervosa (BN) and controls.

REE is one component of the total energy expenditure. It includes basal metabolic rate, which refers to the minimum part of energy required to maintain the organisms' basic functions, and it is related to the amount of energy utilized when the body is at complete rest. It can be measured by means of indirect calorimetry, and it is often used in clinical settings. REE alterations found in eating disorders could be partly explained with the quantitative changes in cell mass<sup>[11,13]</sup>. Similarly, Vaisman *et al*<sup>[14]</sup> attributed the lowered resting metabolic rate to the reduction in lean body mass.

Alternatively, previous findings supported the relationship between stress induced cortisol levels and metabolic rate. In eating disorders, increased hypothalamus adrenal axis (HPA) arousal with abnormalities in its regulation is well proven<sup>[15,16]</sup>; HPA axis hyperactivity is well documented in AN and BN especially during the acute phase of the illness, even if the abnormalities showed by

the BN patients are milder<sup>[17]</sup>. The HPA axis alterations can influence other biological systems involved in eating behaviour, such as leptin, endogenous opioids, thyroid, reproductive, immune and sympathetic nervous systems and the abnormalities in these systems could be considered to be involved in the onset and the maintenance of eating disorders<sup>[17]</sup>. HPA axis abnormalities seem to be associated with an history of stressful life events and an excess of traumatic life events, such as sexual and physical abuse are reported in patients with eating disorders. As the relationship between life events and HPA axis is well known<sup>[17-19]</sup>, it is not unexpected that the HPA axis may also be functioning abnormally in eating disorders. Moreover cortisol is considered a catabolic hormone<sup>[20,21]</sup>, and it has been found that weight gain in AN was associated with normalization of plasmatic and urinary cortisol levels<sup>[22]</sup>, HPA functioning and the reduction of cortisol secretory burst<sup>[23]</sup>.

Finally, few studies considered the relationship between eating disorder psychopathology and REE in eating disorders patients, with conflicting results<sup>[13,23,24]</sup>.

The aims of the present study were as follows: (1) to evaluate the pattern of association between body mass index (BMI) and REE in AN and BN; (2) to investigate the possible mechanisms of REE alterations in AN and BN, analyzing the interaction between eating disorder psychopathology, HPA functioning and metabolic status.

## MATERIALS AND METHODS

### Sample and measures

The study was designed as a cross-sectional survey, and it was planned by the Clinic for Eating Disorders of the University of Florence (Italy). The protocol was approved by the Ethics Committee of the Institution. A written informed consent was obtained from each patient after the procedures of the study were fully explained. The study was performed on a series of 61 eating disorders patients attending the Clinic for Eating Disorders between January 2010 and July 2013, provided they met the following inclusion criteria: female, age between 18 and 60 years, diagnosis of AN restricting type and BN Binge/Purging type, assessed by means of the Structured Clinical Interview for Diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV)<sup>[25,26]</sup>. Patients were included if they reported at least 1 year of a stable Eating Disorder diagnosis (at least 1 year with full diagnostic criteria for anorexia nervosa or bulimia nervosa according the DSM-IV criteria and without diagnostic crossover in the same year), at least 6 wk of a stable body weight, no intense physical exercise in the past six months (both assessed by means of a face-to face clinical interview). None of the patients were in a remission or recovery phase of disease. The exclusion criteria were as follows: comorbid schizophrenia or bipolar disorder, illiteracy, mental retardation, severe medical conditions, current use of psychoactive medications that can interfere with HPA Axis response (*i.e.*, antidepressants use could increase human hippocampal neurogenesis by activating

the glucocorticoid receptor<sup>[27]</sup>.

Six patients refused to participate in the study, and 12 patients were excluded because of the following reasons: bipolar disorder (2 patient), illiteracy (1 patient), mental retardation (1 patient), severe medical conditions (3 patients with heart failure, 1 with renal failure), pharmacological treatments (4 patients taking SSRI). The final sample was composed of 43 female subjects, 22 with AN Restricting Type, and 21 with BN Binge/Purging type. The Structured clinical interview for DSM-IV<sup>[26]</sup> axis was used to confirm psychiatric diagnoses.

### Psychological assessment

Psychopathological, behavioural and sociodemographic data were collected through a face-to-face interview on the first day of admission, by two expert psychiatrists (L.L., C.L.S.). The structured clinical interview for DSM IV<sup>[26]</sup> was applied to evaluate diagnoses according to DSM-IV. Anthropometric measurements were made using standard calibrated instruments. Height (m) was measured using a wall-mounted stadiometer, weight (kg) using electronic scales. BMI was calculated. Eating attitudes and behaviour were specifically investigated by means of the eating disorder examination questionnaire (EDE-Q)<sup>[28]</sup>. The self-reported EDE-Q consists of 38 items, assessing the core psychopathological features of eating disorders, and contains 4 subscales: dietary restraint, eating concern, weight concern, and shape concern. Finally, patients were evaluated by means of the symptom checklist (SCL 90-R)<sup>[29]</sup>, a psychometric instrument devoted to the identification of psychopathological distress.

### Blood samples

Blood samples were drawn in the morning (8 am), after an overnight fast, for the determination of cortisol levels (mcg/L), TSH, and thyroid hormones levels.

### Indirect calorimetry

REE was measured by means of indirect calorimetry using a canopy system (MMC Horizon, Sensor Medics, Anaheim, United States in a quiet environment, with the patients in the supine position for 20 min before measurement, because activities of daily living increase REE, but a short rest (20 min) before testing is sufficient for the effect to dissipate) and after a 12 h overnight fast. Measurement duration of 10 min with the first 5 min deleted and the remaining 5 min having a coefficient of variation < 10% gave accurate readings of REE<sup>[30]</sup>. Energy expenditure was derived from CO<sub>2</sub> production and O<sub>2</sub> consumption, with the appropriate Weir's formula, neglecting protein oxidation<sup>[31]</sup>. The inter-day coefficient of variation of such measurements (as determined in six patients on subsequent days) was always less than 3%, without any sequence effect. Basal energy expenditure can be measured a number of different ways, but perhaps the most convenient way is by indirect calorimetry. This method is based on the assumption that metabolism is a reflection of energy expenditure. Since the oxidation

of nutrients requires oxygen, by measuring oxygen consumption and carbon dioxide production, an estimate of energy production in kilocalories can be made. However, if indirect calorimetry is unavailable, the Harris-Benedict equations (multiple linear regression equations derived from a sample of normal individuals), based on height, weight, age, and sex, can be used clinically to estimate basal energy expenditure<sup>[8]</sup>. For females, the equation is: basal energy expenditure =  $655.1 + (9.56 \times \text{body weight in kg}) + (1.84 \times \text{height in cm}) - (4.67 \times \text{age in years})$ <sup>[32,33]</sup>. REE was measured prior to start psychological and pharmacological interventions.

### Statistical analysis

For between-group comparisons (AN *vs* BN),  $\chi^2$  and Independent-Samples *t* test were applied, while Paired Sample *t* test was adopted to compare REE with predicted REE within each group. Correlation analyses (Pearson's correlation), and subsequently linear regression analyses were performed in the whole sample, and within each group, to assess the associations between BMI, cortisol levels, eating specific and general psychopathology, and REE.

Subsequently, moderator and mediator effect analyses were performed. Whereas moderator variables specify when certain effects will hold, mediators consider how or why such effects occur (Baron and Kenny)<sup>[34]</sup>. The moderator function of third variables partitions a focal independent variable into subgroups, that establish its domains of maximal effectiveness in regard to a given dependent variable. Therefore, in order to evaluate whether the relationship between REE and BMI was different within Eating Disorders subgroups (first aim of the study), general linear model (GLM) was used to examine the moderating effect of diagnosis (AN *vs* BN) on the interaction between REE and BMI.

In order to evaluate the possible mechanisms that could explain the associations between REE and BMI (second aim of the study), mediators effect analyses were performed. The mediator function of a third variable represents the generative mechanism through which the focal independent variable is able to influence the dependent variable of interest.

## RESULTS

Table 1 reported demographic, clinical and psychopathological variables for AN and BN patients. No significant difference was detected between AN and BN, with the exception of BMI and FT3, which were higher in BN as compared with AN patients. No significant difference was found between AN and BN, in terms of REE while predicted REE was significant lower in AN. REE was lower than predicted REE, in both AN ( $t = 2.27$ ;  $P = 0.034$ ) and BN ( $t = 5.82$ ;  $P < 0.001$ ) groups. These comparisons retained their significance even when adjusting for FT3 hormones.

GLM analysis (Figure 1; part A) was adopted to evaluate whether the relationship between REE and BMI was different according to EDs subgroups (AN and BN).



**Table 1** General and clinical characteristics of the sample

	Anorexia Nervosa; n: 22	Bulimia Nervosa; n: 21	t
Age (yr)	31.73 ± 9.86	27.86 ± 7.12	1.46
Education (yr)	18.13 ± 3.78	18.66 ± 2.67	0.52
BMI (kg/m <sup>2</sup> )	15.43 ± 2.00	22.96 ± 1.49	-13.8 <sup>b</sup>
REE (kcal/d)	1088 ± 174	1092 ± 207	-0.06
Predicted REE (kcal/d) <sup>a</sup>	1196 ± 85	1421 ± 207	4.53 <sup>b</sup>
Cortisol levels (mcg/L)	513.65 ± 159.52	496.05 ± 133.22	0.37
TSH (mU/L)	2.14 ± 1.57	1.67 ± 0.70	1.22
FT3 (pmol/L)	3.82 ± 0.98	4.75 ± 0.81	-3.35 <sup>b</sup>
FT4 (pmol/L)	14.10 ± 3.43	14.14 ± 2.29	-0.03
SCL-90 GSI	1.59 ± 0.57	1.52 ± 0.70	0.31
EDE-Q total	3.24 ± 1.59	3.24 ± 1.16	0.08
EDE-Q restraint	3.26 ± 2.23	3.19 ± 1.33	0.11
EDE-Q eating concern	2.86 ± 1.65	2.96 ± 1.38	-0.19
EDE-Q weight concern	3.36 ± 1.54	3.10 ± 1.53	0.54
EDE-Q shape concern	3.48 ± 1.66	3.74 ± 1.09	-0.57
Binge eating episodes (month frequency)		9.7 ± 6.4	
Purging behaviours (month frequency)		8.4 ± 5.9	

<sup>a</sup>Calculated by means of Harris-Benedict equation. Data are expressed as mean ± SD deviation; for between groups comparisons: Independent-Sample *t* test; <sup>b</sup>*P* < 0.05; <sup>c</sup>*P* < 0.01. REE: Resting energy expenditure; BMI: Body mass index; SCL-90 GSI: Symptom checklist (SCL 90-R) global severity index; EDE-Q: Eating disorder examination questionnaire.

GLM (age adjusted) showed a significant effect of REE by diagnosis on BMI ( $\beta = 0.32$ ;  $P < 0.001$ ). The significant interaction was confirmed even when adjusting for body surface area. Therefore, when the interaction was broken down (Figure 1; part B), an opposite pattern of association was found in AN and BN: the increased BMI was associated with higher REE in the BN group (age adjusted  $\beta = 0.64$ ,  $P = 0.001$ ), while in AN patients the reduced BMI was associated with higher REE (age adjusted  $\beta = 0.63$ ,  $P = 0.003$ ).

To evaluate the possible underlying mechanisms for maintenance of REE in AN and BN, Person's correlations were performed, considering psychopathological variables, cortisol levels, BMI and REE, within AN and BN patients (Table 2). No significant association was found in BN patients between REE and binge eating or purging behaviours, TSH or thyroid hormones levels. Considering AN patients, a strong positive association was found between REE and EDE-Q shape concern. Moreover, in the same group, significant positive correlations between EDE-Q shape concern and cortisol levels were also observed, as well as between cortisol levels and REE. Conversely, in BN group these associations were lacking or less significant than in AN.

In order to explain the relationship between a more severe eating disorder psychopathology and a higher REE in AN, we hypothesized a possible mediating role of cortisol levels. According to Baron *et al.*<sup>[34]</sup>, we assumed a three variable system represented by a path diagram,

where the dependent variable was REE (Figure 2). It included a direct impact (Path c) of the independent variable (EDE-Q shape concern), the impact of the mediator (cortisol levels; Path b), and the impact of the independent variable on the mediator (Path a). To test for mediation, we regressed the mediator on the independent variable, the dependent variable on the independent variable, the independent variable on the dependent variable (direct impact), and the dependent variable on both the independent variable and on the mediator. Separate coefficients were estimated and reported. According to this model, the following conditions supported mediation hypothesis: (1) variations in levels of the independent variable (EDE-Q shape concern) significantly accounted for variations in the mediator (cortisol levels;  $\beta = 0.47$ ;  $P = 0.01$ ); (2) variations in the mediator significantly accounted for variations in the dependent variable (REE;  $\beta = 0.80$ ;  $P < 0.01$ ); (3) variations in the independent variable significantly accounted for variations in the dependent variable (REE;  $\beta = 0.63$ ;  $P < 0.01$ ); (4) finally, when Paths a and b were controlled, this previously significant relation between EDE-Q shape concern and REE was no longer significant ( $\beta = 0.12$ ;  $P = 0.53$ ), while the relation between cortisol levels and REE retained its significance ( $\beta = 0.75$ ;  $P = 0.002$ ), showing strong evidence for a single, dominant mediator.

In order to calculate the indirect effect of EDE-Q shape concern on REE *via* the mediator (cortisol levels) we performed the Sobel test<sup>[35]</sup>, which resulted to be significant ( $Z = 2.51$ ;  $P = 0.005$ ). The model described above was tested also for EDE-Q total and subscale scores (data not shown). The best fit for the data was represented by the model including EDE-Q shape concern scores.

## DISCUSSION

To the best of our knowledge, this is the first study which evaluated REE in AN and BN, considering eating disorder specific psychopathology and HPA functioning as possible factors involved in the metabolic alterations.

According to our main results: (1) AN and BN patients showed an opposite pattern of association between BMI and REE. In AN patients, a higher REE was negatively associated with BMI, whereas BN patients showed a positive association between these two variables; (2) in AN patients only, a higher REE was associated with a more severe eating disorder specific psychopathology, and cortisol levels represented a possible mediating factor for this relationship.

According to previous findings<sup>[2,9,11,13]</sup>, both AN and BN showed a reduced REE, which was significantly lower than the predicted REE. It has been suggested that dietary restraint places both AN and BN patients in a state of semi-starvation which is responsible for REE reduction, and it is partially compensated by binge eating behaviours in BN<sup>[7]</sup>. However, in the present study, AN patients did not show a reduced REE compared with BN, despite their lower BMI. In AN patients, we found

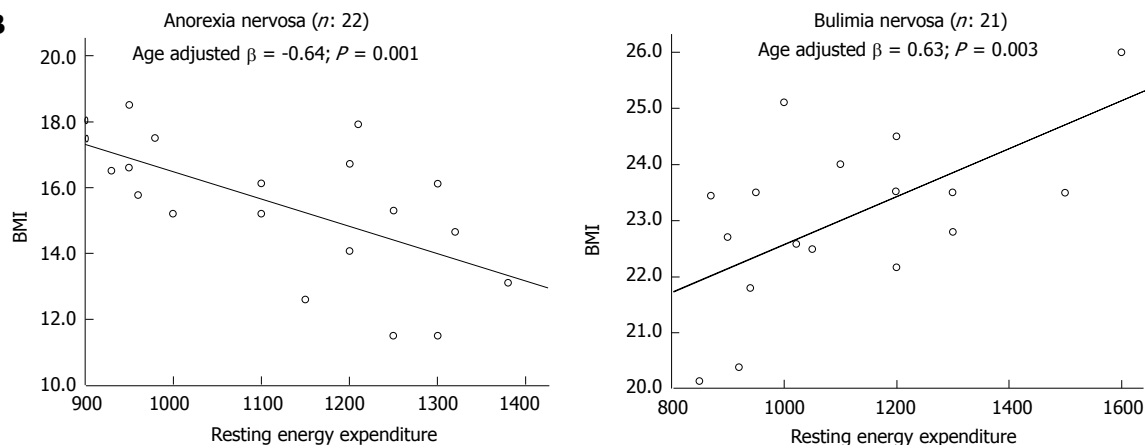
**Table 2** Pearson's correlations

	BMI	REE	Cortisol levels	SCL-90 GSI	EDE-Q total	EDE-Q restraint	EDE-Q EC	EDE-Q WC	EDE-Q SC
Anorexia Nervosa patients; <i>n</i> : 22									
Age	-0.11	-0.07	-0.05	-0.58 <sup>a</sup>	-0.12	0.06	-0.13	-0.12	0.02
BMI		-0.63 <sup>b</sup>	-0.49 <sup>a</sup>	0.13	-0.32	-0.20	-0.42	-0.25	-0.46 <sup>a</sup>
REE			0.83 <sup>b</sup>	0.10	0.39	0.17	0.33	0.45	0.63 <sup>b</sup>
Cortisol levels				0.68	0.33	0.09	0.28	0.34	0.68 <sup>b</sup>
SCL-90 GSI					0.40	0.34	0.35	0.35	0.76 <sup>b</sup>
Bulimia Nervosa patients; <i>n</i> : 21									
Age	-0.16	0.14	-0.04	0.09	0.24	0.38	-0.12	0.42	0.13
BMI		0.59 <sup>b</sup>	0.77	-0.20	0.16	-0.21	0.45 <sup>a</sup>	0.15	0.2
REE			0.24	0.00	0.43	0.33	0.42	0.19	0.46 <sup>a</sup>
Cortisol levels				-0.10	-0.05	0.33	-0.12	0.12	0.23
SCL-90 GSI					0.37	0.28	0.40	-0.02	0.30

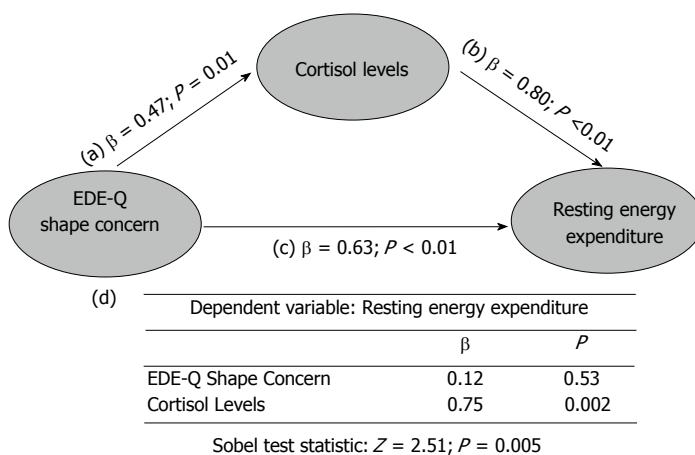
Data are Pearson's correlation coefficients: <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ . REE: Resting energy expenditure; BMI: Body mass index; SCL-90 GSI: Symptom checklist (SCL 90-R) global severity index; EDE-Q: Eating disorder examination questionnaire; EC: Eating concern; WC: Weight concern; SC: Shape concern.

**A**

General linear model-dependent variable: Body mass index (all eating disorders patients included, <i>n</i> : 43)			
	$\beta$	<i>t</i>	<i>P</i>
Age	-0.11	-2.16	0.04
Resting energy expenditure	0.20	3.29	0.002
Diagnosis (AN: 0; BN: 1)	-1.91	-20.54	< 0.001
Resting energy expenditure X Diagnosis interaction	-0.68	-6.83	< 0.001

**B**

**Figure 1** Moderators of the relationship between resting energy expenditure and body mass index. A: General linear model analysis (age adjusted) showed the effect of resting energy expenditure (REE) by diagnosis. Diagnosis was coded as dummy variables: 0 = Anorexia nervosa, 1 = Bulimia nervosa; B: The interaction showed in part A was broken down within diagnoses. Graphs reports results from linear regression analyses (age adjusted) of the opposite pattern of association between REE and body mass index (BMI) in anorexia nervosa and bulimia nervosa subjects.



**Figure 2** Mediator Model for the relationship between shape concern, cortisol levels and resting energy expenditure. The model included a direct impact (Path c) of the independent variable [eating disorder examination questionnaire (EDE-Q) shape concern] on the dependent variable (REE), the impact of the mediator (cortisol levels; Path b), and the impact of the independent variable on the mediator (Path a). The  $\beta$  values of separate regression analyses are reported in the graph. Table (d) section of the figure) reported the regression analysis of the combined effect of EDE-Q shape concern, and cortisol levels on REE. The bottom part of the graph reports the results of the Sobel test, in order to calculate the indirect effect of shape concern on REE via the mediator (cortisol levels).

that a higher REE was associated with a worse clinical condition, including lower BMI and higher eating disorder psychopathology.

It is generally assumed that AN patients maintain their low weight by severely restricting food intake, purging, and engaging in physical activity<sup>[36]</sup>. However, clinical observations consistently suggest that these patients gain weight with great difficulty, and they usually maintain their low body weight for a long time, as well. The physiological maintenance of their low body weight has been inferred from some clinical observations suggesting that AN patients apparently require high energy intake to gain weight<sup>[37]</sup>. This relative increase in energy expenditure could also account for the difficulties of AN subjects to gain weight when desired.

This pilot study provided evidences for possible explaining mechanisms of this phenomenon. We found that a severe underweight status in AN was associated with relative higher REE, and that these two conditions were both associated with an over concern about body shape, which represents the “core psychopathology” of AN.

In order to explain such a relationship, we hypothesized a mediating role of the HPA axis. According to mediation model by Baron *et al.*<sup>[34]</sup>, a given variable may be said to function as a mediator to the extent that it accounts for the relationship between the predictor and the criterion. In this study, the cortisol levels (mediator) explained how eating specific psychopathology accounted for REE variability in AN patients.

As a possible causal mechanisms, it has been supposed that increased plasma cortisol in AN is due to the higher corticotropin releasing hormone (CRH) production in the central nervous system. As already described, stress-which in this study was specifically related with eating psychopathology- may lead to the hypersecretion of CRH, which is known to be a potent anorexic agent<sup>[21]</sup>.

Furthermore, previous findings supported the relationship between stress induced cortisol levels and REE. It has been suggested that, when in excess, cortisol is an overall catabolic hormone, which decreases lean body mass and muscle mass and increase energy expenditure<sup>[20,21]</sup>. Moreover, it increases availability of all fuel substrates by mobilization of glucose<sup>[38]</sup>, free fatty acids<sup>[39]</sup>, and amino acids from endogenous stores<sup>[40]</sup>. Given the cross-sectional design of the study, we are not able to derive final conclusion on causal relationship psychopathology and REE. The present mediation model support a strong mediator role of cortisol, suggesting a possible underlying mechanism. However, it does not explain all the variance of the mentioned association, which could be caused by other metabolic factors related to underweight in AN patients.

Some limitations of this pilot study should be considered. First of all we do not analyze the body composition of the patients, one of the determinant of the REE. Then, the cross-sectional design of the study does not allow any firm conclusion about causal relationships. Tem-

perament and personality disorders information were not available. Finally the sample size is small, and it did not allow the generalization of the main findings. Moreover, it is possible that other psychopathological measures (*e.g.*, EDE-Q weight concern) could reach significant associations with larger samples. Therefore, our results should be considered as preliminary, and larger, prospective researches are warranted in order to confirm or not these findings. The results of the present study supported the hypothesis that Cortisol levels in AN, through changes in REE, could represent a biological substrate for the capacity to maintain low body weight, and for the inefficiency at gaining weight. Safe and effective re-feeding strategies in Eating Disorders should carefully consider the complex mechanisms which can determine the energy balance impairment observed in AN and BN patients.

## COMMENTS

### Background

Metabolic changes in eating disorders patients may partially explain the obstacles of weight regain interventions. One of the reasons can be the resting energy expenditure alteration which appears to be correlated with the disorder severity in terms of both weight loss and eating disorder psychopathology. Cortisol levels, a measure of response to stress appear to be a putative biological mechanism underlying the association between eating psychopathology and resting energy expenditure.

### Research frontiers

The present paper is in line with the recent advanced neurobiological and psychosomatic models based on the complex interaction between psychiatric conditions, stress response and subjective perception of symptoms.

### Innovations and breakthroughs

Resting energy expenditure abnormalities have already been described in Anorexia Nervosa patients, but generally they do not consider the role of psychopathology, as a marker of severity of the disorder. The present study is original and innovative, since it proposes a model which includes resting expenditure, cortisol levels as a marker of stress, as well as eating disorder specific psychopathology.

### Applications

The present study demonstrated that the severity of psychopathology represents not just an indicator of subjective perception of the eating disorder, but also of more compromised metabolic condition which could interfere with treatment process.

### Terminology

Resting Energy Expenditure: it is based on the assumption that metabolism is a reflection of energy expenditure. Since the oxidation of nutrients requires oxygen, by measuring oxygen consumption and carbon dioxide production, an estimate of energy production in kilocalories can be made. Shape concern: it includes all the concerns and behaviors associated with once own body. It is the core psychopathological feature of eating disorders.

### Peer review

This is, in summary, an interesting cross-sectional survey aimed to investigate the relationship between resting energy expenditure, eating psychopathology, and hypothalamus pituitary adrenal-axis functioning in twenty-two patients with eating disorders. The manuscript is interesting and well-written.

## REFERENCES

- 1 Melchior JC, Rigaud D, Rozen R, Malon D, Apfelbaum M. Energy expenditure economy induced by decrease in lean body mass in anorexia nervosa. *Eur J Clin Nutr* 1989; **43**: 793-799 [PMID: 2627927]
- 2 Platte P, Pirke KM, Trimborn P, Pietsch K, Krieg JC, Fichter MM. Resting metabolic rate and total energy expenditure in

- acute and weight recovered patients with anorexia nervosa and in healthy young women. *Int J Eat Disord* 1994; **16**: 45-52 [PMID: 7920580 DOI: 10.1002/1098-108X(199407)16:1<45::AID-EAT2260160104>3.0.CO;2-Z]
- 3 **Schebendach JE**, Golden NH, Jacobson MS, Hertz S, Shenker IR. The metabolic responses to starvation and refeeding in adolescents with anorexia nervosa. *Ann N Y Acad Sci* 1997; **817**: 110-119 [PMID: 9239182 DOI: 10.1111/j.1749-6632.1997.tb48200.x]
  - 4 **Polito A**, Fabbri A, Ferro-Luzzi A, Cuzzolaro M, Censi L, Ciarapica D, Fabbri E, Giannini D. Basal metabolic rate in anorexia nervosa: relation to body composition and leptin concentrations. *Am J Clin Nutr* 2000; **71**: 1495-1502 [PMID: 10837290]
  - 5 **Russell J**, Baur LA, Beumont PJ, Byrnes S, Gross G, Touyz S, Abraham S, Zipfel S. Altered energy metabolism in anorexia nervosa. *Psychoneuroendocrinology* 2001; **26**: 51-63 [PMID: 11070334 DOI: 10.1016/S0306-4530(00)00036-6]
  - 6 **Scalfi L**, Marra M, De Filippo E, Caso G, Pisanis F, Contaldo F. The prediction of basal metabolic rate in female patients with anorexia nervosa. *Int J Obes Relat Metab Disord* 2001; **25**: 359-364 [PMID: 11319633 DOI: 10.1038/sj.ijo.0801547]
  - 7 **de Zwaan M**, Aslam Z, Mitchell JE. Research on energy expenditure in individuals with eating disorders: a review. *Int J Eat Disord* 2002; **32**: 127-134 [DOI: 10.1002/eat.10074]
  - 8 **van Marken Lichtenbelt WD**, Heidendal GA, Westertep KR. Energy expenditure and physical activity in relation to bone mineral density in women with anorexia nervosa. *Eur J Clin Nutr* 1997; **51**: 826-830 [PMID: 9426357 DOI: 10.1038/sj.ejcn.1600492]
  - 9 **Kosmiski L**, Schmiede SJ, Mascolo M, Gaudiani J, Mehler PS. Chronic starvation secondary to anorexia nervosa is associated with an adaptive suppression of resting energy expenditure. *J Clin Endocrinol Metab* 2014; **99**: 908-914 [PMID: 24302748]
  - 10 **Devlin MJ**, Walsh BT, Kral JG, Heymsfield SB, Pi-Sunyer FX, Dantzig S. Metabolic abnormalities in bulimia nervosa. *Arch Gen Psychiatry* 1990; **47**: 144-148 [PMID: 2302026 DOI: 10.1001/archpsyc.1990.01810140044007]
  - 11 **Obazanek E**, Lesem MD, Goldstein DS, Jimerson DC. Reduced resting metabolic rate in patients with bulimia nervosa. *Arch Gen Psychiatry* 1991; **48**: 456-462 [PMID: 2021298 DOI: 10.1001/archpsyc.1991.01810290068013]
  - 12 **Detzer MJ**, Leitenberg H, Poehlman ET, Rosen JC, Silberg NT, Vara LS. Resting metabolic rate in women with bulimia nervosa: a cross-sectional and treatment study. *Am J Clin Nutr* 1994; **60**: 327-332 [PMID: 8074061]
  - 13 **Casper RC**, Schoeller DA, Kushner R, Hnilicka J, Gold ST. Total daily energy expenditure and activity level in anorexia nervosa. *Am J Clin Nutr* 1991; **53**: 1143-1150 [PMID: 1850575]
  - 14 **Vaisman N**, Rossi MF, Goldberg E, Dibden LJ, Wykes LJ, Pencharz PB. Energy expenditure and body composition in patients with anorexia nervosa. *J Pediatr* 1988; **113**: 919-924 [PMID: 3183853 DOI: 10.1016/S0022-3476(88)80032-5]
  - 15 **Brambilla F**, Ferrari E, Brunetta M, Peirone A, Draisci A, Sacerdote P, Panerai A. Immunoendocrine aspects of anorexia nervosa. *Psychiatry Res* 1996; **62**: 97-104 [PMID: 8739119 DOI: 10.1016/0165-1781(96)02992-7]
  - 16 **Monteleone P**, Luisi M, Colurcio B, Casarosa E, Monteleone P, Ioime R, Genazzani AR, Maj M. Plasma levels of neuroactive steroids are increased in untreated women with anorexia nervosa or bulimia nervosa. *Psychosom Med* 2001; **63**: 62-68 [PMID: 11211066 DOI: 10.1097/00006842-200101000-00008]
  - 17 **Lo Sauro C**, Ravaldi C, Cabras PL, Faravelli C, Ricca V. Stress, hypothalamic-pituitary-adrenal axis and eating disorders. *Neuropsychobiology* 2008; **57**: 95-115 [PMID: 18552511 DOI: 10.1159/000138912]
  - 18 **Tyrka AR**, Wier L, Price LH, Ross N, Anderson GM, Wilkinson CW, Carpenter LL. Childhood parental loss and adult hypothalamic-pituitary-adrenal function. *BIOL PSYCHIAT* 2008; **63**: 1247-1254 [DOI: 10.1016/j.biopsych.2008.01.011]
  - 19 **Van Voorhees E**, Scarpa A. The effects of child maltreatment on the hypothalamic-pituitary-adrenal axis. *Trauma Violence Abuse* 2004; **5**: 333-352 [PMID: 15361587 DOI: 10.1177/1524838004269486]
  - 20 **Tataranni PA**, Larson DE, Snitker S, Young JB, Flatt JP, Ravussin E. Effects of glucocorticoids on energy metabolism and food intake in humans. *Am J Physiol* 1996; **271**: E317-E325 [PMID: 8770026]
  - 21 **Christiansen JJ**, Djurhuus CB, Gravholt CH, Iversen P, Christiansen JS, Schmitz O, Weeke J, Jørgensen JO, Møller N. Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. *J Clin Endocrinol Metab* 2007; **92**: 3553-3559 [PMID: 17609300 DOI: 10.1210/jc.2007-0445]
  - 22 **Misra M**, Miller KK, Almazan C, Ramaswamy K, Aggarwal A, Herzog DB, Neubauer G, Breu J, Klibanski A. Hormonal and body composition predictors of soluble leptin receptor, leptin, and free leptin index in adolescent girls with anorexia nervosa and controls and relation to insulin sensitivity. *J Clin Endocrinol Metab* 2004; **89**: 3486-3495 [PMID: 15240636 DOI: 10.1210/jc.2003-032251]
  - 23 **Rigaud D**, Verges B, Colas-Linhart N, Petiet A, Moukaddem M, Van Wymelbeke V, Brondel L. Hormonal and psychological factors linked to the increased thermic effect of food in malnourished fasting anorexia nervosa. *J Clin Endocrinol Metab* 2007; **92**: 1623-1629 [PMID: 17341571 DOI: 10.1210/jc.2006-1319]
  - 24 **Konrad KK**, Carels RA, Garner DM. Metabolic and psychological changes during refeeding in anorexia nervosa. *Eat Weight Disord* 2007; **12**: 20-26 [PMID: 17384526]
  - 25 **American Psychiatric Association**. Diagnostic and statistical manual of mental disorders (4th ed.) Washington, DC: American Psychiatric Association, 1994
  - 26 **First MB**, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV Axis I Disorders-Patient Edition (SCID-I/P version 2.0). New York: Biometrics Research Department, New York State Psychiatric Institute, 1995
  - 27 **Anacker C**, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thure S, Price J, Pariante CM. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol Psychiatry* 2011; **1**-13 [DOI: 10.1038/mp.2011.26]
  - 28 **Fairburn CG**, Beglin SJ. Assessment of eating disorders: interview or self-report questionnaire? *Int J Eat Disord* 1994; **16**: 363-370 [PMID: 7866415]
  - 29 **Derogatis LR**, Lipman RS, Covi L. SCL-90: an outpatient psychiatric rating scale--preliminary report. *Psychopharmacol Bull* 1973; **9**: 13-28 [PMID: 4682398]
  - 30 **Compher C**, Frankenfield D, Keim N, Roth-Yousey L; Evidence Analysis Working Group. Best practice methods to apply to measurement of resting metabolic rate in adults: a systematic review. *J Am Diet Assoc* 2006; **106**: 881-903 [DOI: 10.1016/j.jada.2006.02.009]
  - 31 **Weir JB**. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949; **109**: 1-9 [PMID: 15394301]
  - 32 **Harris JA**, Benedict FG. A biometric study of basal metabolism in man. Washington DC: Carnegie Institute, 1919
  - 33 **Salisbury JJ**, Levine AS, Crow SJ, Mitchell JE. Refeeding, metabolic rate, and weight gain in anorexia nervosa: a review. *Int J Eat Disord* 1995; **17**: 337-345 [PMID: 7620473 DOI: 10.1002/1098-108X(199505)17:4<337::AID-EAT2260170405>3.0.CO;2-Q]
  - 34 **Baron RM**, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 1986; **51**: 1173-1182 [PMID: 3806354 DOI: 10.1037/0022-3514.51.6.1173]
  - 35 **Sobel ME**. Asymptotic confidence intervals for indirect effects in structural equations models. In: Leinhardt S, ed.



Sociological methodology. San Francisco: Jossey-Bass, 1982: 290-312

- 36 **Obarzanek E**, Lesem MD, Jimerson DC. Resting metabolic rate of anorexia nervosa patients during weight gain. *Am J Clin Nutr* 1994; **60**: 666-675 [PMID: 7942571]
- 37 **Kaye WH**, Gwirtsman HE, Obarzanek E, George DT. Relative importance of calorie intake needed to gain weight and level of physical activity in anorexia nervosa. *Am J Clin Nutr* 1988; **47**: 989-994 [PMID: 3376913]
- 38 **Dinneen S**, Alzaid A, Miles J, Rizza R. Metabolic effects of the nocturnal rise in cortisol on carbohydrate metabolism in normal humans. *J Clin Invest* 1993; **92**: 2283-2290 [PMID: 8227343 DOI: 10.1172/JCI116832]
- 39 **Djurhuus CB**, Gravholt CH, Nielsen S, Mengel A, Christiansen JS, Schmitz OE, Møller N. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. *Am J Physiol Endocrinol Metab* 2002; **283**: E172-E177 [PMID: 12067858]
- 40 **Berneis K**, Vosmeer S, Keller U. Effects of glucocorticoids and of growth hormone on serum leptin concentrations in man. *Eur J Endocrinol* 1996; **135**: 663-665 [PMID: 9025709 DOI: 10.1530/eje.0.1350663]

**P- Reviewers:** Akgül S, Inui A, Marchesini G, Serafini G

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Wu HL



# Role of ethnicity in social anxiety disorder: A cross-sectional survey among health science students

Philip De Jager, Sharain Suliman, Soraya Seedat

Philip De Jager, Vrije Universiteit, Faculty of Health Sciences, 1055 VB Amsterdam, The Netherlands

Sharain Suliman, Soraya Seedat, Department of Psychiatry, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa

**Author contributions:** De Jager P conceptualized and conducted the study, wrote the protocol, analyzed and interpreted data and was the first author on the manuscript; Suliman S analyzed and interpreted the data and co-authored the manuscript; Seedat S conceptualised the study, supervised the protocol and study, analyzed and interpreted the data and co-authored and supervised the manuscript.

**Supported by** The South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation

**Correspondence to:** Dr. Sharain Suliman, Department of Psychiatry, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 19063, Tygerberg 7505, South Africa. [sharain@sun.ac.za](mailto:sharain@sun.ac.za)

Telephone: +27-21-9389161 Fax: +27-21-9335790

Received: April 14, 2014 Revised: May 26, 2014

Accepted: June 18, 2014

Published online: July 16, 2014

Disorders Identification Test and Drug Use Disorders Identification Test, and depression with the Centre for Epidemiological Studies Depression Scale.

**RESULTS:** Of 112 students who completed the E-SPIN questionnaire, 54.4% ( $n = 61$ ) met criteria for SAD, with significantly more females than males meeting criteria. Ethnicity had a significant effect on SAD symptomatology, but there was no effect of ethnicity on the rates of drug and alcohol abuse in students with and without SAD. Overall significantly more students with SAD met criteria for depression compared with students without the disorder.

**CONCLUSION:** Among university students, SAD is prevalent regardless of whether interactions are with individuals of the same or different ethnic group. However, ethnicity may be an important determinant of social anxiety for some ethnic groups. SAD was significantly associated with major depression but not significantly associated with drug or alcohol abuse.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

## Abstract

**AIM:** To investigate the influence of ethnicity in social anxiety disorder (SAD), and the relationship with symptom severity, depression and substance use or abuse, in health sciences' students.

**METHODS:** This was a cross-sectional survey of 112 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> year students from the Faculty of Medicine and Health Sciences at Stellenbosch University, Cape Town, South Africa. The self-reported Social Anxiety Spectrum questionnaire was used to assess for SAD. The Social Phobia Inventory (SPIN) was adapted to a version called the E-SPIN (Ethnic-SPIN) in order to evaluate the effects of ethnicity. Two sub-questions per stem question were included to assess whether SAD symptoms in social interactions were ethnicity dependent. Substance use was assessed with the Alcohol Use

**Key words:** Social anxiety; Social phobia; Ethnicity; Students; South Africa

**Core tip:** We investigated the relationship between social anxiety disorder (SAD) and ethnicity, as well as its association with depression and alcohol and drug abuse, among South African students. High levels of social anxiety were present and were significantly associated with major depression but not with drug or alcohol abuse. Ethnicity was found to independently influence social anxiety symptomatology, suggesting that it is an important factor in student interactions in this context. These results contribute to the extant literature by demonstrating that different risk factors may be uniquely associated with SAD for different ethnic/racial groups, and require further exploration given South Africa's historical context.

De Jager P, Suliman S, Seedat S. Role of ethnicity in social anxiety disorder: A cross-sectional survey among health science students. *World J Clin Cases* 2014; 2(7): 265-271 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/265.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.265>

## INTRODUCTION

Social anxiety disorder (SAD), which is characterized by a persistent fear of social or performance situations (such as public speaking) where embarrassment might occur, is a common, psychiatric condition, with a lifetime prevalence ranging from 7% to 13% in the general population<sup>[1]</sup>. Age of onset is generally early; by age 11 in about 50% individuals and by age 20 in approximately 80% of individuals<sup>[2]</sup>. SAD is also highly comorbid with major depression, substance use disorders and other anxiety disorders, and the lifetime prevalence of any two of the aforementioned conditions ranges from 69% to 81%<sup>[3]</sup>. A nationally representative household survey conducted in South Africa between 2002 and 2004 found the most prevalent group of disorders to be anxiety disorders (15.8%). After agoraphobia without panic, SAD was the second most common anxiety disorder. In addition, high lifetime rates of substance abuse (13.5%) and major depression (9.8%) with an early age at onset were documented<sup>[2,4]</sup>.

Psychological treatments and medication have been shown to be effective in the treatment of SAD, with a combination of the two seeming to be most beneficial<sup>[5-7]</sup>. Despite this, the condition remains underdiagnosed and only a small proportion of those in need receive treatment<sup>[8]</sup>, possibly due to factors such as fear of stigmatization, inability to access care due to financial issues, and lack of awareness of the disorder by both patients and service providers.

The student population is diverse and provides many opportunities for social contact and support. Academic activities require social interaction and performance as part of students' learning and assessment, while interpersonal skills are key attributes of student academic success<sup>[9]</sup>. University or college students fall in the age range of increased risk for the onset of SAD. As well as struggling with fundamental issues related to identity and self-management, students are particularly vulnerable to experiences of social anxiety<sup>[10,11]</sup>. Fears of confirming negative stereotypes may also play a significant role in the symptoms of SAD. A related phenomenon is the occurrence of intergroup anxiety, where interracial relations or exchanges carry the potential for intense social anxiety<sup>[12]</sup>. Stephan *et al.*<sup>[13]</sup> term intergroup anxiety as an emotion that involves feelings of uneasiness and awkwardness in the presence of out-group members (people from different ethnic groups than oneself). Recent literature has shown that ethnicity and culture both have a big impact on how anxiety is experienced and how individuals deal with it. In a review by Hofmann *et al.*<sup>[14]</sup>, the authors con-

cluded that an individual's social concerns need to be examined in the context of cultural, racial, and ethnic background to adequately assess the degree and expression of social anxiety and SAD. South Africa is a multicultural and multi-ethnic society and, given the particular circumstances of the country's colonial and apartheid past, it is important to understand the role of ethnicity in social interactions.

This study investigated the influence of ethnicity on social interactions and SAD, and the association of SAD with symptom severity, depression and substance use in a student sample. We hypothesised higher rates of social anxiety and distress in interactions between different ethnic groups compared with same-ethnicity interactions. We further hypothesised that ethnicity would independently predict social anxiety symptomatology and that social anxiety and distress in different-ethnicity interactions would be positively correlated with depression, alcohol and drug abuse symptomatology.

## MATERIALS AND METHODS

We conducted a cross-sectional survey among health science students (medical and allied health science students) at the Stellenbosch University Faculty of Medicine and Health Sciences, Cape Town (South Africa). We sampled 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> year students. The faculty is representative of all the main ethnic groupings in the country (Black, Coloured, Indian, White). The study was approved by the Health Research Ethics Committee of Stellenbosch University and was conducted in accordance with The Declaration of Helsinki and Medical Research Ethical Guidelines on Human Research. After obtaining permission from the respective student departments, an invitation was sent out *via* email to all students inviting them to complete an online questionnaire on a secure online site, SurveyMonkey.com. Carlbring *et al.*<sup>[15]</sup> have demonstrated that anxiety measures completed *via* online questionnaires show similar psychometric properties when compared with questionnaires administered through conventional methods. Survey monkey is a secure service that stores all data in an encrypted, anonymous form. In total three email invitations were sent out. We also made use of other recruitment methods, such as handing out flyers to students after lectures and advertising the survey on the local student website and on television (LCD) screens at the faculty. Students were required to provide informed consent prior to completing the survey. The informed consent form was available online and in the e-mails sent to students, and provided study information (*i.e.*, aims), as well as contact details of investigators and the ethics committee.

We developed a socio-demographic data form that was used to elicit socio-economic status (SES) and socio-demographic profiles. The SES variable was based on questions pertaining to household access to basic needs, number of inhabitants and their educational level, as well as total income. A total score out of 44 was then calculated. Three SES categories were created by dividing the



SES scores into thirds: low: 6-19, medium 20-33, high 34-44. This indicator is similar to that currently used by Statistics South Africa and has been used by others in the South African context<sup>[16]</sup>.

The social phobia inventory (SPIN) is a brief 17-item self-report instrument for measuring SAD severity. A cut off score of 19 distinguishes those with SAD from those without<sup>[17,18]</sup>. The SPIN consists of questions that evaluate fear, avoidance and physiological discomfort. Each of the 17 items is rated on a scale from 0 to 4: not at all, a little bit, somewhat, very much, and extremely (higher scores correspond to greater distress). Scores range from 0-68. The SPIN has proved to be a useful and valid self-rated scale to assess fear, avoidance and physiological aspects of SAD. It validly measures severity of illness, is sensitive to reduction in symptoms over time, and discriminates between treatments<sup>[18]</sup>. The internal consistency (Cronbach's alpha) for individuals with SAD was 0.92 and for combined clinical and non-clinical samples the Cronbach's alpha has been shown to be 0.95<sup>[18]</sup>. For the current study, we adapted the SPIN to evaluate the effects of ethnicity. The E-SPIN or Ethnicity-SPIN includes two sub-questions for each stem question to determine whether respondents experience an exacerbation of SAD symptoms and greater distress when interacting with individuals from a different ethnic group compared to interactions with their own ethnic group.

The Social Anxiety Spectrum Self-Report (SHY-SR) questionnaire is a self-report inventory, used to measure the spectrum of social anxiety. It was derived from the Structured Clinical Interview for Social Anxiety Spectrum, the SCI-SHY, an interview which has previously been validated in psychiatric samples and in control groups in a large Italian multi-center study<sup>[19,20]</sup>. The version of the SHY-SR used in the current study was the "last month" questionnaire. This version includes an appendix on substances and three domains: (1) the interpersonal sensitivity domain, which assesses hypersensitivity to criticism, judgment and refusal, discomfort when the centre of attention, low self-confidence, feeling of inferiority, poor assertiveness, and interpersonal difficulties; (2) the behavioral inhibition and somatic symptoms (BI) domain which explores social behaviour and somatic symptoms associated with social anxiety; and (3) the specific phobias (SP) domain, which assesses situations that may trigger anticipatory anxiety and avoidance behaviours. The items of the SP domain are grouped into 14 subsections, ranging from talking on the phone to dating. These questions are dichotomous (yes/no) and refer to experiences that have occurred in the last month. The instrument is designed for administration in both adults and adolescents. A variety of cut-off scores have been determined using the receiver operating characteristic curve on data used to investigate the validity and reliability of the SCI-SHY. The diagnostic cut-off score of 68, which has a sensitivity and specificity of 84.8% and 85.6%, respectively, was used here<sup>[21]</sup>.

The Center for Epidemiological Studies Depression Scale (CES-D) is a short 20-item questionnaire<sup>[22]</sup>. Each

item is rated on a four-point scale during the last seven-day period. The scales range from "rarely or none of the time" to "most or all of the time". Scores range from 0 to 60, with higher scores indicating more symptoms of depression. CES-D scores of 16 to 26 are considered indicative of mild depression and scores of 27 or more indicative of severe depression<sup>[23]</sup>. The CES-D has been validated in a number of studies in community and primary care populations and has good test-retest reliability<sup>[20]</sup>. The scale has very good internal reliability, with a Cronbach's alpha value of 0.85 in the general population and 0.90 in a psychiatric population<sup>[22]</sup>.

Alcohol Use Disorders Identification Test (AUDIT) detects hazardous and harmful alcohol use<sup>[24]</sup>. The AUDIT contains 10 items referring to alcohol consumption and alcohol-related problems in the past 12-mo period with a cut off score of 8. Responses to each question are scored from 0 to 4, giving a maximum possible score of 40. The AUDIT was designed to measure three domains; consumption (3 items), dependence (3 items) and alcohol-related consequences (4 items). In its original psychometric evaluation, 92% of those diagnosed with alcohol abuse had a score of 8 or more, while 94% of those with non-hazardous consumption had a score of less than 8<sup>[24]</sup>. In a study that assessed the psychometric performance of three alcohol use disorder tools including the AUDIT, the AUDIT had a Cronbach alpha of 0.75<sup>[25]</sup>.

The Drug Use Disorders Identification (DUDIT) (Berman *et al*<sup>[26]</sup>, 2005) is a self-report screening instrument that focuses on current drug-related problems. The eleven items of the DUDIT were chosen to yield information on the level of drug intake and fulfillment of selected criteria for substance abuse/harmful use and dependence according to the International Classification of Diseases, 10<sup>th</sup> edition (ICD-10) and Diagnostic and Statistical Manual of Mental Disorders, 4<sup>th</sup> edition (DSM-IV) diagnostic systems. Responses to each question are scored from 0 to 4 with a maximum score of 44. In the general population, the DUDIT can screen for drug-related problems at a cut-off score of 6 (for men) and 2 (for women). The DUDIT predicts drug dependence with a sensitivity of 90% for both DSM-IV and ICD-10 with a respective specificity of 78% and 88%, and has an internal reliability of 0.80.

### Statistical analysis

Only completed questionnaires were included in the data analysis. Demographic variables were compared between those with SAD and those without SAD on the E-SPIN using cross-tabulations with  $\chi^2$  statistics. The SHY-SR means and standard deviations of the sample were reported using the subscale cut-off scores of the instrument. Owing to missing data, 7 items were omitted from the SH-SR questionnaire. Domain scores were transformed to a 0-100 scale which facilitated comparison of domain scores with other studies. ANOVAs (analysis of variance tests) were conducted to determine differences between groups. Furthermore, we also compared AUDIT, DUDIT and CES-D cut off scores between

**Table 1 Means and standard deviations of the Ethnicity-Social Phobia Inventory (with questions of same and different ethnicity interactions), Social Anxiety Spectrum Self-Report (with subscale groups high, middle and low), Drug Use Disorders Identification Test, Alcohol Use Disorders Identification Test and Center for Epidemiological Studies Depression Scale scores of respondents**

	Mean	SD
E-SPIN	22.03	12.23
Interaction same ethnicity	20.36	11.31
Interaction different ethnicity	22.23	12.84
DUDIT	1.02	2.36
AUDIT	3.31	4.42
CES-D	17.12	12.89
SHY-SR cut-off	51.77	32.12
High > 67	87.79	22.15
Middle 59-67	66.00	1.73
Low < 59	31.48	15.01
SHY-SR sub-scale raw scores		
IPS	14.00	6.49
BI	5.58	4.12
SP	31.51	22.96
Transformed SHY-SR sub-scale scores (1-100 scale)		
IPS	48.3	22.4
BI	34.9	25.8
SP	31.6	23.4

N's vary from 89 to 112 due to missing data. The diagnostic cut-off score for the SHY-SR is 68, the cut-off score of 59 identifies subjects who score high on the social anxiety spectrum but do not meet the diagnostic criteria for the social anxiety disorder (SAD). SHY-SR: Sub-scale domains includes; IPS: Interpersonal sensitivity; BI: Behavioral inhibition; SP: Specific phobia. E-SPIN: Ethnicity-Social Phobia Inventory; DUDIT: Drug Use Disorders Identification Test; AUDIT: Alcohol Use Disorders Identification test; CES-D: Center for Epidemiological Studies Depression Scale; SHY-SR: Social Anxiety Spectrum Self-Report.

students with SAD and those without SAD using  $\chi^2$  tests. Multiple regression analysis was conducted with E-SPIN scores as the dependent variable and ethnicity as the independent variable. Variables such as age, gender, SES were used as covariates in the model. We used a linear regression model to determine if ethnicity predicts E-SPIN scores (*i.e.*, whether ethnicity provides additional explanatory power to explain social anxiety symptom severity). We selected a 5% increase in overall R-squared as our effect size. All statistical analyses were performed using the SPSS 19.0 software package (SPSS Inc., Chicago, IL)<sup>[27]</sup>.

## RESULTS

Of the 958 students invited to participate, responses were received from 120 students (12.5%). Respondents had a mean age of 19.68 years (SD = 2.48) and comprised of 40 (33.3%) males and 80 (66.6%) females. Given that the gender distribution of students at the university is roughly 50/50 this shows that females were more likely to complete the survey. The ethnicity of respondents was similar to that in the general undergraduate student population for Black and Coloured students (16% each), but there were significantly more Indian/Asian respondents (15% *vs* under 3% in the student population), and fewer

White respondents (48% *vs* 65% in the student population).

The majority were studying for a Bachelor of Medicine and Surgery degree 101 (84.2%), with the remainder (15.8%) being Bachelor students in Dietetics, Physiotherapy, Occupational Therapy, and Speech Language and Hearing Therapy. Of this sample, 112 finished the E-SPIN questionnaire (same and different ethnicity interactions), 90 finished the SHY-SR and DUDIT, whereas the AUDIT and CES-D were completed by 89 students. The mean SES score was 33 (range: 19-44) with all ethnic groups falling into the "high" SES category. Despite this, the difference in SES between ethnic groups approached significance, with white and black participants endorsing a higher SES than Coloured and Indian participants, based on our scale [ $F(3,43) = 2.804$ ;  $P = 0.051$ ].

Table 1 differentiates students in the sample based on clinical cut-offs on the various measures of psychopathology, and presents the means and standard deviations of the original and transformed (0-100) scores of the SHY-SR. Of the 90 students who completed the SHY-SR, 28 (31.1%) scored above the diagnostic cut-off score of 68 and had a mean score of 51.77 (SD = 32.12). High scorers (5.6%) had a mean score of 87.79 (SD = 22.15) while low scorers (63.3%) students had a mean score of 31.48 (SD = 15.01). Ethnic groups did not differ significantly on total SHY-SR scores, but there was a significant difference in the SP domain  $F(3,86) = 2.867$ ,  $P = 0.041$ , with Coloured students scoring significantly higher than White students.

Table 2 shows the association of SAD with socio-demographic and psychopathology variables. 54.5% ( $n = 61$ ) of students met criteria for SAD, with significantly more females 63.2% ( $n = 48$ ), than males 36.1% ( $n = 13$ ). More students met criteria for SAD in the context of different ethnic interactions (59.8%,  $n = 67$ ) than in the context of same ethnicity interactions (53.6%,  $n = 60$ ). Gender differences were present with significantly more females than males meeting criteria for SAD, both in same ethnicity [60.5% females ( $n = 46$ ) *vs* 38.9% males ( $n = 14$ ) ( $\chi^2 = 4.598$ ,  $df = 1$ ,  $P < 0.05$ )], and different ethnicity [67.1 % females ( $n = 51$ ) *vs* 44.4% males ( $n = 16$ ) ( $\chi^2 = 5.222$ ,  $df = 3$ ,  $P < 0.05$ )] interactions. Further, there was an association between ethnic group and SAD in the context of same ethnic interactions; Black students experienced significantly more anxiety in interactions with others of ( $\chi^2 = 8.530$ ,  $df = 3$ ,  $P < 0.05$ ).

There was no effect of ethnicity on the rates of drug and alcohol abuse in students with and without SAD. Overall significantly more students with SAD met criteria for depression (73.8%) compared with students without the disorder (26.2%), ( $\chi^2 = 7.512$ ,  $df = 1$ ,  $P < 0.01$ ). This was true both for same ethnicity (73.8% *vs* 26.2%,  $\chi^2 = 10.041$ ,  $df = 1$ ,  $P < 0.01$ ) and different ethnicity (73.8% *vs* 26.2%,  $\chi^2 = 5.751$ ,  $df = 1$ ,  $P < 0.01$ ) interactions (Table 2).

We conducted a multiple linear regression with the E-SPIN total score as the independent variable, ethnicity as the dependent variable, and SES, age and gender as covariates. The adjusted  $R^2$  was 0.074. In subsequent

**Table 2 Social anxiety (Ethnicity-Social Phobia Inventory scores): Socio-demographic variables and associated psychopathology in students with and without social anxiety disorder**

Socio-demographic status:	E-SPIN (same ethnicity)			E-SPIN (different ethnicity)		
	No-SAD <i>n</i> (%)	SAD <i>n</i> (%)	$\chi^2$ ( <i>P</i> )	No-SAD <i>n</i> (%)	SAD <i>n</i> (%)	$\chi^2$ ( <i>P</i> )
Gender						
Male	22 (61.1)	14 (38.9)	4.60 <sup>a</sup>	20 (55.6)	16 (44.4)	5.22 <sup>a</sup>
Female	30 (39.5)	46 (60.5)		25 (32.9)	51 (67.1)	
Ethnicity						
Black	3 (17.6)	14 (82.4)	8.53 <sup>a</sup>	3 (17.6)	14 (82.4)	6.02 (0.11)
White	31 (56.4)	24 (43.6)		27 (49.1)	28 (50.9)	
Indian/Asian	8 (47.1)	9 (52.9)		7 (41.2)	10 (57.8)	
Colored	7 (36.8)	12 (63.2)		6 (31.6)	13 (68.4)	
SES						
Low	1 (50.0)	1 (50.0)	3.84 (0.15)	1 (50.0)	1 (50.0)	5.19 (0.08)
Medium	19 (36.5)	33 (63.5)		15 (28.8)	37 (71.2)	
High	32 (55.2)	26 (44.8)		29 (50.0)	29 (50.0)	
Clinical measures:						
DUDIT						
No drug related problems	31 (41.3)	44 (58.7)	1.76 (0.18)	30 (40.0)	45 (60.0)	0.23 (0.63)
Drug related problems	9 (60.0)	6 (40.0)		5 (33.3)	10 (66.7)	
AUDIT						
No alcohol related problems	32 (42.7)	43 (57.3)	0.26 (0.61)	29 (38.7)	46 (61.3)	0.09 (0.77)
Alcohol related problems	7 (50.0)	7 (50.0)		6 (42.9)	8 (57.1)	
CES-D						
No depression	28 (59.6)	19 (40.4)	10.04 <sup>b</sup>	24 (51.1)	23 (48.9)	5.75 <sup>a</sup>
Depression	11 (26.2)	31 (73.8)		11 (26.2)	31 (73.8)	

<sup>a</sup>*P* < 0.05, 60.5% females (*n* = 46) *vs* 38.9% males (*n* = 14); different ethnicity 67.1 % females (*n* = 51) *vs* 44.4% males; and anxiety in ethnic group and SAD in the context of same-ethnic interactions-Black students *vs* others of their own ethnicity; <sup>b</sup>*P* < 0.01, students with SAD met criteria for depression (73.8%) *vs* students without the disorder (26.2%); same ethnicity 73.8% *vs* 26.2%; and different ethnicity 73.8% *vs* 26.2%. SES Categories Low: 6-19; Medium: 20-33; High: 34-44; E-SPIN: Ethnicity-Social Phobia Inventory; DUDIT: Drug Use Disorders Identification Test; AUDIT: Alcohol Use Disorders Identification Test; CES-D: Center for Epidemiological Studies Depression Scale; SES: Social economic status; SHY-SR: Social Anxiety Spectrum Self-Report Scale.

multiple linear regression with ethnicity excluded, the adjusted *R*<sup>2</sup> was 0.068, (a decrease of 6%). We had selected a 5% change in overall *R*-squared as the effect size, thus ethnicity had sufficient explanatory power in predicting E-SPIN scores, when controlling for age, gender and SES.

## DISCUSSION

We investigated the relationship between SAD and ethnicity in a student sample, as well as its association with depression and alcohol and drug abuse. This is, to our knowledge, the first study of this nature among South African students. South Africa is a multicultural and multi-ethnic society and, given the country's colonial and apartheid past, it is important to understand the role of ethnicity in social interactions. University or college students fall in the age group of increased risk for the onset of SAD. As well as struggling with fundamental issues related to identity and self-management, students are particularly vulnerable to experiences of social anxiety<sup>[10,11]</sup>.

First, more than half of the sample (54.4%) met criteria for SAD. This rate increased to 60.8% in response to questions regarding interactions with different ethnic groups. Although these rates are significantly higher than in the general population, our sample, as a whole, does not appear to suffer more from SAD than other student samples, as former studies have tended to report higher rates using the SPIN in student populations<sup>[1,10,28]</sup>. Stewart *et al*<sup>[29]</sup> also

found a high prevalence of SAD in college students and suggested that normative developmental and contextual issues in the lives of college students may be contributory.

Second, SHY-SR sub-scale domain scores were relatively high, and higher than in an Italian study of 520 high school students (mean age of 18.4 years) in their last year of school<sup>[21]</sup>. Transition from high school to a tertiary setting with the additional academic and social adaptational pressures may partially explain the higher social anxiety symptomatology in the current study.

Third, SAD was more prevalent among females which is consistent with community samples internationally<sup>[3,30]</sup>. However, findings from student samples indicate that gender differences are not common. For instance, there was no significant main effect for gender in a study by Stewart *et al*<sup>[11]</sup>. Further, in a study that compared a clinical sample with a non-clinical undergraduate sample, although women in the clinical sample reported relatively higher fears of criticism/embarrassment and authority than a semi-colon men, suggesting that women with SAD may be more fearful of criticism/embarrassment and more fearful of authority than men, this was not shown in the non-clinical undergraduate sample<sup>[31]</sup>.

Fourth, we found that ethnicity independently influenced severity of social anxiety symptomatology, suggesting that it is an important factor in student interactions in the South African context. Previous research in the area of intercultural communication has suggested that



uncertainty as well as anxiety are important predictors of avoidance behaviour in intercultural encounters<sup>[32,33]</sup>. Given that the expressions of racial bias are no longer socially acceptable<sup>[9]</sup>, research on intergroup prejudice, in particular, indicates that the idea of appearing prejudiced in front of others may elicit strong social anxiety, which may emerge in interracial interactions, as well as in same-race interactions in which an individual fears social sanctions from in-group members for expressing prejudice toward an out-group<sup>[34]</sup>.

Of interest was that Black students appear to fear social disapproval from others of their own ethnicity more than from those of other ethnicities. A possible explanation for this may be that this group of students experienced greater stereotype confirmation concern, a construct described as “a chronic experience of uncertainty and apprehension about appearing to confirm as self-characteristic, a stereotype about ones’ group”<sup>[35]</sup>, among their own ethnic group. Furthermore, Coloured students were found to experience significantly more anxiety in situations that triggered anticipatory anxiety and avoidance behaviours, such as talking on the phone (the SHY-SR specific phobias domain). These results, on the contribution of ethnicity in SAD, are not strongly significant but require further exploration given the historical context, and contribute to the extant literature by demonstrating that different risk factors may be uniquely associated with SAD for different ethnic/racial groups.

SAD was significantly associated with major depression but not significantly associated with drug or alcohol abuse. These findings are consistent with a study on the prevalence of SAD and comorbidities among Nigerian undergraduates, which found that both lifetime and 12 mo depression were significantly associated with lifetime and 12 mo SAD but that there was no significant relationship between SAD and alcohol abuse<sup>[36]</sup>. This suggests that in the student population depression is more likely to be co-morbid with SAD than substance abuse. These findings are further supported by a study of 228 American college students which found that alcohol problems were more directly related to peer influence and social networks than to social anxiety<sup>[37]</sup>. High rates of co-morbidity with depression among university students contribute to further disability (*e.g.*, academic achievement) and quality of life impairments.

Results of this study must be considered preliminary given the small sample size and the fact that self-report measures were used. Participant bias is also an important consideration, as this was a convenience sample and only 112 of a total of 958 first, second and third year students who were invited actually participated. It is therefore plausible that the sample is skewed toward students who were more symptomatic and who chose to participate. This survey could be extended to include health science students at other universities, especially those institutions characterised by greater ethnic diversity. Furthermore, it would be advantageous to explore ways to increase student participation while keeping anonymity intact. It would also be useful to conduct a comparative analysis of

first, second and third year students to elucidate whether SAD prevalence and symptom severity intensifies or is alleviated through the undergraduate student years, particularly with regards to in- and out-group interactions.

## COMMENTS

### Background

Social anxiety disorder (SAD) is a common psychiatric condition that is often comorbid with major depression, substance use disorders and other anxiety disorders. University students fall in the age range of increased risk for the onset of SAD. Recent literature has shown that ethnicity and culture both impact on the experience of anxiety and how individuals deal with it, and indicate that an individual's social concerns need to be examined in the context of cultural, racial, and ethnic background to adequately assess the degree and expression of social anxiety and SAD.

### Research frontiers

South Africa is a multicultural and multi-ethnic society and, given the particular circumstances of the country's colonial and apartheid past, it is important to understand the role of ethnicity in social interactions.

### Innovations and breakthroughs

Although the results on the contribution of ethnicity in SAD are not strongly significant, they do require further exploration given the historical context. This study contributes to the extant literature demonstrating that different risk factors may be uniquely associated with SAD for different ethnic/racial groups.

### Applications

This study indicates that ethnicity has the potential to independently influence severity of social anxiety symptomatology, suggesting that it is an important factor in student interactions, particularly in the South African context, and as such should be considered when assessing for SAD.

### Terminology

SAD or social anxiety disorder is a fairly prevalent anxiety disorder that causes extreme discomfort or fear regarding being judged or evaluated by others in social interactions.

### Peer review

The manuscript aims to investigate the role of ethnic factors in SAD among South African medical students. The topic is interesting and of high scientific and social significance.

## REFERENCES

- 1 **Furmark T.** Social phobia: overview of community surveys. *Acta Psychiatr Scand* 2002; **105**: 84-93 [PMID: 11939957 DOI: 10.1034/j.1600-0447.2002.1r103.x]
- 2 **Stein DJ, Seedat S, Herman A, Moomal H, Heeringa SG, Kessler RC, Williams DR.** Lifetime prevalence of psychiatric disorders in South Africa. *Br J Psychiatry* 2008; **192**: 112-117 [PMID: 18245026 DOI: 10.1192/bjp.bp.106.029280]
- 3 **Fehm L, Wittchen HU.** Comorbidity in Social Anxiety Disorder. In: Bandelow B, Stein DJ, eds. *Social Anxiety Disorder*. New York, NY: Marcel Dekker. 2004: 49-63
- 4 **Herman AA, Stein DJ, Seedat S, Heeringa SG, Moomal H, Williams DR.** The South African Stress and Health (SASH) study: 12-month and lifetime prevalence of common mental disorders. *S Afr Med J* 2009; **99**: 339-344 [PMID: 19588796]
- 5 **Acarturk C, Cuijpers P, van Straten A, de Graaf R.** Psychological treatment of social anxiety disorder: a meta-analysis. *Psychol Med* 2009; **39**: 241-254 [PMID: 18507874 DOI: 10.1017/S0033291708003590]
- 6 **Powers Mark B, Sigmarsson Snorri R, Emmelkamp Paul MG** (2008). A meta-analytic review of psychological treatments for social anxiety disorder. *INT J COGN THER* 2008; **1**: 94-113 [DOI: 10.1521/ijct.2008.1.2.94]
- 7 **Roy-Byrne Peter P, Cowley Deborah S** (2007). Pharmacological treatments for panic disorder, generalized anxiety disorder, specific phobia, and social anxiety disorder. In: *A guide to treatments that work* (3rd ed.). Nathan, Peter E.



- (Ed.); Gorman, Jack M. (Ed.); New York, NY, US: Oxford University Press, 2007: 395-430
- 8 **Wang PS**, Berglund P, Olfson M, Pincus HA, Wells KB, Kessler RC. Failure and delay in initial treatment contact after first onset of mental disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005; **62**: 603-613 [PMID: 15939838 DOI: 10.1001/archpsyc.62.6.603]
  - 9 **Topham P**. Feeling stupid: A survey of university students' experience of social anxiety in learning situations (Project Report). Bristol UK: University of The West of England Bristol, 2009: 2-75
  - 10 **Stewart D**, Johnson E, Walker J and Degen G. Prevalence of social phobia and its relationship with psychopathology and egocentrism in a university setting. Quebec City: The Canadian Psychological Association Annual Conference, 2001
  - 11 **Stewart D**, Walker J, Porpiglia C. Social anxiety on campus: Prevalence in clinical and non-clinical university student samples (2005, May). The Anxiety Disorders Association of Canada Annual Conference, Toronto: ON, 2005
  - 12 **Schlenker BR**, Leary MR. Social anxiety and self-presentation: a conceptualization and model. *Psychol Bull* 1982; **92**: 641-669 [PMID: 7156261 DOI: 10.1037/0033-2909.92.3.641]
  - 13 **Stephan WG**, Stephan CW. Intergroup anxiety. *J Soc Issues* 1985; **55**: 729-743 [DOI: 10.1111/0022-4537.00144]
  - 14 **Hofmann SG**, Anu Asnaani MA, Hinton DE. Cultural aspects in social anxiety and social anxiety disorder. *Depress Anxiety* 2010; **27**: 1117-1127 [PMID: 21132847 DOI: 10.1002/da.20759]
  - 15 **Carlbring P**, Brunt S, Bohman S, Austin D, Richards J, Öst LG, Andersson G. Internet vs. paper and pencil administration of questionnaires commonly used in panic/agoraphobia research. *Computers in Human Behaviour* 2007; **23**: 1421-1434 [DOI: 10.1016/j.chb.2005.05.002]
  - 16 **Myburgh NG**, Solanki GC, Smith MJ, Lalloo R. Patient satisfaction with health care providers in South Africa: the influences of race and socioeconomic status. *Int J Qual Health Care* 2005; **17**: 473-477 [PMID: 15985504 DOI: 10.1093/intqhc/mzi062]
  - 17 **Connor KM**, Davidson JR, Churchill LE, Sherwood A, Foa E, Weisler RH. Psychometric properties of the Social Phobia Inventory (SPIN). New self-rating scale. *Br J Psychiatry* 2000; **176**: 379-386 [PMID: 10827888 DOI: 10.1192/bjp.176.4.379]
  - 18 **Antony MM**, Coons MJ, McCabe RE, Ashbaugh A, Swinson RP. Psychometric properties of the social phobia inventory: further evaluation. *Behav Res Ther* 2006; **44**: 1177-1185 [PMID: 16257387 DOI: 10.1016/j.jbrat.2005.08.013]
  - 19 **Dell'Osso L**, Rucci P, Cassano GB, Maser JD, Endicott J, Shear MK, Sarno N, Sacttoni M, Grochocinski VJ, Frank E. Measuring social anxiety and obsessive-compulsive spectra: comparison of interviews and self-report instruments. *Compr Psychiatry* 2002; **43**: 81-87 [PMID: 11893984 DOI: 10.1053/comp.2002.30795]
  - 20 **Dell'Osso L**, Cassano GB, Sarno N Millanfranchi A, Pfanner C, Gemignani A, Maser JDM, Shear K, Grochocinski VJ, Rucci P, Frank E. Validity and reliability of the Structured Clinical Interview for Obsessive-compulsive Spectrum (SCI-OBS) and of the Structured Clinical Interview for Social Phobia Spectrum (SCI-SHY). *Intern J Methods Psychiat Res* 2000; **9**: 11-24 [DOI: 10.1002/mpr.76]
  - 21 **Dell'Osso L**, Sacttoni M, Papasogli A, Rucci P, Ciapparelli A, Di Poggio AB, Ducci F, Hardoy C, Cassano GB. Social anxiety spectrum: gender differences in Italian high school students. *J Nerv Ment Dis* 2002; **190**: 225-232 [PMID: 11960083 DOI: 10.1097/00005053-200204000-00003]
  - 22 **Radloff LS**. The CES-D Scale: A self-report depression scale for research in the general population. *Appl Psychol Meas* 1977; **1**: 385-401 [DOI: 10.1177/014662167700100306]
  - 23 **Zich JM**, Attkisson CC, Greenfield TK. Screening for depression in primary care clinics: the CES-D and the BDI. *Int J Psychiatry Med* 1990; **20**: 259-277 [PMID: 2265888 DOI: 10.2190/LYKR-7VHP-YJEM-MKM2]
  - 24 **Saunders JB**, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction* 1993; **88**: 791-804 [PMID: 8329970 DOI: 10.1111/j.1360-0443.1993.tb02093.x]
  - 25 **Rumpf HJ**, Hapke U, Meyer C, John U. Screening for alcohol use disorders and at-risk drinking in the general population: psychometric performance of three questionnaires. *Alcohol Alcohol* 2002; **37**: 261-268 [PMID: 12003915 DOI: 10.1093/alcalc/37.3.261]
  - 26 **Berman AH**, Bergman H, Palmstierna T, Schlyter F. Evaluation of the Drug Use Disorders Identification Test (DUDIT) in criminal justice and detoxification settings and in a Swedish population sample. *Eur Addict Res* 2005; **11**: 22-31 [PMID: 15608468 DOI: 10.1159/000081413]
  - 27 SPSS Inc. Chicago, IL SPSS Statistics for Windows Version 19.0 2010. Available from: URL: <http://www-01.ibm.com/software/analytics/spss/>
  - 28 **Radomsky AS**, Ashbaugh AR, Saxe ML, Ouimet AJ, Golden ER, Lavoie SL, O'Connor, KP. Psychometric properties of the French and English versions of the Social Phobia Inventory. *Can J Behav Sci* 2006; **38**: 354-360 [DOI: 10.1037/cjbs2006021]
  - 29 **Stewart DW**, Mandrusiak M. Social Phobia in College Students. *J college stud psychother* 2007; **22**: 65-76 [DOI: 10.1300/J035v22n02\_06]
  - 30 **Grant BF**, Hasin DS, Blanco C, Stinson FS, Chou SP, Goldstein RB, Dawson DA, Smith S, Saha TD, Huang B. The epidemiology of social anxiety disorder in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *J Clin Psychiatry* 2005; **66**: 1351-1361 [PMID: 16420070 DOI: 10.4088/JCP.v66n1102]
  - 31 **Carleton RN**, Collimore KC, Asmundson GJ, McCabe RE, Rowa K, Antony MM. SPINning factors: factor analytic evaluation of the Social Phobia Inventory in clinical and nonclinical undergraduate samples. *J Anxiety Disord* 2010; **24**: 94-101 [PMID: 19818582 DOI: 10.1016/j.janxdis.2009.09.003]
  - 32 **Duronto PM**, Nishida T and Nakayama S. Uncertainty, anxiety, and avoidance in communication with strangers. *Int J Intercult Relat* 2005; **29**: 549-560 [DOI: 10.1016/j.jintrel.2005.08.003]
  - 33 **Hubbert KN**, Gudykunst WB, Guerrero SL. Intergroup communication over time. *Int J Intercult Relat* 1999; **23**: 13-46 [DOI: 10.1016/S0147-1767(98)00024-8]
  - 34 **Ofan RH**, Rubin N, Amodio DM. Situation-based social anxiety enhances the neural processing of faces: evidence from an intergroup context. *SCAN* 2013: 1-7
  - 35 **Contrada RJ**, Ashmore RD, Gary ML, Coups E, Egeth JD, Sewell A, Ewell K, Goyal TM, Chasse V. Measures of Ethnicity-Related Stress: Psychometric properties, ethnic group differences, and associations with well-being. *J Appl Soc Psychol* 2001; **31**: 1775-1820 [DOI: 10.1111/j.1559-1816.2001.tb00205.x]
  - 36 **Bella TT**, Omigbodun OO. Social phobia in Nigerian university students: prevalence, correlates and co-morbidity. *Soc Psychiatry Psychiatr Epidemiol* 2009; **44**: 458-463 [PMID: 18979054 DOI: 10.1007/s00127-008-0457-3]
  - 37 **Ham LS**, Hope DA. Incorporating social anxiety into a model of college problem drinking: replication and extension. *Psychol Addict Behav* 2006; **20**: 348-355 [PMID: 16938075 DOI: 10.1037/0893-164X.20.3.348]

P- Reviewers: Dremencov E, Kravos M S- Editor: Ji FF

L- Editor: A E- Editor: Wu HL



## Cut-off of body mass index and waist circumference to predict hypertension in Indian adults

Tanu Midha, Vinay Krishna, Bhola Nath, Ranjeeta Kumari, Yashwant Kumar Rao, Umeshwar Pandey, Samarjeet Kaur

Tanu Midha, Department of Community Medicine, Government Medical College, Kannauj, Uttar Pradesh 209732, India

Vinay Krishna, Department of Cardiovascular and Thoracic Surgery, LPS Institute of Cardiology, Kanpur, Uttar Pradesh 208002, India

Bhola Nath, Department of Community Medicine, VCSGMS and RI, Srinagar, Garhwal, Uttarakhand 246174, India

Ranjeeta Kumari, Department of Community and Family Medicine, AIIMS, Rishikesh 249201, India

Yashwant Kumar Rao, Department of Pediatrics, GSVM Medical College, Kanpur, Uttar Pradesh 208002, India

Umeshwar Pandey, Department of Cardiology, LPS Institute of Cardiology, Kanpur, Uttar Pradesh 208002, India

Samarjeet Kaur, Department of Community Medicine, GSVM Medical College, Kanpur, Uttar Pradesh 208002, India

**Author contributions:** Midha T, Krishna V, Nath B and Kumari R contributed to conception and design of the study, acquisition of data, or analysis and interpretation of data, drafting the article and final approval of the version to be published; Rao YK, Pandey U and Kaur S helped in conception and design of the study, interpretation of data, revising the article and final approval of the version to be published.

**Correspondence to:** Tanu Midha, Assistant Professor, Department of Community Medicine, Government Medical College, National Highway 91A, Kannauj, Uttar Pradesh 209732, India. [tanumidha2001@gmail.com](mailto:tanumidha2001@gmail.com)

Telephone: +91-933-5828435 Fax: +91-512-2535483

Received: January 9, 2014 Revised: February 25, 2014

Accepted: June 10, 2014

Published online: July 16, 2014

naire was used to elicit the required information from the study participants and the diagnostic criteria for hypertension were taken according to the Seventh Joint National Committee Report on Hypertension (JNC-7). Receiver operating characteristic (ROC) analysis was used to estimate the cut-off values of BMI and waist circumference to predict hypertension.

**RESULTS:** The ROC analysis revealed that BMI is a good predictor of hypertension for both men (area under the ROC curve 0.714) and women (area under the ROC curve 0.821). The cut-off values of BMI for predicting hypertension were identified as  $\geq 24.5$  kg/m<sup>2</sup> in men and  $\geq 24.9$  kg/m<sup>2</sup> in women. Similarly, the ROC analysis for waist circumference showed that it is a good predictor of hypertension both for men (area under the ROC curve 0.784) and women (area under the ROC curve 0.815). The cut-offs for waist circumference for predicting hypertension were estimated as  $\geq 83$  cm for men and  $\geq 78$  cm for women. Adults with high BMI or high waist circumference had a higher prevalence of hypertension, respectively.

**CONCLUSION:** Simple anthropometric measurements such as BMI and waist circumference can be used for screening people at increased risk of hypertension in order to refer them for more careful and early diagnostic evaluation. Policies and programs are required for primary and secondary prevention of hypertension.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

### Abstract

**AIM:** To determine the cut-off values of body mass index (BMI) and waist circumference to predict hypertension in adults in north India.

**METHODS:** A community based cross-sectional study was conducted in 801 subjects in Kanpur, aged 20 years and above, using multistage stratified random sampling technique. A pre-tested structured question-

**Key words:** Anthropometric indices; Body mass index; Waist circumference; Obesity; Hypertension; Adults

**Core tip:** The Receiver operating characteristic analysis for body mass index (BMI) and waist circumference, respectively, showed good discriminatory power for hypertension in both men and women. The cut-off for BMI was identified as  $\geq 24.5$  kg/m<sup>2</sup> in men and  $\geq 24.9$

kg/m<sup>2</sup> in women. The cut-off for waist circumference for screening of hypertension was estimated as  $\geq 83$  cm in men and  $\geq 78$  cm in women. BMI and waist circumference, being simple tools in identifying hypertension, can be used for primordial and primary prevention and can thereby bring about a substantial reduction in cardiovascular morbidity and mortality which occurs as a consequence of hypertension.

Midha T, Krishna V, Nath B, Kumari R, Rao YK, Pandey U, Kaur S. Cut-off of body mass index and waist circumference to predict hypertension in Indian adults. *World J Clin Cases* 2014; 2(7): 272-278 Available from: URL: <http://www.wjg-net.com/2307-8960/full/v2/i7/272.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.272>

## INTRODUCTION

According to World Health Organization (WHO), cardiovascular diseases (CVDs) are the number one cause of death globally: more people die annually from CVDs than from any other cause<sup>[1]</sup>. An estimated 17.3 million people died from CVDs in 2008, representing 30% of all global deaths. The prevalence of hypertension in adults aged 25 years and above, worldwide, was around 40% in 2008<sup>[1]</sup>. Globally, raised blood pressure is estimated to cause 7.5 million deaths, about 12.8% of the total mortality. This, accounts for 57 million disability adjusted life years (DALYS) or 3.7% of total DALYS<sup>[1]</sup>. WHO has estimated that hypertension is directly responsible for about 62% of CVDs and 49% of ischemic heart disease (IHD) worldwide<sup>[2]</sup>.

A meta-analysis of prevalence studies on hypertension in India, from January 2000 to June 2012, revealed a high prevalence of hypertension in the urban (40.8%) as well as rural population (17.9%)<sup>[3]</sup>. India accounts for 17% of the world's population, second largest in the world, thereby contributing largely to the statistics of any disease in the world<sup>[4]</sup>.

Hypertension is a controllable disease and it has been reported that targeted reductions in blood pressure in hypertensives as well as modest population-wide blood pressure reductions are expected to produce large reductions in the burden of cardiovascular disease<sup>[5]</sup>. Miall suggested that genetic influences contribute not more than a third to the variance in blood pressure levels<sup>[6]</sup>. If the remaining two-thirds are environmental in origin, then an understanding of these environmental factors and appropriate preventive measures could help to bring down the burden of hypertension in the world. According to the Seventh Report of the Joint National Committee (JNC-7) on prevention, detection, evaluation, and treatment of high blood pressure, modification of risk factors plays an important role in the prevention and control of high blood pressure<sup>[7]</sup>.

Recent studies show that for every known person with hypertension there are two persons with either undi-

agnosed hypertension or prehypertension<sup>[8]</sup>. Screening the population for hypertension can go a long way in identifying individuals with undiagnosed hypertension and prevent a significant proportion of cardiovascular morbidity and mortality due to inapparent hypertension.

Being overweight in adulthood is well known to increase the risk of CVD especially hypertension<sup>[9]</sup>. It has also been seen that having a body mass index (BMI) outside the normal range significantly worsens risk parameters for CVD in school aged children<sup>[10]</sup>. Recognizing obesity using BMI and waist circumference as a marker, and thereby screening for cardiovascular risk can be a simple and inexpensive method of combating CVDs at the primary health care level. Given the magnitude of the problem of hypertension in India and its grave cardiovascular consequences, accurate estimates of cut-offs of BMI and waist circumference in predicting hypertension in the Indian population are necessary to plan effective control measures.

## MATERIALS AND METHODS

### Study population

The study population consisted of the total population of Kanpur district aged more than 20 years.

### Study design and sample size

This was a population based cross-sectional study. The sample size required ( $N = 372$ ) was calculated taking a prevalence of obesity of 6.2%, as reported in the "Five City study" from Moradabad, with a precision of 2.5% and a confidence level of 95%<sup>[11]</sup>. The formula used was,  $n = Z_{(1-\alpha/2)}^2 pq/d^2$  (where  $Z_{(1-\alpha/2)}$  was taken at 95% confidence;  $p$  = prevalence of obesity,  $q = 100-p$ ;  $d$  = absolute precision). For this study,  $p = 6.2\%$ ;  $q = 93.8\%$ ;  $d = 2.5\%$ . Adding a 10% for incomplete answers, the total number came out to be 409. Since it was a multistage stratified random sampling, a design effect of 2 was included to minimize any error due to inherent variation in the population. The calculated sample size was multiplied by 2 to obtain the sample size of 818. The data was analyzed for 801 subjects only who had provided complete answers.

### Sampling technique

Urban Kanpur has 110 wards and a total population of 2797511 according to 2001 census, and rural Kanpur has 10 blocks and a population of 1370488 which implies a ratio of 2:1 respectively<sup>[12]</sup>. Therefore, applying Probability Proportional to Size (PPS), out of 818 subjects, two-thirds (545) were selected from the urban population and one-third (273) were selected from the rural population. Multistage stratified random sampling technique was used to select representative subjects of Kanpur district. At the first stage, 8 wards were randomly selected to study the urban population<sup>[13]</sup>. Similarly, to study the rural population, 4 blocks were randomly selected<sup>[14]</sup>. At the second stage, 1 urban locality from each ward was randomly selected. Similarly, 1 village from each block was randomly selected. A total of 68 subjects from each



**Table 1** Characteristics of the study population

Parameters	Men (n = 356)	Women (n = 445)	Total (n = 801)
Age (yr)	36.4 ± 13.8	34.8 ± 11.6	35.5 ± 12.6
Weight (kg)	58.6 ± 11.5	51.7 ± 11.7	54.8 ± 12.1
Height (cm)	165.3 ± 7.1	151.7 ± 5.9	157.7 ± 9.3
BMI (kg/m <sup>2</sup> )	21.4 ± 3.9	22.4 ± 4.6	21.9 ± 4.3
Waist circumference (cm)	78.9 ± 11.0	74.4 ± 11.1	76.5 ± 11.3
SBP (mmHg)	127 ± 16	122 ± 18	124 ± 17
DBP (mmHg)	82 ± 9	78 ± 10	80 ± 9

Values are written as mean ± SD. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index.

urban locality/village were interviewed to complete the required sample size.

### Selection of subjects

The households in every urban area/village were selected for the study by systematic sampling. Depending upon the population of the particular urban locality/village, a random number was chosen and every  $n^{\text{th}}$  household was selected for the study. This process was continued till the required sample size was completed. In every household, only one member, aged more than 20 years was randomly selected. Out of the 545 respondents interviewed in the urban area, 10 were excluded owing to incomplete answers, whereas of the 273 respondents interviewed in the rural area 7 were excluded. Therefore, the final analysis included the responses from a total of 801 study subjects. Data collection was done from December 2006 to February 2007.

### Data collection

A pre-tested structured questionnaire was used to elicit the required information from the study participants. A standard mercury sphygmomanometer was used for recording blood pressure. Before the measurement was taken, the patient was seated comfortably for at least 5 min. Care was taken that the arm muscles were relaxed and the arm was supported at heart level. The cuff was applied to the right upper arm and was inflated until the manometer reading was 30 mm Hg above the level at which the radial pulse disappears, and then slowly deflated at a rate of approximately 2 mmHg/s. During this time, the Korotkoff sounds were monitored using a stethoscope placed over the brachial artery. The first (appearance) and the fifth (disappearance) Korotkoff sounds were recorded as the systolic and diastolic blood pressure, respectively. Blood pressures were measured twice and their mean was recorded. According to JNC-7<sup>[15]</sup>, normal blood pressure was defined as a systolic blood pressure (SBP) < 120 mmHg and a diastolic blood pressure (DBP) < 80 mmHg; pre-hypertension as SBP 120-139 mmHg and/or DBP 80-89 mmHg; Stage I hypertension as SBP 140-159 mmHg and/or DBP 90-99 mmHg and stage II hypertension as ≥ 160 mmHg and/or DBP ≥ 100 mmHg. In the present study, subjects in stage I and Stage II were considered as hypertensive. Waist circumference was

measured to the nearest 0.1 cm using a non-extensible tape. Measurement was made at the level of the umbilicus, with the subject in the erect position, breathing silently.

### Statistical analysis

Data was compiled in Microsoft Excel and analysed using MedCalc12.7.5 software. Receiver operating characteristic (ROC) analysis was used to compare the predictive validity, and to determine the optimal cut-off values of anthropometric indices. Area under the curve was also measured to determine the diagnostic power of the test, and to describe the probability that the anthropometric indices would correctly identify subjects with hypertension. Optimal cut-off values were measured by calculating the sensitivity and specificity of the anthropometric measurements at various cut-off points. Youden index was calculated to find out the associated criterion with maximum sensitivity and specificity for predicting hypertension.

## RESULTS

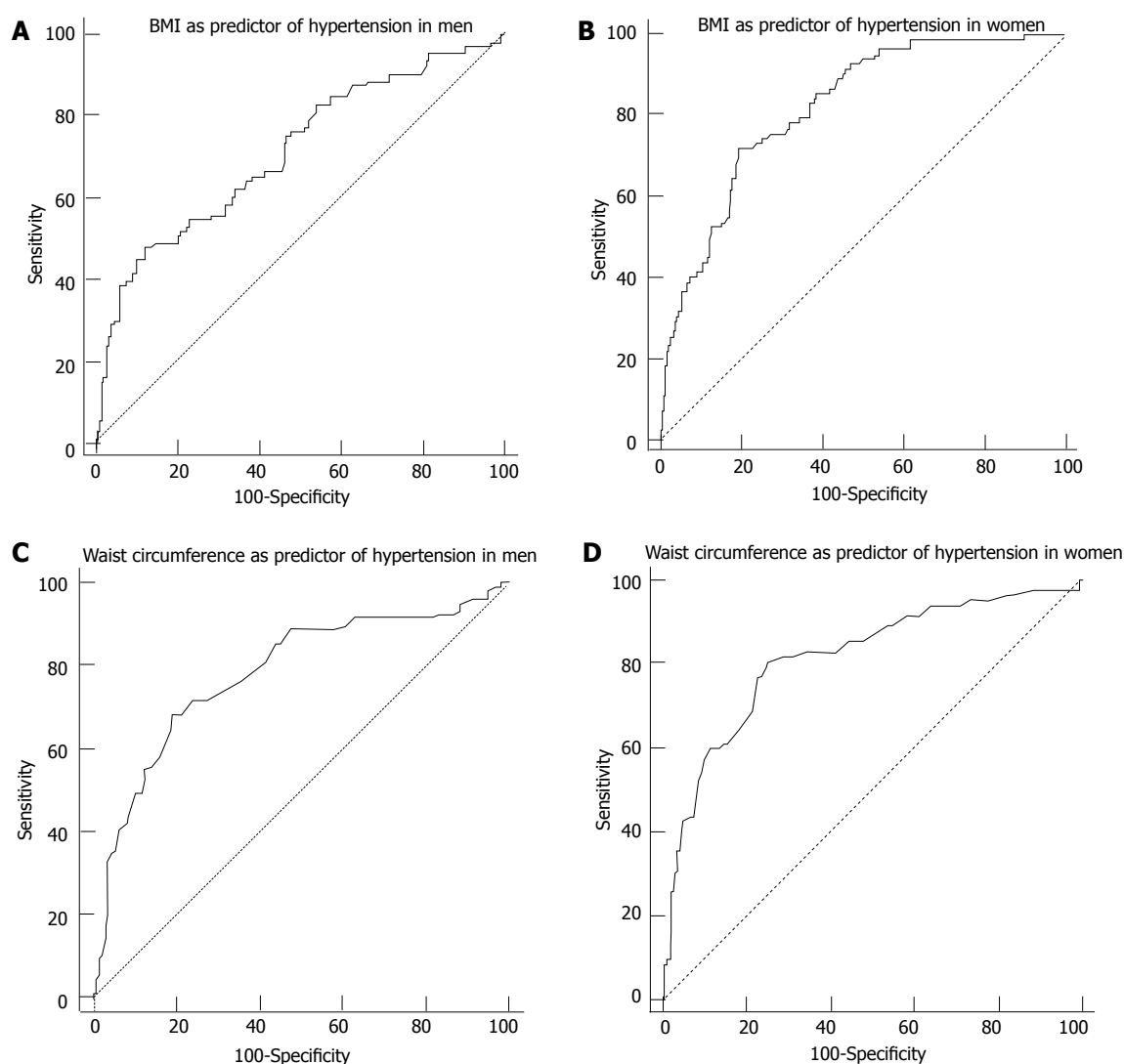
In the present study, the mean age of the study subjects was 35.5 ± 12.6 years, while that of men and women respectively was 36.4 ± 13.8 years and 34.8 ± 11.6 years (Table 1). Average BMI of the study population was 21.9 ± 4.3 kg/m<sup>2</sup>. The mean waist circumference of men was 78.9 ± 11.0 cm and that of women was 74.4 ± 11.1 cm. Average systolic and diastolic blood pressure of the study population was 124 ± 17 mmHg and 80 ± 9 mmHg respectively. The mean systolic blood pressure among men was 127 ± 16 mmHg and that among women was 122 ± 18 mmHg. The mean diastolic blood pressure among men and women was 82 ± 9 mmHg and 78 ± 10 mmHg respectively.

The ROC analysis for BMI showed good discriminatory power for hypertension for both men and women. Area under the ROC curve was 0.714 for men and 0.821 for women respectively (Figure 1 A and B). The cut-off for BMI with better properties for screening of hypertension was identified as ≥ 24.5 kg/m<sup>2</sup> in men and ≥ 24.9 kg/m<sup>2</sup> in women (Table 2). The sensitivity and specificity of cut-off for BMI in men was 48.1% and 87.2% respectively, and that for women was 71.9% and 80.7% respectively.

Similarly, the ROC analysis for waist circumference revealed that it is a predictor of hypertension for both men and women. Area under the ROC curve was 0.784 for men and 0.815 for women respectively (Figure 1C and D). The cut-offs for waist circumference for screening of hypertension was estimated as ≥ 83 cm for men and ≥ 78 cm for women (Table 2). The sensitivity and specificity of cut-off of waist circumference in men was 67.9% and 80.4% respectively while that for women was 81.7% and 71.3% respectively. Adults with high BMI or high waist circumference had a higher prevalence of hypertension, respectively.

ROC analysis was also done after combining the pre-hypertensive and hypertensive subjects as non-normoten-





**Figure 1** Criterion values and coordinates of the receiver operating characteristic curve. A: Receiver operating characteristic (ROC) analysis for body mass index (BMI) as a predictor of hypertension among men revealed area under the curve = 0.714. The optimal cut-off for maximum sensitivity and specificity was 24.5; B: ROC analysis for BMI as a predictor of hypertension among women revealed area under the curve = 0.821. The optimal cut-off for maximum sensitivity and specificity was 24.9; C: ROC analysis for waist circumference as a predictor of hypertension among men revealed area under the curve = 0.784. The optimal cut-off for maximum sensitivity and specificity was 83; D: ROC analysis for waist circumference as a predictor of hypertension among women revealed area under the curve = 0.815. The optimal cut-off for maximum sensitivity and specificity was 78.

sive and the remaining normal subjects as normotensive. It was observed that BMI was not a good predictor of non-normotensive status both in men and women. Area under the curve was 0.452 for BMI among men and 0.540 among women respectively. Similarly it was found that waist circumference did have good discriminatory power for non-normotensive status both in men and women. Area under the curve was 0.429 among men and 0.552 among women respectively. These results were not statistically significant.

Therefore, BMI and waist circumference can be considered good predictors of hypertension but not of pre-hypertension.

## DISCUSSION

In the present study, cut-off for BMI for predicting hypertension was identified as  $\geq 24.5$  kg/m<sup>2</sup> in men and

$\geq 24.9$  kg/m<sup>2</sup> in women. In a study from Malaysia, the mean age of the study subjects was  $44 \pm 14$  years and the cut-off for BMI as predictor of hypertension was 25.5 kg/m<sup>2</sup> in men and 24.9 kg/m<sup>2</sup> in women, which was very similar to our study. Areas under the curves of BMI as a predictor of hypertension were 0.59 and 0.61 in men and women, respectively<sup>[16]</sup>. Area under the curves of 0.6-0.7 are considered to be poor while 0.7-0.8 are considered fair, as seen in our study. Increased CVD risks related to obesity at lower BMIs have been found in Asians<sup>[17,18]</sup>. In addition, Asians are also predisposed to visceral or abdominal obesity<sup>[19]</sup>. Therefore, WHO recently proposed lower BMI values to define overweight and obesity in people of the Asia-Pacific region<sup>[20]</sup>. According to the World Heart Federation, if a person's BMI is more than 30, he/she is obese and at serious risk of cardiovascular disease, whereas in our study, optimal cut-off for BMI for predicting hypertension was identified as  $\geq 24.5$  kg/m<sup>2</sup> in

**Table 2 Receiver operating characteristic analysis for body mass index and waist circumference as predictor of hypertension in men and women**

Indicators	BMI in men	BMI in women	WC in men	WC in women
Area under the ROC curve	0.714	0.821	0.784	0.815
Standard error	0.0312	0.0237	0.0286	0.0284
95% CI	0.664 to 0.760	0.783 to 0.856	0.737 to 0.826	0.776 to 0.850
z statistic	6.857	13.572	9.922	11.117
Significance level	< 0.0001	< 0.0001	< 0.0001	< 0.0001
P (Area = 0.5)				
Youden index J	0.3611	0.5294	0.4912	0.5542
Associated criterion	> 24.5	> 24.9	> 83	> 78

BMI: Body mass index; ROC: Receiver operating characteristic.

men and  $\geq 24.9$  kg/m<sup>2</sup> in women<sup>[21]</sup>.

However, some studies have also revealed that BMI follows a J-shaped curve to predict all case mortality. BMI is a strong predictor of overall mortality both above and below the apparent optimum of about 22.5-25 kg/m<sup>2</sup><sup>[22]</sup>. The progressive excess mortality above this range is mainly due to vascular diseases and is probably causal at large. At 30-35 kg/m<sup>2</sup>, median survival is reduced by 2-4 years; at 40-45 kg/m<sup>2</sup>, it is reduced by 8-10 years (which is comparable with the effects of smoking). The definite excess mortality below 22.5 kg/m<sup>2</sup> is mainly due to smoking-related diseases, and is not fully explained.

In the present study, the optimal cut-offs of waist circumference for screening of hypertension was estimated as  $\geq 83$  cm for men and  $\geq 78$  cm for women. The waist circumference cut-offs for risk of hypertension obtained in this study are comparable to those reported by Snehalatha *et al.*<sup>[23]</sup> for South Indians, which was reported as 85 cm for men and 80 cm for women wherein the mean age of the study population was  $40.4 \pm 14.2$  years. These were also similar to the cut-offs observed by Rao *et al.*<sup>[24]</sup> in Maharashtra as 86 cm in the male population averaging  $42.9 \pm 7.9$  years and 79 cm in the female population averaging  $42.2 \pm 7.8$  years<sup>[23,24]</sup>. The cut-offs in the present study were slightly lower for men when compared to those reported by Misra *et al.*<sup>[25]</sup> for North Indians (90 cm for men averaging  $40.5 \pm 14.7$  years) and slightly higher for women (80 cm for women having mean age of  $38.8 \pm 14.8$  years). In other Asian populations, cut-offs reported by Wildman *et al.*<sup>[26]</sup> for Chinese adults (86 cm for both sexes) and those observed by Lin *et al.*<sup>[27]</sup> for adults from Taiwan as 80.5 cm for men and 71.5 cm for women with mean age  $37.3 \pm 10.9$  years in men and  $37.0 \pm 11.1$  years in women, were on the lower side. Ethnicity plays an important role in determining the predictive power of waist circumference for hypertension. Also, nutrition habits vary among different populations which may be the reason for the difference in waist circumference cut-offs.

According to the World Heart Federation, if the waist circumference is more than 102 cm among men,

the person is at serious risk of CVDs, but for Asian men the cut-off has been set at 90 cm<sup>[21]</sup>. Similarly for women the high risk cut-off is 88 cm whereas in Asian women, it is 80 cm. In our study, the optimal cut-offs for waist circumference for predicting hypertension were  $\geq 83$  cm for men and  $\geq 78$  cm for women, which approximate the Asian cut-offs. In Asians, more than in the Western population, there is a strong association between blood pressure and stroke. It has been estimated that reduction of 3 mmHg in DBP would reduce the number of strokes in Asia by one third. Identification of indicators predicting risk of hypertension therefore has an important implication towards prevention of morbidity and mortality due to CVDs<sup>[28]</sup>.

A major limitation of the study was that the classification of hypertension was based on a single measurement of blood pressure. Secondly, the number of female study subjects was greater than males which might act as a source of bias in studying the difference in the cut-offs for predicting hypertension in men and women, respectively. Although this is not a meta-analysis, it is a useful study with practical application for the prevention and control of hypertension.

The present study reveals that BMI and waist circumference are simple tools in identifying hypertension. Although it is not easy to determine how low the cut-off should be, the findings in this study provide sufficient evidence that BMI and waist circumference can be used as a screening tool for hypertension. Since high blood pressure itself is the entry point to other non-communicable diseases, this emphasizes the need for further research to identify cut-offs of simple anthropometric measurements, which can be calculated by people themselves, for screening of hypertension. Given the risk of CVD associated with high blood pressure, hypertension screening and health education programs regarding weight reduction may be considered as a cost-effective public health approach in dealing with the morbidity attributed to CVDs. This study is only a prelude to the upcoming research in the field of non-communicable diseases especially in the Asian population which is more vulnerable to adverse effects of obesity.

## COMMENTS

### Background

Cardiovascular diseases (CVDs) are the most common cause of mortality in the world; around 30% of all global deaths in 2008 were attributed CVDs. High blood pressure is the entry point to CVDs and other non-communicable diseases. The magnitude of hypertension in adults aged 25 and over was around 40% in the world in 2008. A meta-analysis in 2013 estimated the prevalence of hypertension as 40.8% in the urban and 17.9% in the rural Indian population. Body mass index (BMI) and waist circumference are simple tools in predicting hypertension, which can be calculated by the people themselves. The findings in this study provide cut-off levels of BMI and waist circumference for screening of hypertension in the Indian population. Given the risk of CVDs associated with high blood pressure, hypertension screening and health education programs regarding weight reduction may be considered as a cost-effective public health approach in dealing with the morbidity attributed to CVDs. Therefore, a precise estimate of the cut-offs of BMI and waist circumference specific to the indigenous population of the country is required to assess the magnitude of

the problem that has to be addressed and to design programs and policies for prevention and control.

### Research frontiers

In India, very few studies are available on the cut-offs of BMI and waist circumference for predicting hypertension. Given the variation in anthropometry due to ethnic differences and discrepancies in the nutritional status of different populations, an estimate of the cut-offs for screening of hypertension in the Indian population is required which can help in the development of preventive strategies.

### Innovations and breakthroughs

BMI and waist circumference have good discriminatory power for predicting hypertension in the Indian population and this knowledge will help in shaping primordial and primary level preventive programs for the country.

### Applications

Very few studies on cut-off levels of BMI and waist circumference for screening of hypertension are available in India; therefore, the main application of this study is to provide cut-offs levels for our indigenous population to help develop a strategy for control and prevention of hypertension appropriate for the country.

### Terminology

The ROC curve is a fundamental tool for diagnostic test evaluation using a graphical plot. In a ROC curve the true positive rate (Sensitivity) is plotted as a function of the false positive rate (100-Specificity) for different cut-off points of a parameter. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. The ROC curve estimates and reports all of the combinations of sensitivity and specificity that a diagnostic test is able to provide. The area under the ROC curve (AUC) is a measure of how well a parameter can distinguish between two diagnostic groups (diseased/normal). Accuracy of a diagnostic test is measured by the area under the ROC curve. An area of 1 represents a perfect test; an area of 0.5 represents a worthless test. The associated criterion value corresponding with the Youden index J is the optimal criterion value when disease prevalence is 50% and equal weight is given to sensitivity and specificity.

### Peer review

The study titled "Optimal Cut-off values of BMI and waist circumference to predict hypertension in adults: A cross-sectional study in a north Indian population" has been well thought out. It may be an incremental contribution of the manuscript to the field. Design of the manuscript is also good. It is well-marked that this paper would have a few literature errors formally.

## REFERENCES

- 1 **World Health Organization.** Global Health Repository. Available from: URL: [http://www.who.int/gho/ncd/risk\\_factors/blood\\_pressure\\_prevalence\\_text/en/index.html](http://www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/index.html)
- 2 **World Health Report.** Reducing Risks, Promoting Healthy Life. 2002; Chapter 4; p-12. Available from: URL: [http://www.who.int/whr/2002/en/whr02\\_ch4.pdf](http://www.who.int/whr/2002/en/whr02_ch4.pdf).
- 3 **Midha T, Nath B, Kumari R, Rao YK, Pandey U.** Prevalence of hypertension in India: A meta-analysis. *World J Meta-Anal* 2013; **1**: 83-89 [DOI: 10.13105/wjma.v1.i2.83]
- 4 **World Development Report 2006.** Equity and Development. Washington D.C: A co-publication of the World Bank and Oxford University Press, 2006
- 5 **Rodgers A, Lawes C, MacMahon S.** Reducing the global burden of blood pressure-related cardiovascular disease. *J Hypertens Suppl* 2000; **18**: S3-S6 [PMID: 10939783]
- 6 **Miall WE.** Heredity and hypertension. *Practitioner* 1971; **207**: 20-27 [PMID: 5557040]
- 7 **Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ.** The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289**: 2560-2572 [PMID: 12748199 DOI: 10.1001/jama.289.19.2560]
- 8 **Joshi SR, Saboo B, Vadivale M, Dani SI, Mithal A, Kaul U, Badgandi M, Iyengar SS, Viswanathan V, Sivakadaksham N, Chattopadhyaya PS, Biswas AD, Jindal S, Khan IA, Sethi BK, Rao VD, Dalal JJ.** Prevalence of diagnosed and undiagnosed diabetes and hypertension in India--results from the Screening India's Twin Epidemic (SITE) study. *Diabetes Technol Ther* 2012; **14**: 8-15 [PMID: 22050271 DOI: 10.1089/dia.2011.0243]
- 9 **McPherson K, Marsh T, Brown M.** Tackling obesities: future choices-modelling future trends in obesity and the impact on health: report for Foresight (Government Office for Science, 2007). Available from: URL: [http://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/287937/07-1184x-tackling-obesities-future-choices-report.pdf](http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/287937/07-1184x-tackling-obesities-future-choices-report.pdf)(last accessed 3-7-14)
- 10 **Friedemann C, Heneghan C, Mahtani K, Thompson M, Perera R, Ward AM.** Cardiovascular disease risk in healthy children and its association with body mass index: systematic review and meta-analysis. *BMJ* 2012; **345**: e4759 [PMID: 23015032 DOI: 10.1136/bmj.e4759]
- 11 **Singh RB, Pella D, Mechirova V, Kartikey K, Demeester F, Tomar RS, Beegom R, Mehta AS, Gupta SB, De Amit K, Neki NS, Haque M, Nayse J, Singh S, Thakur AS, Rastogi SS, Singh K, Krishna A.** Prevalence of obesity, physical inactivity and undernutrition, a triple burden of diseases during transition in a developing economy. The Five City Study Group. *Acta Cardiol* 2007; **62**: 119-127 [PMID: 17536599 DOI: 10.2143/AC.62.2.2020231]
- 12 **Government of India.** Census of India 2001, Population Totals, Paper - 1; 2001. Available from: URL: <http://www.censusindia.gov.in/pca/SearchDetails.aspx?Id=163690> (last accessed 3-7-14)
- 13 **Ward-wise List of Slum/Nonslum Areas.** Nagar Nigam: Kanpur District, 2002: 3
- 14 **Blockwise List of Villages.** Kanpur District: Office of Chief Development Office, 2002: 10
- 15 **Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ.** Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003; **42**: 1206-1252 [PMID: 14656957 DOI: 10.1161/01.HYP.0000107251.49515.c2]
- 16 **Zaher ZM, Zambari R, Pheng CS, Muruga V, Ng B, Appannah G, Onn LT.** Optimal cut-off levels to define obesity: body mass index and waist circumference, and their relationship to cardiovascular disease, dyslipidaemia, hypertension and diabetes in Malaysia. *Asia Pac J Clin Nutr* 2009; **18**: 209-216 [PMID: 19713180]
- 17 **Deurenberg-Yap M, Yian TB, Kai CS, Deurenberg P, VAN Staveren WA.** Manifestation of cardiovascular risk factors at low levels of body mass index and waist-to-hip ratio in Singaporean Chinese. *Asia Pac J Clin Nutr* 1999; **8**: 177-183 [PMID: 24394159 DOI: 10.1046/j.1440-6047.1999.00091.x]
- 18 **Hsieh SD, Yoshinaga H, Muto T, Sakurai Y, Kosaka K.** Health risks among Japanese men with moderate body mass index. *Int J Obes Relat Metab Disord* 2000; **24**: 358-362 [PMID: 10757631 DOI: 10.1038/sj.ijo.0801157]
- 19 **Wang J, Russell-Aulet M, Mazariegos M, Burastero S, Thornton J, Lichtman S, Heymsfield SB, Pierson Jr NP.** Body fat by dual photon absorptiometry (DPA): comparisons with traditional methods in Asians, Blacks and Caucasians. *Am J Hum Biol* 1992; **4**: 501-510 [DOI: 10.1002/ajhb.1310040409]
- 20 **World Health Organization.** The Asia-Pacific perspective: redefining obesity and its treatment (WHO: Geneva, 2000). Available from: URL: [http://www.wpro.who.int/nutrition/documents/Redefining\\_obesity/en/](http://www.wpro.who.int/nutrition/documents/Redefining_obesity/en/) (last accessed 3-7-14)
- 21 **World heart federation.** Available from: URL: <http://www.world-heart-federation.org/cardiovascular-health/cardiovascular-disease-risk-factors/obesity/>
- 22 **Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, Qizilbash N, Collins R, Peto R.** Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet* 2009; **373**: 1083-1096 [PMID: 19299006 DOI: 10.1016/S0140-6736(09)60318-4]
- 23 **Snehalatha C, Viswanathan V, Ramachandran A.** Cutoff

- values for normal anthropometric variables in asian Indian adults. *Diabetes Care* 2003; **26**: 1380-1384 [PMID: 12716792 DOI: 10.2337/diacare.26.5.1380]
- 24 **Rao S**, Waingankar PP. Performance of waist circumference relative to BMI in predicting risk of obesity and hypertension among affluent Indian adults. *Health* 2013; **5**: 16-22 [DOI: 10.4236/health.2013.58A3003]
  - 25 **Misra A**, Vikram NK, Gupta R, Pandey RM, Wasir JS, Gupta VP. Waist circumference cutoff points and action levels for Asian Indians for identification of abdominal obesity. *Int J Obes (Lond)* 2006; **30**: 106-111 [PMID: 16189502 DOI: 10.1038/sj.ijo.0803111]
  - 26 **Wildman RP**, Gu D, Reynolds K, Duan X, He J. Appropriate body mass index and waist circumference cutoffs for categorization of overweight and central adiposity among Chinese adults. *Am J Clin Nutr* 2004; **80**: 1129-1136 [PMID: 15531658]
  - 27 **Lin WY**, Lee LT, Chen CY, Lo H, Hsia HH, Liu IL, Lin RS, Shau WY, Huang KC. Optimal cut-off values for obesity: using simple anthropometric indices to predict cardiovascular risk factors in Taiwan. *Int J Obes Relat Metab Disord* 2002; **26**: 1232-1238 [PMID: 12187401 DOI: 10.1038/sj.ijo.0802040]
  - 28 **Cheung YB**, Low L, Osmond C, Barker D, Karlberg J. Fetal growth and early postnatal growth are related to blood pressure in adults. *Hypertension* 2000; **36**: 795-800 [PMID: 11082145 DOI: 10.1161/01.HYP.36.5.795]

**P- Reviewers:** Puddu PE, Talas ZS, Undela K, Xu XH  
**S- Editor:** Wen LL **L- Editor:** A **E- Editor:** Wu HL





## Perirenal extra-adrenal myelolipoma

Ali Hajiran, Chad Morley, Robert Jansen, Stanley Kandzari, Patrick Bacaj, Stanley Zaslau, Jon Cardinal

Ali Hajiran, Chad Morley, Robert Jansen, Stanley Kandzari, Patrick Bacaj, Stanley Zaslau, Jon Cardinal, Division of Urology, West Virginia University, Morgantown, WV 26505, United States

Patrick Bacaj, Department of Pathology, West Virginia University, Morgantown, WV 26505, United States

Jon Cardinal, Division of Surgical Oncology, West Virginia University, Morgantown, WV 26505, United States

Author contributions: Hajiran A, Morley C, Jansen R and Kandzari S wrote the paper; Cardinal J participated in the presented surgical case; Bacaj P provided the histological diagnosis and commentary; Jansen R, Kandzari S, Zaslau S and Cardinal J edited the final paper and provided additional commentary.

Correspondence to: Stanley Zaslau, MD, MBA, FACS, Professor and Chief, Division of Urology, West Virginia University, PO Box 9238, Morgantown, WV 26506.

United States. szaslau@hsc.wvu.edu

Telephone: +1-304-2932706 Fax: +1-304-2932807  
Received: December 11, 2013 Revised: April 22, 2014

Accepted: May 28, 2014

Published online: July 16, 2014

**Core tip:** We report a case of a patient with an incidentally discovered perirenal mass that was initially concerning for a retroperitoneal liposarcoma. Following surgical resection and pathological analysis, the lesion was found to be an extra-adrenal myelolipoma. This case report and review of the literature demonstrates the importance of the proper work-up and management of perirenal lipoma variants while addressing the issues of tissue biopsy, surgical intervention, and pre- and post-operative surveillance.

Hajiran A, Morley C, Jansen R, Kandzari S, Bacaj P, Zaslau S, Cardinal J. Perirenal extra-adrenal myelolipoma. *World J Clin Cases* 2014; 2(7): 279-283 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/279.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.279>

### Abstract

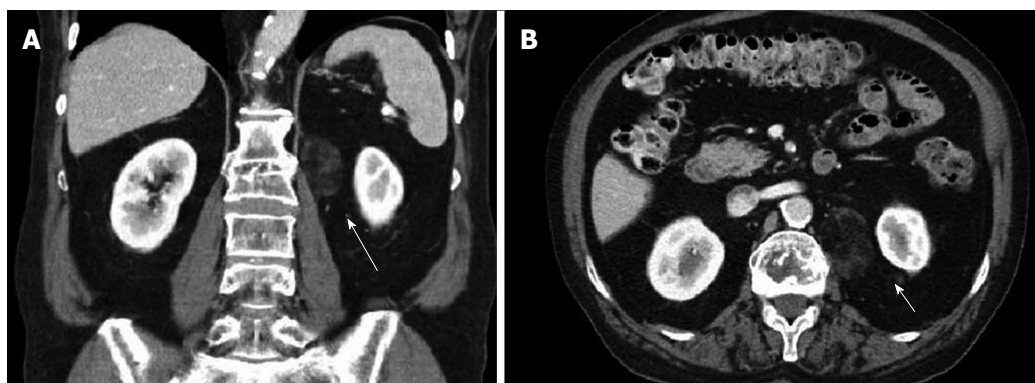
Myelolipomas are rare tumors consisting of both adipose and hematopoietic tissue and are typically found within the adrenal gland. Extra-adrenal involvement is rare, especially those tumors involving the perirenal space and collecting system. We report a case of a patient with an incidentally discovered perirenal mass that was initially concerning for a retroperitoneal liposarcoma. Following surgical resection and pathological analysis, the lesion was found to be an extra-adrenal myelolipoma. This case report and review of the literature demonstrates the importance of the proper work-up and management of perirenal lipoma variants while addressing the issues of tissue biopsy, surgical intervention, and pre- and post-operative surveillance.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

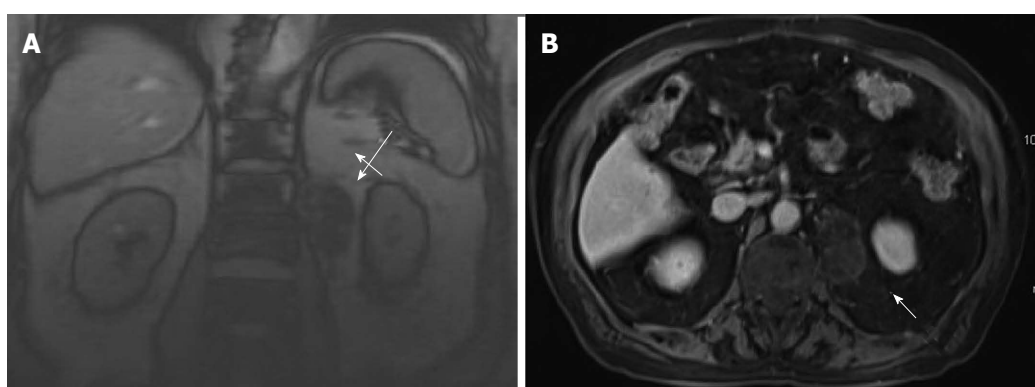
**Key words:** Myelolipoma; Lipoma; Perirenal mass; Nephrectomy; Oncology

### INTRODUCTION

Myelolipomas are mesenchymal tumors which consist of a mixture of mature adipose tissue with hematopoietic cells. This intriguing tumor most commonly occurs within the adrenal gland; however, it has been occasionally found within the pelvis, thorax, retroperitoneal space, and various other sites throughout the body<sup>[1-7]</sup>. There have been less than 60 reported cases of extra-adrenal myelolipomas to this date, with the majority of the literature describing neoplasms found within the pre-sacral space<sup>[2,8-10]</sup>. Perirenal extra-adrenal myelolipomas are especially rare, with only 9 cases previously reported<sup>[11]</sup>. We present a case of a patient with an incidentally discovered perirenal mass which, after having shown interval growth on longitudinal surveillance imaging studies, was surgically resected along with a left nephrectomy for presumed retroperitoneal liposarcoma. On final pathological analysis the lesion was found to be an extra-adrenal myelolipoma.



**Figure 1 Initial computed tomography scan.** Shows incidentally found non-enhancing heterogeneous mass measuring approximately 3.8 cm × 2.3 cm in longitudinal (A) and anterior-posterior dimensions (B), just inferior to the left renal vein (long arrow) and medial to the left kidney (arrow).



**Figure 2 Surveillance magnetic resonance imaging.** Imaging obtained 17 mo after the initial diagnostic computed tomography scan shows that the mass, located just inferior to the left renal vein (long arrow) and medial to the left kidney (arrow), increased in size to 5.0 cm × 3.4 cm in longitudinal (A) and anterior-posterior dimensions (B).

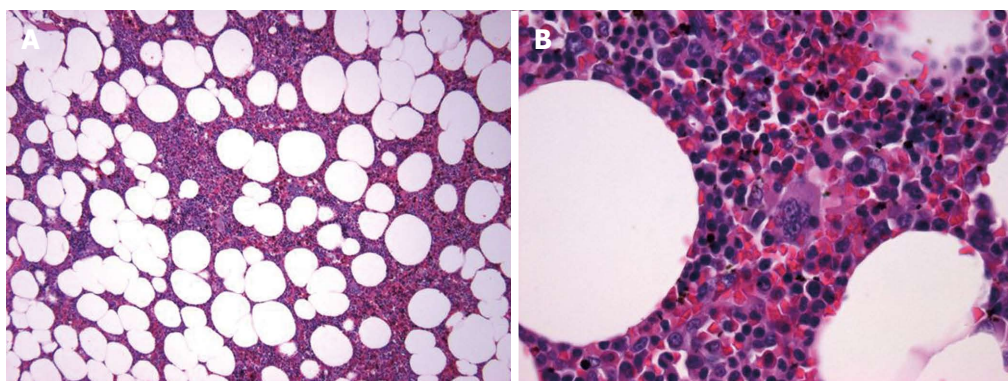
## CASE REPORT

A 78-year-old gentleman presented to the surgical oncology clinic to be evaluated for a left-sided retroperitoneal mass that was incidentally discovered on a computed tomography (CT) scan for a suspected case of acute pancreatitis. His prior medical history included hypertension, hyperlipidemia, acalculous cholecystitis, atrial fibrillation, and coronary artery disease. Initial radiographic findings revealed a non-enhancing heterogeneous mass measuring approximately 3.8 cm × 2.3 cm in longitudinal and anterior-posterior (AP) dimensions, just inferior to the left renal vein and medial to the left kidney (Figure 1). As the lesion appeared to contain mostly adipose with a small amount of soft tissue density, a well differentiated liposarcoma was suspected. The patient was initially offered surgical resection of the lesion, which he refused. Given the small size of the mass and patient's age and health status, the decision was made to closely monitor the lesion with routine cross-sectional surveillance imaging and regular follow-up at 4 to 6 mo.

Throughout the surveillance period, the patient did not complain of any new symptoms. Physical examination repeatedly revealed a soft, non-tender abdomen with no palpable masses or hernias. Repeat cross-sectional

imaging studies, however, did reveal a slowly enlarging left-sided heterogeneous perirenal mass. A magnetic resonance imaging (MRI) obtained 17 mo after initial diagnosis showed that the mass had increased in size to 5.0 cm × 3.4 cm (Figure 2). Four months prior the tumor had remained unchanged. The concern for a progressing malignant lesion prompted the decision to proceed with surgical intervention. Due to its proximity to the renal vessels, as well as the fact that the preoperative diagnosis was liposarcoma, the mass was excised en bloc with the left kidney in an attempt to gain wide surgical margins. The patient tolerated the procedure well and his post-operative course was uneventful. He was discharged home in stable condition on the ninth day following the procedure.

Gross pathology revealed an encapsulated, well-defined, focally hemorrhagic mass measuring 7.2 cm × 4.1 cm × 3.3 cm in size. The tumor did not extend into the renal capsule or adrenal gland. Histology revealed that the mass was composed of mostly mature adipocytes mixed with islands of hematopoietic cells. Trilineage hematopoiesis was present, including nucleated red blood cells and megakaryocytes (Figure 3). Tumor resection margins were free. The above mentioned morphological features were consistent with the diagnosis of “perirenal (extra-



**Figure 3** Hematoxylin and eosin stain at 10 × (A) and 60 × (B) magnification. Shows mature adipose cells with hematopoietic cells including erythroid precursors, granulocytic precursors, and megakaryocytes.

adrenal) myelolipoma”.

## DISCUSSION

Myelolipoma is a unique mesenchymal tumor that is composed of a mixture of adipose and hematopoietic cells. The first case of an adrenal lesion containing both fat and myeloid elements was described by Gierke<sup>[12]</sup> in 1905. The reported incidence of myelolipoma on autopsy ranges from 0.08% to 0.4%<sup>[13]</sup>. This type of tumor is most commonly localized to the adrenal gland; however, there are rare but well-documented cases of extra-adrenal involvement<sup>[1]</sup>. To our knowledge, less than 60 cases of extra-adrenal myelolipomas have been reported<sup>[2,8-10]</sup>, most of them involving the pre-sacral space. Tumors involving the mediastinum, lung, spleen, mandible, and nasal cavity have also been described<sup>[2-7]</sup>. Perirenal extra-adrenal myelolipomas are especially rare, with only 9 cases described so far<sup>[11]</sup>.

A review of the literature shows that extra-adrenal myelolipomas exhibit a slight female predominance and are typically discovered between the ages of 50 to 70 years old<sup>[4,7,11]</sup>. Most tumors are unilateral and have been found to range from 2 to 26 cm in size at the time of diagnosis<sup>[14,15]</sup>. The etiology of extra-adrenal myelolipomas is still to be established. Several theories exist regarding their embryologic origin and pathogenesis. Amin *et al*<sup>[16]</sup> suggest that there may be a relationship between the reactivation of primitive peritoneal foci of extramedullary hematopoiesis under pathological stresses (*i.e.*, severe anemia, sepsis, myeloproliferative disease) and the origin and progression of extra-adrenal myelolipomas. Another theory postulates that myelolipomas originate from metaplasia of previously uncommitted adrenal cortical mesenchymal cells or hematopoietic stem cells that normally migrate to the adrenal gland during intrauterine development<sup>[16]</sup>.

The widespread application of modern imaging techniques has led to a dramatic increase in the detection of extra-adrenal myelolipomas. The majority of patients are asymptomatic at the time of diagnosis, and lesions are discovered incidentally on imaging for alternative medical problems. Typically, physical examination and routine

blood tests fail to yield any conclusive diagnostic findings. Depending on the size and location of the lesion, some patients may present with vague flank or abdominal pain due to hemorrhage, mechanical compression, or tumor infarction<sup>[17]</sup>. CT and MRI have been used to diagnose extra-adrenal myelolipomas. When a myelolipoma is contained within the adrenal gland, the diagnosis is straightforward because it is the only known entity composed of adipose tissue occurring in this location<sup>[18]</sup>. A fatty mass within the retroperitoneal space represents a diagnostic challenge because the differential diagnosis includes an angiomyolipoma, a retroperitoneal teratoma, or a well-differentiated liposarcoma. A study that reviewed the MRI results of 126 consecutively imaged grossly fatty masses found that the sensitivity of MRI in diagnosing well-differentiated liposarcomas is 100%; however, its specificity is merely 83% due to the inability to differentiate between liposarcomas and other lipoma variants<sup>[19]</sup>.

Fine needle biopsy under ultrasound or CT guidance may be useful for the diagnosis of extra-adrenal myelolipoma. Well-differentiated liposarcoma differs from myelolipoma in that the former contains atypical stromal cells, variable-sized adipocytes, some of them with nuclear atypia, and lipoblast which, however, are not diagnostic, being absent in some cases. By contrast, extra-adrenal myelolipomas are composed of mature adipocytes with scattered hematopoietic cells, including megakaryocytes<sup>[20]</sup>. Although these histological differences between the two tumors, in many cases the final diagnosis is difficult, if not impossible, based on tissue biopsy<sup>[11]</sup>. Furthermore, the risks of hemorrhage, rupture, or infection that are associated with biopsy must factor into a clinician's decision to proceed with this invasive diagnostic procedure<sup>[11]</sup>. In our patient, tissue biopsy was deferred due to the patient's preference to forego the procedure.

There is currently no standard treatment for patients with this disease. Daneshmand *et al*<sup>[21]</sup> suggest that small asymptomatic tumors (< 4 cm) should be monitored with routine cross-sectional surveillance imaging, while large symptomatic tumors (> 7 cm) should be surgically removed. Extra-adrenal myelolipomas have been removed using a thoracoabdominal incision, but recently a laparoscopic approach has proven to be just as effective<sup>[10]</sup>.



**Table 1** Review of reported cases of perirenal extra-adrenal myelolipomas

Age at time of diagnosis (yr)	Sex	Presentation	Diagnostic imaging	Biopsy	Gross pathology	Treatment	Ref.
45	Male	Asymptomatic	CT (5 cm × 5 cm)	No	6.0 cm × 3.5 cm × 2.5 cm	Partial nephrectomy	Wagner <i>et al</i> <sup>[23]</sup> , 1997
45	Female	Flank pain Dysuria Frequency Urgency	CT (10 cm × 7 cm)	Yes	9.0 cm × 6.4 cm × 5.5 cm	Laparoscopic mass resection	Beiko <i>et al</i> <sup>[10]</sup> , 2010
60	Male	Abdominal pain	CT (4.2 cm × 3.7 cm)	No	Not reported	Radical nephrectomy	Pascual García <i>et al</i> <sup>[24]</sup> , 2007
63	Male	Asymptomatic	CT (6.5 cm × 5.5 cm)	No	Not reported	Open mass resection	Dan <i>et al</i> <sup>[15]</sup> , 2012
65	Male	Flank pain, Weight loss Hematuria	CT (5.5 cm × 4.5 cm)	No	7.0 cm × 5.0 cm × 1.5 cm	Radical nephrectomy	Talwalkar <i>et al</i> <sup>[9]</sup> , 2006
66	Female	Abdominal distention	CT (20 cm × 20 cm)	No	20 cm × 15 cm × 15 cm	Open mass resection	Brietta <i>et al</i> <sup>[25]</sup> , 1994
67	Male	Asymptomatic	CT (7 cm × 5 cm)	No	Not reported	Radical nephrectomy	Sneiders <i>et al</i> <sup>[26]</sup> , 1993
70	Male	Flank pain Fever	Ultrasound (12 cm × 8.5 cm)	No	17.0 cm × 10.0 cm × 5.0 cm	Open mass resection	Kilinc <sup>[27]</sup> , 2007
77	Male	Abdominal distension Hypertension	CT (Bilateral fat-containing masses)	Yes	Not reported	Follow-up CT 3 mo showed no change	Temizoz <i>et al</i> <sup>[20]</sup> , 2010

CT: Computed tomography.

Early detection and proper management of myelolipomas is important due to the potential for tumor growth and hemorrhage. A study of 86 myelolipomas found that hemorrhage is more common in larger lesions with a diameter measuring greater than 10 cm<sup>[22]</sup>.

A review of 9 reported cases perirenal extra-adrenal myelolipomas, shows that the average age at diagnosis is 62 years of age (Table 1). Perirenal lesions exhibited a male-to-female ratio of 7:2. At the time of diagnosis, patients were either asymptomatic or complained of various symptoms including flank pain, dysuria, frequency, urgency, weight loss, hematuria, or abdominal distention. CT and ultrasound were the imaging modalities used to characterize the masses. Biopsy was used in only 2 of the 9 cases prior to surgical intervention. The average size on imaging is 8.7 cm × 7.4 cm, while the size of the resected masses on gross pathological evaluation is 11.8 cm × 8.0 cm × 5.9 cm. Treatment included open and laparoscopic mass excision with or without nephrectomy or partial nephrectomy depending on concern for adequate surgical margins. Upon reviewing the literature, we felt it was reasonable to monitor the lesion with routine surveillance imaging until the tumor increased in size and to perform a mass resection with a nephrectomy to ensure adequate surgical margins.

Since an extra-adrenal myelolipoma is such a rare entity, a retroperitoneal mass that has imaging characteristics of a well-differentiated liposarcoma should ultimately end up being approached and treated as such. However, this report demonstrates that extra-adrenal myelolipoma should be considered as part of the list of differential diagnoses. In cases in which surgical extirpation of an extra-adrenal myelolipoma is performed, there are no clear

recommendations for post-operative surveillance. Our review did not reveal a case of local recurrence of a retroperitoneal myelolipoma, however, routine radiographic surveillance would certainly be helpful to detect potential locally recurrent disease.

In summary, perirenal extra-adrenal myelolipoma is extremely rare. This neoplasm is typically discovered incidentally on cross-sectional imaging and commonly thought to be a liposarcoma. It can be managed conservatively or surgically depending on the patient's symptoms or level of concern for a malignant lesion. Early detection and proper management of myelolipomas are important due to the potential for tumor growth and hemorrhage.

## COMMENTS

### Case characteristics

This case features a left-sided retroperitoneal mass that was incidentally discovered on a computed tomography (CT) scan for a suspected case of acute pancreatitis.

### Clinical diagnosis

Imaging revealed a non-enhancing heterogeneous mass measuring approximately 3.8 cm × 2.3 cm in longitudinal and anterior-posterior dimensions, just inferior to the left renal vein and medial to the left kidney, and histological evaluation revealed that the mass was composed of mostly mature adipocytes mixed with islands of hematopoietic cells.

### Differential diagnosis

Differential diagnosis was most concerning for liposarcoma, lipoma, malignant fibrous histiocytoma, or a fibrosarcoma.

### Laboratory diagnosis

Laboratory findings were non-contributory to arriving at the final diagnosis.

### Imaging diagnosis

CT and MRI were used to initially detect and follow the progression of the mass.

### Pathological diagnosis

Hematoxylin and eosin (H and E) stain at 10 x and 60 x magnification revealed



mature adipose cells with hematopoietic cells including erythroid precursors, granulocytic precursors, and megakaryocytes.

### Treatment

The mass was excised en bloc with the left kidney in an attempt to gain wide surgical margins.

### Related reports

The list of references to this article contains several related reports to aid readers to further understand this topic.

### Term explanation

*Myelolipoma* is a unique mesenchymal tumor that is composed of a mixture of adipose and hematopoietic cells.

### Experiences and lessons

Perirenal extra-adrenal myelolipomas are neoplasms that are typically discovered incidentally on cross-sectional imaging, they can be managed conservatively or surgically depending on the patient's symptoms or level of concern for a malignant lesion, and early detection and proper management of myelolipomas are critical due to the potential for tumor growth and hemorrhage.

### Peer review

This study describes a lesion which is not a unique phenomenon. Nevertheless, it is well written with a good review of the literature.

## REFERENCES

- 1 Meaglia JP, Schmidt JD. Natural history of an adrenal myelolipoma. *J Urol* 1992; **147**: 1089-1090 [PMID: 1552592]
- 2 Baker KS, Lee D, Huang M, Gould ES. Presacral myelolipoma: a case report and review of imaging findings. *J Radiol Case Rep* 2012; **6**: 1-9 [PMID: 23378876]
- 3 Geng C, Liu N, Yang G, Qi M, Chen W. Primary mediastinal myelolipoma: A case report and review of the literature. *Oncol Lett* 2013; **5**: 862-864 [PMID: 23426140]
- 4 Huang WT, Zhao SJ, Lin DM. Pulmonary-bronchus myelolipoma and review on extra-adrenal myelolipomas in Chinese literature. *Chin Med J (Engl)* 2012; **125**: 3188-3190 [PMID: 22932206]
- 5 Cina SJ, Gordon BM, Curry NS. Ectopic adrenal myelolipoma presenting as a splenic mass. *Arch Pathol Lab Med* 1995; **119**: 561-563 [PMID: 7605177]
- 6 Chiarini L, Bertoldi C, Criscuolo M, Ferronato G. [Myelolipomatosis. A report of a case located in the mandible]. *Minnerva Stomatol* 1992; **41**: 165-172 [PMID: 1461236]
- 7 George SA, Manipadam MT, Thomas R. Primary myelolipoma presenting as a nasal cavity polyp: a case report and review of the literature. *J Med Case Rep* 2012; **6**: 127 [PMID: 22584001 DOI: 10.1186/1752-1947-6-127]
- 8 Bandurski R, Zareba K, Kędra B. Rare case of multifocal (adrenal and extra - adrenal ) myelolipoma. *Pol Przegl Chir* 2013; **85**: 348-350 [PMID: 23828417]
- 9 Talwalkar SS, Shaheen SP. Extra-adrenal myelolipoma in the renal hilum: a case report and review of the literature. *Arch Pathol Lab Med* 2006; **130**: 1049-1052 [PMID: 16831034]
- 10 Beiko D, Roldan H, Sengupta SK, George RL. Laparoscopic excision of a large extra-adrenal perirenal myelolipoma. *Can Urol Assoc J* 2010; **4**: E39-E41 [PMID: 20368880]
- 11 Ghaouti M, Znati K, Jahid A, Zouaia F, Bernoussi Z, Mahassini N. Renal myelolipoma: a rare extra-adrenal tumor in a rare site: a case report and review of the literature. *J Med Case Rep* 2013; **7**: 92 [PMID: 23556993]
- 12 Gierke E. Unusual myeloid tissue in the adrenal gland. *Beitr Pathol Anat* 1905; **3**: 11-25
- 13 Doddi S, Singhal T, Leake T, Sinha P. Management of an incidentally found large adrenal myelolipoma: a case report. *Cases J* 2009; **2**: 8414 [PMID: 19918428 DOI: 10.4076/1757-1626-2-8414]
- 14 Kammen BF, Elder DE, Fraker DL, Siegelman ES. Extraadrenal myelolipoma: MR imaging findings. *AJR Am J Roentgenol* 1998; **171**: 721-723 [PMID: 9725304 DOI: 10.2214/ajr.171.3.9725304]
- 15 Dan D, Bahadursingh S, Hariharan S, Ramjit C, Naraynsingh V, Maharaj R. Extra-adrenal perirenal myelolipoma. A case report and review of literature. *G Chir* 2012; **33**: 62-65 [PMID: 22525547]
- 16 Amin MB, Tickoo SK, Schultz D. Myelolipoma of the renal sinus. An unusual site for a rare extra- adrenal lesion. *Arch Pathol Lab Med* 1999; **123**: 631-634 [PMID: 10388922]
- 17 Olobatuyi FA, MacLennan GT. Myelolipoma. *J Urol* 2006; **176**: 1188 [PMID: 16890722 DOI: 10.1016/j.juro.2006.06.095]
- 18 Butori N, Guy F, Collin F, Benet C, Causeret S, Isambert N. Retroperitoneal extra-adrenal myelolipoma: appearance in CT and MRI. *Diagn Interv Imaging* 2012; **93**: e204-e207 [PMID: 22421286]
- 19 Gaskin CM, Helms CA. Lipomas, lipoma variants, and well-differentiated liposarcomas (atypical lipomas): results of MRI evaluations of 126 consecutive fatty masses. *AJR Am J Roentgenol* 2004; **182**: 733-739 [PMID: 14975977 DOI: 10.2214/ajr.182.3.1820733]
- 20 Temizoz O, Gencellac H, Demir MK, Unlu E, Ozdemir H. Bilateral extra-adrenal perirenal myelolipomas: CT features. *Br J Radiol* 2010; **83**: e198-e199 [PMID: 20846975 DOI: 10.1259/bjr/28801968]
- 21 Daneshmand S, Quek ML. Adrenal myelolipoma: diagnosis and management. *Urol J* 2006; **3**: 71-74 [PMID: 17590837]
- 22 Kenney PJ, Wagner BJ, Rao P, Heffess CS. Myelolipoma: CT and pathologic features. *Radiology* 1998; **208**: 87-95 [PMID: 9646797]
- 23 Wagner JR, Kleiner DE, Walther MM, Linehan WM. Perirenal myelolipoma. *Urology* 1997; **49**: 128-130 [PMID: 9000202 DOI: 10.1016/S0090-4295(97)00368-3]
- 24 Pascual García X, Bujons Tur A, Rodríguez Faba O, Gómez Ruiz JJ, Palou Redorta J, Villavicencio Mavrich H. Extraadrenal perirenal myelolipoma: report of a case and review of the literature. *Actas Urol Esp* 2007; **31**: 932-934 [PMID: 18020221 DOI: 10.1016/S0210-4806(07)73751-8]
- 25 Brietta LK, Watkins D. Giant extra-adrenal myelolipoma. *Arch Pathol Lab Med* 1994; **118**: 188-190 [PMID: 8311663]
- 26 Sneiders A, Zhang G, Gordon BE. Extra-adrenal perirenal myelolipoma. *J Urol* 1993; **150**: 1496-1497 [PMID: 8411435]
- 27 Kilinc N. Extra-adrenal myelolipoma: A case report and review of the literature. *Pak J Med Sci* 2007; **23**: 779-781

P- Reviewers: Fernandez-Pello S, Magro G, Tsamis D  
S- Editor: Ma YJ L- Editor: A E- Editor: Wu HL



## Verrucous carcinoma of the esophagus: A case report and literature review

Chintan Ramani, Neil Shah, Ramasamy Swami Nathan

Chintan Ramani, Neil Shah, Ramasamy Swami Nathan, Department of internal medicine, Hackensack UMC mountainside, Montclair, NJ 07042, United States

Author contributions: Nathan RS performed the endoscopy and diagnosed the case; Ramani C with Shah N and Nathan RS did the literature search and Ramani C wrote a report.

Correspondence to: Ramasamy Swami Nathan, MD, Academic Chief Gastroenterology, Department of internal medicine, Hackensack UMC mountainside, 1 Bay Ave, Montclair, NJ 07042, United States. [chintan.ramani@mountainsidehosp.com](mailto:chintan.ramani@mountainsidehosp.com)

Telephone: +1-973-2398372 Fax: +1-973-2398403

Received: October 28, 2013 Revised: March 23, 2014

Accepted: May 19, 2014

Published online: July 16, 2014

### Abstract

Verrucous carcinoma of the esophagus is a variant of a squamous cell cancer. Our case is a 78-year-old male patient comes in with the dysphagia and weight loss, and on endoscopy (EGD) he is found to have an irregular intraluminal mass at the distal esophagus. With the deep EGD assisted biopsy, diagnosis of the verrucous carcinoma is made. Due to multiple co morbidities and possible infiltration to the pericardium, patient is taken for the esophageal stent placement and is being referred for the chemo-radiation treatment. The diagnosis can be very difficult to make with the superficial biopsies due to very non specific histological changes and requires very high clinical suspicion and deep mucosal biopsies are required for accurate diagnosis of the tumor. Chronic and local disease process is the main risk factor for the development of the verrucous carcinoma of the esophagus. Surgery is the treatment of the choice for the early stage tumor and advanced cases are treated with the palliation and possibly chemo-radiation. The prognosis is usually guarded and needs long term follow up.

**Key words:** Verrucous carcinoma; Hyperkeratosis; Esophageal stent placement; Esophageal carcinoma; Endoscopic Ultrasound

**Core tip:** A verrucous carcinoma is a slow growing, well differentiated, rare form of squamous carcinoma variant. It is associated with chronic, local disease process and it invades locally. On endoscopy (EGD), it appears as an exophytic irregular warty projecting mass, and it is very difficult to diagnose by superficial biopsy due to non specific superficial histological findings. So it requires high index of suspicion and deep biopsy with EGD or endoscopic ultrasonography (EUS). It projects as hypoechoic mucosal thickening on EUS. Early stages of cancers are treated surgically. Advanced cases can be referred for esophageal stent placement for palliation and chemo radiation. It has high morbidity and mortality and requires long term follow up for accurate numbers regarding to the treatment and the follow up.

Ramani C, Shah N, Nathan RS. Verrucous carcinoma of the esophagus: A case report and literature review. *World J Clin Cases* 2014; 2(7): 284-288 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/284.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.284>

### INTRODUCTION

Verrucous carcinoma of the esophagus is a rare form of carcinoma of squamous cell origin<sup>[1-9]</sup>. Majority of the cases are associated with the smoking, reflux esophagitis, alcohol use, human papilloma virus (HPV), achalasia and few other chronic inflammatory conditions<sup>[1-9]</sup>. An incidence rate of such cancer has been shown to be higher in males than females with a ratio of 2:1 and seen in the group from 35 to 80 years<sup>[1-3,6]</sup>. Superficial biopsies of the lesion often show merely chronic inflammation with no

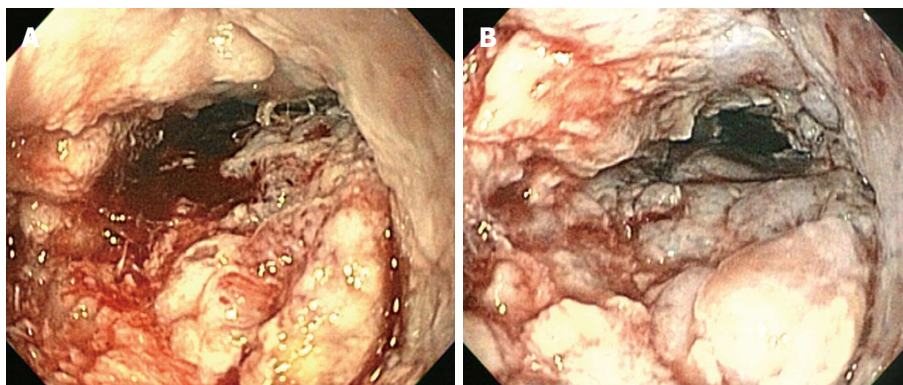


Figure 1 Upper endoscopy showing an irregular velvety appearing intraluminal mass in the esophagus (A and B).

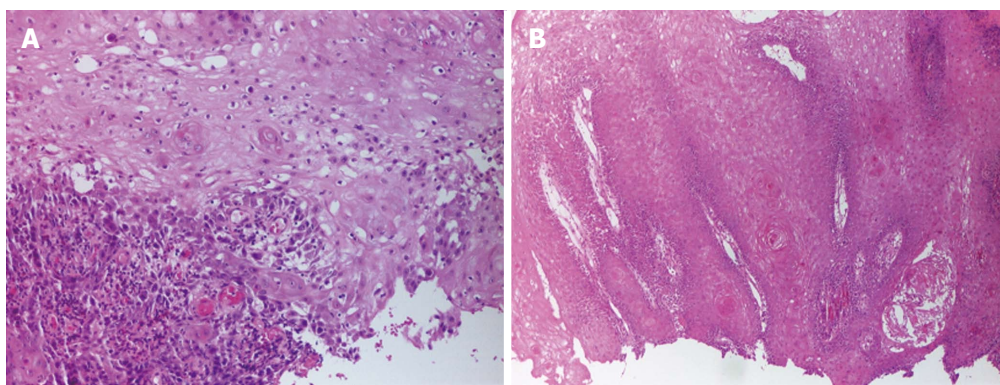


Figure 2 Superficial biopsy of the lesion showing Mild cytological atypia and chronic inflammation and reactive appearing basal layer of sq cell epithelium (A); deep biopsy of the lesion with a jumbo forceps showing Florid proliferation of sq epithelium with verrucous pattern (B).

high grade dysplasia, which makes it difficult to diagnose.

So far, less than 30 cases of such carcinomas are reported in the English literature. We are presenting a case of the verrucous carcinoma of the esophagus with no documented risk factors associated with the carcinoma and a diagnosis is made by deep biopsies of the lesions.

## CASE REPORT

We have recently seen a 78-year-old African American man, presenting with dysphagia, weight loss of 15 Lbs over 4-5 wk and right lower quadrant dull, and non radiating abdominal pain. His past medical history included coronary artery disease status post angioplasty with stent placement, hypertension, dyslipidemia, and diabetes mellitus with complications including diabetic retinopathy and nephropathy. He was non-smoker and non-drinker and denied any history of GERD. And symptoms were gradual in onset over last couple of months. His physical exam showed no abnormality. On lab evaluation, His BUN and serum creatinine were 42 and 2.4 respectively, normal liver function tests; Hemoglobin was 12.4 g/dL with MCV 84.4 fl. All other labs were within normal limits. ultrasonography of the abdomen and computed tomography (CT) scan of the abdomen and pelvis were unremarkable for any hepatobiliary, pancreatic or intestinal pathology. He underwent barium esophagogram, and was

found to have a long, irregular stricture involving the mid and distal esophagus with an apparent intraluminal mass at its proximal end. The endoscopic examination showed a luminal warty appearing mass occupying distal 8 cm of the esophagus (Figure 1). Endoscopic ultrasonography (EUS) examination was performed which showed solid tumor measuring approx 5 cm in greatest dimension, possibly infiltrating into the pericardium (Figure 2). It also showed hypoechoic concentric wall thickening that appeared to be either an inflammatory process or lymphoma.

Initial biopsies were remarkable for squamous epithelial cells with parakeratosis and marked acute and chronic inflammation, ulceration and focal squamous cell with atypia. The repeat endoscopic examination and extensive biopsies with jumbo forceps revealed the diagnosis of verrucous carcinoma of the esophagus. In situ hybridization for both high and low risk HPV was negative.

A CT scan of the chest showed some mediastinal lymph nodes. Bone scan showed no any bone metastasis. In view of the dysphagia, patient underwent endoscopy (EGD) and placement of an esophageal Wallflex stent (Boston scientific partially covered 23 mm wide and 125 mm long).

Due to extensive medical history and fairly advanced stage, surgery was contraindicated and the patient was referred for chemotherapy and radiation.



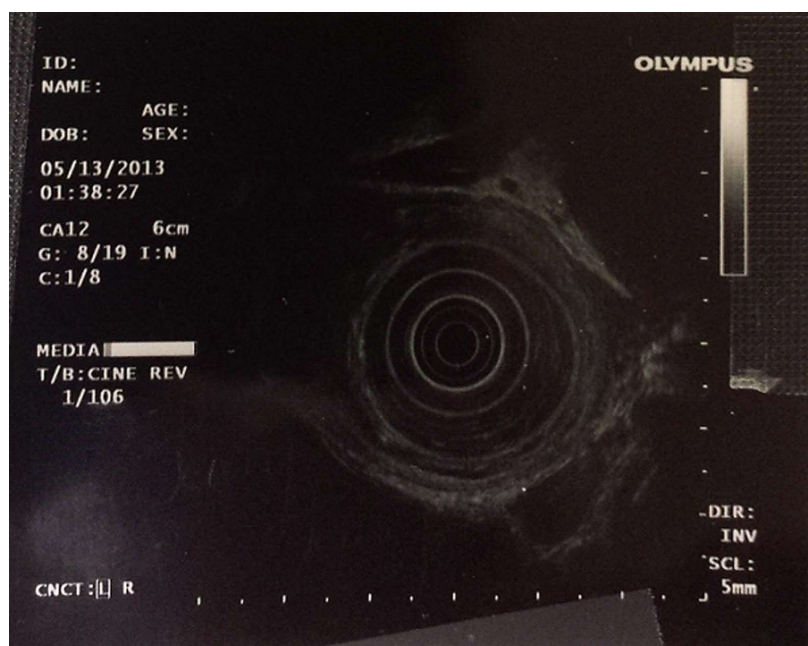


Figure 3 Tumor infiltrating pericardium.

## DISCUSSION

Verrucous carcinomas are a variant of squamous carcinoma. They are slow growing and seen in the oropharynx, larynx, glans penis, scrotum, vulva, vagina, cervix, endometrium, urinary bladder, anorectal region and the sole of the feet<sup>[2,4]</sup>. The Verrucous carcinomas are believed to be associated with the chronic mucosal irritation or inflammation or a long-term disease process<sup>[2,4]</sup>.

Amongst all different sites for verrucous carcinoma, it is rarely seen in the esophagus<sup>[1-9]</sup>. First ever case of such cancer in esophagus was reported by Minelly in 1967<sup>[2,3,4,7]</sup> and thereafter so far less than 30 cases are reported in the English literature<sup>[8]</sup>.

The etiological factors are not clearly delineated in the literature but it seems to be associated with the chronic inflammatory conditions or long term local disease process<sup>[1-9]</sup>. Risk factors may include smoking; alcohol abuse; hiatal hernia; achalasia; esophagitis; caustic injury from lye, battery or kerosene ingestion; esophageal diverticulum or nutcracker esophagus. In the recent years, few reported cases have shown the association with the HPV virus, although the clear association is very unclear<sup>[5,6,8,9]</sup>. Our case was not clearly associated with the any of these documented risk factors. Devlin *et al*<sup>[3]</sup> mentions that the acid inhibition decreases the tumor length and also changes the appearance from polypoid to sessile and warty. This hypothesizes that the long term acid damage could be a contributory for the development of the verrucous carcinoma.

The incidence rate is higher in male as compared to female with a ratio of approx 2:1<sup>[1-3]</sup>. An age distribution of this type of carcinoma ranges from 36 to 79 with a mean age of 61<sup>[1-3]</sup>. Most common presenting

sign and symptoms in the verrucous carcinoma of the esophagus are dysphagia and weight loss, seen in our case as well<sup>[1-3,5,6]</sup>. Other symptoms would be hematemesis, coughing and odynophagia<sup>[2,6]</sup>. Endoscopic appearance of such lesions includes, shaggy, white, exophytic, wart-like, velvety, papillary, spiked, cauliflower like mass<sup>[1-3,7-9]</sup>. The tumor is located mostly in the lower esophagus (70%) with no clear reason but it can involve the upper (23%) or the mid part of the esophagus (7%) as well<sup>[2-9]</sup>.

A diagnosis is usually made by either endoscopy guided deep mucosal biopsy or EUS guided tunnel biopsy or post surgery specimen evaluation. Verrucous carcinoma of the esophagus usually evolves in the sequential fashion from acanthosis, hyperkeratosis, parakeratosis, leukoplakia, verrucous lesions, and papillary hyperplasia to verrucous carcinoma<sup>[2]</sup>. Superficial biopsies of such lesions show only non specific acanthosis, parakeratosis, or hyperkeratosis, with associated acute or chronic inflammation<sup>[2,3,6,8]</sup>, which makes these types of carcinomas are difficult to diagnose and requires high index of suspicion and repeat endoscopic deep biopsies as in our case (Figure 3). Endoscopic biopsies have revealed 46% cases of the verrucous carcinoma, remaining of them have been diagnosed either after surgery or with the use of EUS<sup>[2-9]</sup>. EUS is highly accurate imaging modalities for the diagnosis and staging as well as follow up of esophageal tumors. It also estimates the depth of invasion as well as any lymphadenopathy which is helpful in staging. EUS guided tunnel biopsy of such lesions can be useful as seen in few case reports<sup>[2-4,8]</sup>. A Verrucous carcinoma of the esophagus commonly projects as a diffuse hypoechoic mucosal thickening with varying degree of depth with varying degree of lymphadenopathy in EUS exam<sup>[2-4,7,8]</sup>. It invades as a column of neoplastic



cells in a pushing manner instead of invasion in discrete cells<sup>[3]</sup>, with the 80% invades through and beyond superficial epithelium, 8% limited to the superficial epithelium only and 12% unclear<sup>[2-9]</sup>. More than 50% cases are found have inflammatory infiltrates surrounding the tumor<sup>[3,6]</sup>, and the lymphnode biopsy mostly shows hyperplastic nodes secondary to local inflammation which can prove that the chronic inflammation predisposes to the verrucous carcinoma of the esophagus<sup>[2,7]</sup>. Overall, histologically, it is similar to benign squamous papilloma and the tumor infiltration beyond the superficial mucosa fairly differentiates it from the benign squamous cell papilloma<sup>[2]</sup>.

Despite of its slow growth and high degree of differentiation, it has very poor prognosis. As per literature, there is a delay between the onset of the symptoms and the diagnosis. And at the time of the diagnosis, majority of the cases are locally advanced<sup>[1-9]</sup>. Morbidity and mortality associated with such tumors are mainly due to local invasion or due to surgical complications. There is no any reported case of distant metastasis in the literature. It can spread locally to the lungs, bronchi, pleura and can form fistulas<sup>[1,2,8]</sup>. Our case is the first reported instance of pericardial invasion confirmed with EUS study. With regards to therapy, early stages of the cancer can be treated surgically with esophageal resection or polypectomy/mucosal resection<sup>[1-9]</sup>. More advanced cases, or non surgical candidates can be treated with esophageal stent placement, as done in our case. Due to rarity of such tumors, no clear data are available for any effective chemo- radiation therapy. However, most recent post operative follow up case series have shown better prognosis with cancer free survival ranges from 9 mo to 3 years<sup>[2,3]</sup>. We need a long term follow up with such patients and that can improve the outcome.

A verrucous carcinoma is a slow growing, well differentiated, rare form of squamous carcinoma variant. It is associated with chronic, local disease process and it invades locally. On EGD, it appears as an exophytic irregular warty projecting mass, and it is very difficult to diagnose by superficial biopsy due to non specific superficial histological findings. So it requires high index of suspicion and deep biopsy with EGD or EUS. It projects as hypoechoic mucosal thickening on EUS. Early stages of cancers are treated surgically. Advanced cases can be referred for esophageal stent placement for palliation and chemo radiation. It has high morbidity and mortality and requires long term follow up for accurate numbers regarding to the treatment and the follow up.

## COMMENTS

### Case characteristics

A 78-year-old African American man presented with dysphagia, weight loss of 15 Lbs over 4-5 wk and right lower quadrant dull, and non radiating abdominal pain.

### Clinical Diagnosis

Physical examination was unremarkable.

### Differential diagnosis

Benign squamous papilloma, adenocarcinoma of the esophagus

### Laboratory diagnosis

BUN and serum creatinine were 42 and 2.4 respectively, normal liver function tests; Hemoglobin was 12.4 with MCV 84.4.

### Imaging diagnosis

A barium esophagogram showed a long, irregular stricture involving the mid and distal esophagus with an apparent intraluminal mass at its proximal end. A computed tomography scan of the chest showed some mediastinal lymph nodes. Bone scan showed no any bone metastasis.

### Pathological diagnosis

The endoscopic examination and extensive biopsies with jumbo forceps revealed the diagnosis of verrucous carcinoma of the esophagus. In situ hybridization for both high and low risk HPV was negative.

### Treatment

Due to extensive medical history and fairly advanced stage, surgery was contraindicated and the patient Patient underwent EGD and placement of an esophageal Wallflex stent and was referred to chemotherapy and radiation.

### Related reports

The etiological factors are not clearly delineated in the literature. It has high morbidity and mortality and requires long term follow up for accurate numbers regarding to the treatment and the follow up.

### Experiences and lessons

This report presents a case of rare variant of squamous cell cancer of esophagus, and difficulties associated with the diagnosis and treatment. It is very beneficial for the patients, if it is diagnosed in the early stages. But nonspecific superficial biopsies make it difficult to diagnose and it requires high index of suspicion even without any associated risk factors.

### Peer review

It is indeed a rare form of squamous cell carcinoma. Due to the same reasons, there are not enough studies available in terms of management that can be applied to general population.

## REFERENCES

- 1 **Malik AB**, Bidani JA, Rich HG, McCully KS. Long-term survival in a patient with verrucous carcinoma of the esophagus. *Am J Gastroenterol* 1996; **91**: 1031-1033 [PMID: 8633546]
- 2 **Osborn NK**, Keate RF, Trastek VF, Nguyen CC. Verrucous carcinoma of the esophagus: clinicopathophysiologic features and treatment of a rare entity. *Dig Dis Sci* 2003; **48**: 465-474 [PMID: 12757157]
- 3 **Devlin S**, Falck V, Urbanski SJ, Mitchell P, Romagnuolo J. Verrucous carcinoma of the esophagus eluding multiple sets of endoscopic biopsies and endoscopic ultrasound: a case report and review of the literature. *Can J Gastroenterol* 2004; **18**: 459-462 [PMID: 15229749]
- 4 **Na S**, Choi KD, Yoo C, Chang Y, Song HJ, Lee GH, Jung HY, Cho KJ, Kim JH. Verrucous carcinoma of the esophagus. *Gastrointest Endosc* 2009; **70**: 803-806 [PMID: 19555942 DOI: 10.1016/j.gie.2009.01.048]
- 5 **Tonna J**, Palefsky JM, Rabban J, Campos GM, Theodore P, Ladabaum U. Esophageal verrucous carcinoma arising from hyperkeratotic plaques associated with human papilloma virus type 51. *Dis Esophagus* 2010; **23**: E17-E20 [PMID: 20626449]
- 6 **Macias-Garcia F**, Martinez-Lesquereux L, Fernandez B, Parada P, Larino-Noia J, Sobrino-Faya M, Iglesias-Canle J, Iglesias-Garcia J, Forteza J, Dominguez-Munoz JE. Verrucous carcinoma of the esophagus: a complex diagnosis. *Endoscopy* 2010; **42** Suppl 2: E137-E138 [PMID: 20405383 DOI: 10.1055/s-0029-1244051]
- 7 **Chu Q**, Jaganmohan S, Kelly B, Holey J. Verrucous carcinoma of the esophagus: a rare variant of squamous cell carcinoma for which a preoperative diagnosis can be a difficult one to make. *J La State Med Soc* 2011; **163**: 251-253 [PMID: 22272545]
- 8 **Feinstein AR**. The unit fragility index: an additional ap-

praisal of “statistical significance” for a contrast of two proportions. *J Clin Epidemiol* 1990; **43**: 201-209 [PMID: 2303850 DOI: 10.4321/S1130-01082012000800013]

- 9 **Liberale G**, De Simone P, Snoeck R, Féron P, Gelin M, El Nakadi I. Verrucous carcinoma of the esophagus. A case report. *Minerva Chir* 2005; **60**: 61-65 [PMID: 15902055]

**P- Reviewers:** Eslick GD, Gimeno-Garcia AZ, Natsugoe S  
**S- Editor:** Qi Y **L- Editor:** A **E- Editor:** Wu HL



## 360° fusion for realignment of high grade cervical kyphosis by one step surgery: Case report

Alessandro Landi, Nicola Marotta, Cristina Mancarella, Demo Eugenio Dugoni, Roberto Tarantino, Roberto Delfini

Alessandro Landi, Nicola Marotta, Cristina Mancarella, Demo Eugenio Dugoni, Roberto Tarantino, Roberto Delfini, Department of Neurology and Psychiatry, Division of Neurosurgery, University of Rome, Sapienza, 00100 Rome, Italy  
Author contributions: All authors contributed to this work.

Correspondence to: Alessandro Landi, MD, PhD, Department of Neurology and Psychiatry, Division of Neurosurgery, University of Rome, Sapienza, Viale del Policlinico 155, 00100 Rome, Italy. [dott.alessandro.landi@gmail.com](mailto:dott.alessandro.landi@gmail.com)

Telephone: +39-06-49979105 Fax: +39-06-49979113

Received: November 26, 2013 Revised: April 17, 2014

Accepted: May 16, 2014

Published online: July 16, 2014

Delfini R. 360° fusion for realignment of high grade cervical kyphosis by one step surgery: Case report. *World J Clin Cases* 2014; 2(7): 289-292 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/289.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.289>

### INTRODUCTION

The treatment option for correcting a cervical kyphotic deformity is currently controversial. Lots of studies examined the one-stage combined anterior-posterior treatment, although the rate of fusion and the long term follow up controls are rarely mentioned in the literature<sup>[1-7]</sup>. We present the case of a 67-year-old woman affected by a severe cervical kyphosis. We performed a one-step combined anterior/posterior approach to correct the deformity, getting a good reduction of kyphosis and good stability in a long term follow up. We enclosed that the evidence of motility by dynamic X-rays permits a good anterior decompression and reduction only by discectomy, fusion and plating, without need of multiple corpectomy. Treatment must be completed with posterior fixation and fusion. These strategies could be performed in one step, and shows a good reduction and optimal stability in a long term follow-up. Immobilizing with hard collar and neurophysiological monitoring remains fundamental for the safe and efficacy of this treatment.

### CASE REPORT

A 67-year-old woman affected by a severe cervical kyphotic deformity, came to our attention complaining 4 months of bilateral cervicobrachialgia. She didn't have any significant medical diseases; she denied to have ever suffered of ankylosing spondylitis, osteogenesis imperfecta, rheumatoid arthritis or Larsen syndrome.

Neurological examination showed moderate upper limbs weakness, confirmed by signs of radicular suffering

### Abstract

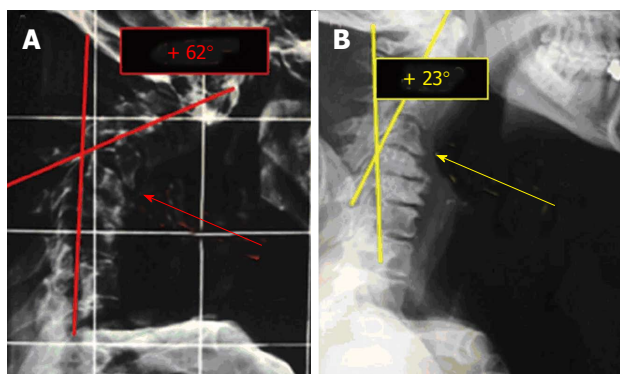
Surgical treatment for cervical kyphotic deformity is still controversial. Circumferential approach has been well described in the literature but long terms outcomes are not well reported. Important to decide the correct treatment option is the preoperative radiological exams to value the type of deformity (flexible or fixed). We report the case of a 67-year-old woman affected by a severe cervical kyphotic deformity who underwent combined anterior/posterior surgical approach, getting a good reduction of the deformity and an optimal stability in a long term follow up.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Cervical deformity; High grade kyphosis; Circumferential fusion; Surgical technique; Degenerative cervical spine

**Core tip:** The choice of the treatment for cervical kyphotic deformity takes into account preoperative radiological exams which allow the classification of the deformity in flexible or fixed.

Landi A, Marotta N, Mancarella C, Dugoni DE, Tarantino R,



**Figure 1 X-ray pictures.** A: Standard X-ray demonstrating a severe cervical kyphosis (preop. Angle of Jackson + 62°); B: A cervical spine X-ray on the bed with a pillow under the shoulders showing a good reduction of kyphosis (+ 23° according to Jackson) due to the motor unit C4-C5 mobility.



**Figure 2 The preoperative computed tomography.** A: Scan control (preop. Angle of Jackson + 62°); B: Showing a good reduction of kyphosis (postop angle of Jackson + 19°).

on the ElectroMioGraphy (EMG). The patient performed standard and flexion/extension cervical spine X-ray demonstrating a severe cervical kyphosis [preop. Ishihara index 64.18% and (8) preop. Angle of Jackson +62°] apparently fixed on the dynamic X-ray. Performed, then, cervical spine computed tomography (CT) and Magnetic Resonance Imaging showing important myeloradicular compression at C5-C6 and C4-C5.

The immediate preoperative exam that the patient had to perform was a cervical spine X-ray on the bed with a pillow under the shoulders in prevision to apply a traction system. This exam showed a good reduction of kyphosis (+23° according to Jackson) due to the motor unit C4-C5 mobility (Figure 1). It was therefore decided not to apply traction and to proceed with combined anterior/posterior surgical approach using neurophysiological monitoring SomatoSensory evoked potentials (SSEP), EMG and motor evoked potentials (MEP).

The first surgical step was the anterior approach, with hyperextension of the neck of the patient. The reduction status of the kyphosis was assessed under fluoroscopic visualization; patient underwent left anterior presternocleidomastoid-precavotid approach, anterior



**Figure 3 A postoperative X-ray control after 6 mo showing a good anterior and posterolateral arthrodesis.**

decompression through microdiscectomy C3-C4 and C4-C5, followed by interbody fusion using a carbon fiber cage in lordosis and anterior plate fixed on C3-C4-C5-C6. The second step was represented by the posterior approach, so the patient was placed in prone position. A C3/C6 posterior stabilization according to magerl was performed followed by posterolateral fusion at all levels. At the end of the procedure a Philadelphia brace was applied. The postoperative CT and X-ray control (Figure 2) and after 3 and 6 mo showed a good reduction of kyphosis (postop. Ishihara index 32.38 % and postop angle of Jackson + 19° with reduction of kyphosis of 31.8% according to Ishihara and 43° according to Jackson), and a good anterior and posterolateral arthrodesis (Figure 3). The patient presented a complete regression of the upper limbs deficit and of the cervicobrachialgia. Six months later the patient is symptoms free.

## DISCUSSION

Cervical kyphosis can be classified into two different groups: type 1 (flexible cervical kyphosis) and type 2 (fixed cervical kyphosis). The treatment for flexible cervical kyphosis (type 1) posturally reducible is usually a posterior stabilization with fusion to guarantee the stability of the cervical spine<sup>[6,7]</sup>. Alternatively, some authors have reported the use of anterior only surgery for flexible cervical kyphosis as discectomy and corpectomy. This approach is useful for anterior column load sharing however it is not required for deformity correction. Fixed cervical kyphosis characterized by postural rigidity needs circumferential approach<sup>[8-10]</sup>. The circumferential approach for the correction of cervical kyphotic deformity is well described in the literature although the long term controls are not always diriment on the real fusion of the correction<sup>[11,12]</sup>. The debate is not whether or not to perform circumferential correction, but if it is more useful to perform a multiple anterior discectomy or multiple corpectomy. In the literature it is described as the execution of multiple discectomies has a greater potential for correction of kyphosis in relation to: (1) a greater kyphosis correction due to the possibility of including more lordotic cages at multiple levels, so to restore a greater



degree of lordosis; and (2) a greater possibility of fusion because of a larger cage-bone interface compared with the use of a Harms mesh or an expansion cage.

In cases of multilevel cervical stenosis, the choice of surgical technique (discectomy *vs* corpectomy) mainly depends on the location of the stenosis. In the case of a kyphotic deformity however, the choice depends on the mobility or less of the bodies involved in the deformity<sup>[10-16]</sup>. It is also important to note that such deformities occur slowly over the time and are frequently the product of a degenerative process that affects the patient for many years: this include a wrong postural attitude that causes a compensatory hypertrophy of the supporting muscles of the neck, which may hide a metameric mobility of kyphosis. In our case, in fact, the dynamic exam in the upright position showed that the kyphosis appeared fixed and cannot be reduced<sup>[10]</sup>.

The X-ray performed in the bed with a pillow under the shoulder, allowed us to appreciate how such kyphosis was actually not fixed on two vertebrae. This made us choose a multiple discectomy and not corpectomy with consequent greater angular correction of kyphosis. Another important aspect in the evaluation of the motor unit motility is the reactive ankylosis of the articular processes. In the severe kyphosis the fusion occurs both at the level of the disc and of the articular masses, thus preventing a good correction of kyphosis after performing the anterior approach. When the articular masses are ankylosed it is necessary a 3-step surgery. The first step is represented by a posterior approach to release the ankylosed articular masses in order to allow the reduction of kyphosis. The second step is the anterior approach with discectomy, and the third step is represented by the posterior approach again to fix and to make arthrodesis<sup>[17-20]</sup>.

To this end, it is crucial to recognize accurately the real motility of the vertebral body, and to do that it is important to perform a dynamic exam without load, to eliminate the reactive contracture of the muscles supporting the neck. Useful for this purpose is to perform radiographic examinations in supine position with supports placed at the base of the neck which put the cervical spine in hyperextension eliminating the analgesic muscle contracture. Another aspect to highlight is the use of intraoperative neurophysiological monitoring, in particular SEPP and MEP; these allow a direct observation of the function of the spinal cord during the entire procedure. The neurophysiological monitoring is important especially during the correction of the spinal deformities because, as these constituted and organized by time, may have led to spinal cord adaptations that may break with the correction maneuvers, resulting in severe neurological deficits. Their use avoid for such eventuality.

In a conclusion, we enclosed that the evidence of motility by dynamic X-rays permits a good anterior decompression and reduction only by discectomy, fusion and plating, without need of multiple corpectomy. Treatment have to be completed with posterior fixation and fusion. These strategies could be performed in one step,

and shows a good reduction and optimal stability in a long term follow-up. Immobilizing with hard collar and neurophysiological monitoring remains fundamental for the safe and efficacy of these treatment.

## COMMENTS

### Case characteristics

The patient complained a 4-mo history of bilateral cervicobrachialgia.

### Clinical diagnosis

At the neurological examination the patient presented moderate upper limb weakness and severe cervical kyphotic deformity.

### Differential diagnosis

Through radiological exams we put cervical kyphotic deformity due to degenerative process in differential diagnosis with neoplastic and infective pathologies.

### Imaging diagnosis

The patient underwent computed tomography scan, magnetic resonance imaging and dynamic X-ray. The most important preoperative exam was X-ray performed in supine position with a pillow under the shoulder.

### Pathological diagnosis

The patient suffered severe cervical kyphotic deformity.

### Treatment

The authors performed combined anterior/posterior surgical approach using neurophysiological monitoring SomatoSensory Evoked Potentials, ElectroMioGrapy and Motor Evoked Potentials.

### Related reports

The choice of surgical treatment depends on the mobility or less of the bodies involved in the deformity.

### Term explanation

Cervical kyphosis is a progressive deformity; circumferential approach means one step combined anterior/posterior approach.

### Experiences and lessons

It is important to recognize the real motility of the vertebral body, and to do that it is necessary to perform a dynamic exam without load, to eliminate the reactive contracture of the muscles supporting the neck.

### Peer review

The author introduces an efficient surgical treatment for severe cervical spine deformity, and to improve the quality of life.

## REFERENCES

- 1 **Abumi K**, Shono Y, Taneichi H, Ito M, Kaneda K. Correction of cervical kyphosis using pedicle screw fixation systems. *Spine* (Phila Pa 1976) 1999; **24**: 2389-2396 [PMID: 10586466]
- 2 **Kanter AS**, Wang MY, Mummaneni PV. A treatment algorithm for the management of cervical spine fractures and deformity in patients with ankylosing spondylitis. *Neurosurg Focus* 2008; **24**: E11 [PMID: 18290737]
- 3 **Ferch RD**, Shad A, Cadoux-Hudson TA, Teddy PJ. Anterior correction of cervical kyphotic deformity: effects on myelopathy, neck pain, and sagittal alignment. *J Neurosurg* 2004; **100**: 13-19 [PMID: 14748568]
- 4 **Zdeblick TA**, Bohlman HH. Cervical kyphosis and myelopathy. Treatment by anterior corpectomy and strut-grafting. *J Bone Joint Surg Am* 1989; **71**: 170-182 [PMID: 2645290]
- 5 **Lin D**, Zhai W, Lian K, Kang L, Ding Z. Anterior versus posterior approach for four-level cervical spondylotic myelopathy. *Orthopedics* 2013; **36**: e1431-e1436 [PMID: 24200449]
- 6 **Spivak J**, Giordano CP. Cervical kyphosis. In: Bridwell KH, DeWald RL, eds. *The Textbook of Spinal Surgery*. 2nd ed. Philadelphia: Lippincott-Raven, 1997: 1027-1038
- 7 **Ganju A**, Ondra SL, Shaffrey CI. Cervical kyphosis. *Tech Orthop* 2003; **17**: 345-354 [DOI: 10.1097/00013611-200209000-00010]
- 8 **Herman JM**, Sonntag VK. Cervical corpectomy and plate fixation for postlaminectomy kyphosis. *J Neurosurg* 1994; **80**: 963-970 [PMID: 8189276]

- 9 Steinmetz MP, Kager CD, Benzel EC. Ventral correction of postsurgical cervical kyphosis. *J Neurosurg (Spine 2)* 2002; **97**: 1-7
- 10 Batzdorf U, Batzdorff A. Analysis of cervical spine curvature in patients with cervical spondylosis. *Neurosurgery* 1988; **22**: 827-836 [PMID: 3380271]
- 11 McAfee PC, Bohlman HH, Ducker TB, Zeidman SM, Goldstein JA. One-stage anterior cervical decompression and posterior stabilization. A study of one hundred patients with a minimum of two years of follow-up. *J Bone Joint Surg Am* 1995; **77**: 1791-1800 [PMID: 8550645]
- 12 Mummaneni PV, Dhall SS, Rodts GE, Haid RW. Circumferential fusion for cervical kyphotic deformity. *J Neurosurg Spine* 2008; **9**: 515-521 [PMID: 19035741]
- 13 Takeshita K, Murakami M, Kobayashi A, Nakamura C. Relationship between cervical curvature index (Ishihara) and cervical spine angle (C2--7). *J Orthop Sci* 2001; **6**: 223-226 [PMID: 11484114]
- 14 Chang SW, Kakarla UK, Maughan PH, DeSanto J, Fox D, Theodore N, Dickman CA, Papadopoulos S, Sonntag VK. Four-level anterior cervical discectomy and fusion with plate fixation: radiographic and clinical results. *Neurosurgery* 2010; **66**: 639-646; discussion 646-647 [PMID: 20305488]
- 15 Chibbaro S, Benvenuti L, Carnesecchi S, Marsella M, Pulerà F, Serino D, Gagliardi R. Anterior cervical corpectomy for cervical spondylotic myelopathy: experience and surgical results in a series of 70 consecutive patients. *J Clin Neurosci* 2006; **13**: 233-238 [PMID: 16503487]
- 16 Hussain M, Nassr A, Natarajan RN, An HS, Andersson GB. Corpectomy versus discectomy for the treatment of multilevel cervical spine pathology: a finite element model analysis. *Spine J* 2012; **12**: 401-408 [PMID: 22572585]
- 17 Kawakami M, Tamaki T, Iwasaki H, Yoshida M, Ando M, Yamada H. A comparative study of surgical approaches for cervical compressive myelopathy. *Clin Orthop Relat Res* 2000; **(381)**: 129-136 [PMID: 11127649]
- 18 Konya D, Ozgen S, Gercek A, Pamir MN. Outcomes for combined anterior and posterior surgical approaches for patients with multilevel cervical spondylotic myelopathy. *J Clin Neurosci* 2009; **16**: 404-409 [PMID: 19153044]
- 19 Lin Q, Zhou X, Wang X, Cao P, Tsai N, Yuan W. A comparison of anterior cervical discectomy and corpectomy in patients with multilevel cervical spondylotic myelopathy. *Eur Spine J* 2012; **21**: 474-481 [PMID: 21826497]
- 20 Jiang SD, Jiang LS, Dai LY. Anterior cervical discectomy and fusion versus anterior cervical corpectomy and fusion for multilevel cervical spondylosis: a systematic review. *Arch Orthop Trauma Surg* 2012; **132**: 155-161 [PMID: 21968573]

P- Reviewers: Tong C, Zhan RY S- Editor: Wen LL  
L- Editor: A E- Editor: Wu HL



## Focal epithelial hyperplasia in a human immuno-deficiency virus patient treated with laser surgery

Alexandros Galanakis, Gaspare Palaia, Gianluca Tenore, Alessandro Del Vecchio, Umberto Romeo

Alexandros Galanakis, Gaspare Palaia, Gianluca Tenore, Alessandro Del Vecchio, Umberto Romeo, Department of Oral and Maxillofacial Science, Sapienza University of Rome, 00161 Rome, Italy

Author contributions: Galanakis A, Tenore G, Del Vecchio A and Romeo U were the attending doctors for the patients; Palaia G and Romeo U designed the report; Galanakis A and Romeo U performed the surgeries; Palaia G organized the report; and Galanakis A wrote the paper.

Correspondence to: Alexandros Galanakis, DDS, PhD, Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, Via Caserta 6, 00161 Rome, Italy. [agalanakis@hotmail.it](mailto:agalanakis@hotmail.it)

Telephone: +39-320-0713294 Fax: +39-064-9976630

Received: December 24, 2013 Revised: April 21, 2014

Accepted: May 15, 2014

Published online: July 16, 2014

virus positive and was a smoker with numerous, asymptomatic oral papules clinically and histologically corresponding to FEH. The labial and buccal mucosa were especially affected by lesions. Surgical treatment was performed using a 532-nm potassium titanyl phosphate laser (SmartLite, Deka, Florence, Italy) in continuous mode with a 300  $\mu$ m fiber and power of 1.4 W (power density 1980.22 W/cm<sup>2</sup>). After anesthesia without vasoconstrictors, the lesions were tractioned with sutures or an Allis clamp and then completely excised. The lesions were preserved in 10% formalin for histological examination, which confirmed the clinical diagnosis of FEH. In this case, the laser allowed excellent control of bleeding, without postoperative sutures, and optimal wound healing.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Lasers; Focal epithelial hyperplasia; Mouth; Human immunodeficiency virus; Oral pathology

### Abstract

Focal epithelial hyperplasia (FEH), or Heck's disease, is a rare disease of the oral mucosa; it is mostly found in children or young adults who are immunosuppressed and who live in regions with low socioeconomic status. It is characterized by asymptomatic papules on the oral mucosa, gingiva, tongue, and lips. Healing can be spontaneous, and treatment is indicated if there are aesthetic or functional complications. Human papillomavirus, especially genotypes 13 and 32, has been associated with FEH and is detected in the majority of lesions. Histopathologically, FEH is characterized by parakeratosis, epithelial hyperplasia, focal acanthosis, and fusion and horizontal outgrowth of epithelial ridges. A 37-year-old male patient was referred to the Department of Oral and Maxillofacial Sciences at the Sapienza University of Rome, complaining of numerous exophytic lesions in his mouth. He stated that the lesions were not painful but he had experienced occasional bleeding after incidental masticatory trauma. He had received no previous treatment for the oral lesions. His medical history revealed that he was human immuno-deficiency

**Core tip:** Focal epithelial hyperplasia (FEH), or Heck's disease, is a rare disease of the oral mucosa, characterized by asymptomatic papules in the oral cavity. Human papillomaviruses have been associated with FEH and have been detected in the majority of lesions. Histopathologically, FEH is characterized by parakeratosis, epithelial hyperplasia, and acanthosis. Here, the case of a 37-year-old male patient, human immuno-deficiency virus-positive, smoker, with numerous asymptomatic oral papules clinically and histologically corresponding to FEH is described. Surgical treatment was performed using a 532-nm potassium-titanyl-phosphate laser. In this case, the laser allowed excellent control of bleeding without postoperative sutures and optimal wound healing.

Galanakis A, Palaia G, Tenore G, Del Vecchio A, Romeo U. Focal epithelial hyperplasia in a human immuno-deficiency virus patient treated with laser surgery. *World J Clin Cases*

## INTRODUCTION

Focal epithelial hyperplasia (FEH), or Heck's disease, is an uncommon, benign disease of the oral mucosa; it is mostly found in children and young adults. It has also been described in some Native American communities in North and South America, as well as in Eskimos of Greenland<sup>[1,2]</sup>. FEH is characterized by numerous nodules or papules, usually painless, with sizes that vary from 1 mm to 1 cm, that are mainly found on the lips, buccal mucosa, tongue and palate<sup>[3]</sup>. Human papilloma-virus (HPV) has been detected in the lesions with both electron microscopy and DNA testing<sup>[4,5]</sup>. The most frequently involved viruses are HPV 13 and HPV 32<sup>[5]</sup>. The case of a young HIV-positive adult affected by FEH is reported below.

## CASE REPORT

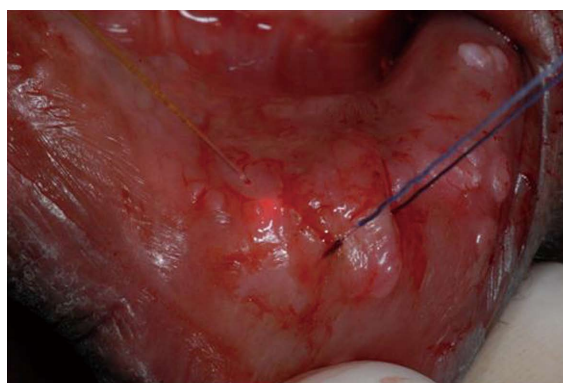
An African male patient, 37-year-old, was referred to the Department of Oral and Maxillofacial Sciences at the Sapienza University of Rome, complaining about numerous exophytic lesions in his mouth. He stated that the lesions were not painful but that he had experienced occasional bleeding after incidental masticatory trauma. He had not received previous treatment for the oral lesions. His medical history revealed that he was human immunodeficiency virus (HIV)-positive. The diagnosis of HIV was made 2 years previously. He was, at the time of the visit, in treatment with lopinavir and ritonavir (Kaletra, Abbott Italy, Campoverde di Aprilia, Italy), emtricitabine and tenofovir (Truvada, Gilead, Foster City, CA, United States), and in prophylaxis with sulfamethoxazole and trimetoprim (Bactrim, Roche, Milan, Italy). He also smoked 10 cigarettes per day, but he did not make use of alcoholic drinks.

According to the classification of the Centers for Disease Control, he was classified as stage A3.

Extraoral examination did not reveal any signs of other diseases. Intraoral examination showed the presence of 17 sessile, soft, normochromic lesions in the oral cavity. The lesions were localized on the lower lip and the buccal mucosa, on both sides (Figure 1). After the examination, a diagnostic hypothesis of FEH was assumed. Excisional biopsy was performed on one of the lesions to confirm the diagnostic hypothesis. The biopsy was performed using a potassium-titanyl-phosphate (KTP) laser with a wavelength of 532 nm (SmartLite, Deka, Florence, Italy) in continuous mode with a 300  $\mu$ m fiber, power 1.4 W (power density 1980.22 W/cm<sup>2</sup>). After anesthesia without vasoconstrictors (Mepivacaina Pierrel 30 mg/mL, Pierrel, Milan, Italy) the lesions were tractioned with sutures or an Allis clamp and then completely excised (Figure 2).



**Figure 1** Clinical aspect of the focal epithelial hyperplasia lesions on the lower lip mucosa.



**Figure 2** Laser excisional biopsy of one of the lesions on the lower lip.

The specimens were preserved in 10% buffered formalin for histological examination, which confirmed the clinical diagnosis of FEH.

In this case, the laser allowed excellent control of bleeding, without postoperative sutures, and optimal wound healing. After the first excisional biopsy, all of the lesions were surgically removed, in several steps, using the same operative approach. The patient was monitored with follow-up visits for one year, during which no recurrence of the pathology was observed (Figure 3), and he was asked to return if any lesions reappeared in the future.

## DISCUSSION

FEH is a rare condition in Italy and Europe. Here, we describe the case of an immunosuppressed African man with FEH. This pathology has been extensively described among native South and North American populations<sup>[1,6-8]</sup>. It seems that there could be a genetic predilection for FEH, as cases seem to be limited to specific ethnic groups in certain geographic regions<sup>[1,6]</sup>. Ledesma-Montes *et al*<sup>[7]</sup> suggested a series of factors that could contribute to the development of FEH: poverty, genetic predisposition (ethnic factors), and a deficient hygienic lifestyle. FEH lesions are most frequently localized on the buccal mucosa, lip, tongue, and commissures; the retromolar area, palate, and mouth floor are rarer local-





**Figure 3** Clinical aspect of the healing after excision of the lesions on the lower lip, 1 yr after treatment.

izations<sup>[7]</sup>. FEH must be included in differential diagnosis with several pathological conditions that can be observed in the oral cavity<sup>[8]</sup>, namely, condylomata acuminata, verrucous carcinoma, inflammatory fibrous hyperplasia, inflammatory papillary hyperplasia, and verruciform xanthoma<sup>[3]</sup>. Condyloma may appear similarly because it is caused by the same virus, but FEH lesions are more numerous and flatter, with typical localizations (buccal mucosa, lip and tongue)<sup>[9]</sup>. Verrucous carcinoma is a malign neoplasm that usually occurs in a different age group, usually in the sixth decade of life, with epidemiological characteristics that are similar to other oral carcinomas<sup>[10]</sup>.

The last three pathologies are reactive lesions that usually occur with an irritating stimulus<sup>[9]</sup>. The diagnosis of FEH can be performed on the basis of clinical observation and can be confirmed by histological examination<sup>[11-13]</sup>.

The histological features of this disease are parakeratosis, acanthosis, elongation of rete ridges, some of which may be anastomosed (the so-called “bronze age battle-axe” appearance<sup>[14]</sup>, and usually koilocytes, as well as other cellular modifications that can indicate viral infection<sup>[5,14]</sup>. Cells with nuclear degeneration, called mitosoid cells, can also appear<sup>[15]</sup>. FEH can be associated with HIV infection, although the relationship between these conditions has not yet been completely clarified<sup>[12]</sup>. Suppression of the immune system leaves the patient vulnerable to opportunistic infections, including HPV infections<sup>[12]</sup>.

There is no agreement in the literature on the potential malignant transformation of FEH lesions in immunocompromised patients. Moerman *et al*<sup>[12]</sup> stated that FEH lesions may have a high risk of malignant transformation in immunocompromised patients. Durso *et al*<sup>[13]</sup> tended to consider FEH a benign condition and stated that to date, no research has demonstrated the potential for malignant transformation of FEH lesions with HPV 13 and 32 subtypes. Only one case of malignant transformation of FEH caused by HPV type 24 has been reported<sup>[16]</sup>. Further studies are required to clarify this point.

Several therapeutic approaches have been proposed throughout the years. Some authors advise against re-

moving the lesions because spontaneous regression can be observed<sup>[17]</sup>, especially in children. Steinhoff *et al*<sup>[18]</sup> successfully treated FEH with topical applications of interferon beta. Other described methods include scalpel surgery, electrocoagulation, electrodesiccation, cryosurgery, and laser surgery<sup>[19,20]</sup>. In this case, laser surgery allowed excellent control of bleeding, without postoperative sutures, and optimal wound healing. Moreover, histological analysis is always possible with laser surgery when the proper parameters and correct surgical technique are used<sup>[21]</sup>.

## COMMENTS

### Case characteristics

A 37-year-old male with a history of human immuno-deficiency virus (HIV) infection presented with numerous asymptomatic lesions in the oral cavity.

### Clinical diagnosis

Seventeen sessile, soft, normochromic lesions on the lower lip and the buccal mucosa, on both sides.

### Differential diagnosis

Condylomata acuminata, verrucous carcinoma, inflammatory fibrous hyperplasia, inflammatory papillary hyperplasia, verruciform xanthoma.

### Laboratory diagnosis

Cluster of differentiation 4 receptors (CD4) 129/μL; HIV RNA < 37 copies/mL; metabolic panel and coagulation within normal limits.

### Pathological diagnosis

Histological examination revealed parakeratosis, acanthosis, presence of koilocytes and mitosoid cells.

### Treatment

Excisional biopsy with a 532-nm potassium titanyl phosphate (KTP) laser.

### Related reports

Focal epithelial hyperplasia (FEH) can be associated with HIV infection, although the relationship between these two conditions has not yet been completely clarified; most likely, suppression of the immune system leaves the patient vulnerable to opportunistic infections, including HPV infections.

### Term explanation

KTP lasers are powerful tools for oral surgery and oral pathology, as are other types of lasers.

### Experiences and lessons

Oral lesions can be a manifestation of more complex systemic diseases; advanced surgical techniques can be useful tools in the management of multiple oral viral lesions.

### Peer review

This paper is worthy of publication as an interesting case report of uncommon oral mucosa disease in HIV infected patient. Particularly, an information of efficiency of KTP laser surgery in treatment of Heck's disease will be useful for clinicians.

## REFERENCES

- 1 Archard HO, Heck JW, Stanley HR. Focal epithelial hyperplasia: an unusual oral mucosal lesion found in Indian children. *oral surg oral med oral pathol* 1965; **20**: 201-212 [PMID: 14322615 DOI: 10.1016/0030-4220(65)90192-1]
- 2 Clausen FP, Mogeltoft M, Roed-Petersen B, Pindborg JJ. Focal epithelial hyperplasia of the oral mucosa in a south-west Greenlandic population. *Scand J Dent Res* 1970; **78**: 287-294 [PMID: 5273696]
- 3 Terezhalmay GT, Riley CK, Moore WS. Focal epithelial hyperplasia (Heck's disease). *Quintessence Int* 2001; **32**: 664-665 [PMID: 11526896]
- 4 Hanks CT, Fishman SL, Guzman MN. Focal epithelial hyperplasia. A light and electron microscopic study. *Oral Surg*

- Oral Med Oral Pathol* 1972; **33**: 934-943 [PMID: 4503460 DOI: 10.1016/0030-4220(72)90185-5]
- 5 **Falaki F**, Amir Chaghmaghi M, Pakfetrat A, Delavarian Z, Mozaffari PM, Pazooki N. Detection of human papilloma virus DNA in seven cases of focal epithelial hyperplasia in Iran. *J Oral Pathol Med* 2009; **38**: 773-776 [PMID: 19453844 DOI: 10.1111/j.1600-0714.2009.00784.x]
- 6 **Witkop CJ**, Niswander JD. Focal epithelial hyperplasia in central and south american indians and ladinos. *Oral Surg Oral Med Oral Pathol* 1965; **20**: 213-217 [PMID: 14319596 DOI: 10.1016/0030-4220(65)90193-3]
- 7 **Ledesma-Montes C**, Garcés-Ortíz M, Hernández-Guerrero JC. Clinicopathological and immunocytochemical study of multifocal epithelial hyperplasia. *J Oral Maxillofac Surg* 2007; **65**: 2211-2217 [PMID: 17954316 DOI: 10.1016/j.joms.2006.11.035]
- 8 **Cubie HA**. Diseases associated with human papillomavirus infection. *Virology* 2013; **445**: 21-34 [PMID: 23932731 DOI: 10.1016/j.virol.2013.06.007]
- 9 **Borborema-Santos CM**, Castro MM, Santos PJ, Talhari S, Astolfi-Filho S. Oral focal epithelial hyperplasia: report of five cases. *Braz Dent J* 2006; **17**: 79-82 [PMID: 16721472 DOI: 10.1590/S0103-64402006000100018]
- 10 **Zhu LK**, Ding YW, Liu W, Zhou YM, Shi LJ, Zhou ZT. A clinicopathological study on verrucous hyperplasia and verrucous carcinoma of the oral mucosa. *J Oral Pathol Med* 2012; **41**: 131-135 [PMID: 21913992 DOI: 10.1111/j.1600-0714.2011.01078.x]
- 11 **Bassioukas K**, Danielides V, Georgiou I, Photos E, Zagorianakou P, Skevas A. Oral focal epithelial hyperplasia. *Eur J Dermatol* 2000; **10**: 395-397 [PMID: 10882951]
- 12 **Moerman M**, Danielides VG, Nousia CS, Van Wanzeele F, Forsyth R, Vermeersch H. Recurrent focal epithelial hyperplasia due to HPV13 in an HIV-positive patient. *Dermatology* 2001; **203**: 339-341 [PMID: 11752826 DOI: 10.1159/000051786]
- 13 **Durso BC**, Pinto JM, Jorge J, de Almeida OP. Extensive focal epithelial hyperplasia: case report. *J Can Dent Assoc* 2005; **71**: 769-771 [PMID: 16324231]
- 14 **Marvan E**, Firth N. Focal epithelial hyperplasia in an HIV positive man. An illustrated case and review of the literature. *Aust Dent J* 1998; **43**: 305-310 [PMID: 9848979 DOI: 10.1111/j.1834-7819.1998.tb00178.x]
- 15 **Segura-Saint-Gerons R**, Toro-Rojas M, Ceballos-Salobreña A, Aparicio-Soria JL, Fuentes-Vaamonde H. Focal epithelial hyperplasia. A rare disease in our area. *Med Oral Patol Oral Cir Bucal* 2005; **10**: 128-131 [PMID: 15735545]
- 16 **Niebrügge B**, Villiers E, Gerlach K, Franke I, Gollnick H. Demonstration of HPV 24 in long-standing Heck's disease with malignant transformation. *Eur J Dermatol* 1999; **9**: 477-479 [PMID: 10491507]
- 17 **Pfister H**, Hettich I, Runne U, Gissmann L, Chliff GN. Characterization of human papillomavirus type 13 from focal epithelial hyperplasia Heck lesions. *J Virol* 1983; **47**: 363-366 [PMID: 6312071]
- 18 **Steinhoff M**, Metze D, Stockfleth E, Luger TA. Successful topical treatment of focal epithelial hyperplasia (Heck's disease) with interferon-beta. *Br J Dermatol* 2001; **144**: 1067-1069 [PMID: 11359400 DOI: 10.1046/j.1365-2133.2001.04201.x]
- 19 **Akyol A**, Anadolu R, Anadolu Y, Ekmekci P, Gürgey E, Akay N. Multifocal papillomavirus epithelial hyperplasia: successful treatment with CO2 laser therapy combined with interferon alpha-2b. *Int J Dermatol* 2003; **42**: 733-735 [PMID: 12956692 DOI: 10.1046/j.1365-4362.2003.01777.x]
- 20 **Said AK**, Leao JC, Fedele S, Porter SR. Focal epithelial hyperplasia - an update. *J Oral Pathol Med* 2013; **42**: 435-442 [PMID: 23061874 DOI: 10.1111/jop.12009]
- 21 **Romeo U**, Palaia G, Del Vecchio A, Tenore G, Gambarini G, Gutknecht N, De Luca M. Effects of KTP laser on oral soft tissues. An in vitro study. *Lasers Med Sci* 2010; **25**: 539-543 [PMID: 20162316 DOI: 10.1007/s10103-010-0756-2]

**P- Reviewers:** Bugaj AM, Kaliyadan F **S- Editor:** Ji FF

**L- Editor:** A **E- Editor:** Wu HL



## Optimal management of a patient with recurrent nasopharyngeal carcinoma

Francesco Perri, Italo Dell'Oca, Paolo Muto, Concetta Schiavone, Corrado Aversa, Franco Fulciniti, Raffaele Solla, Giuseppina Della Vittoria Scarpati, Carlo Buonerba, Giuseppe Di Lorenzo, Francesco Caponigro

Francesco Perri, Francesco Caponigro, Head and Neck Medical Oncology Unit, National Tumor Institute of Naples, 80131 Naples, Italy

Italo Dell'Oca, Radiotherapy Unit, S. Raffaele Hospital of Milan, 20100 Milan, Italy

Paolo Muto, Concetta Schiavone, Radiotherapy Unit, National tumor Institute of Naples, 80131 Naples, Italy

Corrado Aversa, Otolaryngology Unit, National Tumor Institute of Naples, 80131 Naples, Italy

Franco Fulciniti, Pathology Unit, National Tumor Institute of Naples, 80131 Naples, Italy

Raffaele Solla, Institute of Biostructures and Bioimage, National Council of Research (CNR) of Naples, 80131 Naples, Italy

Giuseppina Della Vittoria Scarpati, Oncology and Molecular Biology Research Unit, University of Salerno, 84090 Salerno, Italy

Carlo Buonerba, Giuseppe Di Lorenzo, Medical Oncology, University Federico II of Naples, 80131 Naples, Italy

Author contributions: All authors contributed to this paper.

Correspondence to: Francesco Perri, MD, Head and Neck Medical Oncology Unit, National Tumor Institute of Naples, Via Mariano Semmola, 80131 Naples, Italy. [francesco.perri80@alice.it](mailto:francesco.perri80@alice.it)

Telephone: +39-815-903362 Fax: +39-815-903822

Received: January 15, 2014 Revised: April 24, 2014

Accepted: May 16, 2014

Published online: July 16, 2014

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Cetuximab; Nasopharyngeal carcinoma; Re-irradiation; Surgery of metastases; Undifferentiated

**Core tip:** Recurrent nasopharyngeal carcinoma requires multi-modality therapy based on radiotherapy, surgery and chemotherapy, with a careful evaluation of expected toxicity and patient's quality of life.

Perri F, Dell'Oca I, Muto P, Schiavone C, Aversa C, Fulciniti F, Solla R, Della Vittoria Scarpati G, Buonerba C, Di Lorenzo G, Caponigro F. Optimal management of a patient with recurrent nasopharyngeal carcinoma. *World J Clin Cases* 2014; 2(7): 297-300 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/297.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.297>

### INTRODUCTION

Nasopharyngeal carcinoma (NPC) accounts for less than 1% of all malignancies and is rare in western countries<sup>[1]</sup>. NPC is usually poorly differentiated and is diagnosed in young patients. The prognosis of loco-regional disease is satisfactory, with a 5-year overall survival exceeding 80%. Radiation alone is employed for stage I - II disease, while for locally advanced NPC the combination of chemotherapy and radiotherapy is the standard approach<sup>[1]</sup>. The advent of new radiotherapy techniques, like intensity modulated radiotherapy (IMRT), has further improved prognosis of newly diagnosed NPC patients and contributed to allow re-irradiation of recurrent disease. On the other hand, management of recurrent/metastatic disease remains challenging for medical and radiation oncologists. In addition to cisplatin-based chemotherapy, which is the standard systemic treatment for NPC<sup>[2]</sup>, cetuximab may be in patients with NPC and squamous histology according to guidelines. Importantly, in undifferentiated

### Abstract

Nasopharyngeal carcinoma is rare in western countries, accounting for less than 1% of all malignancies. Despite prognosis is satisfactory for newly diagnosed, non-metastatic disease, management of recurrent disease is challenging, with a survival expectancy of approximately 6 mo with the use of chemotherapy as the sole salvage treatment. We report a case of recurrent nasopharyngeal carcinoma treated with a combination of chemotherapy, radiotherapy and surgery in the context of a multidisciplinary approach. A durable complete response was achieved.

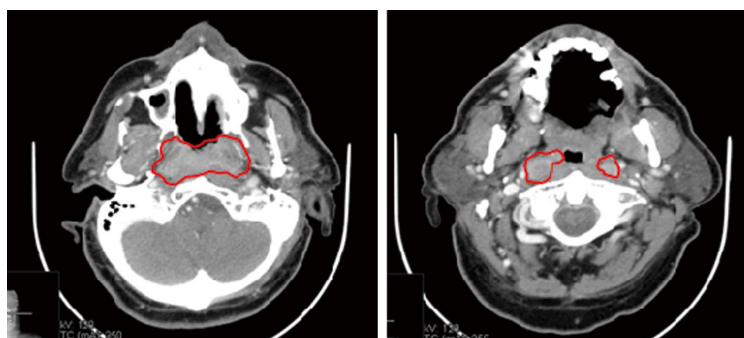


Figure 1 A large lesion arising from the posterior wall of the nasopharynx and bilateral lymphnode metastasis were detected in the parapharyngeal space (October 2009).

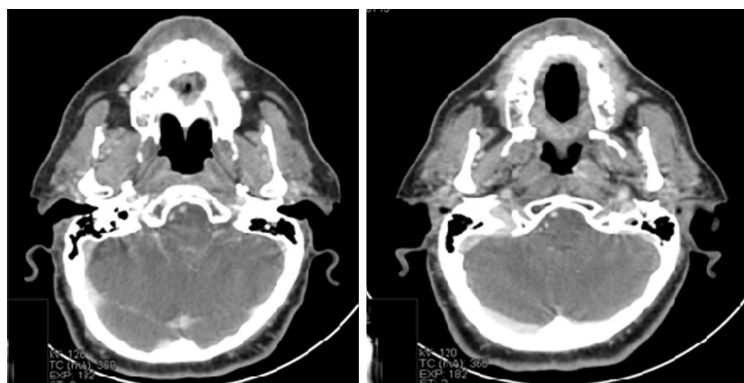


Figure 2 After neoadjuvant TPF followed by cetuximab-radiotherapy, a complete response was obtained (March 2010).

NPC, epithelial growth factor receptor is often overexpressed and k-RAS is never mutated, so cetuximab may provide benefit also in these patients<sup>[5]</sup>. We here report how multidisciplinary management coupled with recent advances in the field allowed us to obtain a durable complete response in a patient with relapsing undifferentiated nasopharyngeal carcinoma.

## CASE REPORT

In September 2009, a 59-year-old male patient was referred to our office for undifferentiated nasopharyngeal carcinoma. Histological diagnosis was made on fiberoptic-guided biopsy. A computed tomography (CT) scan showed a large lesion arising from the posterior wall of the nasopharynx and extending into the paranasal sinuses. The lesion infiltrated the clivus and the oropharynx, and bilateral laterocervical lymphnode metastases were detected. Magnetic resonance imaging (MRI) confirmed CT findings (clinical stage, T4N3M0, IVa AJCC).

A therapeutic strategy based on 3 cycles of neoadjuvant docetaxel, cisplatin and 5-fluorouracil followed by radiotherapy was pursued after written informed consent was obtained. Docetaxel and cisplatin were given at doses of 75 mg/m<sup>2</sup> on day one and 5-Fluorouracil was administered at 750 mg/m<sup>2</sup> daily *via* continuous infusion for four consecutive days every three weeks. In November 2009, CT scan (Figure 1) showed partial response according to RECIST criteria 1.1 (reduction greater than 50% in the sum of the longest diameters of target lesions). From December 2009 until February 2010, a combined cetuximab-RT treatment was delivered. Cetuximab was given at standard doses. A total dose of 70 Gy (2 Gy dose frac-

tions) on clinical target volume was delivered *via* 3D conformational radiotherapy. Toxicity mainly consisted of grade 2 cutaneous rash and grade 2 xerostomia, and did not lead to treatment interruption. Forty-five days after completion of chemo-radiotherapy, a complete response was shown on CT scan (Figure 2) and on PET scan after 60 d.

The patient remained free of disease recurrence until May 2012, when CT scan showed loco-regional recurrence involving the nasopharynx, the left orbital cavity and intraparotid lymph nodes on the left side of the head. Patient presented with visual disturbances and moderate pain. PET scan and cytology confirmed the nature and extension of the recurrence. After multidisciplinary consultation involving the medical oncologist, the radiation oncologist, the pathologist and the surgeon, we decided to use a combined strategy based on an neoadjuvant chemotherapy, followed by re-irradiation and a surgical evaluation of residual disease.

Given the prolonged time to relapse, we deemed the disease to have preserved its sensitivity to taxanes and platinum compounds, so we delivered carboplatin (CB-DCA) AUC 5 (area under curve) on day one every 3 wk, paclitaxel at the dose of 175 mg/m<sup>2</sup> on day one every three weeks and standard doses of weekly cetuximab. Radiological and symptomatic improvement was obtained after three cycles. A CT scan showed disappearance of the nasopharyngeal lesion, shrinkage of the lymphnode mass but no change of the orbital lesion. Toxicity was low, with grade 1 skin rash the most relevant side effect. Re-irradiation was performed from the end of August to the beginning of October 2012 *via* helical IMRT, 18 FDG PET/CT based planning. A total dose of 66 Gy, deliv-



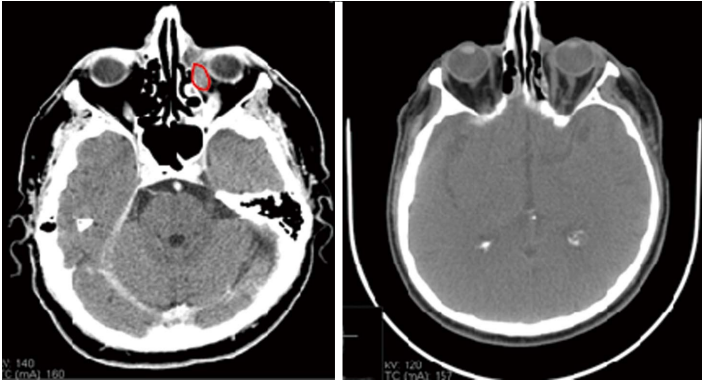


Figure 3 Computed tomography scan showed an intraorbital lesion (May 2012).

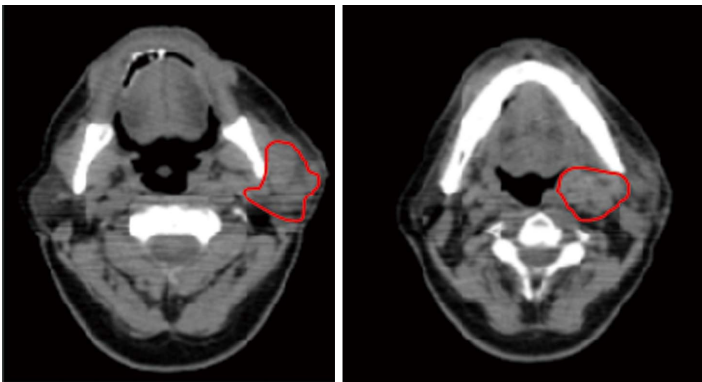


Figure 4 Computed tomography scan showed that the only site of persistent disease was the lymph node mass in the neck (December 2012).

ered in 30 daily fractions of 2.2 Gy, were administered to the site of disease, with the exclusion of the lymph node mass in the neck, with disappearance (Figure 3) of both the orbital lesion and the nasopharynx. After subsequent chemotherapy (three cycles of CBDCA-Pac and cetuximab) CT scan showed that the only site of persistent disease was the lymph node mass in the neck (Figure 4). Patient was scheduled for surgical removal of parathyroid and laterocervical II, III and IV neck levels. In June 2013, CT scan showed no sign of residual disease. As of January 2014, the patient is free of disease. He complains of grade 2 chronic xerostomia due to radiotherapy, and a facial nerve paralysis due to the surgical intervention.

## DISCUSSION

Locally advanced nasopharyngeal carcinoma can be successfully managed by combination platinum-based chemotherapy and radiotherapy<sup>[4]</sup>. Neo-adjuvant chemotherapy is also a valuable option and is especially indicated in patients with large lesions and locally advanced disease, in order to decrease the gross tumor volume<sup>[5]</sup>. Combination of taxanes and platinum compounds is highly active, not only in nasopharyngeal carcinomas but also in other clinical situations, such as head-neck carcinomas of unknown primary<sup>[6]</sup>.

On the other hand, current management of recurrent/metastatic disease is far from yielding satisfactory results, and the prognosis is grim. In addition to cisplatin-based chemotherapy, cetuximab may also be employed in patients with NPC, as shown in some preliminary experiences<sup>[7]</sup>. Our case appears of great interest not only

because cetuximab appeared to improve the outcome at first presentation, but it appeared to have preserved its effectiveness at disease relapse. Cetuximab was well tolerated both at initial presentation and at recurrence. Randomized controlled trials are required to assess efficacy of cetuximab in nasopharyngeal carcinoma before it can be recommended in this setting.

Our case also highlights the importance of a multidisciplinary approach in all patients and especially in those with recurrent disease. The multidisciplinary approach is able to balance toxicity and efficacy associated to combined use of diversified treatments. In recurrent patients, the use of chemotherapy as the sole salvage treatment is associated to a 6-mo prognosis<sup>[8-10]</sup>. With a multidisciplinary approach, we obtained a complete response, and our patient is free of disease recurrence after more than 6 mo and has an acceptable quality of life. Doctor-doctor and patient-doctor communication was of utmost importance for the management of this case, which should set an example of optimal integrated strategy of recurrent nasopharyngeal carcinoma.

## COMMENTS

### Case characteristics

Patient presented with recurrent nasopharyngeal carcinoma treated with chemotherapy, biological therapy, radiation therapy and surgery.

### Clinical diagnosis

Patient was affected by a locally advanced nasopharyngeal tumor, which was shown to be undifferentiated carcinoma on biopsy

### Differential diagnosis

Differential diagnosis included lymphoma and squamous carcinoma.

### Laboratory diagnosis

Patient had normal liver, bone marrow and kidney function at diagnosis and throughout treatment.

### Imaging diagnosis

Patient had locally advanced disease at diagnosis, with a large lesion arising from the posterior wall of the nasopharynx and infiltrating the clivus and bilateral laterocervical lymphnode metastases.

### Pathological diagnosis

Patient was affected by undifferentiated nasopharyngeal carcinoma.

### Treatment

After patient underwent treatment with docetaxel, cisplatin and 5-Fluorouracil and combined cetuximab-radiotherapy, he was treated with carboplatin, paclitaxel and cetuximab, followed by re-irradiation and surgery.

### Term explanation

Clinical target volume.

### Experiences and lessons

Irradiation with cetuximab is advantageous when chemotherapy cannot be delivered.

### Peer review

This paper is a useful clinical case in head and neck diseases.

## REFERENCES

- 1 **Caponigro F**, Longo F, Ionna F, Perri F. Treatment approaches to nasopharyngeal carcinoma: a review. *Anticancer Drugs* 2010; **21**: 471-477 [PMID: 20124988 DOI: 10.1097/CAD.0b013e328337160e]
- 2 **Boussen H**, Cvitkovic E, Wendling JL, Azli N, Bachouchi M, Mahjoubi R, Kalifa C, Wibault P, Schwaab G, Armand JP. Chemotherapy of metastatic and/or recurrent undifferentiated nasopharyngeal carcinoma with cisplatin, bleomycin, and fluorouracil. *J Clin Oncol* 1991; **9**: 1675-1681 [PMID: 1714951]
- 3 **Chan AT**, Hsu MM, Goh BC, Hui EP, Liu TW, Millward MJ, Hong RL, Whang-Peng J, Ma BB, To KF, Mueser M, Amellal N, Lin X, Chang AY. Multicenter, phase II study of cetuximab in combination with carboplatin in patients with recurrent or metastatic nasopharyngeal carcinoma. *J Clin Oncol* 2005; **23**: 3568-3576 [PMID: 15809453 DOI: 10.1200/JCO.2005.02.147]
- 4 **Ma J**, Mai HQ, Hong MH, Min HQ, Mao ZD, Cui NJ, Lu TX, Mo HY. Results of a prospective randomized trial comparing neoadjuvant chemotherapy plus radiotherapy with radiotherapy alone in patients with locoregionally advanced nasopharyngeal carcinoma. *J Clin Oncol* 2001; **19**: 1350-1357 [PMID: 11230478]
- 5 **Perri F**, Bosso D, Buonerba C, Lorenzo GD, Scarpato GD. Locally advanced nasopharyngeal carcinoma: Current and emerging treatment strategies. *World J Clin Oncol* 2011; **2**: 377-383 [PMID: 22171280 DOI: 10.5306/wjco.v2.i12.377]
- 6 **Perri F**, Ionna F, Muto P, Buonerba C, Della Vittoria Scarpato G, Bosso D, Fulciniti F, Daponte A, Argenone A, Sandomenico F, DI Lorenzo G, Caponigro F. Induction docetaxel-cisplatin followed by extended-field radiotherapy in patients with cervical metastases from unknown primary carcinoma. *Anticancer Res* 2013; **33**: 1135-1139 [PMID: 23482792]
- 7 **Smee RI**, Meagher NS, Broadley K, Ho T, Williams JR, Bridger GP. Recurrent nasopharyngeal carcinoma: current management approaches. *Am J Clin Oncol* 2010; **33**: 469-473 [PMID: 19935385 DOI: 10.1097/COC.0b013e3181b4b037]
- 8 **Roeder F**, Zwicker F, Saleh-Ebrahimi L, Timke C, Thieke C, Bischof M, Debus J, Huber PE. Intensity modulated or fractionated stereotactic reirradiation in patients with recurrent nasopharyngeal cancer. *Radiat Oncol* 2011; **6**: 22 [PMID: 21356126 DOI: 10.1186/1748-717X-6-22]
- 9 **Teo PM**, Kwan WH, Chan AT, Lee WY, King WW, Mok CO. How successful is high-dose (> 60 Gy) reirradiation using mainly external beams in salvaging local failures of nasopharyngeal carcinoma? *Int J Radiat Oncol Biol Phys* 1998; **40**: 897-913 [PMID: 9531376 DOI: 10.1016/S0360-3016(97)00854-7]
- 10 **Perri F**, Muto P, Aversa C, Daponte A, Della Vittoria G, Pepe S, Caponigro F. Integrated therapeutic approaches in head and neck cancer: the importance of multidisciplinary team management. *Anticancer Agents Med Chem* 2013; **13**: 834-843 [PMID: 23194421 DOI: 10.2174/18715206113139990110]

P- Reviewers: Ao R, Li YZ S- Editor: Song XX

L- Editor: A E- Editor: Wu HL



## Disseminated infection due to *Mycobacterium bovis* after intravesical BCG instillation

Sara Marquez-Batalla, Esther Fraile-Villarejo, Moncef Belhassen-García, Nieves Gutierrez-Zubiaurre, Miguel Cordero-Sánchez

Sara Marquez-Batalla, Esther Fraile-Villarejo, Servicio de Medicina Interna (CAUSA), IBSAL, Universidad de Salamanca, 37007 Salamanca, Spain

Moncef Belhassen-García, Miguel Cordero-Sánchez, Servicio de Medicina Interna, Sección de Enfermedades Infecciosas, Complejo Asistencial Universitario de Salamanca (CAUSA), Instituto de Investigación Biomedica de Salamanca (IBSAL), Universidad de Salamanca, 37007 Salamanca, Spain

Nieves Gutierrez-Zubiaurre, Servicio de Microbiología, (CAUSA), CIETUS, IBSAL, 37007 Salamanca, Spain

**Author contributions:** Marquez-Batalla S and Fraile-Villarejo E collected the patients clinical data; Gutierrez-Zubiaurre N performed the microbiological analyses; Marquez-Batalla S, Cordero-Sánchez M and Belhassen-García M designed the report and wrote the paper.

**Correspondence to:** Moncef Belhassen-García, MD, PhD, Servicio de Medicina Interna, Sección de Enfermedades Infecciosas, Complejo Asistencial Universitario de Salamanca (CAUSA), Instituto de Investigación Biomedica de Salamanca (IBSAL), Universidad de Salamanca, Paseo San Vicente 58-182, 37007 Salamanca, Spain. [mbelhassen@hotmail.com](mailto:mbelhassen@hotmail.com)  
Telephone: +34-923-291306 Fax: +34-923-291131

Received: February 22, 2014 Revised: April 19, 2014

Accepted: April 25, 2014

Published online: July 16, 2014

**Key words:** Tuberculosis; *Mycobacterium bovis*; Bladder cancer; Bacillus Calmette-Guerin

**Core tip:** Intravesical instillation of bacillus Calmette-Guerin (BCG) is a therapeutic option in bladder cancer. Multi-system effects are a rare complication of this procedure, and certain aspects concerning its diagnosis and treatment are unclear. We report the case of a patient who developed effects on multiple organs after intravesical BCG instillations, and we review current knowledge concerning the diagnosis and management of BCG infection.

Marquez-Batalla S, Fraile-Villarejo E, Belhassen-García M, Gutierrez-Zubiaurre N, Cordero-Sánchez M. Disseminated infection due to *Mycobacterium bovis* after intravesical BCG instillation. *World J Clin Cases* 2014; 2(7): 301-303 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/301.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.301>

### Abstract

Intravesical bacillus Calmette-Guerin (BCG) instillation has been adopted for the treatment of patients with superficial bladder cancer. Severe adverse events due to local instillation of BCG are uncommon, with an overall rate of serious complications of less than 5%. We report the case of an immunocompetent adult patient with multi-system effects, namely pneumonitis, granulomatous hepatitis and meningitis, who responded well to standard treatment for *Mycobacterium bovis*. This case highlights the importance of a thorough assessment of this type of patient.

### INTRODUCTION

Intravesical administration of bacillus Calmette-Guerin (BCG) is an essential tool in the treatment of superficial bladder carcinoma *in situ*<sup>[1]</sup>. This approach is generally well tolerated, although it occasionally leads to severe local and/or systemic complications<sup>[2]</sup>. The most serious complications of intravesical BCG instillation are related to disseminated infection. When disseminated BCG infection occurs, antituberculous therapy with or without glucocorticoids should be administered. We report a case of disseminated infection due to intravesical BCG instillations, resulting in pneumonitis, granulomatous hepatitis and meningitis.

### CASE REPORT

We present the case of a 64-year-old male patient with a



**Figure 1** A thoracic computed tomography scan revealed a pulmonary micronodular pattern.



**Figure 2** An abdominal computed tomography scan revealed hepatic granulomas and hepatosplenomegaly.

history of urothelial vesical neoplasm, benign prostatic hyperplasia and chronic obstructive pulmonary disease. He was being treated with tamsulosin, tiotropium bromide and salbutamol. Six months before, he had received six weekly doses of intravesical BCG instillation as an induction treatment, and one month before, he had started maintenance therapy with three doses per week. At the time of presentation, he reported weakness, weight loss and a slight fever with three weeks of evolution. Physical examination revealed hepatosplenomegaly. An analysis showed hepatic cholestasis (alkaline phosphatase 358 U/L, gamma-glutamyl transpeptidase 306 U/L), lactate dehydrogenase (LDH) 547 U/L and C-reactive protein 1.27 mg/dL. A complete blood count and urine sediment were normal. A thoracic X-ray showed multiple micronodular opacities. A thoraco-abdominal CT scan revealed a pulmonary micronodular pattern, hepatic granulomas and hepatosplenomegaly (Figures 1 and 2). Blood and urine cultures for bacteria were negative, as was a serological analysis (for HIV, HBV, HCV and *Treponema pallidum*). The urinalysis was positive for the species *Mycobacterium tuberculosis*, and *Mycobacterium bovis* (*M. bovis*) BCG grew with resistance to cycloserine and pyrazinamide. A genetic study detected the pnc A C169G (H57D) mutation, and we identified the species as *M. bovis*. The patient started treatment with isoniazid, rifampicin and etham-



**Figure 3** Magnetic resonance imaging showed thickening and linear meningeal enhancement.

butol. After one week of treatment, the patient showed dizziness and instability while standing still and walking. The cerebrospinal fluid (CSF) showed red blood cells 4500/ $\mu$ L, leukocytes 16  $\mu$ L (polymorphonuclear 25%, mononuclear 75%), proteins 82 mg/mL, glucose 67 mg/mL (capillary glycemia 144 mg/dL), LDH: 35 IU/L and adenosine deaminase 5.6 U/L (0-5). Magnetic resonance imaging of the brain (Figure 3) showed thickening and linear meningeal enhancement. Microbiological analysis of the CSF was negative. Given the meningeal involvement, anti-tuberculosis treatment was administered for one year, with good clinical and radiological responses.

## DISCUSSION

The adverse effects of intravesical BCG instillations can appear early or several years after the treatment. Although there are certain common local effects, such as cystitis (91%)<sup>[3]</sup> (this condition can be difficult to differentiate from other urinary tract infections), systemic complications are rare. The frequency of appearance ranges from 2.9% in cases with fever to 0.3% in cases with skin exanthema<sup>[4]</sup>. The most severe symptoms are pneumonitis, hepatitis, sepsis and pancytopenia. No differences in the incidence of complications were observed when comparing different BCG preparations or doses<sup>[5]</sup>. The spreading mechanism is not exhaustively known. Certain authors think that hematogenous spread occurs from the bladder<sup>[3]</sup>, whereas others believe that the spread is due to a type IV hypersensitivity-related mechanism<sup>[6]</sup>. The response to glucocorticoids administered along with antituberculous drugs has also supported the notion of a hypersensitivity response. There are no established effective measures to prevent a disseminated infection with BCG<sup>[7]</sup>, although the risk increases when instillations are temporally close to surgery or to traumatic catheterization<sup>[8]</sup>. The genetic study of the urine sample of our patient allowed us to identify *M. bovis* and to confirm its classic resistance profile. Given the meningeal involvement in the patient in our case, the treatment was extended for one year, with combination with corticoids in the first weeks.



In conclusion, intravesical BCG instillation can induce disseminated infection. Molecular techniques can help in early diagnosis because a delay in management can be lethal. In our patient, standard triple therapy with steroids led to complete recovery.

## COMMENTS

### Case characteristics

A 64-year-old male patient with an urothelial-vesical neoplasm treated by intravesical bacillus Calmette-Guérin (BCG) instillations.

### Clinical diagnosis

Hepatosplenomegaly and later dizziness and instability.

### Differential diagnosis

Neoplasm and central nervous system infection.

### Laboratory diagnosis

Alkaline phosphatase 358 U/L, gamma-glutamyl transpeptidase 306 U/L, lactate dehydrogenase 547 U/L and C-reactive protein 1.27 mg/dL. A complete blood count and urine sediment were normal.

### Imaging diagnosis

A thoracic X-ray showed multiple micronodular opacities. A thoraco-abdominal computed tomography scan revealed a pulmonary micronodular pattern, hepatic granulomas and hepatosplenomegaly. Magnetic resonance imaging of the brain showed thickening and linear meningeal enhancement.

### Pathological diagnosis

A urinalysis was positive for *Mycobacterium bovis*.

### Treatment

Anti-tuberculosis treatment was administered for one year.

### Related reports

Severe adverse events due to local instillation of BCG are uncommon, and disseminated BCG infection can simulate several diseases.

### Term explanation

BGG is a low-virulence mycobacterium that originates from successive cultures of *Mycobacterium bovis*. Pnc A is a gene encoding the mycobacterial enzyme pyrazinamidase.

### Experiences and lessons

BCG can induce disseminated disease with multi-system failure.

### Peer review

This paper reports the case of serious complications in a variety of organs after intravesical BCG instillation. The manuscript is basically well written.

## REFERENCES

- 1 **Malmström PU**, Sylvester RJ, Crawford DE, Friedrich M, Krege S, Rintala E, Solsona E, Di Stasi SM, Witjes JA. An individual patient data meta-analysis of the long-term outcome of randomised studies comparing intravesical mitomycin C versus bacillus Calmette-Guérin for non-muscle-invasive bladder cancer. *Eur Urol* 2009; **56**: 247-256 [PMID: 19409692 DOI: 10.1016/j.eururo.2009.04.038]
- 2 **McParland C**, Cotton DJ, Gowda KS, Hoepfner VH, Martin WT, Weckworth PF. Miliary Mycobacterium bovis induced by intravesical bacille Calmette-Guérin immunotherapy. *Am Rev Respir Dis* 1992; **146**: 1330-1333 [PMID: 1443892 DOI: 10.1164/ajrccm/146.5\_Pt\_1.1330]
- 3 **Gonzalez OY**, Musher DM, Brar I, Furgeson S, Boktour MR, Septimus EJ, Hamill RJ, Graviss EA. Spectrum of bacille Calmette-Guérin (BCG) infection after intravesical BCG immunotherapy. *Clin Infect Dis* 2003; **36**: 140-148 [PMID: 12522745 DOI: 10.1086/344908]
- 4 **Miranda S**, Verdet M, Héron F, Vittecoq O, Levesque H, Marie I. [Acute reactive arthritis after intravesical instillation of bacillus Calmette-Guérin. Two case reports and literature review]. *Rev Med Interne* 2010; **31**: 558-561 [PMID: 20494494 DOI: 10.1016/j.revmed.2010.01.010]
- 5 **Lamm DL**. Efficacy and safety of bacille Calmette-Guérin immunotherapy in superficial bladder cancer. *Clin Infect Dis* 2000; **31** Suppl 3: S86-S90 [PMID: 11010830 DOI: 10.1086/314064]
- 6 **Elkabani M**, Greene JN, Vincent AL, VanHook S, Sandin RL. Disseminated Mycobacterium bovis after intravesicular bacillus calmette-Guérin treatments for bladder cancer. *Cancer Control* 2000; **7**: 476-481 [PMID: 11000618]
- 7 **Damiano R**, De Sio M, Quarto G, Di Lorenzo G, Perdonà S, Palumbo IM, Azzarito G, Giugliano F, Autorino R. Short-term administration of prulifloxacin in patients with non-muscle-invasive bladder cancer: an effective option for the prevention of bacillus Calmette-Guérin-induced toxicity? *BJU Int* 2009; **104**: 633-639 [PMID: 19298412 DOI: 10.1111/j.1464-410X.2009.08469.x]
- 8 **Nadasy KA**, Patel RS, Emmett M, Murillo RA, Tribble MA, Black RD, Sutker WL. Four cases of disseminated Mycobacterium bovis infection following intravesical BCG instillation for treatment of bladder carcinoma. *South Med J* 2008; **101**: 91-95 [PMID: 18176300 DOI: 10.1097/SMJ.0b013e31815d4047]

P- Reviewers: Hsu WH, Nagata T S- Editor: Song XX  
L- Editor: A E- Editor: Wu HL



## One-stage revision in two cases of *Salmonella* prosthetic hip infection

Kimberly TV Jeroense, Jesse WP Kuiper, Sascha Colen, Rogier P Schade, Rachid Saouti

Kimberly TV Jeroense, Rachid Saouti, Department of Orthopaedic Surgery, VU University Medical Centre, Amsterdam 1081 HV, Noord-Holland, The Netherlands

Jesse WP Kuiper, Centre for Orthopaedic Research Alkmaar, Medical Centre Alkmaar, Alkmaar 1815 JD, Noord-Holland, The Netherlands

Sascha Colen, Department of Orthopedic Surgery and Traumatology, Sint Bonifatius Hospital, Lingen, 49808, Ems, Germany

Rogier P Schade, Department of Medical Microbiology and Infection Control, VU University Medical Centre, Amsterdam 1081 HV, Noord-Holland, The Netherlands

Author contributions: Jeroense KTV, Kuiper JWP, Colen S, Schade RP and Saouti R designed the report; Kuiper JWP, Jeroense KTV and Saouti R collected the patient's clinical data; Jeroense KTV and Kuiper JWP wrote the paper; all authors revised the manuscript.

Correspondence to: Jesse WP Kuiper, Orthopaedic Surgery Resident, Centre for Orthopaedic Research Alkmaar, Medical Centre Alkmaar, Wilhelminalaan 12, Alkmaar 1815 JD, The Netherlands. [jwp.kuiper@gmail.com](mailto:jwp.kuiper@gmail.com)

Telephone: +31-72-5484342 Fax: +31-72-5482168

Received: December 28, 2013 Revised: April 17, 2014

Accepted: May 28, 2014

Published online: July 16, 2014

as well. According to the latest guidelines, one-stage revision has comparable success rates and less morbidity compared to two-stage treatment, when selecting the right patients. In our opinion, PJI caused by *Salmonella* should be treated just as PJI caused by other bacteria, with consideration of the selection criteria as mentioned in several treatment guidelines. As illustrated by these two cases, one-stage revision can be successful in both early and late *Salmonella* PJI of the hip.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** *Salmonella*; Prosthetic joint infection; One-stage revision; Two-stage revision; Treatment

**Core tip:** Prosthetic joint infection (PJI) of the hip by *Salmonella* species is rare. There is an ongoing debate whether treatment of prosthetic joint infection should consist of a one- or two-stage approach and also whether or not PJI caused by *Salmonella* should be treated similarly to PJI caused by other bacteria. We report two cases of *Salmonella* PJI, one early and one late infection, successfully treated by one-stage revision.

### Abstract

We describe two cases of prosthetic joint infection (PJI) of the hip due to *Salmonella*. The first patient presented with an early infection 5 d after being discharged following a total hip replacement and the second patient presented at the emergency ward with a late infection, thirteen years following a total hip replacement. Both cases occurred within one month of each other at our institution and both were successfully treated with a one-stage revision. PJI caused by *Salmonella* species is very rare: so far only 20 *Salmonella* PJIs of the hip have been described. Therefore, full consensus on the best treatment approach has not yet been reached. An aggressive two-stage approach is advised because of the virulence of *Salmonella*, although a limited number of successful one-stage approaches have been described

Jeroense KTV, Kuiper JWP, Colen S, Schade RP, Saouti R. One-stage revision in two cases of *Salmonella* prosthetic hip infection. *World J Clin Cases* 2014; 2(7): 304-308 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/304.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.304>

### INTRODUCTION

*Salmonella* infections are usually associated with food consumption, specifically raw egg and related products, which account for at least one third of all outbreaks in the United States<sup>[1]</sup>. In the Netherlands, a Salmonellosis incidence of around 300/100000 is seen, mostly manifesting as gastroenteritis<sup>[2]</sup>. Although a general decline in human Salmonellosis has occurred in the last two

decades<sup>[1,2]</sup>, because of demographic changes, the excess mortality due to *Salmonella* infections was predicted to double in the next 50 years<sup>[3]</sup>.

The same demographic changes, i.e. the relatively and absolutely growing elderly population, will account for an increase in the number of total hip arthroplasties (THAs), and subsequently prosthetic joint infections (PJIs) of these hips<sup>[4]</sup>. PJI occurs in around 1%-2% of all THAs<sup>[5]</sup>, and is one of the most severe and costly complications, usually requiring additional surgery, a prolonged hospital stay and administration of antibiotic agents, and (temporary) decreased function and quality of life<sup>[6,7]</sup>.

PJI caused by *Salmonella* is nevertheless rare: only 28 patients (30 joints) have been described in the literature, of which 20 were prosthetic hip infections<sup>[8]</sup>. In the case of *Salmonella typhi*, the most common serotype, the prevalence of involvement of the bones or joints is only 1% or even less<sup>[9]</sup>. A higher frequency of *Salmonella* infections is seen in patients with sickle cell disease, systemic lupus erythematosus (SLE) and other immunocompromised states<sup>[5,10]</sup>, and in children aged under five<sup>[3,11,12]</sup>. Most cases of *Salmonella* joint infections are caused by hematogenous spread<sup>[5,13]</sup>.

An aggressive approach with two-stage revision is advised in cases of *Salmonella* PJI, because of the bacterial virulence<sup>[8]</sup>.

We describe two cases of *Salmonella* prosthetic hip infection, both occurring within one month of each other, treated with one-stage revision.

## CASE REPORT

### Case 1

A 68-year-old female patient visited our outpatient clinic for severe osteoarthritis of the right hip. Her medical history further included clubfoot correction in her early youth, pneumonectomy for carcinoid tumor, hypophysectomy after pituitary adenoma, hypertension, renal failure grade 3, heart failure grade 3, atrial flutter and a stroke. She was using anticoagulants (acenocoumarol) and steroids (hydrocortisone) among other medication. She also mentioned an allergy for cephalosporins. In accord with the patient THA was planned.

Four days prior to surgery, her anticoagulants were replaced by therapeutic low molecular weight heparins (LMWH, nadroparin 5700 IE two times daily). Peri-operatively, prophylactic clindamycin was administered. A non-cemented Trident cup with polyethylene insert (Trident system, Stryker Orthopaedics, Mahwah, New Jersey, United States) and a cemented Exeter stem (Exeter Total Hip system, Stryker Orthopaedics, Mahwah, New Jersey, United States) using Simplex P bone cement with Tobramycin (1 g tobramycin, Stryker Orthopaedics, Mahwah, New Jersey, United States) were implanted.

After an uncomplicated rehabilitation course of 5 d the patient was discharged. However, she was readmitted at her local hospital just 2 d later, with anemia (hemoglobin of 4.1 mmol/L, reference value 7.5-10.0 mmol/L). She was given 2 units of packed red blood cells (PRBC)

and was transferred to our center.

Due to a sudden onset of right flank pain and elevated liver enzymes, an abdominal computed tomography was performed, which revealed a retroperitoneal hematoma, possibly with ongoing bleeding. Administration of nadroparin was discontinued, the patient received another 4 units of PRBC and after fluid resuscitation her hemodynamic status remained stable.

Gram staining of joint aspirate and superficial wound cultures revealed gram negative rods, while blood parameters showed a C-reactive protein (CRP of 334 mg/L (reference value < 8 mg/L). DAIR (debridement, antibiotics, irrigation and retention) was performed soon after. Intra-operatively 5 cultures were gathered, the insert and prosthetic head were exchanged, and 3 resorbable gentamicin sponges (130 mg gentamicin per sponge; Garacol, EUSA Pharma, Oxford, United Kingdom) were left in the surgical area. Afterwards, intravenous ciprofloxacin was started at 400 mg 3 times daily combined with intravenous vancomycin 1000 mg once daily.

After one week the cultures taken intra-operatively yielded Group E *Salmonella* species. At that moment the vancomycin was stopped while intravenous ciprofloxacin was continued for another week. This was followed by 4 wk of 750 mg ciprofloxacin orally twice a day. Then, due to her existing renal failure, the dosage of ciprofloxacin was lowered to 500 mg twice a day for one week and 500mg once a day for yet another week.

Due to sanguinous wound drainage, the dosage of nadroparin was changed to the prophylactic dosage (0.3 mL = 2850 IE, once a day). Because of the continuing wound drainage and increasing CRP from 119 to 147 mg/L, it was decided to proceed with a one-stage revision, nearly two months after the initial arthroplasty.

Intra-operatively, all components and cement were removed and the wound was thoroughly debrided and irrigated. Subsequently the medullary canal was reamed and an uncemented Restoration Modular stem (Restoration Modular System, Stryker Orthopaedics, Mahwah, New Jersey, United States) and Trident cup were inserted. Antibiotic treatment was continued with ciprofloxacin, according to the antibiogram from the cultures taken previously.

A subsequent debridement, antibiotics and implant retention (DAIR) procedure was performed because of persistent leakage, which appeared to be due to a fracture of the proximal femur around the stable stem. After wiring of this fracture and after exchanging the insert and prosthetic head of THA again, the patient recovered quickly and wound leakage ceased.

The 5 cultures taken during the one-stage revision, as well as the 2 cultures from the second DAIR procedure, all taken during antibiotic treatment, turned out negative. After the second DAIR procedure oral ciprofloxacin dose was administered at 750 mg 2 times daily again. After 5 wk this was lowered back to 500 mg once a day (because of the renal failure).

At the first follow up, two months since the one-stage revision, the patient was walking with a walker. The wound still showed a little redness, but CRP had declined

to 26 mg/L and erythrocyte sedimentation rate (ESR) was 43 mm/h (reference value < 20 mm/h).

At 5 mo follow-up, antibiotic treatment was stopped, the wound showed no signs of infection and ESR had normalized.

## Case 2

A 59-year-old man presented at the emergency department with pain in the right groin since four days, along with a progressive fever and nausea. At the age 16 he underwent screw osteosynthesis, because of epiphysiolysis capitis femoris, which was followed by THA in 1999 at the age of 46. No other comorbidities were present.

On physical examination of the hip the patient showed a painless function of 100 degrees flexion, 0 degrees extension, 20 degrees endorotation, and 30 degrees exorotation. Pressure in the groin was painful, however. The psoas sign was negative.

Additional blood tests showed a C-reactive protein of 193 mg/L and ESR 23 mm/h. On X-ray the prosthetic head appeared not to be centered (sign of polyethylene wear), though there were no signs of loosening of the prosthesis. Ultrasound of the groin revealed a 5.5 cm × 7.5 cm abscess. Aspiration produced some drops of pus, along with serosanguinous synovial fluid. After culturing the aspirated fluid revealed *Salmonella enteritidis*.

Antibiotic treatment was postponed until after intra-operative cultures could be taken, as the patient was not septic.

Six days after admission the patient underwent a one-stage revision. The decision to proceed with a one-stage revision rather than performing a DAIR procedure, was made intra-operatively, when osteolytic lesions were found around the femoral stem. A Trident cup and Omnifit stem (Omnifit system, Stryker Orthopaedics, Mahwah, New Jersey, United States) were inserted. Ciprofloxacin was started at 400 mg intravenously three times daily.

After the operation, CRP declined to 49 mg/L (from 245 mg/L at the day of operation) and the incision showed no redness or wound drainage. All 5 cultures obtained intra-operatively showed *Salmonella enteritidis*. The patient was discharged with a regimen of oral ciprofloxacin (750 mg twice a day) after having received ciprofloxacin intravenously for 8 d.

At follow up, two weeks after discontinuing the antibiotic treatment (3 mo regime in total) and a total of 3 mo after discharge, the patient had no infectious signs or symptoms. Blood results showed a CRP of 5 mg/L, and an ESR of 9 mm/h. At 6 mo follow up the clinical and radiological findings were normal.

## DISCUSSION

PJIs are a severe complication seen in 1%-2% of cases after arthroplasty, causing additional costs and morbidity, including serious impairment in quality of life for the patient<sup>[7]</sup>. PJI due to *Salmonella* is especially uncommon.

Although most patients having a *Salmonella* infection are suffering from gastro-intestinal complaints, in the

presented cases no overt gastro-intestinal symptoms were present (one patient presented only with mild nausea), indicating that PJI by *Salmonella* can occur without general gastro-intestinal complaints as has been previously stated<sup>[12]</sup>.

Even though our cases presented within the same month, the course of infection was different. In the first case, symptoms occurred only 2 wk after initial THA, classifying this as an early infection<sup>[14,15]</sup> suggesting intra-operative contamination, although *Salmonella* usually spreads *via* the hematogenous route<sup>[16]</sup>. Another possibility is a carrier state of *Salmonella* species, however, we did not take fecal cultures to rule this out. Hematogenous spread in the early postoperative phase, although unlikely, is of course also possible.

The second PJI would be classified as a late infection<sup>[14,15]</sup> occurring 13 years after initial surgery.

When comparing this with other case-reports, there seems to be no outspoken trend in time since THA before infection: out of the 20 cases of *Salmonella* PJI in THA, 9 were late (> 24 mo)<sup>[11,14,17-22]</sup>, 5 were delayed (3-24 mo)<sup>[23-26]</sup> and 5 were early infections (< 3 mo)<sup>[10,15,17,27]</sup>. In one case time until infection was not specified<sup>[28]</sup>.

In bone infections the most encountered serotypes of *Salmonella* are *S. typhimurium* (group D) and *S. enteritidis* (group B)<sup>[15]</sup>. In the first case PJI was caused by group E *Salmonella*, which has not been reported before. The other patient's culture turned out to be the more common *Salmonella enteritidis*, reported previously in 5 PJIs of the hip<sup>[8,11,20,23,28]</sup> and in 6 PJIs of the knee<sup>[8,10,23,29-31]</sup>.

For both our patients, the original treatment plan was to perform a DAIR procedure. In the first patient however, DAIR treatment failed, and a one-stage revision was performed.

During surgery in the second patient we proceeded with a one-stage revision rather than a DAIR procedure because of the encountered osteolysis. Because of good previous experiences by the surgeon, we opted for a one-stage revision rather than a two-stage revision. Only one case treated with a one-stage revision has been described before. In that case it was performed instead of the preferred two-stage procedure, because of the patient's comorbidity<sup>[11]</sup>.

In 2004, Zimmerli *et al*<sup>[15]</sup> already set ground rules for choosing between retention or resection of the prosthesis. This choice is based on duration of symptoms, (absence of) prosthetic loosening, tissue status and bacterial susceptibility.

In a recent systematic review by Leonard *et al*<sup>[32]</sup>, functional outcome and reinfection rates were compared between one- and two-stage revision for PJI of the hip. There seems to be a trend toward better functional outcome in single-stage surgery, whereas reinfection rates turn out to be comparable between the two approaches. Besides this, a two-stage revision is associated with a significantly higher morbidity and mortality, and tissue changes associated with a period without a hip implant can lead to important functional deficits after reimplantation<sup>[32]</sup>.



Furthermore, if the selection criteria, mentioned by Zimmerli *et al.*<sup>[15]</sup> and by multiple other articles as summarized in the infectious diseases society of America (IDSA) guidelines<sup>[14]</sup>, are strictly followed, retention and debridement and one-stage revision have high success rates, with less morbidity, in selected patients.

Nevertheless, despite commonly accepted directives and reported good results of one-stage revision in general PJI, both Tóth *et al.*<sup>[11]</sup> and De la Torre *et al.*<sup>[8]</sup> advocate a two-stage approach in patients with a *Salmonella* PJI.

De la Torre *et al.*<sup>[8]</sup> propagate the aggressive treatment approach, because the virulence of *Salmonella* infections, difficulty in re-revision, and results of debridement procedures (based on a meta-analysis of studies published between 1977 and 1999)<sup>[8,33]</sup>. The virulence of *Salmonella* infections will generally cause a quick onset of symptoms. This means patients will present with symptoms quickly, and treatment can be started early (surgically and medically). If the bacterium has good susceptibility, a high cure rate can be expected, just like the guidelines propagate (Osmon 2013)<sup>[14]</sup>.

In a recent study by Papavasileiou *et al.*<sup>[34]</sup>, the antimicrobial resistance of *Salmonella enteritidis* was compared between the planktonic form and the biofilm form in multiple antibiotics<sup>[34]</sup>. It appeared that the best results were obtained with ciprofloxacin and moxifloxacin<sup>[34]</sup>. None of the previously described case-reports in which ciprofloxacin was used reported recurrence of PJI<sup>[11,23,29,30,35]</sup>. This includes the one case treated with a one-stage revision<sup>[11]</sup>.

So far neither of our patients, both treated with a one-stage approach and ciprofloxacin, show signs of reinfection. However, the first patient had undergone a DAIR procedure prior to, and after the one-stage approach, which might have influenced the outcome in a positive way: the IDSA guidelines describe that the thoroughness of debridement positively affects the success rate of a single stage surgery<sup>[14]</sup>, and in our opinion, this might be true for multiple debridements as well.

In conclusion, good results can be achieved with one-stage revision, taking into consideration the guidelines for selecting the right patients<sup>[14,15]</sup>, in combination with the use of appropriate antibiotics with a good activity against the causative micro-organism. One-stage revision is, in selected cases, a better alternative than the two-stage approach, causing less morbidity, less mortality and a much smaller burden of disease for the patient. In our opinion, *Salmonella* PJI could and should be treated as other bacterial PJIs, depending on the factors mentioned in the guidelines, and therefore one-stage revision could also be performed more often in these particular cases.

## COMMENTS

### Case characteristics

Case 1: A 68-year-old female with a history of severe osteoarthritis of the right hip was readmitted with anemia 5 d after right total hip arthroplasty; Case 2: A 59-year-old male with a history of total hip arthroplasty presented at the emergency department with pain in the right groin.

### Clinical diagnosis

Case 1: Anemia and sudden onset of right flank pain shortly after right total hip arthroplasty; Case 2: Mildly declined function of the hip, painful pressure in the groin, along with fever and nausea.

### Differential diagnosis

Case 1: Post-operative bleeding, periprosthetic joint infection, loosening of the prosthesis, periprosthetic fissure or fracture, total hip arthroplasty (THA) dislocation, intraabdominal pathology; Case 2: Periprosthetic joint infection, loosening of the prosthesis, THA dislocation, heterotopic ossification, hernia inguinalis

### Laboratory diagnosis

Case 1: Hemoglobin 4.1 mmol/L; C-reactive protein (CRP) 334 mg/L; elevated liver enzymes; Case 2: CRP 193 mg/L; erythrocyte sedimentation rate 23 mm/h.

### Imaging diagnosis

Case 1: Computed tomography revealed a retroperitoneal hematoma; Case 2: On X-ray the prosthetic head appeared not to be centered, without signs of loosening of the prosthesis, while an ultrasound of the groin revealed a 5.5 cm × 7.5 cm abscess.

### Pathological diagnosis

Case 1: Intraoperatively taken cultures yielded group E *Salmonella* species; Case 2: Joint aspiration fluid revealed *Salmonella enteritidis*.

### Treatment

Case 1: After one failed debridement, antibiotics and implant retention-procedure, one-stage revision was performed followed by ciprofloxacin; Case 2: Because of encountered osteolysis the patient was treated with a one-stage revision, followed by ciprofloxacin.

### Related reports

There is an ongoing debate whether prosthetic joint infection of the hip is best treated by one- or two-stage revision surgery, but also whether *Salmonella* infections should be treated similarly to periprosthetic joint infections due to other bacteria.

### Experiences and lessons

This case report illustrates that one-stage revision of periprosthetic joint infections of the hip can be a successful treatment even when infection is due to *Salmonella* species.

### Peer review

This is a report of two case with a prosthetic joint infection cause by *Salmonella* treated with one-stage revision. The paper is very well presented with a clear message.

## REFERENCES

- 1 Braden CR. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. *Clin Infect Dis* 2006; **43**: 512-517 [PMID: 16838242 DOI: 10.1086/505973]
- 2 RIVM (Netherlands National Institute for Public Health and the Environment), Available from: URL: <http://www.rivm.nl/Onderwerpen/S/Salmonellose>
- 3 Bouwknegt M, van Pelt W, Havelaar AH. Scoping the impact of changes in population age-structure on the future burden of foodborne disease in the Netherlands, 2020-2060. *Int J Environ Res Public Health* 2013; **10**: 2888-2896 [PMID: 23851976 DOI: 10.3390/ijerph10072888]
- 4 Kurtz S, Ong K, Lau E, Mowat F, Halpern M. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Joint Surg Am* 2007; **89**: 780-785 [PMID: 17403800 DOI: 10.2106/JBJS.F.00222]
- 5 Musante DB, Ogden WS. *Salmonella* infection in joint arthroplasty. *Orthopedics* 2004; **27**: 770-772 [PMID: 15315049]
- 6 Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. *N Engl J Med* 2009; **361**: 787-794 [PMID: 19692690 DOI: 10.1056/NEJMc0905029]
- 7 Cahill JL, Shadbolt B, Scarvell JM, Smith PN. Quality of life after infection in total joint replacement. *J Orthop Surg (Hong Kong)* 2008; **16**: 58-65 [PMID: 18453662]
- 8 De la Torre B, Tena D, Arias M, Romanillos O. Recurrent prosthetic joint infection due to *Salmonella enteritidis*: case report and literature review. *Eur J Orthop Surg Traumatol* 2012;

- 22 (Suppl 1): S89-S97 [DOI: 10.1007/s00590-012-0955-6]
- 9 **Huang DB**, DuPont HL. Problem pathogens: extra-intestinal complications of *Salmonella enterica* serotype Typhi infection. *Lancet Infect Dis* 2005; **5**: 341-348 [PMID: 15919620 DOI: 10.1016/S1473-3099(05)70138-9]
- 10 **Day LJ**, Qayyum QJ, Kauffman CA. *Salmonella* prosthetic joint septic arthritis. *Clin Microbiol Infect* 2002; **8**: 427-430 [PMID: 12199853]
- 11 **Tóth K**, Janositz G, Kovács G, Sisák K, Rudner E. Successful treatment of late *Salmonella* infections in total hip replacement - report of two cases. *BMC Infect Dis* 2010; **10**: 160 [PMID: 20529326 DOI: 10.1186/1471-2334-10-160]
- 12 **Oe K**, Wada T, Ohno H, Kushida T, Iida H. *Salmonella* septic arthritis following total knee arthroplasty for rheumatoid arthritis in a patient receiving etanercept. *J Orthop Sci* 2011; **16**: 258-262 [PMID: 21301900 DOI: 10.1007/s00776-011-0023-9]
- 13 **Cohen JL**, Bartlett JA, Corey GR. Extra-intestinal manifestations of *salmonella* infections. *Medicine* (Baltimore) 1987; **66**: 349-388 [PMID: 3306260 DOI: 10.1097/00005792-198709000-00003]
- 14 **Osmon DR**, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR. Executive summary: diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2013; **56**: 1-10 [PMID: 23230301 DOI: 10.1093/cid/cis966]
- 15 **Zimmerli W**, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004; **351**: 1645-1654 [PMID: 15483283 DOI: 10.1056/NEJMra040181]
- 16 **Langenskiöld A**, Riska EB. Haematogenous *salmonella* infection around a metal hip endoprosthesis. *Acta Orthop Scand* 1967; **38**: 220-225 [PMID: 6033415 DOI: 10.3109/17453676708989635]
- 17 **Samra Y**, Shaked Y, Maier MK. Nontyphoid salmonellosis in patients with total hip replacement: report of four cases and review of the literature. *Rev Infect Dis* 1986; **8**: 978-983 [PMID: 3541130 DOI: 10.1093/clinids/8.6.978]
- 18 **Sherman JW**, Conte JE. Ceftriaxone treatment of multidrug-resistant *Salmonella* osteomyelitis. *Am J Med* 1987; **83**: 137-138 [PMID: 3605165 DOI: 10.1016/0002-9343(87)90508-0]
- 19 **Widmer AF**, Colombo VE, Gächter A, Thiel G, Zimmerli W. *Salmonella* infection in total hip replacement: tests to predict the outcome of antimicrobial therapy. *Scand J Infect Dis* 1990; **22**: 611-618 [PMID: 2259871 DOI: 10.3109/00365549009027105]
- 20 **Creisson A**, Martinot C, Fuzibet JG, Taillan B, Verdier JM, Dujardin P. *Salmonella enteritidis* infection at the site of an articular prosthesis. *Presse Med* 1991; **20**: 1290 [PMID: 1832770]
- 21 **Tattevin P**, Crémieux AC, Joly-Guillou ML, Carbon C. First case of *Salmonella hirschfeldii* (paratyphi C) infection of a prosthetic hip. *Clin Microbiol Infect* 1998; **4**: 228-230 [PMID: 11864333 DOI: 10.1111/j.1469-0691.1998.tb00676.x]
- 22 **Fu TS**, Ueng SW. Two-staged revision total hip arthroplasty due to *Salmonella* infection: case report. *Chang Gung Med J* 2001; **24**: 202-207 [PMID: 11355089]
- 23 **Arda B**, Sipahi OR, Yamazhan T, Emircan I, Aksu K, Ulusoy S. *Salmonella enteritidis* related prosthetic joint infection. *West Indian Med J* 2006; **55**: 454-455 [PMID: 17691246 DOI: 10.1590/S0043-31442006000600018]
- 24 **Ahlberg A**, Carlsson AS, Lindberg L. Hematogenous infection in total joint replacement. *Clin Orthop Relat Res* 1978; **69**: 69-75 [PMID: 743846]
- 25 **Ortiz-Neu C**, Marr JS, Cherubin CE, Neu HC. Bone and joint infections due to *Salmonella*. *J Infect Dis* 1978; **138**: 820-828 [PMID: 368264 DOI: 10.1093/infdis/138.6.820]
- 26 **Chong PY**, Sporer SM. Case report: *Salmonella* infection following total hip arthroplasty. *Iowa Orthop J* 2005; **25**: 42-43 [PMID: 16089070]
- 27 **Cheng N**, Mulier JC. *Salmonella* osteomyelitis in total hip replacement. A case report of hematogenous infection from gastro-intestinal tract. *Arch Orthop Trauma Surg* 1982; **99**: 281-283 [PMID: 7092526 DOI: 10.1007/BF00381408]
- 28 **Chen CM**, Lu TC, Lo WH, Chiu FY. *Salmonella* infection in total hip replacement--report of successful reimplantation and review of the literature. *Zhonghua Yixue Zazhi* (Taipei) 1999; **62**: 472-476 [PMID: 10418184]
- 29 **Kobayashi H**, Hall GS, Tuohy MJ, Knothe U, Procop GW, Bauer TW. Bilateral periprosthetic joint infection caused by *Salmonella enterica* serotype Enteritidis, and identification of *Salmonella* sp using molecular techniques. *Int J Infect Dis* 2009; **13**: e463-e466 [PMID: 19269872 DOI: 10.1016/j.ijid.2008.12.015]
- 30 **Madan S**, Abbas D, Jowett RL, Mounce K. *Salmonella enteritidis* infection in total knee replacement. *Rheumatology (Oxford)* 2001; **40**: 112-113 [PMID: 11157155 DOI: 10.1093/rheumatology/40.1.112]
- 31 **Miron D**, Zuker M, Lev-El A. [*Salmonella* prosthetic knee septic arthritis successful retention of the prosthesis with prolonged suppressive therapy]. *Harefuah* 2006; **145**: 261-23, 319 [PMID: 16642625]
- 32 **Leonard HA**, Liddle AD, Burke O, Murray DW, Pandit H. Single- or two-stage revision for infected total hip arthroplasty? A systematic review of the literature. *Clin Orthop Relat Res* 2014; **472**: 1036-1042 [PMID: 24057192 DOI: 10.1007/s11999-013-3294-y]
- 33 **Silva M**, Tharani R, Schmalzried TP. Results of direct exchange or debridement of the infected total knee arthroplasty. *Clin Orthop Relat Res* 2002; **(404)**: 125-131 [PMID: 12439250]
- 34 **Papavasileiou K**, Papavasileiou E, Tseleni-Kotsovoli A, Bersimis S, Nicolaou C, Ioannidis A, Chatzipanagiotou S. Comparative antimicrobial susceptibility of biofilm versus planktonic forms of *Salmonella enterica* strains isolated from children with gastroenteritis. *Eur J Clin Microbiol Infect Dis* 2010; **29**: 1401-1405 [PMID: 20640867 DOI: 10.1007/s10096-010-1015-y]
- 35 **Carlile GS**, Elvy J, Toms AD. *Salmonella* infection of a total knee replacement. *Knee* 2010; **17**: 356-358 [PMID: 19897369 DOI: 10.1016/j.knee.2009.10.003]

P- Reviewer: Drosos GI S- Editor: Song XX L- Editor: A  
E- Editor: Wu HL



## Passage of nasogastric tube through tracheo-esophageal fistula into stomach: A rare event

Ravikiran Shankar Kamble, Rahul Kumar Gupta, Abhaya Gupta, Paras Kothari, K Vishesh Dikshit, Krishnakumar Kesan, Kedar Mudkhedkar

Ravikiran Shankar Kamble, Rahul Kumar Gupta, Abhaya Gupta, Paras Kothari, K Vishesh Dikshit, Krishnakumar Kesan, Kedar Mudkhedkar, Department of Pediatric Surgery, Lokamany Tilak Municipal Medical College and Government Hospital, Maharashtra 400022, India

Author contributions: Kamble RS, Gupta R and Gupta A evaluated the patient and performed the surgery and compiled the manuscript; Kothari P guided for surgery and follow up; Dikshit KV and Mudkhedkar K collected data; Kesan K edited the manuscript.

Correspondence to: Dr. Ravikiran Shankar Kamble, Department of Pediatric Surgery, Lokamany Tilak Municipal Medical College and Government Hospital, Sion, Mumbai, Maharashtra 400022, India. [drkambleravi80@gmail.com](mailto:drkambleravi80@gmail.com)

Telephone: +91-22-24042190

Received: February 16, 2014 Revised: May 14, 2014

Accepted: May 28, 2014

Published online: July 16, 2014

### Abstract

Esophageal atresia with tracheo-oesophageal fistula (TEF) occurs in 1 in 3500 live births. Anorectal malformation is found to be associated with 14% of TEF. Esophageal atresia with TEF is a congenital anomaly which classically presents as excessive frothing from the mouth and respiratory distress. Rarely gastric position of the feeding tube in a case of TEF can be obtained delaying the diagnosis of TEF. We had an uncommon situation where a nasogastric tube reached the stomach through the trachea and tracheo-esophageal fistula, leading to misdiagnosis in a case of esophageal atresia with tracheoesophageal fistula. By using a stiff rubber catheter instead of a soft feeding tube for the diagnosis of esophageal atresia and TEF, such situation can be avoided.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Esophageal atresia; Tracheoesophageal

fistula; Nasogastric tube; Red rubber catheter; Misdiagnosis

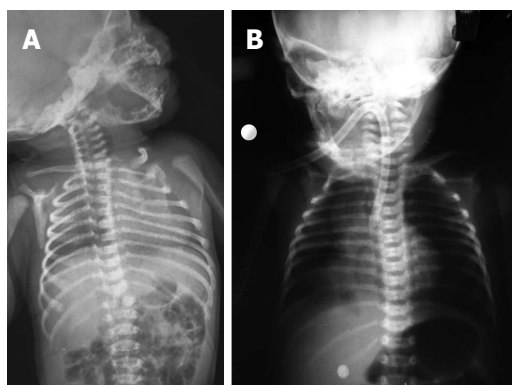
**Core tip:** Esophageal atresia with tracheoesophageal fistula is congenital anomaly which presents as excessive frothing from mouth and respiratory distress. It can be suspected when a nasogastric tube difficult to insert into stomach or radiographically presence coiled nasogastric tube in pharynx. We had an uncommon situation where a nasogastric tube reached the stomach through the trachea and tracheo-esophageal fistula, leading to misdiagnosis in a case of esophageal atresia with tracheoesophageal fistula. Similar clinical situations can be avoided by using a stiff rubber catheter instead of a soft feeding tube for the diagnosis of esophageal atresia and tracheo-oesophageal fistula.

Kamble RS, Gupta R, Gupta A, Kothari P, Dikshit KV, Kesan K, Mudkhedkar K. Passage of nasogastric tube through tracheo-esophageal fistula into stomach: A rare event. *World J Clin Cases* 2014; 2(7): 309-310 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/309.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.309>

### INTRODUCTION

Esophageal atresia with tracheoesophageal fistula (TEF) is a congenital anomaly which classically presents as excessive frothing from the mouth and respiratory distress. It is diagnosed by inability to pass a catheter into the stomach which usually gets stuck at 10 to 12 cm from the mouth. We had an uncommon situation where a nasogastric tube reached the stomach through the trachea and tracheo-esophageal fistula, leading to misdiagnosis in a case of esophageal atresia with tracheoesophageal fistula.





**Figure 1 Radiograph.** A: Showing tip of nasogastric tube in stomach; B: Showing red rubber catheter in upper esophageal pouch.

## CASE REPORT

A one day old, full term, male child (2.7 kg) was referred from a peripheral Hospital as a case of imperforate anus with cleft lip and palate. At the peripheral hospital a 7 Fr nasogastric tube was inserted which had bilious aspirate. A chest X-ray showed the nasogastric tube in the stomach (Figure 1A). The baby had excessive oral secretions and bilateral chest crepitations along with cleft lip and palate. Prone crosstable X-ray was suggestive of high anorectal malformation. The patient was taken for diverting colostomy.

During intubation the anesthetist noticed that the nasogastric tube was passing through the trachea. Findings were reconfirmed by laryngoscopy and laryngotracheo-esophageal cleft was ruled out. We tried to pass a number 10 stiff red rubber catheter through esophagus but were unable to pass beyond 10 cm. A diagnosis of esophageal atresia (EA) with tracheoesophageal fistula (TEF) was suspected and X-ray chest was repeated with the red rubber catheter *in situ* which confirmed the diagnosis (Figure 1B). Right posterolateral thoracotomy was done. The patient had tracheoesophageal fistula type III with a Wide fistula, short upper pouch and a long gap. The fistula was ligated and a Left sided cervical oesophagostomy with feeding gastrostomy was done. Transverse colostomy was done for imperforate anus.

The baby was shifted on ventilator and was successfully weaned off at post operative day 5. The baby is now 4 mo old and is doing well on follow up. He is awaiting definitive management for anorectal malformation, esophageal replacement, cleft lip and cleft palate repair.

## DISCUSSION

Esophageal atresia with TEF occurs in 1 in 3500 live births<sup>[1]</sup>. Anorectal malformation is found to be associated with 14% of TEF<sup>[1]</sup>. TEF classically presents as excessive frothing from the mouth and regurgitation, choking and coughing after feed. There is a routine prac-

tice of passing a 5Fr or 6Fr infant feeding tube through the nose in patients of imperforate anus to decompress the obstructed intestinal tract and also to rule out associated esophageal atresia. If the radiograph shows a coiled catheter in the upper esophageal pouch one can suspect esophageal atresia.

Rarely gastric position of the feeding tube in a case of TEF can be obtained delaying the diagnosis of TEF<sup>[2,3]</sup>. In this case we did not suspect an esophageal atresia as the patient came with the IFT in the stomach with bilious aspirate and this had been confirmed radio graphically. The feeding tube could reach the stomach from the upper pouch then into the tracheal route and then through the TEF. A peculiar pathological anatomy and a weak cough reflex made this occurrence possible. Only three such cases have been reported so far in literature<sup>[2,3]</sup>. If an esophageal atresia is suspected on clinical grounds the ideal test would be to pass a stiff red rubber catheter through the mouth and note the resistance. Radiographs should be taken with a red rubber catheter *in situ* which will show the position of the catheter tip. Barium esophagography is not usually advised due to the risk of aspiration pneumonia. Instead a small amount of air can be used as contrast<sup>[1]</sup>.

In a conclusion, all neonates with excessive frothing and respiratory distress should be evaluated for TEF. Similar clinical situations can be avoided by using a stiff rubber catheter instead of a soft feeding tube for the diagnosis of EA and TEF.

## COMMENTS

### Case characteristics

Authors' came across a uncommon situation, a neonate referred from peripheral hospital with nasogastric tube passed through tracheo-esophageal fistula into the stomach.

### Clinical diagnosis

The baby had excessive oral secretions and bilateral chest crepitations along with cleft lip and palate.

### Differential diagnosis

Prone crosstable X-ray was suggestive of high anorectal malformation.

### Peer review

The case report is interesting and well written, the field of the report is focused on pediatric.

## REFERENCES

- 1 **Carroll MH**, Arnold GC. Congenital anomalies of Esophagus. In: Arnold GC, Editor. *Pediatric Surgery*, 7th Edition. Philadelphia: Elsevier Saunders, 2012: 893-918
- 2 **Hombalkar NN**, Dhanawade S, Hombalkar P, Vaze D. Esophageal atresia with tracheo-esophageal fistula: Accidental transtracheal gastric intubation. *J Indian Assoc Pediatr Surg* 2009; **14**: 224-225 [PMID: 20419027 DOI: 10.4103/0971-9261.59608]
- 3 **Rani RS**, Shenoi A, Nagesh NK, Ramachandra C. Inadvertent passage of infant feeding tube into the stomach through a tracheo-esophageal fistula. *Indian Pediatr* 2000; **37**: 96-97 [PMID: 10745397]

P- Reviewer: Lisotti A S- Editor: Wen LL L- Editor: A  
E- Editor: Wu HL





## Diagnostic pitfall of sebaceous gland metaplasia of the esophagus

King-Wah Chiu, Cheng-Kun Wu, Long-Sheng Lu, Hock-Liew Eng, Shue-Shian Chiou

King-Wah Chiu, Cheng-Kun Wu, Long-Sheng Lu, Shue-Shian Chiou, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University, College of Medicine, Kaohsiung 83305, Taiwan

Hock-Liew Eng, Department of Pathology, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University, College of Medicine, Kaohsiung 83305, Taiwan

Author contributions: Chiu KW designed the report; Chiou SS was attending doctors for the patients; Eng HL performed pathological examinations; Wu CK and Lu LS were performed image diagnosis; Chiu KW and Chiou SS organized the report; and Chiu KW wrote paper.

Correspondence to: King-Wah Chiu, MD, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kaohsiung Chang Gung Memorial Hospital, Chang Gung University, College of Medicine, 123, Ta-Pei Road, Niao-Sung District, Kaohsiung 83305, Taiwan. [kwchiu@adm.cgmh.org.tw](mailto:kwchiu@adm.cgmh.org.tw)  
Telephone: +886-7-73171238190 Fax: +886-7-7336856

Received: October 12, 2013

Revised: March 6, 2014

Accepted: May 16, 2014

Published online: July 16, 2014

### Abstract

We investigated the sebaceous gland metaplasia (SGM) of the esophagus and clarified the evidence of misdiagnosis and its diagnosis pitfall. Cases of pathologically proven SGM were enrolled in the clinical analysis and reviewed description of endoscope. In the current study, we demonstrated that SGM is very rare esophageal condition with an incidence around 0.00465% and an occurrence rate of 0.41 per year. There were 57.1% of senior endoscopists identified 8 episodes of SGM. In contrast, 7.7% of junior endoscopists identified SGM in only 2 episodes. Moreover, we investigated the difference in endoscopic biopsy attempt rate between the senior and junior endoscopist ( $P = 0.0001$ ). The senior endoscopists had more motivation to look for SGM than did junior endoscopists ( $P = 0.01$ ). We concluded that SGM of the esophagus is rare condition that is easily and not recognized in endoscopy studies omitting

pathological review.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

**Key words:** Esophagus; Sebaceous gland; Metaplasia; Endoscopy; Endoscopist

**Core tip:** Cases of pathologically proven sebaceous gland metaplasia (SGM) of the esophagus were enrolled in the clinical analysis and reviewed the description of endoscope. It is very rare esophageal condition with an incidence around 0.00465% and an occurrence rate of 0.41 per year. There are 57.1% of senior endoscopists identified 8 episodes of SGM and 7.7% of junior endoscopists identified SGM in only 2 episodes. The senior endoscopist had more motivation to look for SGM than did junior endoscopists. We concluded SGM of the esophagus is rare condition that is easily and not recognized in endoscopy studies omitting pathological review.

Chiu KW, Wu CK, Lu LS, Eng HL, Chiou SS. Diagnostic pitfall of sebaceous gland metaplasia of the esophagus. *World J Clin Cases* 2014; 2(7): 311-315 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v2/i7/311.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v2.i7.311>

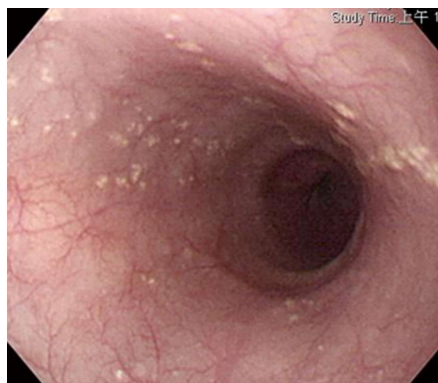
### INTRODUCTION

Sebaceous gland metaplasia (SGM) tends to be found incidentally during autopsy or esophageal resection<sup>[1,2]</sup>. From the point of differential diagnosis of esophageal lesions, SGM becomes of scientific interest during endoscopic studies. Endoscopists should take the first look at unusual lesions<sup>[3]</sup>. Although several reports have attempted to determine whether SGM is the result of a metaplastic process or a congenital anomaly, histological examination of endoscopic biopsies is traditionally used to make a pathological diagnosis in clinical practice<sup>[2]</sup>. Biopsied tis-

**Table 1** The clinical profile, pre-biopsy diagnosis, biopsy diagnosis, and pathological review of the 6 cases of sebaceous gland metaplasia

Case	Sex	Age	1 <sup>st</sup> visit	Source	Symptom	1 <sup>st</sup> ESP	1 <sup>st</sup> End. Dx	Biopsy	Review	2 <sup>nd</sup> ESP	2 <sup>nd</sup> End. Dx	Biopsy	Review
1	M	46	1998	Outpatient	Peptic	Senior 1	Xanthoma	SGM	+				
2	M	71	1999	Health screen	Denied	Senior 1	SGM	SGM	+				
3	M	60	2002	Outpatient	Peptic	Senior 1	SGM	SGM	+	Senior 2	Xanthoma	-	-
4	M	65	2002	Health screen	Denied	Senior 2	Papilloma	SGM	-	Senior 4	Papilloma	-	-
5	F	49	2008	Health screen	Denied	Junior 2	Candidiasis	SGM	-	Junior 1	Negative	-	-
6	F	55	2012	Health screen	Denied	Senior 3	Candidiasis	-	-	Senior 3	Xanthoma	SGM	+

ESP: Endoscopist; End. Dx: Endoscopic diagnosis; Review: Pathological review; SGM: Sebaceous gland metaplasia.



**Figure 1** Sebaceous gland metaplasia in the esophagus. Numerous tiny round yellowish lesions clustering distribution at the submucosa of the middle and lower esophagus.

sues can be taken for histological and marker studies of SGM<sup>[4,5]</sup>. Due to the benign nature of SGM-containing endoscopic readings<sup>[6]</sup>, the diagnosis is commonly missed when tissue biopsies are not reviewed. Therefore, the aim of this study was to clarify the incidence of SGM and identify the hallmarks of SGM in endoscopic review.

## CASE REPORT

### Method

From January 1, 1998 to June 30, 2012, a total of 215046 patients underwent endoscopic procedures with 35302 tissue biopsies taken by 33 endoscopists in the endoscopy unit of Kaohsiung Chang Memorial Hospital. The endoscopic procedures included 864 esophagoscopies and 214182 gastroscopies (include 650 nasoendoscopies). Cases of endoscopic ultrasound of the upper gastrointestinal tract, endoscopic retrograde cholangiopancreatography, and double-balloon enteroscopy *via* an oral route were excluded from this study. The cases of pathologically proven SGM were enrolled in the clinical analysis and the endoscopic characteristics were reviewed.

By definition, an endoscopist with more than 20 years of experience was defined as a senior endoscopist, while an endoscopist with less than 20 years of experience was defined as a junior endoscopist. Accordingly, 7 senior and 26 junior endoscopists were identified. The senior endoscopists performed 110022 (51.2%) endoscopic studies and 16012 (14.5%) endoscopic biopsies, and the junior

endoscopists performed 105046 (48.8%) endoscopic studies and 19290 (18.4%) endoscopic biopsies. Histological examinations of the endoscopic biopsies were performed by an experienced pathologist.

Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL, United States). Comparisons of the endoscopic biopsy parameters and SGM frequency between the senior and junior endoscopists were performed using the  $\chi^2$  test and Fisher's exact test. *P* values less than 0.05 were considered statistically significant.

### Results

Among the 215046 endoscopic studies performed during the study period, there were 6 cases of pathologically documented SGM in 10 endoscopic studies (Table 1). The incidence and occurrence rates were 0.00465% was 0.41 per year, respectively. The male to female ratio was 3:1, and the mean age was 57.6 years (range 46-71 years). Four of the 6 cases with SGM came from a health screening center, and the other 2 came from outpatient clinics. All of the cases had numerous tiny yellowish lesions with histopathological examination identified heterotopic sebaceous gland located in the middle-lower esophagus (Figure 1).

The primary endoscopic impression was xanthoma (by senior 1, 2 and 3) in 3 cases, candidiasis (by junior 2 and senior 3) in 2 cases, papilloma (by senior 2 and senior 4) in 2 cases, and a negative description (by junior 1) in 1 case (Table 1). No cases of SGM were recognized by the senior endoscopists 1, 2, 3 and 4 or junior endoscopists 1 and 2 in the primary endoscopic study, and no cases (0/3) of SGM were recognized by senior endoscopists 2 or 4 or junior endoscopist 1 in the secondary endoscopic impression without tissue pathology review (Table 1). The rate improved after pathological review, there was a 100% (2/2) positive diagnosis experienced in the case 2 and case 3 in the primary endoscopic impression by senior 1, and only 3.03% (1/33) of our endoscopists did it.

Among the 6 pathologically confirmed cases of SGM, 5 were diagnosed in the first endoscopic study by tissue biopsy, while the sixth case was diagnosed in the second endoscopic study by tissue biopsy (Table 1). Among the 7 senior endoscopists who performed 110022 (51.2%) endoscopies, 16012 (14.5%) endoscopic biopsies were performed, an average of 2287 biopsies per senior en-

**Table 2** Rates of identification of sebaceous gland metaplasia by the senior and junior endoscopists

ESP (n)	End. study	End. biopsy (%)	End. biopsy/ESP	No. of ESP identifying SGM (%)	Identification episodes
Senior (7)	110022	16012 (14.5) <sup>1</sup>	2287.4	4 (57.1) <sup>2</sup>	8 <sup>3</sup>
Junior (26)	105024	19290 (18.4) <sup>1</sup>	741.9	2 (7.7) <sup>2</sup>	2 <sup>4</sup>
Total (33)	215046	35302 (16.4)	1069.8	6 (18.2)	10

<sup>1</sup>P = 0.0001 using the  $\chi^2$  test; <sup>2</sup>P = 0.01 using Fisher's exact test; <sup>3</sup>senior 1 in 3, senior 2 in 2, senior 3 in 2, and senior 4 in 1 meet; <sup>4</sup>junior 1 in 1 and junior 2 in 1 case of identified SG. SGM: Sebaceous gland metaplasia; ESP: Endoscopist; End: Endoscopic.

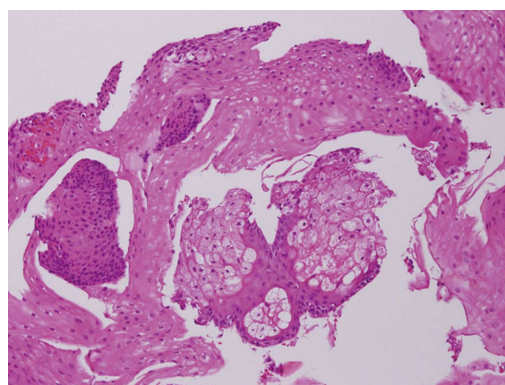
doscopist and 4 (57.1%) cases diagnosed of SGM in 8 episodes by senior 1 of 3 times, senior 2 of 2 times, senior 3 of 2 times, and senior 4 in 1 time. In contrast, of the 105024 (48.8%) endoscopies performed by 26 junior endoscopists, 19290 (18.4%) endoscopic biopsies were performed, an average of 741.9 biopsies per junior endoscopists, and identified-2 (7.7%) cases SGM in only 2 episodes by junior 1 and junior 2 respectively. Significantly fewer endoscopic biopsies were performed by the senior endoscopists than by the junior endoscopists ( $P = 0.0001$ ), and significantly more cases of HSGM were identified by senior endoscopists than by junior endoscopists ( $P = 0.01$ ) (Table 2).

Endoscopic biopsy showed multiple light yellow plaques 2-5 mm in diameter with clustering distribution that embedded the surface of the esophagus (Figure 1). In 100% (6/6) of cases, sebaceous glands were located at the lower to middle esophagus. Pathological analysis revealed stratified squamous epithelium with lobules of sebaceous glands abutting the lower epithelium. No associated polymorphic nuclear or cellular infiltration was noted (Figure 2).

## DISCUSSION

The present study demonstrated that SGM is a very rare esophageal condition with an incidence around 0.00465% and an occurrence rate of 0.41 per year. There was no doubt of the pathological diagnosis of SGM<sup>[7,8]</sup>, but endoscopic biopsy should make an important impact on the incidence of SGM. In the real world, endoscopic biopsy is performed by endoscopists for 2 possible reasons: a suspected malignant lesion, or a lesion that is difficult to identify under endoscopic study. Such lesions look benign, especially in narrow band imaging<sup>[9,10]</sup>, we believed that endoscopists does not perform biopsy, if they macroscopically diagnosed the lesion for benign. It may be a reason for the incidence of SGM is likely underestimated.

In the current study, senior endoscopist 1 encountered SGM 3 times (cases 1, 2 and 3) in 1998, 1999 and 2002. After the endoscopic biopsy and pathological review in the first case, a 100% (2/2) endoscopic diagnosis rate of senior endoscopist 1 was noted in cases 2 and 3. In contrast, senior endoscopist 2 missed an endoscopic diagnosis in a xanthoma due to a lack of pathological re-

**Figure 2** Esophageal squamous epithelial with sebaceous glands (HE stained  $\times 400$ ).

view despite the first case being proven. Senior endoscopist 2 also missed an endoscopic diagnosis in a papilloma that was biopsied but not pathologically reviewed.

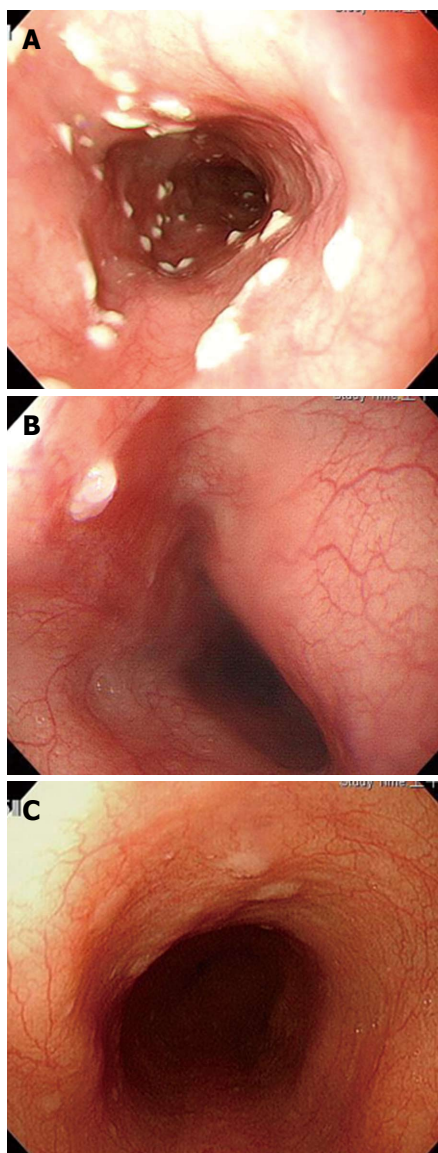
In the meantime, senior endoscopist 4 also missed an endoscopic diagnosis in a papilloma in case 4 due to lack of pathological review. In SGM cases 5 and 6, the primary endoscopic diagnosis of candida esophagitis was made by junior endoscopist 2 and senior endoscopist 3. Junior endoscopist 2 had performed an endoscopic biopsy in case 5, but senior endoscopist 3 did not in case 6.

Due to the lack of a pathological review, junior endoscopist 1 made a negative endoscopic diagnosis in case 5 during a scheduled health screen. In contrast, senior endoscopist 2 also missed the endoscopic diagnosis in SGM case 6 due to the lack of a response to candida infection treatment in the follow-up clinic. Because endoscopic biopsy and pathological review were both performed, 6 cases of SGM were documented in our series.

Our series showed that 66.7% of the cases of SGM came from health screening centers, and for these cases the pathology reports were not returned to the ordering endoscopist. In addition, the interpreting doctor at the health screening center was not the original endoscopist, and the patients did not return to the outpatient clinic due to there being no evidence of malignancy. To overcome the diagnostic underestimation demonstrated here, endoscopists need to actively follow up each pathological report after an endoscopic biopsy, or a computer management system should send the final pathological report to the original endoscopist on a weekly basis. Therefore, the accurate diagnosis of SGM requires both endoscopic biopsy and pathological review<sup>[11]</sup>.

Studies have reported that candida infection (Figure 3A) is the most common endoscopic diagnosis to appear as SGM<sup>[7,8]</sup>. In the present study (Table 1), 10 episodes of SGM were found at a rate of 3 in xanthomas, 2 in candida infections, and 2 in papillomas (Figure 3B). The same situation was the first impression as candida infection of the esophagus but no medication response to it in our case 6<sup>[12]</sup>. SGM case 6 was determined by a subsequent pathological review. For most endoscopists, the first impression would be glycogenic acanthosis (Figure 3C), potentially with minor atypical features. All of the





**Figure 3 Photograph.** A: Candida infection of the esophagus. Multiple small brightly whitish elevated patches at the upper and middle esophagus; B: Papilloma of the esophagus. A single round whitish elevated nodule at the middle esophagus; C: Glycogenic acanthosis of the esophagus. Some small round lucent to lightly whitish nodules at the upper and middle esophagus.

above situations belong to the benign nature of the etiology of SGM, and the symptoms of SGM are not alarming enough to warrant an endoscopic biopsy. Therefore, missing endoscopic diagnosis occurs easily in situations in which both endoscopic biopsy and pathological review are not both performed.

An interesting finding in the current study was the difference in endoscopic biopsy attempts between the senior and junior endoscopists. More cases were examined by the senior endoscopists than by the junior endoscopists (51.2% *vs* 48.8%), in contrast to the endoscopic biopsy rate (14.5% *vs* 18.4%). The large difference in the number of biopsies taken by senior (21.2%) *vs* junior endoscopists (78.8%) might give a false impression in statistical analysis. Senior endoscopists had a 3-fold higher number of endoscopic biopsies compared to the junior

endoscopists (2287.4 *vs* 741.9 endoscopic biopsies per endoscopist). Senior endoscopists had more motivation to look for SGM than the junior endoscopists. Anyway, SGM is a very rare endoscopically indistinct benign finding in the esophagus. The histogenesis of ectopic sebaceous glands in the esophagus is unknown; whilst it could be a congenital abnormality, a majority of authors defined it like an acquired metaplastic process. No malignant transformation has yet been reported. From the pathologists' point of view an inflammatory or neoplastic process has to be excluded as the cause of the non-distinctive endoscopic findings<sup>[8,13]</sup>. In our recent study found that senior endoscopists are more interested than junior endoscopist, in look for the esophagus SGM cells as well as the attempt for endoscopic biopsy<sup>[14]</sup>.

In conclusion, asymptomatic esophageal SGM is a rare condition that occurs in old age (> 50 years) and is male dominant. A differential diagnosis of a benign non-inflammatory nature should be kept in mind in daily practice with endoscopic biopsies and pathological review but may be never seen clinically.

## COMMENTS

### Case characteristics

Sebaceous gland metaplasia of esophagus tends to be found incidentally because of the usually no symptoms.

### Clinical diagnosis

It is hard to make a clinical diagnosis due to the silent disease.

### Differential diagnosis

Endoscopic biopsy with pathological review is very important for the differential diagnosis from the other esophageal pathologies.

### Laboratory diagnosis

Histological examination with HE stained showed characteristic sebaceous differentiation.

### Imaging diagnosis

Endoscopy demonstrated numerous tiny rounded, elevated, white-yellowish lesions distributed at the middle and lower esophagus.

### Pathological diagnosis

Histopathological examination identified numerous sebaceous glands were located in the lamina propria, revealed lobules of cells that showed characteristic sebaceous differentiation.

### Treatment

Because there were no esophageal symptoms or/and eating problems, the patient did not require endoscopic surgery or other treatment.

### Term explanation

A sebaceous cell of presumed ectodermal origin, in the esophageal mucosa, which is of endodermal origin, is of scientific interest. Different theories may explain the existence of this peculiarity by sebaceous gland metaplasia is the most plausible.

### Experiences and lessons

Sebaceous gland metaplasia tends to be found incidentally during autopsy or esophageal resection.

### Peer review

This is a well designed and well written case report which may be interesting for gastroenterologists and other clinicians.

## REFERENCES

- 1 Zak FG, Lawson W. Sebaceous glands in the esophagus. First case observed grossly. *Arch Dermatol* 1976; **112**: 1153-1154 [PMID: 952538 DOI: 10.1001/archderm.1976.01630320059018]
- 2 Nakanishi Y, Ochiai A, Shimoda T, Yamaguchi H, Tachi-



- mori Y, Kato H, Watanabe H, Hirohashi S. Heterotopic sebaceous glands in the esophagus: histopathological and immunohistochemical study of a resected esophagus. *Pathol Int* 1999; **49**: 364-368 [PMID: 10365859 DOI: 10.1046/j.1440-1827.1999.00874.x]
- 3 **Bambirra EA**, de Souza Andrade J, Hooper de Souza LA, Savi A, Ferreira Lima G, Affonso de Oliveira C. Sebaceous glands in the esophagus. *Gastrointest Endosc* 1983; **29**: 251-252 [PMID: 6618133 DOI: 10.1016/S0016-5107(83)72605-2]
- 4 **Nakada T**, Inoue F, Iwasaki M, Nagayama K, Tanaka T. Ectopic sebaceous glands in the esophagus. *Am J Gastroenterol* 1995; **90**: 501-503 [PMID: 7532913]
- 5 **van Esch E**, Brennan S. Sebaceous gland metaplasia in the oesophagus of a cynomolgus monkey (*Macaca fascicularis*). *J Comp Pathol* 2012; **147**: 248-252 [PMID: 22305858 DOI: 10.1016/j.jcpa.2011.11.201]
- 6 **Grube-Pagola P**, Vicuña-González RM, Rivera-Salgado I, Alderete-Vázquez G, Remes-Troche JM, Valencia-Romero AM. [Ectopic sebaceous glands in the esophagus. Report of three cases]. *Gastroenterol Hepatol* 2011; **34**: 75-78 [PMID: 21339017 DOI: 10.1016/j.gastrohep.2010.10.016]
- 7 **Thalheimer U**, Wright JL, Maxwell P, Firth J, Millar A. Sebaceous glands in the esophagus. *Endoscopy* 2008; **40** Suppl 2: E57 [PMID: 18633905 DOI: 10.1055/s-2007-967059]
- 8 **Marín-Serrano E**, Jaquotot-Herranz M, Casanova-Martínez L, Tur-González R, Segura-Cabral JM. Ectopic sebaceous glands in the esophagus. *Rev Esp Enferm Dig* 2010; **102**: 141-142 [PMID: 20361850 DOI: 10.4321/S1130-01082010000200009]
- 9 **Ide E**, Maluf-Filho F, Chaves DM, Matuguma SE, Sakai P. Narrow-band imaging without magnification for detecting early esophageal squamous cell carcinoma. *World J Gastroenterol* 2011; **17**: 4408-4413 [PMID: 22110267 DOI: 10.3748/wjg.v17.i39.4408]
- 10 **Kawai T**, Takagi Y, Yamamoto K, Hayama Y, Fukuzawa M, Yagi K, Fukuzawa M, Kataoka M, Kawakami K, Itoi T, Moriyasu F, Matsubayashi J, Nagao T. Narrow-band imaging on screening of esophageal lesions using an ultrathin transnasal endoscopy. *J Gastroenterol Hepatol* 2012; **27** Suppl 3: 34-39 [PMID: 22486869 DOI: 10.1111/j.1440-1746.2012.07068.x]
- 11 **Fukuchi M**, Tsukagoshi R, Sakurai S, Kiriya S, Horiuchi K, Yuasa K, Suzuki M, Yamauchi H, Tabe Y, Fukasawa T, Naitoh H, Kuwano H. Ectopic Sebaceous Glands in the Esophagus: Endoscopic Findings over Three Years. *Case Rep Gastroenterol* 2012; **6**: 217-222 [PMID: 22701398 DOI: 10.1159/000338651]
- 12 **Hoshika K**, Inoue S, Mizuno M, Iida M, Shimizu M. Endoscopic detection of ectopic multiple minute sebaceous glands in the esophagus. Report of a case and review of the literature. *Dig Dis Sci* 1995; **40**: 287-290 [PMID: 7851191 DOI: 10.1007/BF02065411]
- 13 **Suttorp AC**, Heike M, Fährndrich M, Reis H, Lorenzen J. Talgdrüsenheterotopie im Ösophagus. *Der Pathologe* 2013; **34**: 162-164 [PMID: 23111754 DOI: 10.1007/s00292-012-1714-5]
- 14 **Chiu KW**, Chiu SS. Endoscopic biopsy as a quality assurance for the endoscopic service. *Plos one* 2013; **11**: e78557 [PMID: 24265698 DOI: 10.1371/journal.pone.0078557]

**P- Reviewers:** Alsolaiman M, Bugaj AM, Huerta-Franco MR

**S- Editor:** Wen LL **L- Editor:** A **E- Editor:** Wu HL





## INSTRUCTIONS TO AUTHORS

### GENERAL INFORMATION

*World Journal of Clinical Cases* (World J Clin Cases, WJCC, online ISSN 2307-8960, DOI: 10.12998) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

#### Aim and scope

The primary task of WJCC is to rapidly publish high-quality Autobiography, Case Report, Clinical Case Conference (Clinicopathological Conference), Clinical Management, Diagnostic Advances, Editorial, Field of Vision, Frontier, Medical Ethics, Original Articles, Clinical Practice, Meta-Analysis, Minireviews, Review, Therapeutics Advances, and Topic Highlight, in the fields of allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, geriatrics and gerontology, hematology, immunology, infectious diseases, internal medicine, obstetrics and gynecology, oncology, ophthalmology, orthopedics, otolaryngology, pathology, pediatrics, peripheral vascular disease, psychiatry, radiology, rehabilitation, respiratory medicine, rheumatology, surgery, toxicology, transplantation, and urology and nephrology.

WJCC is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher.

#### Columns

The columns in the issues of WJCC will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more

than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers); (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, etc.; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in clinical research; (12) Clinical Practice: To briefly report the novel and innovative findings in clinical practice; (13) Meta-Analysis: Covers the systematic review, mixed treatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, e.g., the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in WJCC, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of clinical medicine; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

#### Name of journal

*World Journal of Clinical Cases*

#### ISSN

ISSN 2307-8960 (online)

#### Launch date

April 16, 2013

## Instructions to authors

### Frequency

Monthly

### Editors-in-Chief

**Giuseppe Di Lorenzo, MD, PhD, Professor,** Genitourinary Cancer Section and Rare-Cancer Center, University Federico II of Napoli, Via Sergio Pansini, 5 Ed. 1, 80131, Naples, Italy

**Jan Jacques Michiels, MD, PhD, Professor,** Primary Care, Medical Diagnostic Center Rijnmond Rotterdam, Bloodcoagulation, Internal and Vascular Medicine, Erasmus University Medical Center, Rotterdam, Goodheart Institute and Foundation, Erasmus Tower, Veenmos 13, 3069 AT, Erasmus City, Rotterdam, The Netherlands

**Sandro Vento, MD,** Department of Internal Medicine, University of Botswana, Private Bag 00713, Gaborone, Botswana

**Shuhei Yoshida, MD, PhD,** Division of Gastroenterology, Beth Israel Deaconess Medical Center, Dana 509, Harvard Medical School, 330 Brookline Ave, Boston, MA 02215, United States

### Editorial office

Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director

*World Journal of Clinical Cases*

Room 903, Building D, Ocean International Center,

No. 62 Dongsihuan Zhonglu, Chaoyang District,

Beijing 100025, China

Telephone: +86-10-85381891

Fax: +86-10-85381893

E-mail: [editorialoffice@wjgnet.com](mailto:editorialoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

### Publisher

Baishideng Publishing Group Inc

8226 Regency Drive,

Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

### Instructions to authors

Full instructions are available online at [http://www.wjgnet.com/2307-8960/g\\_info\\_20100722180909.htm](http://www.wjgnet.com/2307-8960/g_info_20100722180909.htm).

### Indexed and Abstracted in

Digital Object Identifier.

## SPECIAL STATEMENT

All articles published in journals owned by the BPG represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

### Biostatistical editing

Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Riddit, 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, *WJCC* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in 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](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 conform 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. Book 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 BPG, 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 insti-

tution 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 ICMJE 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.wjnet.com/esps/>. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS ([http://www.wjnet.com/2307-8960/g\\_info\\_20100722180909.htm](http://www.wjnet.com/2307-8960/g_info_20100722180909.htm)) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to [wjcc@wjnet.com](mailto:wjcc@wjnet.com), or by telephone: +86-10-85381892. 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 ICMJE, 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](mailto: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 on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

### Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/..."), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*,  $6.92 \pm 3.86$  vs  $3.61 \pm 1.67$ ,  $P < 0.001$ ), and 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.

### Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

### Text

For articles of these sections, original articles and brief articles, 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.

### 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. 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 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



## Instructions to authors

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. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  should be noted ( $P > 0.05$  should not be noted). If there are other series of  $P$  values, <sup>c</sup> $P < 0.05$  and <sup>d</sup> $P < 0.01$  are used. A third series of  $P$  values can be expressed as <sup>e</sup> $P < 0.05$  and <sup>f</sup> $P < 0.01$ . Other notes in tables or under illustrations should be expressed as <sup>1</sup>F, <sup>2</sup>F, <sup>3</sup>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<sup>[1,2]</sup>". If references are cited directly in the text, they should be put together within the text, for example, "From references<sup>[19,22-24]</sup>, 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

Please 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/Simple-TextQuery/>, 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 bold-faced 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 bold-faced 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-diarhoea. *Shijie Huaren Xiaobua 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 PMID: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. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

### Books

*Personal author(s)*

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary 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**, Schorffheide AM. Adolescent pregnancy. 2nd ed. Wiczorek 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  $\pm$  SD or mean  $\pm$  SE.

### Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as  $\chi^2$  (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (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)  $6.4 \pm 2.1$  mmol/L; blood CEA mass concentration, *p* (CEA) = 8.6  $24.5 \mu\text{g/L}$ ; CO<sub>2</sub> volume fraction, 50 mL/L CO<sub>2</sub>, not 5% CO<sub>2</sub>; 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 quantum numbers can be found at: [http://www.wjgnet.com/2307-8960/g\\_info\\_20100725073806.htm](http://www.wjgnet.com/2307-8960/g_info_20100725073806.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: *EcoRI*, *HindI*, *BamHI*, *Kho I*, *Kpn I*, *etc.*

Biology: *H. pylori*, *E. coli*, *etc.*

### Examples for paper writing

All types of articles' writing style and requirement will be found in the link: <http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15>

## RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Inc. All rights reserved. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to [esps@wjgnet.com](mailto:esps@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.

### Copyright assignment form

Please download a Copyright assignment form from [http://www.wjgnet.com/2307-8960/g\\_info\\_20100725073726.htm](http://www.wjgnet.com/2307-8960/g_info_20100725073726.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/2307-8960/g\\_info\\_20100725073445.htm](http://www.wjgnet.com/2307-8960/g_info_20100725073445.htm).

### Proof of financial support

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

## STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

## PUBLICATION FEE

WJCC is an international, peer-reviewed, OA 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 and format, 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. Publication fee: 698 USD per article. All invited articles are published free of charge.



Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

