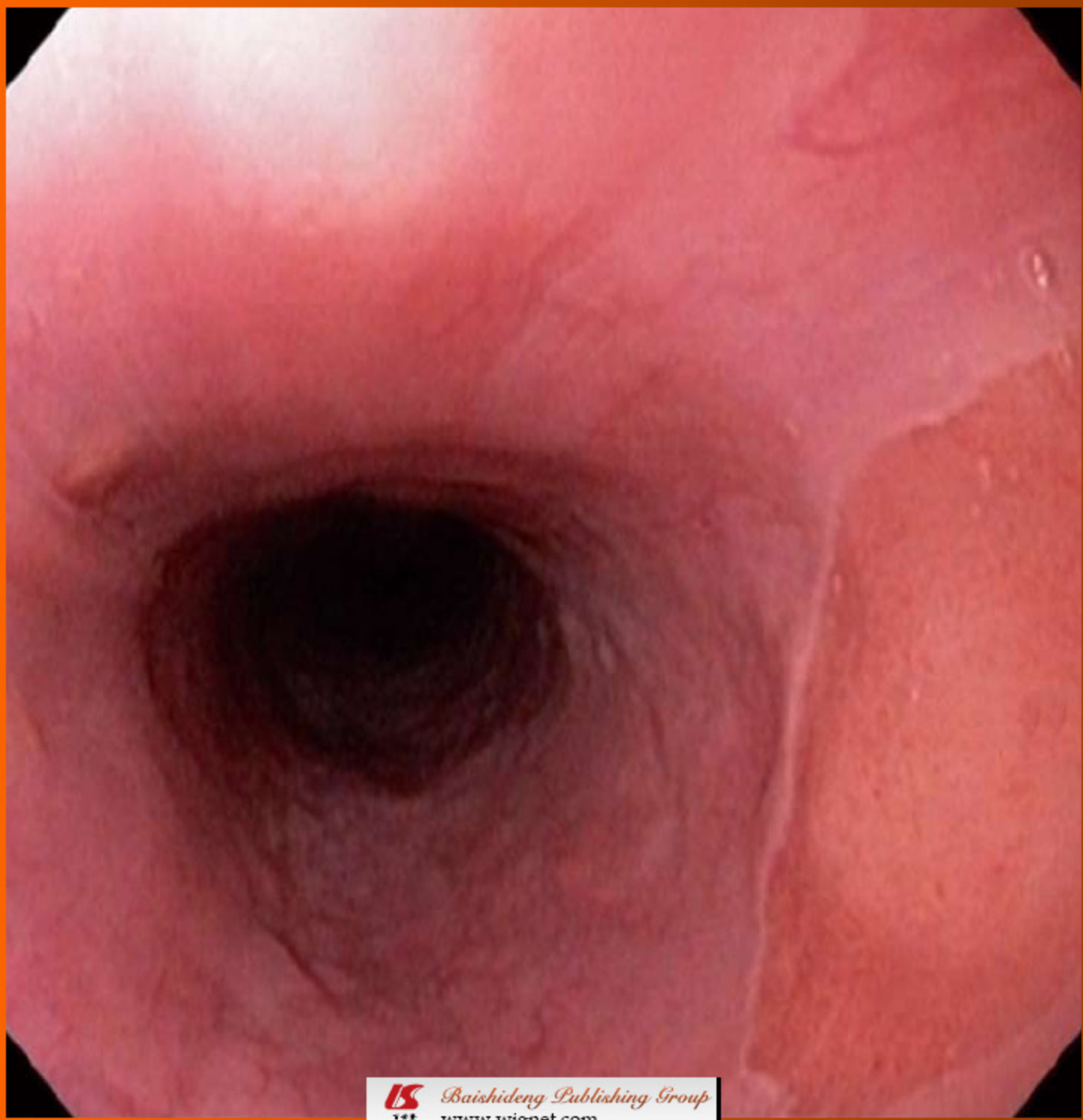
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2012 May 28; 18(20): 2443-2590



Baishideng Publishing Group
www.wjgnet.com

WJG World Journal of Gastroenterology

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

Editorial Board

2010-2013

The *World Journal of Gastroenterology* Editorial Board consists of 1352 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 64 countries, including Albania (1), Argentina (8), Australia (33), Austria (15), Belgium (14), Brazil (13), Brunei Darussalam (1), Bulgaria (2), Canada (21), Chile (3), China (82), Colombia (1), Croatia (2), Cuba (1), Czech (6), Denmark (9), Ecuador (1), Egypt (4), Estonia (2), Finland (8), France (29), Germany (87), Greece (22), Hungary (11), India (32), Indonesia (2), Iran (10), Ireland (6), Israel (13), Italy (124), Japan (140), Jordan (2), Kuwait (1), Lebanon (4), Lithuania (2), Malaysia (1), Mexico (11), Morocco (1), Moldova (1), Netherlands (32), New Zealand (2), Norway (13), Pakistan (2), Poland (11), Portugal (6), Romania (4), Russia (1), Saudi Arabia (3), Serbia (3), Singapore (11), Slovenia (1), South Africa (3), South Korea (46), Spain (43), Sri Lanka (1), Sweden (17), Switzerland (12), Thailand (1), Trinidad and Tobago (1), Turkey (30), United Arab Emirates (2), United Kingdom (95), United States (285), and Uruguay (1).

Phillip S Oates, *Perth*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Philip G Dinning, *Koagarah*
Christopher Christophi, *Melbourne*
Cuong D Tran, *North Adelaide*
Shan Rajendra, *Tasmania*
Rajvinder Singh, *Adelaide*
William Kemp, *Melbourne*
Phil Sutton, *Melbourne*
Richard Anderson, *Victoria*
Vance Matthews, *Melbourne*
Alexander G Heriot, *Melbourne*
Debbie Trinder, *Fremantle*
Ian C Lawrance, *Perth*
Adrian G Cummins, *Adelaide*
John K Olynyk, *Fremantle*
Alex Boussioutas, *Melbourne*
Emilia Prakoso, *Sydney*
Robert JL Fraser, *Daw Park*

Austria

Wolfgang Mikulits, *Vienna*
Alfred Gangl, *Vienna*
Dietmar Öfner, *Salzburg*
Georg Roth, *Vienna*
Herwig R Cerwenka, *Graz*
Ashraf Dahaba, *Graz*
Markus Raderer, *Vienna*
Alexander M Hirschl, *Wien*
Thomas Wild, *Kapellerfeld*
Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Michael Trauner, *Vienna*
Stefan Riss, *Vienna*

Belgium

Rudi Beyaert, *Gent*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Benedicte Y De Winter, *Antwerp*
Etienne M Sokal, *Brussels*
Marc Peeters, *De Pintelaan*
Eddie Wisse, *Keerbergen*
Jean-Yves L Reginster, *Liège*
Mark De Ridder, *Brussel*
Freddy Penninckx, *Leuven*
Kristin Verbeke, *Leuven*
Lukas Van Oudenhove, *Leuven*
Leo van Grunsven, *Brussels*
Philip Meuleman, *Ghent*

Brazil

Heitor Rosa, *Goiania*
Roberto J Carvalho-Filho, *Sao Paulo*
Damiao Carlos Moraes Santos, *Rio de Janeiro*
Marcelo Lima Ribeiro, *Braganca Paulista*
Eduardo Garcia Vilela, *Belo Horizonte*
Jaime Natan Eisig, *São Paulo*
Andre Castro Lyra, *Salvador*
José Liberato Ferreira Caboclo, *Brazil*
Yukie Sato-Kuwabara, *São Paulo*
Raquel Rocha, *Salvador*
Paolo R Salvalaggio, *Sao Paulo*
Ana Cristina Simões e Silva, *Belo Horizonte*
Joao Batista Teixeira Rocha, *Santa Maria*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Zahariy Krastev, *Sofia*
Mihaela Petrova, *Sofia*

Canada

Eldon Shaffer, *Calgary*
Nathalie Perreault, *Sherbrooke*
Philip H Gordon, *Montreal*
Ram Prakash Galwa, *Ottawa*
Baljinder Singh Salh, *Vancouver*
Claudia Zwingmann, *Montreal*
Alain Bitton, *Montreal*
Pingchang Yang, *Hamilton*
Michael F Byrne, *Vancouver*
Andrew L Mason, *Alberta*
John K Marshall, *Hamilton Ontario*
Kostas Pantopoulos, *Montreal*
Waliul Khan, *Ontario*
Eric M Yoshida, *Vancouver*
Geoffrey C Nguyen, *Toronto*
Devendra K Amre, *Montreal*
Tedros Bezabeh, *Winnipeg*
Wangxue Chen, *Ottawa*
Qiang Liu, *Saskatoon*

Chile

De Aretxabala Xabier, *Santiago*
Marcelo A Beltran, *La Serena*
Silvana Zanlungo, *Santiago*

China

Chi-Hin Cho, *Hong Kong*
Chun-Qing Zhang, *Jinan*
Ren Xiang Tan, *Nanjing*
Fei Li, *Beijing*
Hui-Jie Bian, *Xi'an*
Xiao-Peng Zhang, *Beijing*
Xing-Hua Lu, *Beijing*
Fu-Sheng Wang, *Beijing*
An-Gang Yang, *Xi'an*
Xiao-Ping Chen, *Wuhan*
Zong-Jie Cui, *Beijing*
Ming-Liang He, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
Qin Su, *Beijing*
Jian-Zhong Zhang, *Beijing*
Paul Kwong-Hang Tam, *Hong Kong*
Wen-Rong Xu, *Zhenjiang*
Chun-Yi Hao, *Beijing*
San-Jun Cai, *Shanghai*
Simon Law, *Hong Kong*
Yuk Him Tam, *Hong Kong*
De-Liang Fu, *Shanghai*
Eric WC Tse, *Hong Kong*
Justin CY Wu, *Hong Kong*
Nathalie Wong, *Hong Kong*
Jing Yuan Fang, *Shanghai*
Yi-Min Mao, *Shanghai*
Wei-Cheng You, *Beijing*
Xiang-Dong Wang, *Shanghai*
Xuan Zhang, *Beijing*
Zhao-Shen Li, *Shanghai*
Guang-Wen Cao, *Shanghai*
En-min Li, *Shantou*
Yu-Yuan Li, *Guangzhou*
Fook Hong Ng, *Hong Kong*
Hsiang-Fu Kung, *Hong Kong*
Wai Lun Law, *Hong Kong*
Eric CH Lai, *Hong Kong*
Jun Yu, *Hong Kong*
Ze-Guang Han, *Shanghai*
Bian zhao-xiang, *Hong Kong*
Wei-Dong Tong, *Chongqing*

Colombia

Germán Campuzano-Maya, *Medellín*

Croatia

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

Cuba

Damian C Rodriguez, *Havana*

Czech

Milan Jirsa, *Praha*
Pavel Trunečka, *Prague*
Jan Bures, *Hradec Kralove*
Marcela Kopacova, *Hradec Kralove*
Ondrej Slaby, *Brno*
Radan Bruha, *Prague*

Denmark

Asbjørn M Drewes, *Aalborg*
Leif Percival Andersen, *Copenhagen*
Jan Mollenhauer, *Odense C*
Morten Frisch, *Copenhagen S*
Jorgen Rask-Madsen, *Skodsborg*
Morten Hylander Møller, *Holte*
Søren Rafaelsen, *Vejle*
Vibeke Andersen, *Aabenraa*
Ole Haagen Nielsen, *Herlev*

Ecuador

Fernando E Sempértegui, *Quito*

Egypt

Zeinab Nabil Ahmed Said, *Cairo*
Hussein M Atta, *El-Minia*
Asmaa Gaber Abdou, *Shebein Elkom*

Maha Maher Shehata, *Mansoura*

Estonia

Riina Salupere, *Tartu*
Tamara Vorobjova, *Tartu*

Finland

Saila Kauhanen, *Turku*
Pauli Antero Puolakkainen, *Turku*
Minna Nyström, *Helsinki*
Juhani Sand, *Tampere*
Jukka-Pekka Mecklin, *Jyvaskyla*
Lea Veijola, *Helsinki*
Kaija-Leena Kolho, *Helsinki*
Thomas Kietzmann, *Oulu*

France

Boris Guiu, *Dijon*
Baumert F Thomas, *Strasbourg*
Alain L Servin, *Châtenay-Malabry*
Patrick Marcellin, *Paris*
Jean-Jacques Tuech, *Rouen*
Francoise L Fabiani, *Angers*
Jean-Luc Faucheron, *Grenoble*
Philippe Lehours, *Bordeaux*
Stephane Supiot, *Nantes*
Lionel Bueno, *Toulouse*
Flavio Maina, *Marseille*
Paul Hofman, *Nice*
Abdel-Majid Khatib, *Paris*
Annie Schmid-Alliana, *Nice cedex 3*
Frank Zerbib, *Bordeaux Cedex*
Rene Gerolami Santandera, *Marseille*
Sabine Colnot, *Paris*
Catherine Daniel, *Lille Cedex*
Thabut Dominique, *Paris*
Laurent Huwart, *Paris*
Alain Braillon, *Amiens*
Bruno Bonaz, *Grenoble*
Evelyne Schvoerer, *Strasbourg*
M Coeffier, *Rouen*
Mathias Chamaillard, *Lille*
Hang Nguyen, *Clermont-Ferrand*
Veronique Vitton, *Marseille*
Alexis Desmoulière, *Limoges*
Juan Iovanna, *Marseille*

Germany

Hans L Tillmann, *Leipzig*
Stefan Kubicka, *Hannover*
Elke Cario, *Essen*
Hans Scherubl, *Berlin*
Harald F Teutsch, *Ulm*
Peter Konturek, *Erlangen*
Thilo Hackert, *Heidelberg*
Jurgen M Stein, *Frankfurt*
Andrej Khandoga, *Munich*
Karsten Schulmann, *Bochum*
Jutta Elisabeth Lüttges, *Riegelsberg*
Wolfgang Hagmann, *Heidelberg*
Hubert Blum, *Freiburg*
Thomas Bock, *Berlin*
Christa Buechler, *Regensburg*
Christoph F Dietrich, *Bad Mergentheim*
Ulrich R Fölsch, *Kiel*
Nikolaus Gassler, *Aachen*
Markus Gerhard, *Munich*
Dieter Glebe, *Giessen*
Klaus R Herrlinger, *Stuttgart*
Eberhard Hildt, *Berlin*
Joerg C Hoffmann, *Ludwigshafen*
Joachim Labenz, *Siegen*
Peter Malfertheiner, *Magdeburg*
Sabine Mihm, *Göttingen*
Markus Reiser, *Bochum*
Steffen Rickes, *Magdeburg*
Andreas G Schreyer, *Regensburg*
Henning Schulze-Bergkamen, *Heidelberg*
Ulrike S Stein, *Berlin*
Wolfgang R Stremmel, *Heidelberg*
Fritz von Weizsäcker, *Berlin*
Stefan Wirth, *Wuppertal*
Dean Bogoevski, *Hamburg*
Bruno Christ, *Halle/Saale*
Peter N Meier, *Hannover*
Stephan Johannes Ott, *Kiel*
Arndt Vogel, *Hannover*
Dirk Haller, *Freising*
Jens Standop, *Bonn*
Jonas Mudter, *Erlangen*
Jürgen Büning, *Lübeck*
Matthias Ocker, *Erlangen*
Joerg Trojan, *Frankfurt*
Christian Trautwein, *Aachen*
Jorg Kleeff, *Munich*
Christian Rust, *Munich*
Claus Hellerbrand, *Regensburg*
Elke Roeb, *Giessen*
Erwin Biecker, *Siegburg*
Ingmar Königsrainer, *Tübingen*
Jürgen Borlak, *Hannover*
Axel M Gressner, *Aachen*
Oliver Mann, *Hamburg*
Marty Zdichavsky, *Tübingen*
Christoph Reichel, *Bad Brückenau*
Nils Habbe, *Marburg*
Thomas Wex, *Magdeburg*
Frank Ulrich Weiss, *Greifswald*
Manfred V Singer, *Mannheim*
Martin K Schilling, *Homburg*
Philip D Hard, *Giessen*
Michael Linnebacher, *Rostock*
Ralph Graeser, *Freiburg*
Rene Schmidt, *Freiburg*
Robert Obermaier, *Freiburg*
Sebastian Mueller, *Heidelberg*
Andrea Hille, *Goettingen*
Klaus Mönkemüller, *Bottrop*
Elfriede Bollschweiler, *Köln*
Siegfried Wagner, *Deggendorf*
Dieter Schilling, *Mannheim*
Joerg F Schlaak, *Essen*
Michael Keese, *Frankfurt*
Robert Grützmann, *Dresden*
Ali Canbay, *Essen*
Dirk Domagk, *Muenster*
Jens Hoeppner, *Freiburg*
Frank Tacke, *Aachen*
Patrick Michl, *Marburg*
Alfred A Königsrainer, *Tübingen*
Kilian Weigand, *Heidelberg*
Mohamed Hassan, *Duesseldorf*
Gustav Paumgartner, *Munich*
Philipe N Khalil, *Munich*
Martin Storr, *Munich*

Greece

Andreas Larentzakis, *Athens*
Tsianos Epameinondas, *Ioannina*
Elias A Kouroumalis, *Heraklion*
Helen Christopoulou-Aletra, *Thessaloniki*
George Papatheodoridis, *Athens*
Ioannis Kanellos, *Thessaloniki*
Michael Koutsilieris, *Athens*
T Choli-Papadopoulou, *Thessaloniki*
Emanuel K Manesis, *Athens*
Evangelos Tsiambas, *Ag Paraskevi Attiki*
Konstantinos Mimidis, *Alexandroupolis*
Spilios Manolakopoulos, *Athens*
Spiros Sgouros, *Athens*
Ioannis E Koutroubakis, *Heraklion*
Stefanos Karagiannis, *Athens*
Spiros Ladas, *Athens*
Elena Vezali, *Athens*
Dina G Tiniakos, *Athens*
Ekaterini Chatzaki, *Alexandroupolis*
Dimitrios Roukos, *Ioannina*
George Sgourakis, *Athens*
Maroulio Talieri, *Athens*

Hungary

Peter L Lakatos, *Budapest*
Yvette Mándi, *Szeged*
Ferenc Sipos, *Budapest*
György M Buzás, *Budapest*
László Czakó, *Szeged*
Peter Hegyi, *Szeged*
Zoltan Rakonczay, *Szeged*
Gyula Farkas, *Szeged*
Zsuzsa Szondy, *Debrecen*
Gabor Veres, *Budapest*
Zsuzsa Schaff, *Budapest*

India

Philip Abraham, *Mumbai*
Sri P Misra, *Allahabad*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*
Jai Dev Wig, *Chandigarh*
Uday C Ghoshal, *Lucknow*
Pramod Kumar Garg, *New Delhi*
Barjesh Chander Sharma, *New Delhi*
Gopal Nath, *Varanasi*
Bhupendra Kumar Jain, *Delhi*
Devinder Kumar Dhawan, *Chandigarh*
Ashok Kumar, *Lucknow*
Benjamin Perakath, *Tamil Nadu*
Debidas Ghosh, *Midnpore*
Pankaj Garg, *Panchkula*
Samiran Nundy, *New Delhi*
Virendra Singh, *Chandigarh*
Bikash Medhi, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Vandana Panda, *Mumbai*
Vineet Ahuja, *New Delhi*
SV Rana, *Chandigarh*

Deepak N Amarapurkar, *Mumbai*
Abhijit Chowdhury, *Kolkata*
Jasbir Singh, *Kurukshetra*
B Mittal, *Lucknow*
Sundeep Singh Saluja, *New Delhi*
Pradyumna Kumar Mishra, *Mumbai*
Runu Chakravarty, *Kolkata*
Nagarajan Perumal, *New Delhi*

Indonesia

David handojo Muljono, *Jakarta*
Andi Utama, *Tangerang*

Iran

Seyed-Moayed Alavian, *Tehran*
Reza Malekzadeh, *Tehran*
Peyman Adibi, *Isfahan*
Alireza Mani, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Mohammad Abdollahi, *Tehran*
Majid Assadi, *Bushehr*
Arezoo Aghakhani, *Tehran*
Marjan Mohammadi, *Tehran*
Fariborz Mansour-Ghanaei, *Rasht*

Ireland

Ross McManus, *Dublin*
Billy Bourke, *Dublin*
Catherine Greene, *Dublin*
Ted Dinan, *Cork*
Marion Rowland, *Dublin*

Israel

Abraham R Eliakim, *Haifa*
Simon Bar-Meir, *Tel Hashomer*
Ami D Sperber, *Beer-Sheva*
Boris Kirshtein, *Beer Sheva*
Mark Pines, *Bet Dagan*
Menachem Moshkowitz, *Tel-Aviv*
Ron Shaoul, *Haifa*
Shmuel Odes, *Beer Sheva*
Sigal Fishman, *Tel Aviv*
Alexander Becker, *Afula*
Assy Nimer, *Safed*
Eli Magen, *Ashdod*
Amir Shlomai, *Tel-Aviv*

Italy

Mauro Bortolotti, *Bologna*
Gianlorenzo Dionigi, *Varese*
Fiorucci Stefano, *Perugia*
Roberto Berni Canani, *Naples*
Ballarin Roberto, *Modena*
Bruno Annibale, *Roma*
Vincenzo Stanghellini, *Bologna*
Giovanni B Gaeta, *Napoli*
Claudio Bassi, *Verona*
Mauro Bernardi, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Dario Conte, *Milano*
Francesco Costa, *Pisa*
Giovanni D De Palma, *Naples*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Edoardo G Giannini, *Genoa*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Pietro Invernizzi, *Milan*
Ezio Laconi, *Cagliari*
Giuseppe Montalto, *Palermo*
Giovanni Musso, *Torino*
Gerardo Nardone, *Napoli*
Valerio Nobili, *Rome*
Raffaele Pezzilli, *Bologna*
Alberto Piperno, *Monza*
Anna C Piscaglia, *Roma*
Piero Portincasa, *Bari*
Giovanni Tarantino, *Naples*
Cesare Tosetti, *Porretta Terme*
Alessandra Ferlini, *Ferrara*
Alessandro Ferrero, *Torino*
Donato F Altomare, *Bari*
Giovanni Milito, *Rome*
Giuseppe Sica, *Rome*
Guglielmo Borgia, *Naples*
Giovanni Latella, *L'Aquila*
Salvatore Auricchio, *Naples*
Alberto Biondi, *Rome*
Alberto Tommasini, *Trieste*
Antonio Basoli, *Roma*
Giuliana Decorti, *Trieste*
Marco Silano, *Roma*
Michele Reni, *Milan*
Pierpaolo Sileri, *Rome*
Achille Iolascon, *Naples*
Alessandro Granito, *Bologna*
Angelo A Izzo, *Naples*
Giuseppe Currò, *Messina*
Pier Mannuccio Mannucci, *Milano*
Marco Vivarelli, *Bologna*
Massimo Levrero, *Rome*
Massimo Rugge, *Padova*
Paolo Angeli, *Padova*
Silvio Danese, *Milano*
Antonello Trecca, *Rome*
Antonio Gasbarrini, *Rome*
Cesare Ruffolo, *Treviso*
Massimo Falconi, *Verona*
Fausto Catena, *Bologna*
Francesco Manguso, *Napoli*
Giancarlo Mansueto, *Verona*
Luca Morelli, *Trento*
Marco Scarpa, *Padova*
Mario M D'Elios, *Florence*
Francesco Luzza, *Catanzaro*
Franco Roviello, *Siena*
Guido Torzilli, *Rozzano Milano*
Luca Frulloni, *Verona*
Lucia Malaguarnera, *Catania*
Lucia Ricci Vitiani, *Rome*
Mara Massimi, *L'Aquila*
Mario Pescatori, *Rome*
Mario Rizzetto, *Torino*
Mirko D'Onofrio, *Verona*
Nadia Peparini, *Rome*
Paola De Nardi, *Milan*
Paolo Aurello, *Rome*
Piero Amodio, *Padova*
Riccardo Nascimbeni, *Brescia*
Vincenzo Villanacci, *Brescia*
Vittorio Ricci, *Pavia*
Silvia Fargion, *Milan*
Luigi Bonavina, *Milano*
Oliviero Riggio, *Rome*
Fabio Pace, *Milano*
Gabrio Bassotti, *Perugia*
Giulio Marchesini, *Bologna*
Roberto de Franchis, *Milano*
Giovanni Monteleone, *Rome*
C armelo Scarpignato, *Parma*
Luca VC Valenti, *Milan*
Urgesi Riccardo, *Rome*
Marcello Persico, *Naples*
Antonio Moschetta, *Bari*
Luigi Muratori, *Bologna*
Angelo Zullo, *Roma*
Vito Annese, *Florence*
Simone Lanini, *Rome*
Alessandro Grasso, *Savona*
Giovanni Targher, *Verona*
Domenico Girelli, *Verona*
Alessandro Cucchetti, *Bologna*
Fabio Marra, *Florence*
Michele Milella, *Rome*
Francesco Franceschi, *Rome*
Giuseppina De Petro, *Brescia*
Salvatore Leonardi, *Catania*
Cristiano Simone, *Santa Maria Imbaro*
Bernardino Rampone, *Salerno*
Francesco Crea, *Pisa*
Walter Fries, *Messina*
Antonio Craxì, *Palermo*
Gerardo Rosati, *Potenza*
Mario Guslandi, *Milano*
Gianluigi Giannelli, *Bari*
Paola Loria, *Modena*
Paolo Sorrentino, *Avellino*
Armando Santoro, *Rozzano*
Gabriele Grassi, *Trieste*
Antonio Orlacchio, *Rome*

Japan

Tsuneo Kitamura, *Chiba*
Katsutoshi Yoshizato, *Higashihiroshima*
Masahiro Arai, *Tokyo*
Shinji Tanaka, *Hiroshima*
Keiji Hirata, *Kitakyushu*
Yoshio Shirai, *Niigata*
Susumu Ohmada, *Maebashi*
Kenichi Ikejima, *Tokyo*
Masatoshi Kudo, *Osaka*
Yoshiaki Murakami, *Hiroshima*
Masahiro Tajika, *Nagoya*
Kentaro Yoshika, *Toyoake*
Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Hitoshi Asakura, *Tokyo*
Mitsuhiro Fujishiro, *Tokyo*
Toru Hiyama, *Higashihiroshima*
Yutaka Inagaki, *Kanagawa*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Yoshiaki Iwasaki, *Okayama*
Terumi Kamisawa, *Tokyo*

Norihiro Kokudo, *Tokyo*
Shin Maeda, *Tokyo*
Yasushi Matsuzaki, *Ibaraki*
Kenji Miki, *Tokyo*
Hiroto Miwa, *Hyogo*
Yoshiharu Motoo, *Kanazawa*
Kunihiko Murase, *Tusima*
Atsushi Nakajima, *Yokohama*
Yuji Naito, *Kyoto*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Mikio Nishioka, *Niihama*
Hirohide Ohnishi, *Akita*
Kazuichi Okazaki, *Osaka*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Yasushi Sano, *Kobe*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Toyama*
Shinji Shimoda, *Fukuoka*
Masayuki Sho, *Nara*
Hidekazu Suzuki, *Tokyo*
Shinji Togo, *Yokohama*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Akihito Nagahara, *Tokyo*
Hiroaki Takeuchi, *Kochi*
Keiji Ogura, *Tokyo*
Kotaro Miyake, *Tokushima*
Mitsunori Yamakawa, *Yamagata*
Naoaki Sakata, *Sendai*
Naoya Kato, *Tokyo*
Satoshi Mamori, *Hyogo*
Shogo Kikuchi, *Aichi*
Shoichiro Sumi, *Kyoto*
Susumu Ikehara, *Osaka*
Taketo Yamaguchi, *Chiba*
Tokihiko Sawada, *Tochigi*
Tomoharu Yoshizumi, *Fukuoka*
Toshiyuki Ishiwata, *Tokyo*
Yasuhiro Fujino, *Akashi*
Yasuhiro Koga, *Isehara city*
Yoshihisa Takahashi, *Tokyo*
Yoshitaka Takuma, *Okayama*
Yutaka Yata, *Maebashi-city*
Itaru Endo, *Yokohama*
Kazuo Chijiiwa, *Miyazaki*
Kouhei Fukushima, *Sendai*
Masahiro Iizuka, *Akita*
Mitsuyoshi Urashima, *Tokyo*
Munechika Enjoji, *Fukuoka*
Takashi Kojima, *Sapporo*
Takumi Kawaguchi, *Kurume*
Yoshiyuki Ueno, *Sendai*
Yuichiro Eguchi, *Saga*
Akihiro Tamori, *Osaka*
Atsushi Masamune, *Sendai*
Atsushi Tanaka, *Tokyo*
Hitoshi Tsuda, *Tokyo*
Takashi Kobayashi, *Tokyo*
Akimasa Nakao, *Nagogya*
Hiroyuki Uehara, *Osaka*
Masahito Uemura, *Kashihara*
Satoshi Tanno, *Sapporo*
Toshinari Takamura, *Kanazawa*
Yohei Kida, *Kainan*

Masanori Hatakeyama, *Tokyo*
Satoru Kakizaki, *Gunma*
Shuhei Nishiguchi, *Hyogo*
Yuichi Yoshida, *Osaka*
Manabu Morimoto, *Japan*
Mototsugu Kato, *Sapporo*
Naoki Ishii, *Tokyo*
Noriko Nakajima, *Tokyo*
Nobuhiro Ohkohchi, *Tsukuba*
Takanori Kanai, *Tokyo*
Kenichi Goda, *Tokyo*
Mitsugi Shimoda, *Mibu*
Zenichi Morise, *Nagoya*
Hitoshi Yoshiji, *Kashihara*
Takahiro Nakazawa, *Nagoya*
Utaroh Motosugi, *Yamanashi*
Nobuyuki Matsuhashi, *Tokyo*
Yasuhiro Kodera, *Nagoya*
Takayoshi Ito, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Haruhiko Sugimura, *Hamamatsu*
Hiroki Yamaue, *Wakayama*
Masao Ichinose, *Wakayama*
Takaaki Arigami, *Kagoshima*
Nobuhiro Zaima, *Nara*
Naoki Tanaka, *Matsumoto*
Satoru Motoyama, *Akita*
Tomoyuki Shibata, *Toyoake*
Tatsuya Ide, *Kurume*
Tsutomu Fujii, *Nagoya*
Osamu Kanauchi, *Toky*
Atsushi Irisawa, *Aizuwakamatsu*
Hikaru Nagahara, *Tokyo*
Keiji Hanada, *Onomichi*
Keiichi Mitsuyama, *Fukuoka*
Shin Maeda, *Yokohama*
Takuya Watanabe, *Niigata*
Toshihiro Mitaka, *Sapporo*
Yoshiki Murakami, *Kyoto*
Tadashi Shimoyama, *Hirosaki*

Jordan

Ismail Matalka, *Irbid*
Khaled Jadallah, *Irbid*

Kuwait

Islam Khan, *Safat*

Lebanon

Bassam N Abboud, *Beirut*
Rami Moucari, *Beirut*
Ala I Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania

Giedrius Barauskas, *Kaunas*
Limas Kupcinskas, *Kaunas*

Malaysia

Andrew Seng Boon Chua, *Ipoh*

Mexico

Saúl Villa-Trevio, *México*
Omar Vergara-Fernandez, *Mexico*
Diego Garcia-Compean, *Monterrey*
Arturo Panduro, *Jalisco*
Miguel Angel Mercado, *Distrito Federal*
Richard A Awad, *Mexico*
Aldo Torre Delgadillo, *México*
Paulino Martínez Hernández Magro, *Celaya*
Carlos A Aguilar-Salinas, *Mexico*
Jesus K Yamamoto-Furusho, *Mexico*

Morocco

Samir Ahboucha, *Khouribga*

Moldova

Igor Mishin, *Kishinev*

Netherlands

Ulrich Beuers, *Amsterdam*
Albert Frederik Pull ter Gunne, *Tilburg*
Jantine van Baal, *Heidelberglaan*
Wendy Wilhelmina Johanna de Leng, *Utrecht*
Gerrit A Meijer, *Amsterdam*
Lee Bouwman, *Leiden*
J Bart A Crusius, *Amsterdam*
Frank Hoentjen, *Haarlem*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Karel van Erpecum, *Utrecht*
BW Marcel Spanier, *Arnhem*
Misha Luyer, *Sittard*
Pieter JF de Jonge, *Rotterdam*
Robert Christiaan Verdonk, *Groningen*
John Plukker, *Groningen*
Maarten Tushuizen, *Amsterdam*
Wouter de Herder, *Rotterdam*
Erwin G Zoetendal, *Wageningen*
Robert J de Knegt, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Annemarie de Vries, *Rotterdam*
Astrid van der Velde, *Ede*
Lodewijk AA Brosens, *Utrecht*
James CH Hardwick, *Leiden*
Loes van Keimpema, *Nijmegen*
WJ de Jonge, *Amsterdam*
Zuzana Zelinkova, *Rotterdam*
LN van Steenbergen, *Eindhoven*
Frank G Schaap, *Amsterdam*
Jeroen Maljaars, *Leiden*

New Zealand

Andrew S Day, *Christchurch*
Max S Petrov, *Auckland*

Norway

Espen Melum, *Oslo*

Trine Olsen, *Tromsø*
Eyvind J Paulssen, *Tromsø*
Rasmus Goll, *Tromsø*
Asle W Medhus, *Oslo*
Jon Arne Søreide, *Stavanger*
Kjetil Soreide, *Stavanger*
Reidar Fossmark, *Trondheim*
Trond Peder Flaten, *Trondheim*
Olav Dalgard, *Oslo*
Ole Høie, *Arendal*
Magdy El-Salhy, *Bergen*
Jørgen Valeur, *Oslo*

Pakistan

Shahab Abid, *Karachi*
Syed MW Jafri, *Karachi*

Poland

Beata Jolanta Jablońska, *Katowice*
Halina Cichoż-Lach, *Lublin*
Tomasz Brzozowski, *Cracow*
Hanna Gregorek, *Warsaw*
Marek Hartleb, *Katowice*
Stanislaw J Konturek, *Krakow*
Andrzej Dabrowski, *Bialystok*
Jan Kulig, *Kraków*
Julian Swierczynski, *Gdansk*
Marek Bebenek, *Wroclaw*
Dariusz M Lebensztejn, *Bialystok*

Portugal

Ricardo Marcos, *Porto*
Guida Portela-Gomes, *Estoril*
Ana Isabel Lopes, *Lisboa Codex*
Raquel Almeida, *Porto*
Rui Tato Marinho, *Lisbon*
Ceu Figueiredo, *Porto*

Romania

Dan L Dumitrascu, *Cluj*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Anca Trifan, *Iasi*

Russia

Vasiliy I Reshetnyak, *Moscow*

Saudi Arabia

Ibrahim A Al Mofleh, *Riyadh*
Abdul-Wahed Meshikhes, *Qatif*
Faisal Sanai, *Riyadh*

Serbia

Tamara M Alempijevic, *Belgrade*
Dusan M Jovanovic, *Sremska Kamenica*
Zoran Krivokapic, *Belgrade*

Singapore

Brian Kim Poh Goh, *Singapore*
Khek-Yu Ho, *Singapore*
Fock Kwong Ming, *Singapore*
Francis Seow-Choen, *Singapore*
Kok Sun Ho, *Singapore*
Kong Weng Eu, *Singapore*
Madhav Bhatia, *Singapore*
London Lucien Ooi, *Singapore*
Wei Ning Chen, *Singapore*
Richie Soong, *Singapore*
Kok Ann Gwee, *Singapore*

Slovenia

Matjaz Homan, *Ljubljana*

South Africa

Rosemary Joyce Burnett, *Pretoria*
Michael Kew, *Cape Town*
Roland Ndip, *Alice*

South Korea

Byung Chul Yoo, *Seoul*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Marie Yeo, *Suwon*
Jeong Min Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Joong-Won Park, *Goyang*
Hoon Jai Chun, *Seoul*
Myung-Gyu Choi, *Seoul*
Sang Kil Lee, *Seoul*
Sang Yeoup Lee, *Gyeongsangnam-do*
Won Ho Kim, *Seoul*
Dae-Yeul Yu, *Daejeon*
Donghee Kim, *Seoul*
Sang Geon Kim, *Seoul*
Sun Pyo Hong, *Geonggi-do*
Sung-Gil Chi, *Seoul*
Yeun-Jun Chung, *Seoul*
Ki-Baik Hahm, *Incheon*
Ji Kon Ryu, *Seoul*
Kyu Taek Lee, *Seoul*
Yong Chan Lee, *Seoul*
Seong Gyu Hwang, *Seongnam*
Seung Woon Paik, *Seoul*
Sung Kim, *Seoul*
Hong Joo Kim, *Seoul*
Hyoung-Chul Oh, *Seoul*
Nayoung Kim, *Seongnam-si*
Sang Hoon Ahn, *Seoul*
Seon Hahn Kim, *Seoul*
Si Young Song, *Seoul*
Young-Hwa Chung, *Seoul*
Hyo-Cheol Kim, *Seoul*
Kwang Jae Lee, *Swon*
Sang Min Park, *Seoul*
Young Chul Kim, *Seoul*
Do Hyun Park, *Seoul*
Dae Won Jun, *Seoul*
Dong Wan Seo, *Seoul*
Soon-Sun Hong, *Incheon*
Hoguen Kim, *Seoul*
Ho-Young Song, *Seoul*
Joo-Ho Lee, *Seoul*
Jung Eun Lee, *Seoul*
Jong H Moon, *Bucheon*

Spain

Eva Vaquero, *Barcelona*
Andres Cardenas, *Barcelona*
Laureano Fernández-Cruz, *Barcelona*
Antoni Farré, *Spain*
Maria-Angeles Aller, *Madrid*
Raul J Andrade, *Málaga*
Fernando Azpiroz, *Barcelona*
Josep M Bordas, *Barcelona*
Antoni Castells, *Barcelona*
Vicente Felipo, *Valencia*
Isabel Fabregat, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
María IT López, *Jaén*
Jesús M Prieto, *Pamplona*
Mireia Miquel, *Sabadell*
Ramon Bataller, *Barcelona*
Fernando J Corrales, *Pamplona*
Julio Mayol, *Madrid*
Matias A Avila, *Pamplona*
Juan Macías, *Seville*
Juan Carlos Laguna Egea, *Barcelona*
Juli Busquets, *Barcelona*
Belén Beltrán, *Valencia*
José Manuel Martin-Villa, *Madrid*
Lisardo Boscá, *Madrid*
Luis Grande, *Barcelona*
Pedro Lorenzo Majano Rodriguez, *Madrid*
Adolfo Benages, *Valencia*
Domínguez-Muñoz JE, *Santiago de Compostela*
Gloria González Aseguinolaza, *Navarra*
Javier Martin, *Granada*
Luis Bujanda, *San Sebastián*
Matilde Bustos, *Pamplona*
Luis Aparisi, *Valencia*
José Julián calvo Andrés, *Salamanca*
Benito Velayos, *Valladolid*
Javier Gonzalez-Gallego, *León*
Ruben Ciria, *Córdoba*
Francisco Rodriguez-Frias, *Barcelona*
Manuel Romero-Gómez, *Sevilla*
Albert Parés, *Barcelona*
Joan Roselló-Catafau, *Barcelona*

Sri Lanka

Arjuna De Silva, *Kelaniya*

Sweden

Stefan G Pierzynowski, *Lund*
Hanns-Ulrich Marschall, *Stockholm*
Lars A Pahlman, *Uppsala*
Helena Nordenstedt, *Stockholm*
Bobby Tingstedt, *Lund*
Evangelos Kalaitzakis, *Gothenburg*
Lars Erik Agréus, *Huddinge*
Annika Lindblom, *Stockholm*

Roland Andersson, *Lund*
Zongli Zheng, *Stockholm*
Mauro D'Amato, *Huddinge*
Greger Lindberg, *Stockholm*
Pär Erik Myrelid, *Linköping*
Sara Lindén, *Göteborg*
Sara Regnér, *Malmö*
Åke Nilsson, *Lund*

Switzerland

Jean L Frossard, *Geneva*
Andreas Geier, *Zürich*
Bruno Stieger, *Zürich*
Pascal Gervaz, *Geneva*
Paul M Schneider, *Zurich*
Felix Stickel, *Berne*
Fabrizio Montecucco, *Geneva*
Inti Zlobec, *Basel*
Michelangelo Foti, *Geneva*
Pascal Bucher, *Geneva*
Andrea De Gottardi, *Berne*
Christian Toso, *Geneva*

Thailand

Weekitt Kittisupamongkol, *Bangkok*

Trinidad and Tobago

Shivananda Nayak, *Mount Hope*

Turkey

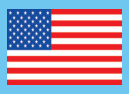
Tarkan Karakan, *Ankara*
Yusuf Bayraktar, *Ankara*
Ahmet Tekin, *Mersin*
Aydin Karabacakoglu, *Konya*
Osman C Ozdogan, *Istanbul*
Özlem Yilmaz, *Izmir*
Bülent Salman, *Ankara*
Can GONEN, *Kutahya*
Cuneyt Kayaalp, *Malatya*
Ekmel Tezel, *Ankara*
Eren Ersoy, *Ankara*
Hayrullah Derici, *Balıkesir*
Mehmet Refik Mas, *Etlik-Ankara*
Sinan Akay, *Tekirdag*
A Mithat Bozdayi, *Ankara*
Metin Basaranoglu, *Istanbul*
Mesut Tez, *Ankara*
Orhan Sezgin, *Mersin*
Mukaddes Esrefoglu, *Malatya*
Ilker Tasci, *Ankara*
Kemal Kismet, *Ankara*
Selin Kapan, *Istanbul*
Seyfettin Köklü, *Ankara*
Murat Sayan, *Kocaeli*
Sabahattin Kaymakoglu, *Istanbul*
Yucel Ustundag, *Zonguldak*
Can Gonen, *Istanbul*
Yusuf Yilmaz, *Istanbul*
Müge Tecder-Ünal, *Ankara*
İlhami Yüksel, *Ankara*

United Arab Emirates

Fikri M Abu-Zidan, *Al-Ain*
Sherif M Karam, *Al-Ain*

United Kingdom

Anastasios Koulaouzidis, *Edinburgh*
Sylvia LF Pender, *Southampton*
Hong-Xiang Liu, *Cambridge*
William Dickey, *Londonderry*
Simon D Taylor-Robinson, *London*
James Neuberger, *Birmingham*
Frank I Tovey, *London*
Kevin Robertson, *Glasgow*
Chew Thean Soon, *Manchester*
Geoffrey Burnstock, *London*
Vamsi R Velchuru, *United Kingdom*
Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Lesley A Anderson, *Belfast*
Anthony TR Axon, *Leeds*
Jim D Bell, *London*
Alastair D Burt, *Newcastle*
Tatjana Crnogorac-Jurcevic, *London*
Daniel R Gaya, *Edinburgh*
William Greenhalf, *Liverpool*
Indra N Guha, *Southampton*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
Janusz AZ Jankowski, *Oxford*
Peter Karayiannis, *London*
Patricia F Lalor, *Birmingham*
Giorgina Mieli-Vergani, *London*
D Mark Pritchard, *Liverpool*
Marco Senzolo, *Padova*
Roger Williams, *London*
M H Ahmed, *Southampton*
Christos Paraskeva, *Bristol*
Emad M El-Omar, *Aberdeen*
A M El-Tawil, *Birmingham*
Anne McCune, *Bristol*
Charles B Ferguson, *Belfast*
Chin Wee Ang, *Liverpool*
Clement W Imrie, *Glasgow*
Dileep N Lobo, *Nottingham*
Graham MacKay, *Glasgow*
Guy Fairbairn Nash, *Poole*
Ian Lindsey, *Oxford*
Jason CB Goh, *Birmingham*
Jeremy FL Cobbold, *London*
Julian RF Walters, *London*
Jamie Murphy, *London*
John Beynon, *Swansea*
John B Schofield, *Kent*
Anil George, *London*
Aravind Suppiah, *East Yorkshire*
Basil Ammori, *Salford*
Catherine Walter, *Cheltenham*
Chris Briggs, *Sheffield*
Jeff Butterworth, *Shrewsbury*
Nawfal Hussein, *Nottingham*
Patrick O'Dwyer, *Glasgow*
Rob Glynne-Jones, *Northwood*
Sharad Karandikar, *Birmingham*
Venkatesh Shanmugam, *Derby*
Yeng S Ang, *Wigan*
Alberto Quaglia, *London*
Andrew Fowell, *Southampton*
Gianpiero Gravante, *Leicester*
Piers Gatenby, *London*
Kondragunta Rajendra Prasad, *Leeds*
Sunil Dolwani, *Cardiff*
Andrew McCulloch Veitch, *Wolverhampton*
Brian Green, *Belfast*
Noriko Suzuki, *Middlesex*
Richard Parker, *North Staffordshire*
Shahid A Khan, *London*
Akhilesh B Reddy, *Cambridge*
Jean E Crabtree, *Leeds*
John S Leeds, *Sheffield*
Paul Sharp, *London*
Sumita Verma, *Brighton*
Thamara Perera, *Birmingham*
Donald Campbell McMillan, *Glasgow*
Kathleen B Bamford, *London*
Helen Coleman, *Belfast*
Eyad Elkord, *Manchester*
Mohammad Ilyas, *Nottingham*
Simon R Carding, *Norwich*
Ian Chau, *Sutton*
Claudio Nicoletti, *Norwich*
Hendrik-Tobias Arkenau, *London*
Muhammad Imran Aslam, *Leicester*
Giuseppe Orlando, *Oxford*
John S Leeds, *Aberdeen*
S Madhusudan, *Nottingham*
Amin Ibrahim Amin, *Dunfermline*
David C Hay,*Edinburgh*
Alan Burns, *London*



United States

Tauseef Ali, *Oklahoma City*
George Y Wu, *Farmington*
Josef E Fischer, *Boston*
Thomas Clancy, *Boston*
John Morton, *Stanford*
Luca Stocchi, *Cleveland*
Kevin Michael Reavis, *Orange*
Shiu-Ming Kuo, *Buffalo*
Gary R Lichtenstein, *Philadelphia*
Natalie J Torok, *Sacramento*
Scott A Waldman, *Philadelphia*
Georgios Papachristou, *Pittsburgh*
Carla W Brady, *Durham*
Robert CG Martin, *Louisville*
Eugene P Ceppa, *Durham*
Shashi Bala, *Worcester*
Imran Hassan, *Springfield*
Klaus Thaler, *Columbia*
Andreas M Kaiser, *Los Angeles*
Shawn D Safford, *Norfolk*
Massimo Raimondo, *Jacksonville*
Kazuaki Takabe, *Richmond VA*
Stephen M Kavic, *Baltimore*
T Clark Gamblin, *Pittsburgh*
BS Anand, *Houston*
Ananthanarayanan M, *New York*
Anthony J Bauer, *Pittsburgh*
Edmund J Bini, *New York*
Xian-Ming Chen, *Omaha*
Ramsey Chi-man Cheung, *Palo Alto*
Parimal Chowdhury, *Arkansas*
Mark J Czaja, *New York*

Conor P Delaney, *Cleveland*
Sharon DeMorrow, *Temple*
Bijan Eghtesad, *Cleveland*
Alessandro Fichera, *Chicago*
Glenn T Furuta, *Aurora*
Jean-Francois Geschwind, *Baltimore*
Shannon S Glaser, *Temple*
Ajay Goel, *Dallas*
James H Grendell, *New York*
Anna S Gukovskaya, *Los Angeles*
Jamal A Ibdah, *Columbia*
Atif Iqbal, *Omaha*
Hajime Isomoto, *Rochester*
Hartmut Jaeschke, *Kansas*
Leonard R Johnson, *Memphis*
Rashmi Kaul, *Tulsa*
Ali Keshavarzian, *Chicago*
Miran Kim, *Providence*
Burton I Korelitz, *New York*
Richard A Kozarek, *Seattle*
Alyssa M Krasinskas, *Pittsburgh*
Ming Li, New *Orleans*
Zhiping Li, *Baltimore*
Chen Liu, *Gainesville*
Michael R Lucey, *Madison*
James D Luketich, *Pittsburgh*
Patrick M Lynch, *Houston*
Willis C Maddrey, *Dallas*
Mercedes Susan Mandell, *Aurora*
Wendy M Mars, *Pittsburgh*
Laura E Matarese, *Pittsburgh*
Lynne V McFarland, *Washington*
Stephan Menne, *New York*
Didier Merlin, *Atlanta*
George Michalopoulos, *Pittsburgh*
James M Millis, *Chicago*
Pramod K Mistry, *New Haven*
Emiko Mizoguchi, *Boston*
Peter L Moses, *Burlington*
Masaki Nagaya, *Boston*
Robert D Odze, *Boston*
Stephen JD O'Keefe, *Pittsburgh*
Zhiheng Pei, *New York*
Raymund R Razonable, *Minnesota*
Basil Rigas, *New York*
Richard A Rippe, *Chapel Hill*
Philip Rosenthal, *San Francisco*
Stuart Sherman, *Indianapolis*
Christina Surawicz, *Seattle*
Wing-Kin Syn, *Durham*
Yvette Taché, *Los Angeles*
K-M Tchou-Wong, *New York*
George Triadafilopoulos, *Stanford*
Chung-Jyi Tsai, *Lexington*
Andrew Ukleja, *Florida*
Arnold Wald, *Wisconsin*
Irving Waxman, *Chicago*
Steven D Wexner, *Weston*
Jackie Wood, *Ohio*
Jian Wu, *Sacramento*
Zobair M Younossi, *Virginia*
Liqing Yu, *Winston-Salem*
Ruben Zamora, *Pittsburgh*
Michael E Zenilman, *New York*
Michael A Zimmerman, *Colorado*
Beat Schnüriger, *California*
Clifford S Cho, *Madison*

R Mark Ghobrial, *Texas*
Anthony T Yeung, *Philadelphia*
Chang Kim, *West Lafayette*
Balamurugan N Appakalai, *Minneapolis*
Aejaz Nasir, *Tampa*
Ashkan Farhadi, *Irvine*
Kevin E Behrns, *Gainesville*
Joseph J Cullen, *Iowa City*
David J McGee, *Shreveport*
Anthony J Demetris, *Pittsburgh*
Dimitrios V Avgerinos, *New York*
Dong-Hui Li, *Houston*
Eric S Hungness, *Chicago*
Giuseppe Orlando, *Winston Salem*
Hai-Yong Han, *Phoenix*
Huanbiao Mo, *Denton*
Jong Park, *Tampa*
Justin MM Cates, *Nashville*
Charles P Heise, *Madison*
Craig D Logsdon, *Houston*
Ece A Mutlu, *Chicago*
Jessica A Davila, *Houston*
Rabih M Salloum, *Rochester*
Amir Maqbul Khan, *Marshall*
Bruce E Sands, *Boston*
Chakshu Gupta, *Saint Joseph*
Ricardo Alberto Cruciani, *New York*
Mariana D Dabeva, *Bronx*
Edward L Bradley III, *Sarasota*
Martín E Fernández-Zapico, *Rochester*
Henry J Binder, *New Haven*
John R Grider, *Richmond*
Ronnie Fass, *Tucson*
Dinesh Vyas, *Washington*
Wael El-Rifai, *Nashville*
Craig J McClain, *Louisville*
Christopher Mantyh, *Durham*
Daniel S Straus, *Riverside*
David A Brenner, *San Diego*
Eileen F Grady, *San Francisco*
Ekihiro Seki, *La Jolla*
Fang Yan, *Nashville*
Fritz Francois, *New York*
Giamila Fantuzzi, *Chicago*
Guang-Yin Xu, *Galveston*
Jianyuan Chai, *Long Beach*
JingXuan Kang, *Charlestown*
Le Shen, *Chicago*
Lin Zhang, *Pittsburgh*
Mitchell L Shiffman, *Richmond*
Douglas K Rex, *Indianapolis*
Bo Shen, *Cleveland*
Edward J Ciaccio, *New York*
Jean S Wang, *Saint Louis*
Bao-Ting Zhu, *Kansas*
Tamir Miloh, *Phoenix*
Eric R Kallwitz, *Chicago*
Yujin Hoshida, *Cambridge*
C Chris Yun, *Atlanta*
Alan C Moss, *Boston*
Oliver Grundmann, *Gainesville*
Linda A Feagins, *Dallas*
Chanjuan Shi, *Nashville*
Xiaonan Han, *Cincinnati*
William R Brugge, *Boston*
Richard W McCallum, *El Paso*
Lisa Ganley-Leal, *Boston*
Lin-Feng Chen, *Urbana*

Elaine Y Lin, *New York*
Julian Abrams, *New York*
Arun Swaminath, *New York*
Huiping Zhou, *Richmond*
Korkut Uygun, *Boston*
Anupam Bishayee, *Signal Hill*
C Bart Rountree, *Hershey*
Avinash Kambadakone, *Boston*
Courtney W Houchen, *Oklahoma*
Joshua R Friedman, *Philadelphia*
Justin H Nguyen, *Jacksonville*
Sophoclis Alexopoulos, *Los Angeles*
Suryakanth R Gurudu, *Scottsdale*
Wei Jia, *Kannapolis*
Yoon-Young Jang, *Baltimore*
Ourania M Andrisani, *West Lafayette*
Roderick M Quiros, *Bethlehem*
Timothy R Koch, *Washington*
Adam S Cheifetz, *Boston*
Lifang Hou, *Chicago*
Thiru vengadam Muniraj, *Pittsburgh*
Dhiraj Yadav, *Pittsburgh*
Ying Gao, *Rockville*
John F Gibbs, *Buffalo*
Aaron Vinik, *Norfolk*
Charles Thomas, *Oregon*
Robert Jensen, *Bethesda*
John W Wiley, *Ann Arbor*
Jonathan Strosberg, *Tampa*
Randeep Singh Kashyap, *New York*
Kaye M Reid Lombardo, *Rochester*
Lygia Stewart, *San Francisco*
Martin D Zielinski, *Rochester*
Matthew James Schuchert, *Pittsburgh*
Michelle Lai, *Boston*
Million Mulugeta, *Los Angeles*
Patricia Sylla, *Boston*
Pete Muscarella, *Columbus*
Raul J Rosenthal, *Weston*
Robert V Rege, *Dallas*
Roberto Bergamaschi, *New York*
Ronald S Chamberlain, *Livingston*
Alexander S Rosemurgy, *Tampa*
Run Yu, *Los Angeles*
Samuel B Ho, *San Diego*
Sami R Achem, *Florida*
Sandeep Mukherjee, *Omaha*
Santhi Swaroop Vege, *Rochester*
Scott Steele, *Fort Lewis*
Steven Hochwald, *Gainesville*
Udayakumar Navaneethan, *Cincinnati*
Radha Krishna Yellapu, *New York*
Rupjyoti Talukdar, *Rochester*
Shi-Ying Cai, *New Haven*
Thérèse Tuohy, *Salt Lake City*
Tor C Savidge, *Galveston*
William R Parker, *Durham*
Xiaofa Qin, *Newark*
Zhang-Xu Liu, *Los Angeles*
Adeel A Butt, *Pittsburgh*
Dean Y Kim, *Detroit*
Denesh Chitkara, *East Brunswick*
Mohamad A Eloubeidi, *Alabama*
JiPing Wang, *Boston*
Oscar Joe Hines, *Los Angeles*
Jon C Gould, *Madison*
Kirk Ludwig, *Wisconsin*
Mansour A Parsi, *Cleveland*

World Journal of Gastroenterology

Contents

Weekly Volume 18 Number 20 May 28, 2012

World Journal of Gastroenterology
Volume 18 Number 20 May 28, 2012

Contents

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN AND EISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

SPONSOR
Taiyuan Research and Treatment Center for Digestive Diseases, 77 Shuangta Xijie, Taiyuan 030001, Shanxi Province, China

EDITING
Editorial Board of *World Journal of Gastroenterology*
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

EDITORIAL OFFICE
Jian-Xia Cheng, Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpg@baishideng.com
http://www.wjgnet.com

PRINT SUBSCRIPTION
RMB 300 Yuan for each issue, RMB 14400 Yuan for one year.

PUBLICATION DATE
May 28, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/1007-9327office/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2443

World J Gastroenterol 2012 May 28; 18(20): 2443-2451
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

T cell immunopathogenesis and immunotherapeutic strategies for chronic hepatitis B virus infection

Yukihiro Shimizu

Yukihiro Shimizu, Gastroenterology Unit, Takaoka City Hospital, 4-1 Takara-machi, Takaoka city, Toyama 933-8550, Japan
Author contributions: Shimizu Y designed and wrote the manuscript.
Correspondence to: Yukihiro Shimizu, MD, PhD, Gastoenterology Unit, Takaoka City Hospital, 4-1 Takara-machi, Takaoka city, Toyama 933-8550, Japan. rsf14240@nifty.com
Telephone: +81-766-230204 Fax: +81-766-262882
Received: October 17, 2011 Revised: February 8, 2012
Accepted: February 26, 2012
Published online: May 28, 2012

Abstract

Hepatitis B is caused by the host immune response and T cells play a major role in the immunopathogenesis. More importantly, T cells not only destroy hepatocytes infected by hepatitis B virus (HBV), but also control HBV replication or eradicate HBV in a noncytolytic manner. Therefore, analysis of T cell immune response during acute and chronic HBV infection is important to develop a strategy for successful viral control, which could lead to immunotherapy for terminating persistent HBV infection. There have been many attempts at immunotherapy for chronic HBV infection, and some have shown promising results. High viral load has been shown to suppress antiviral immune responses and immunoinhibitory signals have been recently elucidated, therefore, viral suppression by nucleos(t)ide analogs, stimulation of antiviral immune response, and suppression of the immunoinhibitory signals must be combined to achieve desirable antiviral effects.

Key words: T cells; Immunopathogenesis; Immunotherapy; Hepatitis B virus infection

Peer reviewer: Dr. Huiping Zhou, Department of Microbiology and Immunology, Virginia Commonwealth University, 1217 East Marshall Street MB#533, Richmond, VA 23298, United States

Shimizu Y. T cell immunopathogenesis and immunotherapeutic strategies for chronic hepatitis B virus infection. *World J Gastroenterol* 2012; 18(20): 2443-2451 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2443.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2443

INTRODUCTION

Hepatitis B virus (HBV) is not cytopathic, and hepatitis B is caused by the host immune response, mainly T-cell-mediated, against virus-related peptides expressed on hepatocytes in conjunction with human leukocyte antigens (HLAs). In acute self-limiting hepatitis, a broad T-cell immune response occurs that is strong enough to eradicate the virus or suppress viral replication[1]. However, there are many mechanisms that hamper the antiviral immune response, leading to persistent infection. To develop an optimal strategy to stimulate antiviral immune response with therapeutic potential, extensive analyses of immune mechanisms for successful viral eradication and immunosuppressive mechanisms induced by viral infection during persistent infection are required. In this review, I focus on T cell immune response during HBV infection, and summarize attempted immunotherapeutic approaches against persistent HBV infection.

T CELL RESPONSE IN ACUTE HBV INFECTION

Immunological analysis has been extensively performed in transgenic and chimpanzee models of acute HBV infection. In one model, transgenic mice, in which infectious HBV virions replicate in the liver with expression of all HBV-related antigens, were injected with hepatitis B surface antigen (HBsAg)-specific cytotoxic T lymphocytes (CTLs) that had been induced in nontransgenic mice. The injected CTLs produced interferon (IFN)-γ and tumor necrosis factor (TNF)-α, which purged viral

RNA and DNA without destroying infected hepatocytes[2-4]. Importantly, this noncytolytic clearance of intracellular HBV is more efficient at controlling HBV replication than the killing of infected hepatocytes. In this sense, hepatitis is not only a harmful event but also represents an effective mechanism by which CTLs suppress HBV. Noncytolytic viral eradication can account for recovery from acute HBV infection in that most HBV is cleared from hepatocytes with only a fraction of the hepatocytes being destroyed. This was confirmed in a chimpanzee infection model; HBV DNA level was markedly decreased in the liver and blood of acutely infected chimpanzees before peak serum alanine aminotransferase (ALT) concentrations were reached[5], suggesting that this noncytopathic T cell effector mechanism results in early viral inhibition or eradication, whereas a cytopathic T cell effector mechanism is required to eliminate the remaining virus by destroying infected hepatocytes.

In humans, the HBV-specific T cell response during incubation phase of acute hepatitis B has been analyzed extensively using HLA class I tetramer and cytokine staining[6]. The data showed that maximal reduction in HBV DNA in the serum occurred before the peak of ALT elevation; again indicating that suppression of HBV replication occurs without hepatocyte injury. Moreover, infiltration of HBV-specific $CD8^+$ T cells into the liver has been observed several weeks before the peak of liver injury, suggesting that HBV-specific T cell infiltration occurs at an early stage of infection, resulting in suppression of HBV replication. Thereafter, recruitment of mostly nonspecific cells induced by cytokines or chemokines produced by HBV-specific T cells contributes to significant liver damage. Interestingly, in the HBV transgenic mouse model of acute hepatitis, administration of antibodies against the chemokines, IFN-inducible protein (IP-10) and monokine induced by interferon-(Mig), reduced the recruitment of mostly antigen-nonspecific mononuclear cells into the liver that had been induced by cytokines and chemokines produced by injected CTLs, leading to a reduction in the severity of hepatitis without affecting the antiviral activity of the CTLs[7]. These observations have important therapeutic implications, because suppression of antigen-nonspecific mononuclear cell recruitment may suppress hepatitis, while retaining the antiviral function of the CTLs.

The overall data from studies in chimpanzees and humans are essentially the same, and indicate that a sufficient T cell response to HBV at an early phase of infection is important for eradication of virus infection, and that an insufficient T cell response may lead to persistent viral infection.

The contributions of $CD4^+$ and $CD8^+$ T cells to the control of viral infection have been analyzed in a chimpanzee model of acute hepatitis B by depleting either T cell population with monoclonal antibodies. The data show that $CD8^+$ T cells are the main effector cells responsible for virus elimination[8].

Antigen specificity of T cell response in acute HBV infection

The antigen specificity of the T cell response to HBV in acute hepatitis has been analyzed, and it is clear that acute viral hepatitis involves a vigorous CTL response to multiple epitopes in the viral nucleocapsid, envelope, and polymerase proteins, whereas these are not seen in patients with chronic hepatitis[1]. Although multispecificity of the CTL response is characteristic in acute hepatitis, there is known to be a hierarchy of epitope-specific $CD8^+$ T cell responses determined by cytokine production after peptide stimulation. In acute hepatitis B, $CD8^+$ T cell response to HBc18-27 (HLA-A2 restricted epitope) is dominant followed by the response to polymerase epitope (455-463), whereas envelope epitopes are always subdominant[9]. The hierarchy is clearly distinct from that observed in chronic hepatitis, in which the $CD8^+$ T cell response to envelope epitope (183-191) is always dominant. Interestingly, chronic hepatitis patients with lower HBV DNA levels in the serum show greater responses to HBc18-27 than those with high HBV DNA. These findings imply that the T cell response to hepatitis B core antigen (HBcAg) is important for viral control, which is important for designing peptide vaccines for the treatment of chronic HBV infection.

Long-lasting T cell immune response after resolution of acute hepatitis B

In humans, most HBV is cleared after resolution of acute hepatitis. However, it has been shown that trace amounts of HBV DNA can be detected for several years after resolution of acute hepatitis, and the long-lasting memory T cell response is maintained by persistent replication of HBV[10], indicating that low levels of HBV replication could continue in most patients even in the convalescent phase of acute hepatitis in balance with immunological pressure.

T CELL RESPONSE IN CHRONIC HBV INFECTION

In peripheral blood, HBV-specific helper T lymphocytes and CTLs are barely detectable in patients with chronic hepatitis B (CHB)[11], possibly due to exhaustion by high viral load or tolerance to HBV.

In contrast, several studies have characterized intrahepatic $CD4^+$ and $CD8^+$ T lymphocytes in CHB. Intrahepatic $CD4^+$ T lymphocytes in patients with CHB have been found to contain T helper (Th)0 cells, which produce not only IFN-γ, but also interleukin (IL)-4 and IL-5, thus differing from cells in the livers of patients with chronic hepatitis C, which are mostly Th1 cells[12]. $CD4^+$ T lymphocytes that produce IL-17 infiltrate into the livers of patients with CHB and are involved in liver inflammation[13].

Livers of patients with low HBV replication contain intralobular $CD8^+$ T lymphocytes[14], and the percent-

ages of virus-specific T lymphocytes in the liver have been clarified by immunohistochemical staining with peptide-MHC tetramer. The proportion of $CD8^+$ T lymphocytes in the livers of patients with chronic HBV specific for HBc18-27, a major HBV epitope, has been found to range from 0.18% to 1.28%[15]. Maini *et al*[16] have reported that the number of HBc18-27-specific $CD8^+$ T cells, detected using tetramers, was the same in livers with low HBV DNA/ALT as in those with high HBV DNA/ALT. Hence, HBV-specific T cells recognize HBV antigens and carry out immune surveillance in the liver. Thus, they have an important role in controlling HBV replication in the liver without causing hepatic necroinflammation in low DNA/ALT anti-HBe^+ HBV carriers. It remains unknown why HBV-specific T cells fail to control effectively HBV replication in the liver with chronic hepatitis. However, recent advances in immunology have given some insight into the mechanism as described below.

IMMUNOSUPPRESSIVE MECHANISM RESPOSIBLE FOR PERSISTENT HBV INFECTION

Regulatory T cells

Regulatory T (Treg) cells expressing the forkhead family transcription factor, FoxP3, are specialized cells that exert negative control on a variety of physiological and pathological immune responses, resulting in maintenance of immunological self-tolerance[17]. They show diverse phenotypes, occurring in both $CD4^+$ and $CD8^+$ T cell subsets, and express CD25 (IL-2 receptor chain) and/or cytotoxic T-lymphocyte antigen 4 (CTLA-4) in addition to Foxp3.

In HBV infection, hepatitis B e antigen (HBeAg)-positive patients with high HBV DNA levels in the serum show elevated numbers of $CD4^+CD25^+$ Treg cells in the blood compared to patients with acute and chronic hepatitis C virus (HCV) infection[18]. Significant accumulation of $CD4^+CD25^+FoxP3^+$ Treg cells in the liver is found in patients with chronic HBV infection. Moreover, patients with high viral load have a higher proportion of Treg cells in the liver[19], suggesting that intrahepatic Treg cells suppress antiviral immune responses in the liver in chronic HBV infection.

Th cells that produce IL-17 (Th17 cells) have recently been identified as the third subset of effector T cells[20], which produce IL-17A, IL-17F, IL-22 and IL-21[21]. Recently, IL-6 has been shown to induce the generation of Th17 cells from naïve T cells together with transforming growth factor (TGF)-β and inhibits TGF-induced Treg cell differentiation[22]. Importantly, there is a reciprocal relationship between Th17 and Treg cells; not only in development, but also in their effector function, indicating that the Treg/Th17 balance may determine the quality and magnitude of immune responses in the liver[20]. Unexpectedly, the increases in circulating and intrahepatic Th17 cells are positively correlated with HBV DNA in the serum, serum ALT levels, and histological activity index of the livers with CHB, suggesting that activation of Th17 cells does not exert antiviral function in CHB[23].

Programmed death-1

Programmed death-1 (PD-1) is a surface receptor critical for the regulation of T cell function[24,25]. Binding to PD-1 by its ligands PD-L1 and PD-L2 results in the antigen-specific inhibition of T cell proliferation, cytokine production, and cytolytic function, leading to exhaustion of T cells. In the liver, PD-1 is expressed on lymphocytes; PD-L1 is expressed on lymphocytes, hepatocytes and sinusoidal endothelial cells; and PD-L2 is expressed on Kupffer cells and dendritic cells (DCs)[26]. HBeAg-positive patients with high HBV DNA levels in the serum show increased PD-1 and CTLA-4 expression on HBV-specific $CD8^+$ T cells[27]. Moreover, PD-1 expression on $CD4^+$ T cells is correlated positively with serum HBV DNA load in CHB patients[28]. Intrahepatic HBV-specific $CD8^+$ T cells express higher levels of PD-1, and upregulation of intrahepatic PD-1/PD-L1 is associated with liver inflammation and ALT elevation[29]. Although the mechanism underlying the upregulation of PD-1 on $CD8^+$ T cells in the inflamed liver is unknown, signals from PD-1 inhibit HBV-specific T cells, resulting in insufficient antiviral responses, leading to failure of viral control and persistent liver inflammation. Importantly, PD-1/PD-L1 blockade increased $CD8^+$ T cell proliferation and enhanced IFN-γ and IL-2 production by intrahepatic lymphocytes[29]. These findings suggest that inhibition of PD-1/PD-L1 may have therapeutic potential for the control of hepatitis B.

IL-10

IL-10 is an important cytokine with anti-inflammatory properties, and is produced by activated monocytes/macrophages and T cell subsets, including Treg and Th1 cells[30]. Immunosuppression by IL-10 is associated with functional exhaustion of memory T cells in chronic lymphocytic choriomeningitis virus (LCMV) infection, and blockade of IL-10 receptors could terminate chronic LCMV infection[31]. In chronic HBV infection, HBcAg stimulates the production of IL-10, which negatively regulates HBcAg-specific Th17 cell responses in CHB patients[32].

T cell immunoglobulin- and mucin-domain-containing molecule-3

It has been reported that not all exhausted T cells show upregulation of PD-1 and downregulation of CD127 (IL-7 receptor), and blockade of the PD-1/PD-L1 signaling pathway does not always restore proliferation and cytokine production[33]. Recently, another inhibitory molecule, T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3), has been reported. A high frequency of Tim3-expressing $CD4^+$ and $CD8^+$ T cells are found in chronic HBV infection, and the frequency of $Tim\text{-}3^+$ T cells is positively correlated with the severity of liver inflammation, and negatively correlated with plasma IFN-γ levels[34].

Table 1 Immunotherapeutic approaches for animal models of hepatitis B virus infection

Animal model	Immunotherapy	Results	Ref.
Peptide vaccination			
HBV transgenic mice	A synthesized fusion peptide, consisting HBcAg18-27 and HIV Tat49-57	Decrease in serum HBV DNA levels and the expression levels of HBsAg and HBcAg in the liver	[40]
Protein vaccination			
HBV transgenic mice	HBsAg vaccine	Most of the mice showed reduction of HBV DNA levels and disappearance of HBeAg and HBsAg	[41]
Woodchuck hepatitis virus infection	Combination of vaccine of HBV large surface protein and clevudine	Restoration of T-cell response to Pre-S and S region	[42]
DNA immunization			
Acute DHBV infection	DNA vaccine expressing DHBc and Pre-S/S and entecavir Boosted with fowl poxvirus vectors expressing DHBc and Pre-S/S	Clearance of DHBV infection at a rate of 100%	[43]
Chronic DHBV infection	DNA vaccine encoding the HBV large envelope and/or core protein with or without lamivudine	Reduction of viremia and liver DHBV cccDNA in 33% of ducks Seroconversion to anti-pre S in 67% of ducks showing cccDNA clearance	[44]
DC immunization			
HBV transgenic mice	Activated bone marrow-derived DCs	Break CTL tolerance to HBsAg	[45]
HBV transgenic mice	Anti-CD40 agonistic monoclonal Ab	Induction of noncytopathic inhibition of HBV replication mediated by antiviral cytokines (IL-12 and TNF-α) produced by activated intrahepatic APCs	[46]
HBV transgenic mice	HBV-specific peptide-pulsed DCs	Reductions in the serum HBsAg and HBV DNA	[47]
Cytokines and adjuvants			
HBV transgenic mice	Recombinant IL-12	Marked inhibition of HBV replication in the liver	[48]
HBV transgenic mice	α-galactosylceramide that can activate NK T cells	Complete inhibition of HBV replication	[49]
HBV transgenic mice	Recombinant IL-18	Inhibition of HBV replication noncytopathically, mediated by activation of resident intrahepatic NK cells and NK T cells	[50]
Gene therapy			
HBsAg transgenic mice	Lentivectors expressing HBsAg and IgFc fusion Ag	Induction of seroconversion to anti-HBs	[51]

HBV: Hepatitis B virus; DHBV: Duck HBV; DC: Dendritic cell; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core antigen; HIV: Human immunodeficiency virus; APC: Antigen-presenting cell; IL: Interleukin; NK: Natural killer; CTL: Cytotoxic T lymphocyte; TNF: Tumor necrosis factor; cccDNA: Covalently closed circular DNA; Ab: Antibody; Ag: Antigen.

Dysfunction of DCs

DCs are specialized antigen-presenting cells that orchestrate immune responses. They stimulate innate and acquired immune responses, but also act as tolerogenic cells for immune responses in a variety of situations. In viral hepatitis, dysfunction of DCs from peripheral blood has been reported. In patients with CHB, maturation of DCs from peripheral blood of patients after incubation with cytokines is lower than that of normal subjects with lower expression of HLA-DR and co-stimulatory molecules in the former population[35], leading to low allostimulatory function of DCs from CHB patients. The mechanism of impairment of DC function in patients with CHB is unclear, but both HBV particles and purified HBsAg may have immunomodulatory capacity and may directly contribute to the dysfunction of myeloid DCs[36]. Interestingly, impaired function of monocyte-derived DCs from patients with CHB could be reversed by inhibiting viral replication with nucleos(t)ide analogs such as lamivudine[37]. Type 2 precursor plasmacytoid dendritic cells (pDCs), which are the most important cells in antiviral innate immunity, are also reported to have quantitative and qualitative impairment in patients with chronic HBV infection[38]. Recently, HBV itself was shown to inhibit the functions of pDCs[39]. These data indicate that DCs in patients with CHB have impaired function leading to insufficient T cell response to HBV, which could be the mechanism responsible for persistent viral infection.

IMMUNOTHERAPY FOR VIRAL HEPATITIS

In chronic HBV infection, strong long-term viral suppression can now be achieved with various nucleoside or nucleotide analogs. However, there are some problems that must be solved in the near future. One of the problems with treatment with nucleos(t)ide analogs is a low rate of HBe seroconversion even after long-term administration in $HBeAg^+$ patients. Moreover, reactivation rate of HBV replication is high in both $HBeAg^+$ and $HBeAg^-$ patients after cessation of treatment, although drug-free viral controls would be better than long-term administration of the drugs in terms of control of medical costs and avoidance of adverse effects of these agents. It could be possible to achieve long-term viral eradication even after cessation of nucleos(t)ide analogs, if viral suppression with nucleos(t)ide analogs could be combined with efficient immunotherapies.

Previous animal studies and human trials in HBV infection are listed in Tables 1 and 2, respectively.

IMMUNOTHERAPEUTIC APPROACHES FOR HBV INFECTION

Immunotherapeutic strategies for CHB include suppres-

Table 2 Immunotherapeutic trials for chronic hepatitis B virus infection in humans

Immunotherapy	Results	Ref.
Peptide vaccination		
A vaccine with HBc18-27 peptide comprised of a T-helper cell epitope and two palmitic acid residues	Low levels of CTL activity were induced but no significant changes in liver biochemistry or viral serology were observed	[52]
Protein vaccination		
PreS2/S (GenHevac B) or S (Recombivax)	HBe/anti-HBe seroconversion in 13% and HBV DNA negativity in 16% of the treated patients	[53]
Intradermal HBsAg vaccine and laimvudine in combination with IL-2	Induction of significant HBV DNA loss in the serum in two of five the treated patients	[54]
Oral administration of HBV envelope proteins (HBsAg + preS1 + preS2)	Induction of histological improvement in 30%, HBeAg negativity in 26.3% and HBsAg-specific T cell proliferation in 78% of the treated patients	[55]
IFN-α-2b monotherapy (9 mo) or IFN-α-2b plus pre-S2/S vaccine	Greater reduction in HBV DNA in patients with combination HBV therapy than those who received IFN-α-2b monotherapy	[56]
The combination with lamivudune and HBsAg vaccine in $HBeAg^+$ cases	No improvement of HBe seroconversion rate in comparison with lamivudine therapy alone	[57]
Combination of lamivudine and HBsAg vaccine	Induction of sustained negativity of HBV DNA in 1/4 of patients	[58]
Combination of lamivudine and HBsAg vaccine	HBV DNA became undetectable in 64% of the patients, and was decreased in the remaining patients	[59]
DNA immunization		
DNA vaccine encoding HBV envelope protein	Induction of an increase in HBV-specific IFN-γ-secreting T cells in nonresponders to conventional therapies, and HBV DNA levels were transiently decreased in 50% of vaccinated patients	[60]
DNA vaccine encoding PreS and S in patients with lamivudine breakthrough	Development of IFN-γ-producing T cells specific for preS or S antigen; Two of 10 patients showed seroconversion to anti-HBe	[61]
DC immunization		
Peripheral blood-derived DCs, activated with GM-CSF and IL-4 pulsed with HBsAg	Both patients with normal and elevated ALT responded equally to DC vaccine and 53% of the patients showed induction of HBeAg negativity	[62]
Activated DCs from PBL with GM-CSF and IL-4, pulsed with two peptides, HBc18-27 and PreS2 44-53	Undetectable HBV DNA was achieved in 46.3% and 3.1% of $HBeAg^-$ and HBeAg+ patients, respectively. ALT normalization was observed in 69% and 30.5% of HBeAg- and HBeAg+ patients, respectively	[63]
Cytokines		
GM-CSF	Safe and tolerable up to 1.0 μg/kg body weight, and induced HBV DNA negativity in 4/8 patients	[64]
Combination therapy with GM-CSF and HBsAg vaccine in HBV carrier children	Significant reduction of serum HBV DNA	[65]
High dose of IL-12 (0.5 μg/kg)	HBV DNA clearance was observed in 25% of the patients	[66]
Combination of IL-12 and lamivudine	Stimulation of T cell response to HBV with IFN-γ production. However, IL-12 was unable to suppress re-elevation of HBV DNA after cessation of lamivudine	[67]
Combination of IL-12 and IL-18	Stimulation of IFN-γ production by CD4+ T cells isolated from peripheral blood in response to HBcAg, and the effect was greater than those observed with either cytokine alone	[68]
α-galactosylceramide	Poorly tolerated and showed no clear suppressive effect on serum HBV DNA or ALT levels	[69]
Tα1		
Combination of Tα1 and IFN-α	No statistically significant differences as compared with IFN-α monotherapy with respect to HBeAg seroconversion, changes in histology, normalization of ALT or loss of HBV DNA	[70]
Tα1 alone	At 12 mo after cessation of therapy, 36.4% of patients treated with 1.6 mg of Tα1 achieved ALT normalization, 15% achieved HBV DNA clearance by transcription-mediated amplification, and 22.8% achieved clearance of HBeAg	[71]
Comparative effect of Tα1 and IFN-α	Tα1 treatment was more effective in achieving ALT normalization and HBV DNA negativity at the end of the follow-up period than IFN-α	[72]
Combination of Tα1 and lamivudine	No any additional antiviral effect compared with lamivudine monotherapy as determined by HBe seroconversion and the emergence of viral breakthrough	[73]
Combination therapy with lamivudine and Tα1	Induction of significantly higher rates of ALT normalization, virological response, and HBeAg seroconversion than lamivudine monotherapy	[74]

HBV: Hepatitis B virus; Tα1: Thymosin α1; IFN: Interferon; ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; HBsAg: Hepatitis B surface antigen; HBcAg: Hepatitis B core antigen; PBL: Peripheral blood lymphocytes.

sion of viral replication, stimulation of T cell immune response to hepatitis virus, activation of nonspecific cells, and administration of cytokines with antiviral activity (Tables 1 and 2).

Suppression of viral replication

High viral load has been shown to suppress $CD4^+$ and $CD8^+$ T cells in addition to induction of Treg cells, which could be reversed by antiviral therapy in CHB[75].

Therefore, immunotherapy followed by restoration of virus-specific T cell response with antiviral therapy could be more efficient in CHB.

Stimulation of immune response to HBV

Peptide immunization: A peptide vaccine containing highly immunogenic HBc18-27 has been developed and administered to CHB patients[52], but the results were disappointing because there was no induction of a significant antiviral T cell response.

Protein immunization: In a model of HBV in transgenic mice, vaccine on the base of surface antigen in complete Freund's adjuvant once monthly for 1 year induced reduction in HBV DNA, and the disappearance of HBeAg and HBsAg in most mice treated[41]. Moreover, it is important to note that some mice developed anti-HBs in the sera. However, several human trials with HBsAg vaccine showed limited efficacy if used as monotherapy.

Recently, hepatitis B vaccine containing not only S protein but also preS has been used with increased immunogenicity[53,55], or has been combined with lamivudine or IFN-α[56], leading to potential improvement of clinical efficacy. However, analysis of the T cell epitope hierarchy has indicated that the most important epitope for viral control is HBc18-27, and not the HBsAg epitope in HLA-A2 patients[9], suggesting the necessity to reconsider antigen selection for vaccination that could lead to better viral control.

DNA immunization: Injection of plasmid DNA has been shown to elicit strongly both cellular and humoral immune responses, and is now known to be safe and well-tolerated both in mice and humans. In a model of duck HBV infection, DNA vaccine encoding HBV large envelope and/or core protein was shown to induce reduction in not only viremia but also covalently closed circular DNA (cccDNA) in the liver in one thirds of ducks receiving DNA monotherapy or combination treatment with lamivudine[44]. This finding is encouraging because clearance of cccDNA from the liver is the goal of treatment for HBV infection, but is difficult to achieve using IFN-α or nucleos(t)ide analogs. Clinical trials have also been performed in HBV infection with some encouraging results, which remain to be confirmed by future randomized large-scale trials.

DC immunization: DCs are specialized antigen-presenting cells that can induce strong immune responses in T and B cells. We have previously shown that activated bone-marrow-derived DCs can break CTL tolerance to HBsAg in HBV transgenic mice[45]. Thereafter, several immunotherapies with activated DCs have been applied in both animals and humans. In a recent study performed in HBV transgenic mice, peptide-pulsed DCs were shown to reduce significantly the concentrations of serum HBsAg and HBV DNA[47], indicating therapeutic potential in chronic HBV infection. Recently, DCs treated with peptide inhibitors of IL-10 have been shown to induce strong anti-HCV T cell responses in HCV transgenic mice[76], suggesting a strategy to augment the immunogenic function of DCs. Moreover, when intrahepatic antigen-presenting cells, including DCs, are activated by injection of an anti-CD40 agonistic antibody, HBV replication is inhibited by a noncytopathic mechanism, possibly through production of antiviral cytokines such as TNF-α and IL-12[46]. Although no CTL response against HBV antigens was reported in this study, the *in vivo* activation of DCs could be an alternative way for inducing antiviral immune responses, including possible activation of CTLs against HBV. In humans, injection of activated DCs loaded with HBV peptide or protein has achieved a reduction in HBV DNA level in some patients[62,63]. HBeAg negativity was achieved in more than half of the treated patients in one study[62]. Although preparation of activated and mature DCs incurs financial costs and requires experienced researchers, immunotherapy with DCs is a promising method.

Natural killer T cells: A single injection of α-galactosylceramide abolished HBV replication by activating natural killer (NK) T cells in the liver in HBV transgenic mice[49]. However, α-galactosylceramide was poorly tolerated in humans and showed no clear antiviral effect[69], possibly due to smaller numbers of NKT cells in the human liver than in the mouse liver.

Cytokines and thymosin-1: Cytokines such as IL-12[48] and IL-18[50] have been shown to inhibit HBV replication noncytopathically in HBV transgenic mice. In humans, granulocyte–macrophage colony-stimulating factor[64,65] and IL-12[66,67] have been used for treatment with some antiviral effects. They have been used as monotherapy or in combination with hepatitis B vaccine or lamivudine.

Thymosin (T)α1, a synthetic 28-amino acid peptide, is able to enhance the Thl immune response and also exerts a direct antiviral mechanism of action. It has been used for the treatment of chronic HBV infection in humans[70-73], and has shown some antiviral efficacy. Although antiviral effect by the addition of Tα1 to lamivudine or IFN-α therapy was controversial, a meta-analysis has demonstrated that combination therapy with lamivudine and Tα1 shows significantly higher rates of ALT normalization, virological response, and HBeAg seroconversion as compared with lamivudine monotherapy[74]. It is of note that HBeAg seroconversion rate was 45% in the combination group, which was significantly higher than that with lamivudine monotherapy (15%).

Blockade of immunoinhibitory signals

Recently, there have been several basic attempts to improve the efficacy of immunotherapy. Among these reports, augmentation or restoration of T cell response by blocking the inhibitory signals has been extensively analyzed *in vitro*. It has been demonstrated that exhausted T

cells express not only PD-1, but also CTLA-4[77], CD244[78] or Tim-3[33], and blocking of these molecules in combination could be better than blocking any single molecule to achieve full activation of the exhausted T cells.

CONCLUSION

There have been several attempts to apply immunotherapy for the control of chronic HBV infection, and some of the data are promising. Viral suppression, stimulation of antiviral immune response with cytokines or immunization with peptide, protein, DNA or DCs, and suppression of the immunoinhibitory signals must be combined to achieve desirable antiviral effects, although further studies are required to explore the best protocols and their most efficient combinations.

REFERENCES

1 **Rehermann B**. Immunopathogenesis of viral hepatitis. *Baillieres Clin Gastroenterol* 1996; **10**: 483-500
2 **Guidotti LG**, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 1996; **4**: 25-36
3 **Chisari FV**. Cytotoxic T cells and viral hepatitis. *J Clin Invest* 1997; **99**: 1472-1477
4 **Guidotti LG**, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; **19**: 65-91
5 **Guidotti LG**, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science* 1999; **284**: 825-829
6 **Webster GJ**, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, Brown D, Amlot PL, Williams R, Vergani D, Dusheiko GM, Bertoletti A. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 2000; **32**: 1117-1124
7 **Kakimi K**, Lane TE, Wieland S, Asensio VC, Campbell IL, Chisari FV, Guidotti LG. Blocking chemokine responsive to gamma-2/interferon (IFN)-gamma inducible protein and monokine induced by IFN-gamma activity in vivo reduces the pathogenetic but not the antiviral potential of hepatitis B virus-specific cytotoxic T lymphocytes. *J Exp Med* 2001; **194**: 1755-1766
8 **Thimme R**, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol* 2003; **77**: 68-76
9 **Webster G**, Bertoletti A. Quantity and quality of virus-specific CD8 cell response: relevance to the design of a therapeutic vaccine for chronic HBV infection. *Mol Immunol* 2001; **38**: 467-473
10 **Penna A**, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, Chisari FV, Rehermann B, Del Prete G, Fiaccadori F, Ferrari C. Long-lasting memory T cell responses following self-limited acute hepatitis B. *J Clin Invest* 1996; **98**: 1185-1194
11 **Ferrari C**, Penna A, Bertoletti A, Valli A, Antoni AD, Giuberti T, Cavalli A, Petit MA, Fiaccadori F. Cellular immune response to hepatitis B virus-encoded antigens in acute and chronic hepatitis B virus infection. *J Immunol* 1990; **145**: 3442-3449
12 **Bertoletti A**, D'Elios MM, Boni C, De Carli M, Zignego AL, Durazzo M, Missale G, Penna A, Fiaccadori F, Del Prete G, Ferrari C. Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. *Gastroenterology* 1997; **112**: 193-199
13 **Zhang JY**, Zhang Z, Lin F, Zou ZS, Xu RN, Jin L, Fu JL, Shi F, Shi M, Wang HF, Wang FS. Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B. *Hepatology* 2010; **51**: 81-91
14 **Tang TJ**, Kwekkeboom J, Laman JD, Niesters HG, Zondervan PE, de Man RA, Schalm SW, Janssen HL. The role of intrahepatic immune effector cells in inflammatory liver injury and viral control during chronic hepatitis B infection. *J Viral Hepat* 2003; **10**: 159-167
15 **Shimada N**, Yamamoto K, Kuroda MJ, Terada R, Hakoda T, Shimomura H, Hata H, Nakayama E, Shiratori Y. HBcAg-specific CD8 T cells play an important role in virus suppression, and acute flare-up is associated with the expansion of activated memory T cells. *J Clin Immunol* 2003; **23**: 223-232
16 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191**: 1269-1280
17 **Miyara M**, Sakaguchi S. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. *Immunol Cell Biol* 2011; **89**: 346-351
18 **Xu D**, Fu J, Jin L, Zhang H, Zhou C, Zou Z, Zhao JM, Zhang B, Shi M, Ding X, Tang Z, Fu YX, Wang FS. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006; **177**: 739-747
19 **Stoop JN**, Claassen MA, Woltman AM, Binda RS, Kuipers EJ, Janssen HL, van der Molen RG, Boonstra A. Intrahepatic regulatory T cells are phenotypically distinct from their peripheral counterparts in chronic HBV patients. *Clin Immunol* 2008; **129**: 419-427
20 **Zhao L**, Qiu de K, Ma X. Th17 cells: the emerging reciprocal partner of regulatory T cells in the liver. *J Dig Dis* 2010; **11**: 126-133
21 **Hu Y**, Shen F, Crellin NK, Ouyang W. The IL-17 pathway as a major therapeutic target in autoimmune diseases. *Ann N Y Acad Sci* 2011; **1217**: 60-76
22 **Kitani A**, Xu L. Regulatory T cells and the induction of IL-17. *Mucosal Immunol* 2008; **1** Suppl 1: S43-S46
23 **Zhang JY**, Song CH, Shi F, Zhang Z, Fu JL, Wang FS. Decreased ratio of Treg cells to Th17 cells correlates with HBV DNA suppression in chronic hepatitis B patients undergoing entecavir treatment. *PLoS One* 2010; **5**: e13869
24 **Francisco LM**, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 2010; **236**: 219-242
25 **Fife BT**, Pauken KE. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann N Y Acad Sci* 2011; **1217**: 45-59
26 **Chen J**, Wang XM, Wu XJ, Wang Y, Zhao H, Shen B, Wang GQ. Intrahepatic levels of PD-1/PD-L correlate with liver inflammation in chronic hepatitis B. *Inflamm Res* 2011; **60**: 47-53
27 **Peng G**, Luo B, Li J, Zhao D, Wu W, Chen F, Chen Z. Hepatitis B e-antigen persistency is associated with the properties of HBV-specific CD8 T cells in CHB patients. *J Clin Immunol* 2011; **31**: 195-204
28 **Nan XP**, Zhang Y, Yu HT, Li Y, Sun RL, Wang JP, Bai XF. Circulating CD4+CD25high regulatory T cells and expression of PD-1 and BTLA on CD4+ T cells in patients with chronic hepatitis B virus infection. *Viral Immunol* 2010; **23**: 63-70
29 **Fisicaro P**, Valdatta C, Massari M, Loggi E, Biasini E, Sacchelli L, Cavallo MC, Silini EM, Andreone P, Missale G, Ferrari C. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology* 2010; **138**: 682-693, 693.e1-4
30 **Sabat R**, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, Geginat J. Biology of interleukin-10. *Cytokine Growth Factor Rev* 2010; **21**: 331-344

31 **Ejrnaes M**, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, von Herrath MG. Resolution of a chronic viral infection after interleukin-10 receptor blockade. *J Exp Med* 2006; **203**: 2461-2472

32 **Li J**, Wu W, Peng G, Chen F, Bai M, Zheng M, Chen Z. HBcAg induces interleukin-10 production, inhibiting HBcAg-specific Th17 responses in chronic hepatitis B patients. *Immunol Cell Biol* 2010; **88**: 834-841

33 **Golden-Mason L**, Palmer BE, Kassam N, Townshend-Bulson L, Livingston S, McMahon BJ, Castelblanco N, Kuchroo V, Gretch DR, Rosen HR. Negative immune regulator Tim-3 is overexpressed on T cells in hepatitis C virus infection and its blockade rescues dysfunctional CD4+ and CD8+ T cells. *J Virol* 2009; **83**: 9122-9130

34 **Ju Y**, Hou N, Zhang XN, Zhao D, Liu Y, Wang JJ, Luan F, Shi W, Zhu FL, Sun WS, Zhang LN, Gao CJ, Gao LF, Liang XH, Ma CH. Blockade of Tim-3 pathway ameliorates interferon-gamma production from hepatic CD8+ T cells in a mouse model of hepatitis B virus infection. *Cell Mol Immunol* 2009; **6**: 35-43

35 **Wang FS**, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2001; **7**: 537-541

36 **Op den Brouw ML**, Binda RS, van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, Woltman AM. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. *Immunology* 2009; **126**: 280-289

37 **Beckebaum S**, Cicinnati VR, Zhang X, Ferencik S, Frilling A, Grosse-Wilde H, Broelsch CE, Gerken G. Hepatitis B virus-induced defect of monocyte-derived dendritic cells leads to impaired T helper type 1 response in vitro: mechanisms for viral immune escape. *Immunology* 2003; **109**: 487-495

38 **Duan XZ**, Wang M, Li HW, Zhuang H, Xu D, Wang FS. Decreased frequency and function of circulating plasmocytoid dendritic cells (pDC) in hepatitis B virus infected humans. *J Clin Immunol* 2004; **24**: 637-646

39 **Woltman AM**, Ter Borg MJ, Binda RS, Sprengers D, von Blomberg BM, Scheper RJ, Hayashi K, Nishi N, Boonstra A, van der Molen R, Janssen HL. Alpha-galactosylceramide in chronic hepatitis B infection: results from a randomized placebo-controlled Phase I/II trial. *Antivir Ther* 2009; **14**: 809-818

40 **Wang S**, Han Q, Zhang N, Chen J, Liu Z, Zhang G, Li Z. HBcAg18-27 epitope fused to HIV-Tat 49-57 adjuvanted with CpG ODN induces immunotherapeutic effects in transgenic mice. *Immunol Lett* 2010; **127**: 143-149

41 **Akbar SM**, Kajino K, Tanimoto K, Kurose K, Masumoto T, Michitaka K, Horiike N, Onji M. Placebo-controlled trial of vaccination with hepatitis B virus surface antigen in hepatitis B virus transgenic mice. *J Hepatol* 1997; **26**: 131-137

42 **Menne S**, Tennant BC, Gerin JL, Cote PJ. Chemoimmunotherapy of chronic hepatitis B virus infection in the woodchuck model overcomes immunologic tolerance and restores T-cell responses to pre-S and S regions of the viral envelope protein. *J Virol* 2007; **81**: 10614-10624

43 **Miller DS**, Boyle D, Feng F, Reaiche GY, Kotlarski I, Colonno R, Jilbert AR. Antiviral therapy with entecavir combined with post-exposure "prime-boost" vaccination eliminates duck hepatitis B virus-infected hepatocytes and prevents the development of persistent infection. *Virology* 2008; **373**: 329-341

44 **Thermet A**, Buronfosse T, Werle-Lapostolle B, Chevallier M, Pradat P, Trepo C, Zoulim F, Cova L. DNA vaccination in combination or not with lamivudine treatment breaks humoral immune tolerance and enhances cccDNA clearance in the duck model of chronic hepatitis B virus infection. *J Gen Virol* 2008; **89**: 1192-1201

45 **Shimizu Y**, Guidotti LG, Fowler P, Chisari FV. Dendritic cell immunization breaks cytotoxic T lymphocyte tolerance in hepatitis B virus transgenic mice. *J Immunol* 1998; **161**: 4520-4529

46 **Kimura K**, Kakimi K, Wieland S, Guidotti LG, Chisari FV. Activated intrahepatic antigen-presenting cells inhibit hepatitis B virus replication in the liver of transgenic mice. *J Immunol* 2002; **169**: 5188-5195

47 **Jiang WZ**, Fan Y, Liu X, Zhang YL, Wen JJ, Hao WL, Qian M. Therapeutic potential of dendritic cell-based immunization against HBV in transgenic mice. *Antiviral Res* 2008; **77**: 50-55

48 **Cavanaugh VJ**, Guidotti LG, Chisari FV. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. *J Virol* 1997; **71**: 3236-3243

49 **Kakimi K**, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med* 2000; **192**: 921-930

50 **Kimura K**, Kakimi K, Wieland S, Guidotti LG, Chisari FV. Interleukin-18 inhibits hepatitis B virus replication in the livers of transgenic mice. *J Virol* 2002; **76**: 10702-10707

51 **Hong Y**, Peng Y, Mi M, Xiao H, Munn DH, Wang GQ, He Y. Lentivector expressing HBsAg and immunoglobulin Fc fusion antigen induces potent immune responses and results in seroconversion in HBsAg transgenic mice. *Vaccine* 2011; **29**: 3909-3916

52 **Heathcote J**, McHutchison J, Lee S, Tong M, Benner K, Minuk G, Wright T, Fikes J, Livingston B, Sette A, Chestnut R. A pilot study of the CY-1899 T-cell vaccine in subjects chronically infected with hepatitis B virus. The CY1899 T Cell Vaccine Study Group. *Hepatology* 1999; **30**: 531-536

53 **Pol S**, Nalpas B, Driss F, Michel ML, Tiollais P, Denis J, Brécho C. Efficacy and limitations of a specific immunotherapy in chronic hepatitis B. *J Hepatol* 2001; **34**: 917-921

54 **Dahmen A**, Herzog-Hauff S, Böcher WO, Galle PR, Löhr HF. Clinical and immunological efficacy of intradermal vaccine plus lamivudine with or without interleukin-2 in patients with chronic hepatitis B. *J Med Virol* 2002; **66**: 452-460

55 **Safadi R**, Israeli E, Papo O, Shibolet O, Melhem A, Bloch A, Rowe M, Alper R, Klein A, Hemed N, Segol O, Thalenfeld B, Engelhardt D, Rabbani E, Ilan Y. Treatment of chronic hepatitis B virus infection via oral immune regulation toward hepatitis B virus proteins. *Am J Gastroenterol* 2003; **98**: 2505-2515

56 **Helvaci M**, Kizilgunesler A, Kasirga E, Ozbal E, Kuzu M, Sozen G. Efficacy of hepatitis B vaccination and interferon-alpha-2b combination therapy versus interferon-alpha-2b monotherapy in children with chronic hepatitis B. *J Gastroenterol Hepatol* 2004; **19**: 785-791

57 **Vandepapelière P**, Lau GK, Leroux-Roels G, Horsmans Y, Gane E, Tawandee T, Merican MI, Win KM, Trepo C, Cooksley G, Wettendorff M, Ferrari C. Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. *Vaccine* 2007; **25**: 8585-8597

58 **Senturk H**, Tabak F, Ozaras R, Erdem L, Canbakan B, Mert A, Yurdakul I. Efficacy of pre-S-containing HBV vaccine combined with lamivudine in the treatment of chronic HBV infection. *Dig Dis Sci* 2009; **54**: 2026-2030

59 **Al-Mahtab M**, Rahman S, Akbar SM, Khan SI, Uddin H, Karim F, Ahmed F. Combination therapy with antiviral drugs and hepatitis B vaccine in incidentally-detected and asymptomatic chronic hepatitis virus B carriers at Bangladesh. *Viral Immunol* 2010; **23**: 335-338

60 **Mancini-Bourgine M**, Fontaine H, Scott-Algara D, Pol S, Bréchot C, Michel ML. Induction or expansion of T-cell responses by a hepatitis B DNA vaccine administered to chronic HBV carriers. *Hepatology* 2004; **40**: 874-882

61 **Mancini-Bourgine M**, Fontaine H, Bréchot C, Pol S, Michel ML. Immunogenicity of a hepatitis B DNA vaccine administered to chronic HBV carriers. *Vaccine* 2006; **24**: 4482-4489

62 **Chen M**, Li YG, Zhang DZ, Wang ZY, Zeng WQ, Shi XF, Guo Y, Guo SH, Ren H. Therapeutic effect of autologous dendritic cell vaccine on patients with chronic hepatitis B: a clinical study. *World J Gastroenterol* 2005; **11**: 1806-1808

63 **Luo J**, Li J, Chen RL, Nie L, Huang J, Liu ZW, Luo L, Yan XJ. Autologus dendritic cell vaccine for chronic hepatitis B carriers: a pilot, open label, clinical trial in human volunteers. *Vaccine* 2010; **28**: 2497-2504

64 **Martin J**, Bosch O, Moraleda G, Bartolome J, Quiroga JA, Carreño V. Pilot study of recombinant human granulocyte-macrophage colony-stimulating factor in the treatment of chronic hepatitis B. *Hepatology* 1993; **18**: 775-780

65 **Wang J**, Zhu Q, Zhang T, Yu H. A pilot study on the combined therapy of granulocyte-macrophage colony-stimulating factor and hepatitis B vaccine on chronic hepatitis B virus carrier children. *Chin Med J* (Engl) 2002; **115**: 1824-1828

66 **Zeuzem S**, Carreño V. Interleukin-12 in the treatment of chronic hepatitis B and C. *Antiviral Res* 2001; **52**: 181-188

67 **Rigopoulou EI**, Suri D, Chokshi S, Mullerova I, Rice S, Tedder RS, Williams R, Naoumov NV. Lamivudine plus interleukin-12 combination therapy in chronic hepatitis B: antiviral and immunological activity. *Hepatology* 2005; **42**: 1028-1036

68 **Szkaradkiewicz A**, Jopek A, Wysocki J. Effects of IL-12 and IL-18 on HBcAg-specific cytokine production by CD4 T lymphocytes of children with chronic hepatitis B infection. *Antiviral Res* 2005; **66**: 23-27

69 **Woltman AM**, Ter Borg MJ, Binda RS, Sprengers D, von Blomberg BM, Scheper RJ, Hayashi K, Nishi N, Boonstra A, van der Molen R, Janssen HL. Alpha-galactosylceramide in chronic hepatitis B infection: results from a randomized placebo-controlled Phase I/II trial. *Antivir Ther* 2009; **14**: 809-818

70 **Arase Y**, Tsubota A, Suzuki Y, Suzuki F, Kobayashi M, Someya T, Akuta N, Hosaka T, Saitoh S, Ikeda K, Kobayashi M, Kumada H. A pilot study of thymosin alpha1 therapy for chronic hepatitis B patients. *Intern Med* 2003; **42**: 941-946

71 **Iino S**, Toyota J, Kumada H, Kiyosawa K, Kakumu S, Sata M, Suzuki H, Martins EB. The efficacy and safety of thymosin alpha-1 in Japanese patients with chronic hepatitis B; results from a randomized clinical trial. *J Viral Hepat* 2005; **12**: 300-306

72 **You J**, Zhuang L, Cheng HY, Yan SM, Yu L, Huang JH, Tang BZ, Huang ML, Ma YL, Chongsuvivatwong V, Sriplung H, Geater A, Qiao YW, Wu RX. Efficacy of thymosin alpha-1 and interferon alpha in treatment of chronic viral hepatitis B: a randomized controlled study. *World J Gastroenterol* 2006; **12**: 6715-6721

73 **Lee HW**, Lee JI, Um SH, Ahn SH, Chang HY, Park YK, Hong SP, Moon YM, Han KH. Combination therapy of thymosin alpha-1 and lamivudine for HBeAg positive chronic hepatitis B: A prospective randomized, comparative pilot study. *J Gastroenterol Hepatol* 2008; **23**: 729-735

74 **Zhang YY**, Chen EQ, Yang J, Duan YR, Tang H. Treatment with lamivudine versus lamivudine and thymosin alpha-1 for e antigen-positive chronic hepatitis B patients: a meta-analysis. *Virol J* 2009; **6**: 63

75 **Boni C**, Penna A, Ogg GS, Bertoletti A, Pilli M, Cavallo C, Cavalli A, Urbani S, Boehme R, Panebianco R, Fiaccadori F, Ferrari C. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. *Hepatology* 2001; **33**: 963-971

76 **Díaz-Valdés N**, Manterola L, Belsúe V, Riezu-Boj JI, Larrea E, Echeverria I, Llópiz D, López-Sagaseta J, Lerat H, Pawlotsky JM, Prieto J, Lasarte JJ, Borrás-Cuesta F, Sarobe P. Improved dendritic cell-based immunization against hepatitis C virus using peptide inhibitors of interleukin 10. *Hepatology* 2011; **53**: 23-31

77 **Nakamoto N**, Cho H, Shaked A, Olthoff K, Valiga ME, Kaminski M, Gostick E, Price DA, Freeman GJ, Wherry EJ, Chang KM. Synergistic reversal of intrahepatic HCV-specific CD8 T cell exhaustion by combined PD-1/CTLA-4 blockade. *PLoS Pathog* 2009; **5**: e1000313

78 **Raziorrouh B**, Schraut W, Gerlach T, Nowack D, Grüner NH, Ulsenheimer A, Zachoval R, Wächtler M, Spannagl M, Haas J, Diepolder HM, Jung MC. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. *Hepatology* 2010; **52**: 1934-1947

S- Editor Gou SX **L- Editor** Kerr C **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2452

World J Gastroenterol 2012 May 28; 18(20): 2452-2461
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Luis Bujanda, PhD, Professor, *Series Editor*

Serrated polyposis syndrome: Molecular, pathological and clinical aspects

Carla Guarinos, Cristina Sánchez-Fortún, María Rodríguez-Soler, Cristina Alenda, Artemio Payá, Rodrigo Jover

Carla Guarinos, María Rodríguez-Soler, Research Unit, Hospital General Universitario de Alicante, 03010 Alicante, Spain
Cristina Sánchez-Fortún, María Rodríguez-Soler, Rodrigo Jover, Unidad de Gastroenterología, Hospital General Universitario de Alicante, 03010 Alicante, Spain
Cristina Alenda, Artemio Payá, Department of Pathology, Hospital General Universitario de Alicante, 03010 Alicante, Spain
Author contributions: Guarinos C, Sánchez-Fortún C and Rodríguez-Soler M made substantial contributions to conception, design and drafting of the article and revised it critically for important intellectual content; Alenda C and Payá A critically revised the article and made important contributions to its final format; Jover R designed and conceived the article and also critically revised it; and all authors approved the version to be published.
Supported by Grants from Instituto de Salud Carlos Ⅲ, INT09/208 and PI08/0726, to Jover R; Fundación de la CV para la Investigación en el Hospital General Universitario de Alicante, to Alenda C, Payá A and Jover R; a predoctoral grant from Conselleria d'Educació de la Generalitat Valenciana, VALi+d. EXP ACIF/2010/018, to Guarinos C; a grant from Fundación de la CV para la Investigación en el Hospital General Universitario de Alicante, to Rodríguez-Soler M
Correspondence to: Dr. Rodrigo Jover, Unidad de Gastroenterología, Hospital General Universitario de Alicante, Pintor Baeza, 12, 03010 Alicante, Spain. jover_rod@gva.es
Telephone: +34-96-5933468 Fax: +34-96-5933468
Received: September 3, 2011 Revised: February 8, 2012
Accepted: February 26, 2012
Published online: May 28, 2012

Abstract

Hyperplastic polyps have traditionally been considered not to have malignant potential. New pathological classification of serrated polyps and recent discoveries about the serrated pathway of carcinogenesis have revolutionized the concepts and revitalized the research in this area. Until recently, it has been thought that most colorectal cancers arise from conventional adenomas *via* the traditional tumor suppressor pathway initiated by a mutation of the *APC* gene, but it has been found that this pathway accounts for only approximately 70%-80% of colorectal cancer (CRC) cases. The majority of the remaining colorectal cancer cases follow an alternative pathway leading to CpG island methylator phenotype carcinoma with BRAF mutation and with or without microsatellite instability. The mechanism of carcinomas arising from this alternative pathway seems to begin with an activating mutation of the *BRAF* oncogene. Serrated polyposis syndrome is a relatively rare condition characterized by multiple and/or large serrated polyps of the colon. Clinical characteristics, etiology and relationship of serrated polyposis syndrome to CRC have not been clarified yet. Patients with this syndrome show a high risk of CRC and both sporadic and hereditary cases have been described. Clinical criteria have been used for diagnosis and frequent colonoscopy surveillance should be performed in order to prevent colorectal cancer. In this review, we try to gather new insights into the molecular pathogenesis of serrated polyps in order to understand their possible clinical implications and to make an approach to the management of this syndrome.

Key words: Colorectal cancer; Hyperplastic polyps; CpG island methylator phenotype; Serrated polyposis; Serrated pathway

Peer reviewers: Michael Linnebacher, PhD, Molecular Oncology and Immunotherapy, University of Rostock, Schillingallee 35, 18055 Rostock, Germany; Lodewijk AA Brosens, MD, PhD, Department of Pathology, University Medical Center Utrecht, Postbus 85500, 3508 GA Utrecht, The Netherlands

Guarinos C, Sánchez-Fortún C, Rodríguez-Soler M, Alenda C, Payá A, Jover R. Serrated polyposis syndrome: Molecular, pathological and clinical aspects. *World J Gastroenterol* 2012; 18(20): 2452-2461 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2452.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2452

INTRODUCTION

Colorectal cancer (CRC) is a common and lethal disease. It is a major health issue in western countries where it represents the second most common fatal malignancy after lung cancer[1]. Until recently, it has been thought that most CRCs arise from conventional adenomas *via* the traditional tumor suppressor pathway initiated with a mutation of the *APC* gene, but it has been found that this pathway accounts for only approximately 70%-80% of CRC cases[2-4]. The majority of the remaining CRC cases follow an alternative pathway leading to CpG island methylator phenotype (CIMP+) carcinoma with *BRAF* mutation and with or without microsatellite instability. This pathway is called the serrated pathway of colorectal carcinogenesis[3]. The mechanism of carcinomas arising from this alternative pathway seems to begin with an activating mutation of the *BRAF* oncogene. This *BRAF* mutation provokes the development of serrated lesions that are mainly microvesicular hyperplastic polyps or sessile serrated polyps[5]. These lesions are prone to methylation of CpG islands in the promoter regions of genes resulting in their epigenetic silencing. The best characterized gene silenced by this mechanism is *MLH1*. This gene is one of the mismatch repair genes and its epigenetic silencing results in sporadic tumors with microsatellite instability (MSI). However, other genes such as *P16*, *MGMT*, or *IGFBP7* may also be epigenetically inactivated. The serrated polyposis syndrome (SPS) is a relatively rare condition characterized by multiple and/or large serrated polyps of the colon. Diagnosis of this disease is made by the fulfillment of any of the World Health Organization's (WHO) clinical criteria[6] (Table 1). SPS exhibits an increased risk of CRC[7], which occurs on average in subjects aged between 50 to 60 years. There is a high incidence of synchronous cancers[8] and CRC shows a trend to be located in the proximal colon[9]. These patients and their relatives should receive strict surveillance strategies because of the high risk of CRC. This review focuses on the SPS, its genetics and management.

SERRATED POLYPOSIS SYNDROME

Serrated polyposis syndrome is the paradigm of the serrated pathway of carcinogenesis and an excellent and interesting human model for the study of the features that drive progression from hyperplastic polyps (HP) to serrated carcinoma (Figure 1). These patients show clinical, pathological and molecular features that are very useful for expanding the knowledge of this particular and alternative carcinogenetic pathway.

Diagnostic criteria

Diagnostic criteria of SPS were first defined by Burt and Jass in 2000 for the WHO. These criteria have been recently redefined and this entity is now called Serrated Polyposis[6]. A patient is diagnosed with SPS if at least one of the following criteria is met: (1) At least five serrated polyps proximal to the sigmoid colon, two of which are greater than 10 mm in diameter; (2) Any number of serrated polyps occurring proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis; and (3) More than 20 serrated polyps of any size distributed throughout the colon (Table 1). This arbitrary definition has been considered over the years somewhat restrictive. Moreover, SPS probably comprises a heterogeneous group of patients that includes several phenotypes of serrated polyposis. However, until the molecular basis of this syndrome is better understood, this clinical definition is applicable.

Table 1 The World Health Organization's clinical criteria for the identification of serrated polyposis

Criterion A	At least five serrated polyps proximal to the sigmoid colon, two of which are greater than 10 mm in diameter
Criterion B	Any number of serrated polyps occurring proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis
Criterion C	More than 20 serrated polyps of any size distributed throughout the colon

A diagnosis of serrated polyposis syndrome can be made if a patient fulfils any of these criteria.

Table 2 Summary findings from publications including patients that fulfil World Health Organization criteria of serrated polyposis syndrome

Author	Patients (*n*)	Age at diagnosis (median, yr)	CRC (%)	CRC family history (%)
Lage *et al*[12]	14	54	43	36
Ferrández *et al*[10]	15	52	7	0
Rubio *et al*[13]	10	61	70	10
Chow *et al*[9]	38	44	26	50
Boparai *et al*[7]	77	56	35	NR

CRC: Colorectal cancer; NR: Not reported.

Clinical characteristics

Characteristics of patients with SPS have been defined mainly based on the publication of series of cases[7,9-13] (Table 2). There is no sex predominance and the mean age at diagnosis is around 55 years. SPS has largely been considered a genetic disease, but the pattern of inheritance remains unknown: both autosomal recessive and autosomal dominant patterns have been suggested. Published case series report that between 10%-50% of patients meeting SPS criteria have a family history of CRC[9,11-13]. In this way, Boparai *et al*[14] have recently described an increased risk of CRC [relative risk (RR) = 5.4] and SPS (RR = 39) in first-degree relatives of probands diagnosed with SPS compared to the general population.

It is important to point out that conventional adenomas may coexist with serrated polyps in patients with SPS[7,9-13]. Some authors have suggested the existence of various phenotypes within the SPS definition. Kalady *et al*[11] described three phenotypic patterns in a series of 115 patients with multiple serrated polyps: (1) The patients presented a right-sided phenotype with large sessile serrated adenomas (SSAs)

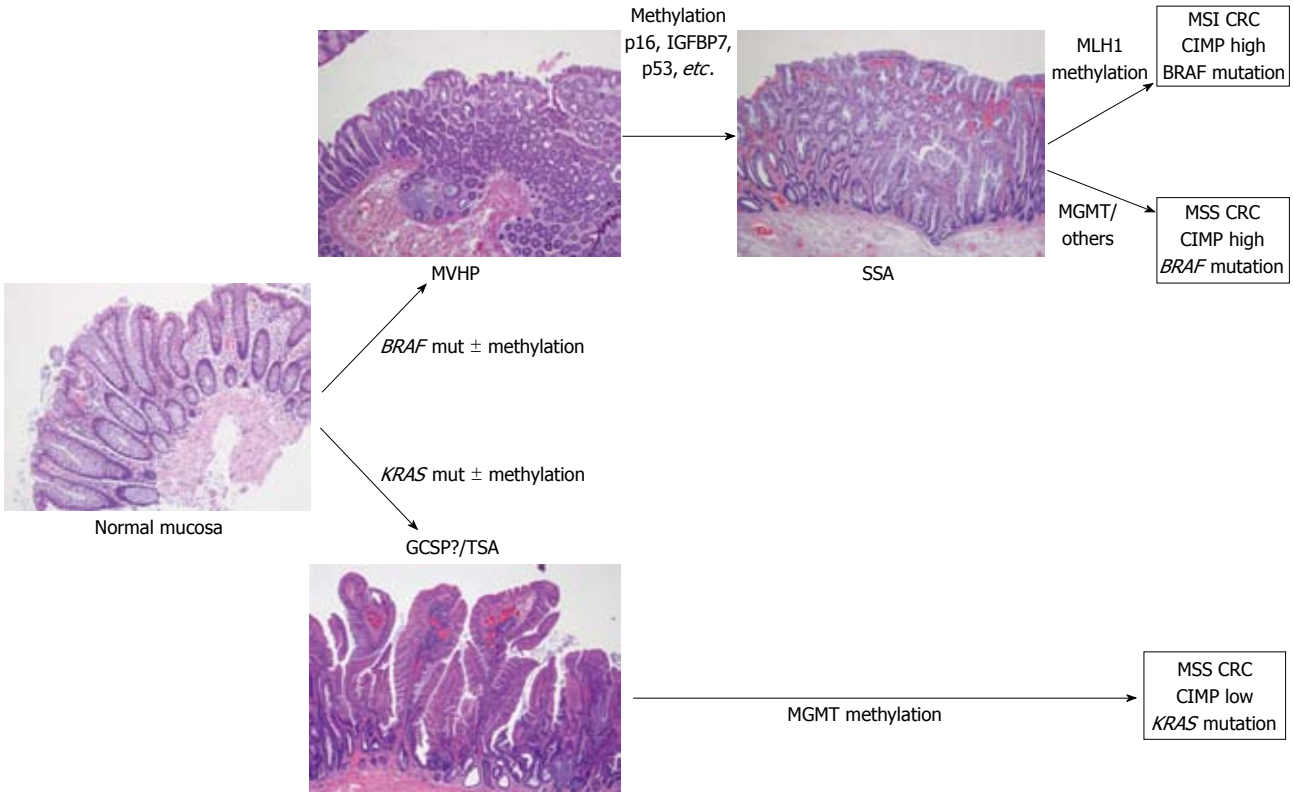


Figure 1 Model of serrated pathway of colorectal carcinogenesis. MVHP: Microvesicular hyperplastic polyp; SSA: Sessile serrated adenoma; MGMT: Methylguanine methyltransferase; MSI: Microsatellite instability; MSS: Microsatellite stable; CRC: Colorectal cancer; CIMP: CpG island methylator phenotype; GCSP: Goblet cell serrated polyp; TSA: Traditional sessile adenomas.

and with a CRC onset in younger individuals (48%); (2) Left-sided phenotype with a greater amount of small polyps (16%); and (3) Mixed phenotype with shared features of the previous phenotypes (37%). These different patterns should be revised in future studies.

Environmental factors could be partially responsible for the phenotypic differences and model the unknown pattern of inheritance. Smoking, being overweight and some drugs have been postulated as potential risk factors of HPs. Samowitz *et al*[15] described a statistically significant dose-response association between CIMP+ CRC and smoking. Moreover, Walker *et al*[16] found a strong association between cigarette smoking and SPS (odds ratio = 8.3; 95% CI: 3.0-22.9) in a case-control study comparing SPS patients with a population-based registry. Wallace *et al*[17], using the data of multicenter chemoprevention trials, came upon the association of some environmental factors with an increased risk of colonic serrated polyps (not necessary SPS criteria). On the one hand, in the left colon, obesity, smoking, increased dietary fat and red meat intake were linked with serrated polyps. On the other hand, in the right colon, the risk factors were folate intake and family history of polyps, whereas aspirin treatment was shown as a protective factor. These results should be confirmed by targeted studies.

Somatic molecular characteristics of polyps in patients with serrated polyposis syndrome

Molecular heterogeneity among polyps from patients with SPS has been described[18]. In fact, although a mixed phenotype has been identified[11], SPS patients can be molecularly classified into two defined groups[19]. The first group is characterized by the presence of relatively few large right-sided polyps which show *BRAF* mutation while the other group presents with many small left-sided polyps associated with *KRAS* mutation[11,19]. Mutations in *KRAS* and *BRAF* are more common in HPs from SPS patients as well as in younger cases. The frequency of *BRAF* mutations in SPS patients is higher than *KRAS* mutations[19].

The combined incidence of *BRAF* and *KRAS* mutations in serrated polyps ranges from 64% to 75%[19,20]. The presence of epithelial dysplasia is associated with higher rates (90%) of mutation in either *BRAF* or *KRAS*, indicating the importance of the activation of the RAS-RAF-MAP kinase pathway in the pathogenesis of the serrated lesions[20]. Furthermore, nearly 90% of all CIMP+ CRCs have either *BRAF* or *KRAS* mutations[21]. Serrated polyps from patients with SPS have different frequencies of *BRAF* mutation and it is higher in those lesions that show typical features of SSA[21-23]. However, there are differences between studies due to the methodology used for the detection of *BRAF* mutation[22] or because of the lack of consensus about the diagnostic terms for serrated lesions[24,25].

Carvajal-Carmona *et al*[19] proposed molecular criteria that could complement the clinical WHO criteria for SPS. They recommended that SPS should be diagnosed

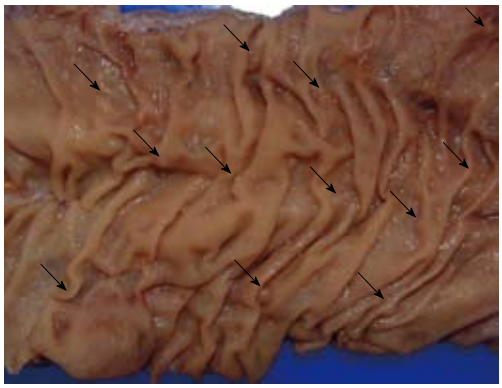


Figure 2 Segment of colectomy in a case of serrated polyposis. Polyps are frequently small (arrows) and flat, making their endoscopic detection difficult.

if *BRAF* or *KRAS* mutations are present at a significantly higher frequency in a patient's polyps than in sporadic HPs. In addition, SPS could be excluded if both *BRAF* and *KRAS* mutations are present in less than 10% of HPs from one patient, or if less than 5% of HPs are MSI.

Genetic predisposition in patients with serrated polyposis syndrome

SPS is a very heterogeneous condition[26] and it has been suggested that each phenotype may result from different underlying genetic causes[11]. Familial cases of SPS have been reported[19,26]. Although the genetic basis of SPS remains unknown, both recessive and dominant transmission patterns have been proposed[9,23,26]. Young *et al*[27] provided evidence for a syndrome of familial CRC distinct from hereditary nonpolyposis colorectal cancer by describing 11 families, of which 6 met the Amsterdam I criteria, with multiple members across several generations with CRC with variable MSI phenotype, *BRAF* mutation in 70% and hypermethylation of MINT31 in 80%. Moreover CRCs showed early age at diagnosis and were more likely to show a serrated architecture. Frazier *et al*[28] observed that patients whose CRC show methylation in *p16*, *MINT1*, *MINT31* and *MLH1* are 14 times more likely to have a family history of cancer than patients with methylation at none of the four loci. Taking into account these studies and the fact that extensive DNA methylation in normal colorectal mucosa has been described in patients with SPS[19,29,30], it has been postulated that the hypermethylation of gene promoters is due to genetic predisposition[23].

On the other hand, patients meeting criteria for hereditary nonpolyposis colorectal cancer may also fulfil criteria of SPS[31]. Occasional HPs have also been described in MYH-associated polyposis (MAP) patients and some of them met the criteria for SPS. Moreover HPs and SSAs can also be considered a phenotypic expression of MAP[32] and pathogenic biallelic *MYH* mutations were detected in 1 patient with SPS[9]. For that reason *MYH* mutations should be studied in SPS patients, especially when adenomas occur simultaneously with HPs in the same patient[9]. *PTEN* mutations have also been identified in patients with a combination of hyperplastic and adenomatous polyps[33].

A recent study from Roberts *et al*[34] has showed linkage to 2q32.2-q33.3 in approximately half of the SPS families studied. Sequencing of coding regions and exon-intron boundaries of five potential candidate genes in this region did not reveal any variants segregating with disease.

Together these data support the existence of more than one genetic cause of SPS. Identification of the underlying genetic defect of SPS will help to improve management of these patients and may identify therapeutic targets for the treatment of CRC associated with this disease.

Risk of cancer in serrated polyposis syndrome

Serrated polyposis syndrome has been associated with an increased incidence of CRC. In the published series[7,9,10,12,13], about 25%-70% of patients with SPS had CRC at time of diagnosis or during follow-up. In the largest series with patients meeting WHO criteria for SPS[7], 35% of patients had CRC (28.5% at initial endoscopy and 6.5% during the mean follow-up of 5.6 years). In this study, increased number of polyps and the presence of serrated adenomas were associated with CRC. The results of the larger published series are summarized in Table 2. In addition, first degree relatives of SPS patients have an increased risk for both CRC and SPS compared to the general population[14].

Recommendations for treatment and surveillance

The management of patients with SPS should be based on regular screening colonoscopies in order to remove potential premalignant lesions. It is important to point out that it could be difficult to detect these serrated polyps and colonoscopy should be done under high quality conditions (Figure 2). Serrated polyps are less likely than adenomas to bleed, so fecal occult blood test could be less suitable for an early diagnosis. Surveillance recommendations can be done as follows[4]: (1) Colonoscopy with pancolonic chromoendoscopy every 1-2 years with removal of all polyps. It is recommended that this resection be performed at a tertiary centre, if possible; (2) If colonoscopy does not allow the total control of colonic polyps because of their size or number or the patient does not wish to have such frequent colonoscopies or cancer is detected, colectomy with ileorectal anastomosis should be indicated; and (3) First-degree relatives should be offered 1-2 years screening colonoscopy from 10 years younger than the index case and if it is possible by pancolonic chromoendoscopy.

Pedunculated polyps can be removed by conventional electrocautery snare polypectomy. The technique of choice for removal of flat and large HPs is endoscopic mucosal resection. Besides, it may be advisable to apply argon plasma coagulation in the lesion borders in order to reduce the risk of recurrence[35].

SERRATED PATHWAY OF CARCINOGENESIS

In 1999, Iino *et al*[36] suggested that a proportion of

hyperplastic polyps may serve as precursors of some CRC cases. Now, there is increasing evidence showing that, in some conditions, hyperplastic polyps can be the initial premalignant lesion in the serrated pathway of carcinogenesis. Some studies have reported the existence of *BRAF* mutations in sporadic MSI CRCs which show CIMP[21,23,27,37-41] suggesting the existence of this alternative pathway. *BRAF* mutations and DNA methylation would be early events in this pathway with serrated polyps as precursor lesions[4,21,27,41]. The lack of adenoma-specific mutations such as *APC, KRAS* and *TP53* in sporadic MSI CRCs, and the fact that *BRAF* mutation and methylation of CpG islands are exceptional in classic adenomas[42] supports the existence of this pathway[43]. Tumors following this pathway show some specific characteristics, being more frequent in females and located in the right colon[4]. Moreover, some preliminary studies suggest that these tumors could be unresponsive to 5-fluorouracil chemotherapy[44].

Molecular characteristics of serrated polyps

As mentioned above, *BRAF* mutations and DNA methylation would be early events in this pathway. In fact, epigenetic changes in normal mucosa in patients with SPS have been described[29]. The first role of *BRAF* in the serrated pathway is probably to allow the apoptosis evasion[21,45]. Then, under normal conditions, these cells are eliminated by regular senescence. However, the silencing of key cell cycle regulatory genes such as *p16*, *IGFBP7* or *p53* through promoter methylation allows the cell to escape from senescence[4], facilitating its proliferation (Figure 1). When cells acquire other mutations, activated *BRAF* itself could also drive proliferation[21] and facilitate the maintenance of an invasive phenotype[45]. *BRAF* mutation has even been observed in serrated hyperplastic aberrant crypt foci, suggesting that these lesions are probably the earliest histological evident lesions in the serrated pathway[4,46].

There are several lines of evidence suggesting the existence of two parallel serrated pathways depending on the oncogene involved: *BRAF* or *KRAS* (Figure 1). The serrated pathway that involves *BRAF* mutations usually leads to CIMP tumors[4,29,43,47] and tumors are located in the proximal colon[38,47,48]. These tumors will be MSI or microsatellite stable (MSS) depending on the involvement of *MLH1*. As has been already stated, SSA seems to be the precursor lesion in the *BRAF* serrated pathway. In contrast, serrated tumors with *KRAS* mutations are more frequently MSI-low or MSS and are frequently associated with *MGMT* silencing[47,49]. These tumors are predominantly located in the left colorectum[4,29,47,48]. Differently, traditional sessile adenomas (TSA) would be the intermediate lesion in the *KRAS* serrated pathway.

CpG island methylator phenotype in colorectal cancer

CpG islands are 0.5- to 2-kb regions rich in cytosine guanine dinucleotides and are present in the 5' region of approximately 50% of human genes[21,29]. CIMP is characterized by methylation of CpG islands within the promoter regions of multiple genes resulting in the silencing of gene expression[18,21,29,45]. It might be assumed that methylation of CpG islands in most cancers arises stochastically[18,23,50]. This phenomenon can alter the expression of genes which are known to be important in neoplastic development, such as *p16*, *MGMT*, and the mismatch repair gene *MLH1*. However, the role of some genes affected by hypermethylation is not associated with colorectal carcinogenesis suggesting that not all de novo events are subject to growth selection. Taking into account that particular sequence motifs are significantly overrepresented among promoters vulnerable to CIMP, it is not surprising that some CpG islands are more likely to undergo hypermethylation than others[23]. The balance between DNA methyltransferases and the transcriptional machinery will determine the extent of methylation. Moreover, an active transcription may provide protection from de novo methylation[50]. The CIMP pathway is heterogeneous with respect to MSI status[27] and appears to be responsible for approximately 30% of all sporadic CRC[27,39].

There are different studies showing that a high proportion of polyps in SPS are CIMP+[18,29]. Chan *et al*[29] also observed that 75% of serrated polyps from patients with SPS showed CIMP frequently and had methylation of the *p16* gene. Moreover, extensive DNA methylation in normal colorectal mucosa has been described in patients with SPS[19,29,30], suggesting a field defect in epigenetic regulation and, consequently, a possible underlying genetic predisposition to extensive and early onset of DNA methylation. Furthermore, this phenomenon would be associated with a predisposition to CRC that would arise through the serrated pathway[51].

Endoscopic characteristics of serrated polyps

Serrated and hyperplastic polyps present endoscopic features that could help to differentiate them from adenomatous polyps. HPs appear pale, glistening, and very similar to the surrounding mucosa and usually covered by mucus. The vascular network is weak, in contrast to that of hypervascular adenomas. In addition, serrated polyps, mainly SSAs, are typically sessile or flat, making their detection even more difficult[48] (Figures 2 and 3, panels A and B). Since the malignant potential of these lesions, particularly in the context of SPS, has been shown, early endoscopic detection becomes more important. In this regard, the new advanced endoscopic techniques such as chromoendoscopy and narrow-band imaging (NBI) (Figure 3, panels C and D) become significant.

Chromoendoscopy in the SPS should be carried out by spraying contrast over the entire surface of the colon and using a magnification endoscope. The most widely used contrast is indigo carmine, which accumulates in pits and innominate grooves of the colonic mucosa, outlining the limits of flat lesions and drawing the described Kudo patterns[52]. Hyperplastic and serrated polyps typically show Kudo type I (normal) or type II ("stellate" or

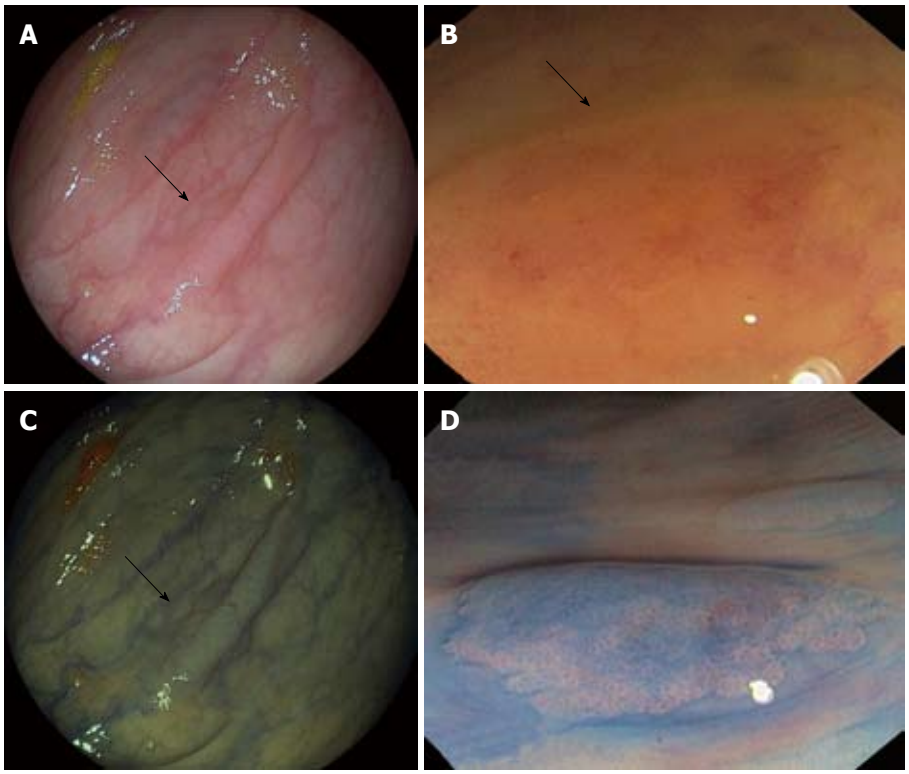


Figure 3 Endoscopic appearance of serrated polyps. A and B: Sessile serrated adenoma (SSA) (arrows) as flat polyp on conventional optical colonoscopy; C: Narrow-band imaging appearance of polyp (arrow) seen in panel A; D: Chromoendoscopy image of SSA revealing Kudo II pattern (Images courtesy of Dr. Adolfo Parra, Hospital Central de Asturias, Oviedo, Spain).

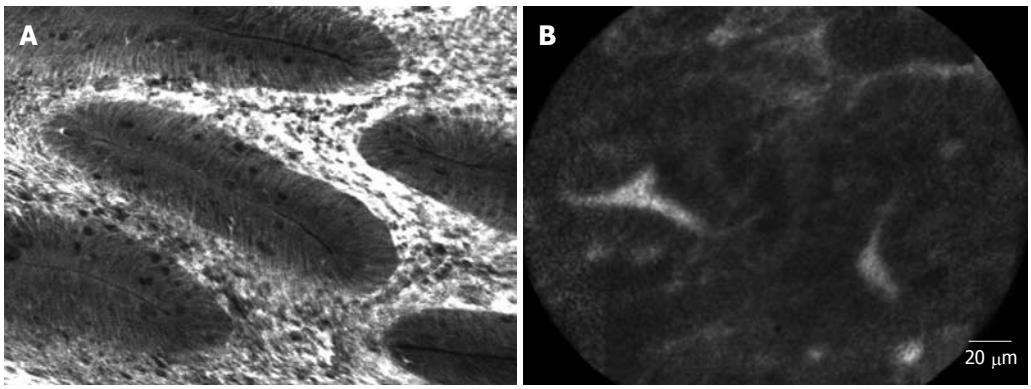


Figure 4 Confocal endomicroscopy of (A) tubular adenoma, low-grade dysplasia and (B) serrated polyp. Both types of polyps show different shape as well as differences in the cellular structures (Images courtesy of Dr. Maria Pellisé, Hospital Clinic, Barcelona, Spain).

"papillary") (Figure 3, panel D). Published randomized trials have shown that pancolonic chromoendoscopy almost doubles the rate of detection of sporadic serrated polyps compared to conventional endoscopy[53-56]. In these studies, HPs were found in 20% of patients using chromoendoscopy *vs* 10% of patients with conventional endoscopy, and this difference was statistically significant. New endoscopic technologies, such as NBI and confocal laser endomicroscopy (CLE) should also be taken into consideration (Figure 4).

It is accepted that the vascular pattern evaluation by chromoendoscopy or NBI could be an appropriate method to differentiate adenomas from HPs[57,58], but it has not been specifically studied in the SPS. In this way, Boparai *et al*[59] ran a prospective series including 7 patients with SPS who underwent a colonoscopy with trimodal imaging (high resolution, AFI and NBI): they obtained an unsatisfactory diagnostic accuracy for differentiate between HPs and SSAs but distinguishing adenomas from HPs was possible with NBI (accuracy 94%). Highest accuracy (76%) was achieved by the combination of a size of 3 mm or larger and a proximal location. Comparing CLE with virtual chromoendoscopy, it was shown that CLE demonstrated higher sensitivity (91% *vs* 77%; $P = 0.010$) with similar specificity in histological classification of colorectal polyps. However, further studies are needed to implement the CLE in clinical practice. The limited field of view and the horizontal sections of CLE hinder the detection of architectural distortion of sessile polyps (Figure 4).

Pathological characteristics of serrated polyps

Confirmation of the serrated character of polyps can only be made by pathological study. Serrated polyps are defined as epithelial lesions that show serrated appearance on histological section due to infolding of crypt epithelium. There are different types of serrated polyps (Table 3). HPs, considered for a long time as a benign and non-

Table 3 Characteristics of serrated polyps

Polyp name	Alternative terminology	Morphology and significance	Predominant location	Molecular features
Hyperplastic polyp, goblet type	Type 1 hyperplastic polyp	Subtype of hyperplastic polyp with conspicuous goblet cells and showing the least morphologic deviation from normal; Described as goblet-cell rich type	Distal colon: Sigmoid and rectum	Frequent *KRAS* mutation (54%)
Hyperplastic polyp, microvesicular type	Type 2 hyperplastic polyp	Variant of hyperplastic polyp in which columnar cells have mucin-filled vesicles within the apical cytoplasm and goblet cells are relatively inconspicuous	Right colon and distal colon	Frequent *BRAF* mutation (76%) and CIMP (68%)
Sesile serrated adenoma	Sessile serrated polyp; Serrated polyp with atypical proliferation	Advanced type of serrated polyp with abnormalities of architecture and proliferation but lacking the classic features of epithelial dysplasia (intraepithelial neoplasia)	Right colon	Frequent *BRAF* mutation (75%-82%) and CIMP (92%)
Sessile serrated adenoma with cytological dysplasia	Mixed polyp	Rare serrated polyp that includes two separate components: Nondysplastic (usually SSA) and either traditional adenoma or serrated adenoma	Right and left colon	Frequent *BRAF* mutation, (89%)
Serrated adenoma	Mixed hyperplastic adenomatous polyp; Atypical hyperplastic polyp; TSA	Relatively rare neoplastic polyp having a serrated architecture reminiscent of hyperplastic polyp but with unequivocal traditional adenomatous dysplasia; Comprises < 5% of serrated polyps	Left colon	Marked molecular heterogeneity; May have either *KRAS* or *BRAF* mutation

SSA: Sessile serrated adenoma; TSA: Traditional serrated adenoma; CIMP: CpG island methylator phenotype.

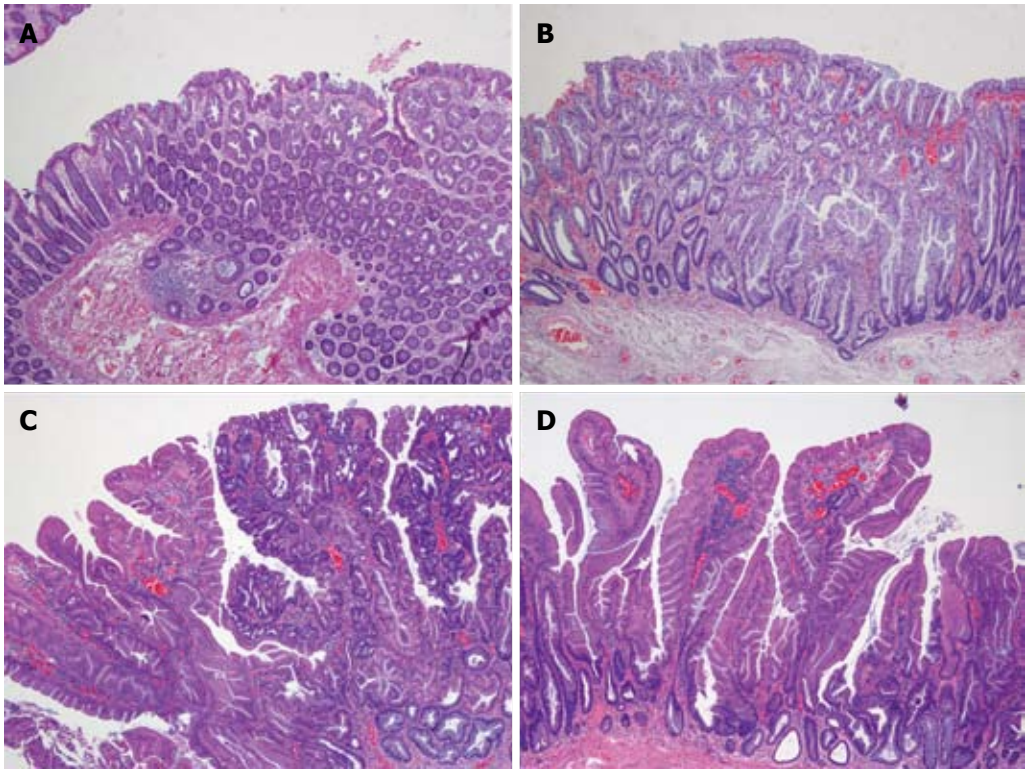


Figure 5 Pathological types of serrated polyps. A: Microvesicular serrated polyp; B: Sessile serrated adenomas; C: Traditional serrated adenoma; D: Mixed polyp.

premalignant colorectal lesion, SSA, mixed polyps (MP), and TSA are included in this group[4,48,60,61] (Figure 5).

HPs are the most common colorectal polyps. Sporadic HPs are usually small (2-5 mm)[10], multiple and mainly distributed in the rectum and sigmoid colon[10,47]. HPs have been divided into two main histological subtypes: microvesicular serrated polyps (MVSPs) (Figure 5, panel A), in which columnar cells have mucin-filled vesicles within atypical cytoplasm, and goblet cell serrated polyps (GCSPs) with conspicuous goblet cells that are predominantly found in the distal colon[4,23,47]. MVSPs seem to be the precursor lesion of SSA, especially when located in the right colon. In fact, both have the same molecular genetic abnormalities such as mutations in *BRAF* and CIMP. MVSPs show large and regular stellate pit openings. However, the large GCSPs are likely to have *KRAS* mutation, which is infrequently found in SSA. There is some evidence that large GCSPs are potential precursors of dysplastic serrated polyps which show *KRAS* mutations[47,61,62]. A third type of HP has been added, mucin poor type, but its frequency and importance is lower than the two main HP types[6].

SSA is an atypical HP variant described by Torlakovic and Snover in 1996[63]. SSAs are larger than (usually greater than 1 cm) HPs and more frequently located in the right colon[10]. Histologically, SSAs are distinguished from

typical HPs by the presence of inverted T- or L-shaped crypt bases that reflect disordered proliferation (Figure 5, panel B). Other features include dilated crypts and serration extending into the lower third of the crypt. Focal nuclear stratification, mild nuclear atypia, or dystrophic goblet cells may be seen in the crypt bases[47,60,61]. Moreover, SSAs show increased mucin production, absence of enteroendocrine cells, and absence of a thickened basement membrane under the surface[43]. Other less common features include small foci of pseudostratification and eosinophilic change (identical to that seen in TSAs) of the surface epithelium. Small prominent nucleoli, open chromatin, and irregular nuclear contours also might be present, along with mitoses in the upper third of the crypts or on the surface itself[61]. SSAs are thought to represent approximately 2% of all colonoscopically removed polyps, over 8% of all polyps that were previously diagnosed as HPs and around 18% of all serrated polyps[60].

MP, also called SSA with cytological dysplasia include two separate hyperplastic and adenomatous components (Figure 5, panel C)[21,23]. One component is usually SSA (nondysplastic) whereas the second dysplastic component is either adenoma or TSA.

TSAs, usually present on distal location, are dysplastic serrated polyps which lack SSA patterns and more closely resemble conventional adenoma with tubulovillous architecture (Figure 5, panel D)[4,24,47,60]. Ectopic crypt formation, defined by the presence of crypts with bases not seated adjacent to the muscularis mucosae, is a feature that makes it possible to distinguish between TSAs and SSAs[4]. Columnar cells from the epithelium show eosinophilic cytoplasm, centrally placed elongated nuclei that are hyperchromatic and display pseudostratification[46].

There is no strong morphological evidence suggesting that SSAs are the precursor of TSAs, otherwise there are some histological and epidemiologic differences for keeping these lesions apart in different categories[4,61]. SSAs have been associated with proximal CRCs, high level of CIMP, *BRAF* mutations and MSI-high[47,48]. TSAs have been associated with distal location and MSS, CIMP-low CRCs with KRAS mutations[48]. SSAs, TSAs and MPs are described as "advanced serrated polyps" and represent approximately 5%-15% of all serrated polyps found in colonoscopy patients[23].

FUTURE DIRECTIONS

Advances in the knowledge about the serrated pathway of carcinogenesis are making it possible to differentiate a new type of CRC with different natural history, prognosis and response to chemotherapy treatment. For this reason it is important to be able to easily identify this kind of colorectal tumor and its precursors. Identification of molecular markers in both polyps and cancers that follow this pathway will provide the opportunity of a better understanding of how these tumours grow and how we could explain differences in clinical presentation, evolution and symptoms in different types of CRC. These molecular markers will also allow improvement in the identification of patients with serrated polyposis, moving forward the currently used clinical criteria, and will give us better rationale for appropriate management and surveillance intervals for patients and their relatives.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917
2 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767
3 **Jass JR**. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. *Histopathology* 2007; **50**: 113-130
4 **Leggett B**, Whitehall V. Role of the serrated pathway in colorectal cancer pathogenesis. *Gastroenterology* 2010; **138**: 2088-2100
5 **Torlakovic E**, Skovlund E, Snover DC, Torlakovic G, Nesland JM. Morphologic reappraisal of serrated colorectal polyps. *Am J Surg Pathol* 2003; **27**: 65-81
6 **Snover DC**, Ahnen DJ, Burt RW, Odze RD. Serrated polyps of the colon and rectum and serrated ("hyperplastic") polyposis. In: Bosman ST, Carneiro F, Hruban RH, Theise ND. WHO Classification of tumours of the digestive system. Berlin: Springer-Verlag, 2010
7 **Boparai KS**, Mathus-Vliegen EM, Koornstra JJ, Nagengast FM, van Leerdam M, van Noesel CJ, Houben M, Cats A, van Hest LP, Fockens P, Dekker E. Increased colorectal cancer risk during follow-up in patients with hyperplastic polyposis syndrome: a multicentre cohort study. *Gut* 2010; **59**: 1094-1100
8 **Yeoman A**, Young J, Arnold J, Jass J, Parry S. Hyperplastic polyposis in the New Zealand population: a condition associated with increased colorectal cancer risk and European ancestry. *N Z Med J* 2007; **120**: U2827
9 **Chow E**, Lipton L, Lynch E, D'Souza R, Aragona C, Hodgkin L, Brown G, Winship I, Barker M, Buchanan D, Cowie S, Nasioulas S, du Sart D, Young J, Leggett B, Jass J, Macrae F. Hyperplastic polyposis syndrome: phenotypic presentations and the role of MBD4 and MYH. *Gastroenterology* 2006; **131**: 30-39
10 **Ferrández A**, Samowitz W, DiSario JA, Burt RW. Phenotypic characteristics and risk of cancer development in hyperplastic polyposis: case series and literature review. *Am J Gastroenterol* 2004; **99**: 2012-2018
11 **Kalady MF**, Jarrar A, Leach B, LaGuardia L, O'Malley M, Eng C, Church JM. Defining phenotypes and cancer risk in hyperplastic polyposis syndrome. *Dis Colon Rectum* 2011; **54**: 164-170
12 **Lage P**, Cravo M, Sousa R, Chaves P, Salazar M, Fonseca R, Claro I, Suspiro A, Rodrigues P, Raposo H, Fidalgo P, Nobre-Leitão C. Management of Portuguese patients with hyperplastic polyposis and screening of at-risk first-degree relatives: a contribution for future guidelines based on a clinical study. *Am J Gastroenterol* 2004; **99**: 1779-1784
13 **Rubio CA**, Stemme S, Jaramillo E, Lindblom A. Hyperplastic polyposis coli syndrome and colorectal carcinoma. *Endoscopy* 2006; **38**: 266-270
14 **Boparai KS**, Reitsma JB, Lemmens V, van Os TA, Mathus-Vliegen EM, Koornstra JJ, Nagengast FM, van Hest LP, Keller JJ, Dekker E. Increased colorectal cancer risk in first-degree relatives of patients with hyperplastic polyposis syndrome. *Gut* 2010; **59**: 1222-1225
15 **Samowitz WS**, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, Wolff RK, Slattery ML. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. *J Natl Cancer Inst* 2006; **98**: 1731-1738

16 **Walker RG**, Landmann JK, Hewett DG, Worthley DL, Buttenshaw RL, Knight N, Webb PM, Whiteman DC, Whitehall VL, Leggett BA. Hyperplastic polyposis syndrome is associated with cigarette smoking, which may be a modifiable risk factor. *Am J Gastroenterol* 2010; **105**: 1642-1647

17 **Wallace K**, Grau MV, Ahnen D, Snover DC, Robertson DJ, Mahnke D, Gui J, Barry EL, Summers RW, McKeown-Eyssen G, Haile RW, Baron JA. The association of lifestyle and dietary factors with the risk for serrated polyps of the colorectum. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2310-2317

18 **Wynter CV**, Walsh MD, Higuchi T, Leggett BA, Young J, Jass JR. Methylation patterns define two types of hyperplastic polyp associated with colorectal cancer. *Gut* 2004; **53**: 573-580

19 **Carvajal-Carmona LG**, Howarth KM, Lockett M, Polanco-Echeverry GM, Volikos E, Gorman M, Barclay E, Martin L, Jones AM, Saunders B, Guenther T, Donaldson A, Paterson J, Frayling I, Novelli MR, Phillips R, Thomas HJ, Silver A, Atkin W, Tomlinson IP. Molecular classification and genetic pathways in hyperplastic polyposis syndrome. *J Pathol* 2007; **212**: 378-385

20 **Chan TL**, Zhao W, Leung SY, Yuen ST. BRAF and KRAS mutations in colorectal hyperplastic polyps and serrated adenomas. *Cancer Res* 2003; **63**: 4878-4881

21 **Kambara T**, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, Barker MA, Arnold S, McGivern A, Matsubara N, Tanaka N, Higuchi T, Young J, Jass JR, Leggett BA. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004; **53**: 1137-1144

22 **Beach R**, Chan AO, Wu TT, White JA, Morris JS, Lunagomez S, Broaddus RR, Issa JP, Hamilton SR, Rashid A. BRAF mutations in aberrant crypt foci and hyperplastic polyposis. *Am J Pathol* 2005; **166**: 1069-1075

23 **Young J**, Jass JR. The case for a genetic predisposition to serrated neoplasia in the colorectum: hypothesis and review of the literature. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1778-1784

24 **Lazarus R**, Junttila OE, Karttunen TJ, Mäkinen MJ. The risk of metachronous neoplasia in patients with serrated adenoma. *Am J Clin Pathol* 2005; **123**: 349-359

25 **Lindor NM**. Hereditary colorectal cancer: MYH-associated polyposis and other newly identified disorders. *Best Pract Res Clin Gastroenterol* 2009; **23**: 75-87

26 **Jasperson KW**, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. *Gastroenterology* 2010; **138**: 2044-2058

27 **Young J**, Barker MA, Simms LA, Walsh MD, Biden KG, Buchanan D, Buttenshaw R, Whitehall VL, Arnold S, Jackson L, Kambara T, Spring KJ, Jenkins MA, Walker GJ, Hopper JL, Leggett BA, Jass JR. Evidence for BRAF mutation and variable levels of microsatellite instability in a syndrome of familial colorectal cancer. *Clin Gastroenterol Hepatol* 2005; **3**: 254-263

28 **Frazier ML**, Xi L, Zong J, Viscofsky N, Rashid A, Wu EF, Lynch PM, Amos CI, Issa JP. Association of the CpG island methylator phenotype with family history of cancer in patients with colorectal cancer. *Cancer Res* 2003; **63**: 4805-4808

29 **Chan AO**, Issa JP, Morris JS, Hamilton SR, Rashid A. Concordant CpG island methylation in hyperplastic polyposis. *Am J Pathol* 2002; **160**: 529-536

30 **Minoo P**, Baker K, Goswami R, Chong G, Foulkes WD, Ruszkiewicz AR, Barker M, Buchanan D, Young J, Jass JR. Extensive DNA methylation in normal colorectal mucosa in hyperplastic polyposis. *Gut* 2006; **55**: 1467-1474

31 **Jarrar AM**, Church JM, Fay S, Kalady MF. Is the phenotype mixed or mistaken? Hereditary nonpolyposis colorectal cancer and hyperplastic polyposis syndrome. *Dis Colon Rectum* 2009; **52**: 1949-1955

32 **Boparai KS**, Dekker E, Van Eeden S, Polak MM, Bartelsman JF, Mathus-Vliegen EM, Keller JJ, van Noesel CJ. Hyperplastic polyps and sessile serrated adenomas as a phenotypic expression of MYH-associated polyposis. *Gastroenterology* 2008; **135**: 2014-2018

33 **Sweet K**, Willis J, Zhou XP, Gallione C, Sawada T, Alhopuro P, Khoo SK, Patocs A, Martin C, Bridgeman S, Heinz J, Pilarski R, Lehtonen R, Prior TW, Frebourg T, Teh BT, Marchuk DA, Aaltonen LA, Eng C. Molecular classification of patients with unexplained hamartomatous and hyperplastic polyposis. *JAMA* 2005; **294**: 2465-2473

34 **Roberts A**, Nancarrow D, Clendenning M, Buchanan DD, Jenkins MA, Duggan D, Taverna D, McKeone D, Walters R, Walsh MD, Young BW, Jass JR, Rosty C, Gattas M, Pelzer E, Hopper JL, Goldblatt J, George J, Suthers GK, Phillips K, Parry S, Woodall S, Arnold J, Tucker K, Muir A, Drini M, Macrae F, Newcomb P, Potter JD, Pavluk E, Lindblom A, Young JP. Linkage to chromosome 2q32.2-q33.3 in familial serrated neoplasia (Jass syndrome). *Fam Cancer* 2011; **10**: 245-254

35 **Brooker JC**, Saunders BP, Shah SG, Thapar CJ, Suzuki N, Williams CB. Treatment with argon plasma coagulation reduces recurrence after piecemeal resection of large sessile colonic polyps: a randomized trial and recommendations. *Gastrointest Endosc* 2002; **55**: 371-375

36 **Iino H**, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y, Watanabe H. DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: a mild mutator pathway for colorectal cancer? *J Clin Pathol* 1999; **52**: 5-9

37 **Goel A**, Nagasaka T, Arnold CN, Inoue T, Hamilton C, Niedzwiecki D, Compton C, Mayer RJ, Goldberg R, Bertagnolli MM, Boland CR. The CpG island methylator phenotype and chromosomal instability are inversely correlated in sporadic colorectal cancer. *Gastroenterology* 2007; **132**: 127-138

38 **Hawkins NJ**, Ward RL. Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. *J Natl Cancer Inst* 2001; **93**: 1307-1313

39 **Lee S**, Cho NY, Choi M, Yoo EJ, Kim JH, Kang GH. Clinicopathological features of CpG island methylator phenotype-positive colorectal cancer and its adverse prognosis in relation to KRAS/BRAF mutation. *Pathol Int* 2008; **58**: 104-113

40 **Tanaka H**, Deng G, Matsuzaki K, Kakar S, Kim GE, Miura S, Sleisenger MH, Kim YS. BRAF mutation, CpG island methylator phenotype and microsatellite instability occur more frequently and concordantly in mucinous than non-mucinous colorectal cancer. *Int J Cancer* 2006; **118**: 2765-2771

41 **Velho S**, Moutinho C, Cirnes L, Albuquerque C, Hamelin R, Schmitt F, Carneiro F, Oliveira C, Seruca R. BRAF, KRAS and PIK3CA mutations in colorectal serrated polyps and cancer: primary or secondary genetic events in colorectal carcinogenesis? *BMC Cancer* 2008; **8**: 255

42 **Jass JR**. Colorectal polyposes: from phenotype to diagnosis. *Pathol Res Pract* 2008; **204**: 431-447

43 **Jass JR**. Hyperplastic polyps and colorectal cancer: is there a link? *Clin Gastroenterol Hepatol* 2004; **2**: 1-8

44 **Jover R**, Nguyen TP, Pérez-Carbonell L, Zapater P, Payá A, Alenda C, Rojas E, Cubiella J, Balaguer F, Morillas JD, Clofent J, Bujanda L, Reñé JM, Bessa X, Xicola RM, Nicolás-Pérez D, Castells A, Andreu M, Llor X, Boland CR, Goel A. 5-Fluorouracil adjuvant chemotherapy does not increase survival in patients with CpG island methylator phenotype colorectal cancer. *Gastroenterology* 2011; **140**: 1174-1181

45 **Minoo P**, Moyer MP, Jass JR. Role of BRAF-V600E in the serrated pathway of colorectal tumourigenesis. *J Pathol* 2007; **212**: 124-133

46 **Rosenberg DW**, Yang S, Pleau DC, Greenspan EJ, Stevens RG, Rajan TV, Heinen CD, Levine J, Zhou Y, O'Brien MJ. Mutations in BRAF and KRAS differentially distinguish ser-

rated versus non-serrated hyperplastic aberrant crypt foci in humans. *Cancer Res* 2007; **67**: 3551-3554
47 **Huang CS**, Farraye FA, Yang S, O'Brien MJ. The clinical significance of serrated polyps. *Am J Gastroenterol* 2011; **106**: 229-240; quiz 241
48 **East JE**, Saunders BP, Jass JR. Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management. *Gastroenterol Clin North Am* 2008; **37**: 25-46, v
49 **Whitehall VL**, Walsh MD, Young J, Leggett BA, Jass JR. Methylation of O-6-methylguanine DNA methyltransferase characterizes a subset of colorectal cancer with low-level DNA microsatellite instability. *Cancer Res* 2001; **61**: 827-830
50 **Hesson LB**, Hitchins MP, Ward RL. Epimutations and cancer predisposition: importance and mechanisms. *Curr Opin Genet Dev* 2010; **20**: 290-298
51 **Worthley DL**, Whitehall VL, Buttenshaw RL, Irahara N, Greco SA, Ramsnes I, Mallitt KA, Le Leu RK, Winter J, Hu Y, Ogino S, Young GP, Leggett BA. DNA methylation within the normal colorectal mucosa is associated with pathway-specific predisposition to cancer. *Oncogene* 2010; **29**: 1653-1662
52 **Kudo S**, Tamura S, Nakajima T, Yamano H, Kusaka H, Watanabe H. Diagnosis of colorectal tumorous lesions by magnifying endoscopy. *Gastrointest Endosc* 1996; **44**: 8-14
53 **Brooker JC**, Saunders BP, Shah SG, Thapar CJ, Thomas HJ, Atkin WS, Cardwell CR, Williams CB. Total colonic dye-spray increases the detection of diminutive adenomas during routine colonoscopy: a randomized controlled trial. *Gastrointest Endosc* 2002; **56**: 333-338
54 **Hurlstone DP**, Cross SS, Slater R, Sanders DS, Brown S. Detecting diminutive colorectal lesions at colonoscopy: a randomised controlled trial of pan-colonic versus targeted chromoscopy. *Gut* 2004; **53**: 376-380
55 **Lapalus MG**, Helbert T, Napoleon B, Rey JF, Houcke P, Ponchon T. Does chromoendoscopy with structure enhancement improve the colonoscopic adenoma detection rate? *Endoscopy* 2006; **38**: 444-448
56 **Le Rhun M**, Coron E, Parlier D, Nguyen JM, Canard JM, Alamdari A, Sautereau D, Chaussade S, Galmiche JP. High resolution colonoscopy with chromoscopy versus standard colonoscopy for the detection of colonic neoplasia: a randomized study. *Clin Gastroenterol Hepatol* 2006; **4**: 349-354
57 **Rastogi A**, Bansal A, Wani S, Callahan P, McGregor DH, Cherian R, Sharma P. Narrow-band imaging colonoscopy--a pilot feasibility study for the detection of polyps and correlation of surface patterns with polyp histologic diagnosis. *Gastrointest Endosc* 2008; **67**: 280-286
58 **Tischendorf JJ**, Wasmuth HE, Koch A, Hecker H, Trautwein C, Winograd R. Value of magnifying chromoendoscopy and narrow band imaging (NBI) in classifying colorectal polyps: a prospective controlled study. *Endoscopy* 2007; **39**: 1092-1096
59 **Boparai KS**, van den Broek FJ, van Eeden S, Fockens P, Dekker E. Hyperplastic polyposis syndrome: a pilot study for the differentiation of polyps by using high-resolution endoscopy, autofluorescence imaging, and narrow-band imaging. *Gastrointest Endosc* 2009; **70**: 947-955
60 **Liang JJ**, Alrawi S, Tan D. Nomenclature, molecular genetics and clinical significance of the precursor lesions in the serrated polyp pathway of colorectal carcinoma. *Int J Clin Exp Pathol* 2008; **1**: 317-324
61 **Sandmeier D**, Benhattar J, Martin P, Bouzourene H. Serrated polyps of the large intestine: a molecular study comparing sessile serrated adenomas and hyperplastic polyps. *Histopathology* 2009; **55**: 206-213
62 **Hiraoka S**, Kato J, Fujiki S, Kaji E, Morikawa T, Murakami T, Nawa T, Kuriyama M, Uraoka T, Ohara N, Yamamoto K. The presence of large serrated polyps increases risk for colorectal cancer. *Gastroenterology* 2010; **139**: 1503-1510
63 **Torlakovic E**, Snover DC. Serrated adenomatous polyposis in humans. *Gastroenterology* 1996; **110**: 748-755

S- Editor Gou SX **L- Editor** O'Neill M **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2462

World J Gastroenterol 2012 May 28; 18(20): 2462-2471
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Globus pharyngeus: A review of its etiology, diagnosis and treatment

Bong Eun Lee, Gwang Ha Kim

Bong Eun Lee, Gwang Ha Kim, Department of Internal Medicine, Pusan National University School of Medicine and Biomedical Research Institute, Pusan National University Hospital, Busan 602-739, South Korea
Author contributions: Lee BE and Kim GH contributed equally to this manuscript; Kim GH proposed the main idea of the study and contributed to the study design; Lee BE contributed to the study design, performed the literature search, and wrote the manuscript.
Supported by A Pusan National University Hospital Clinical Research Grant (2011); a grant from the National R and D Program for Cancer Control, Ministry for Health, Welfare and Family Affairs, Republic of Korea, No. 0920050
Correspondence to: Gwang Ha Kim, MD, Professor, Department of Internal Medicine, Pusan National University School of Medicine and Biomedical Research Institute, Pusan National University Hospital, 1-10 Ami-dong, Seo-gu, Busan 602-739, South Korea. doc0224@pusan.ac.kr
Telephone: +82-51-2407869 Fax: +82-51-2408180
Received: December 21, 2011 Revised: January 30, 2012
Accepted: March 9, 2012
Published online: May 28, 2012

Abstract

Globus is a persistent or intermittent non-painful sensation of a lump or foreign body in the throat. It is a commonly encountered clinical condition that is usually long-lasting, difficult to treat, and has a tendency to recur. Furthermore, due to the uncertain etiology of globus, it remains difficult to establish standard investigation and treatment strategies for affected patients. As a first step for managing globus, careful history taking and nasolaryngoscopy are essential. Given the benign nature of the condition and the recent notion that gastroesophageal reflux disease is a major cause of globus, empirical therapy with a high dose of proton pump inhibitors is reasonable for patients with typical globus. If patients are nonresponsive to this therapy, definitive assessments such as endoscopy, multichannel intraluminal impedance/pH monitoring, and manometry should be considered. Speech and language therapy, anti-depressants, and cognitive-behavioral therapy can be helpful in patients whose symptoms persist despite negative investigations.

Key words: Diagnosis; Gastroesophageal reflux disease; Globus; Proton pump inhibitor; Treatment

Peer reviewers: Justin Che Yuen Wu, Professor, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, 9/F Clinical Science Building, Prince of Wales Hospital, Shatin, Hong Kong, China; Diego Garcia-Compean, Faculty of Medicine, Autonomous University of Nuevo Leon, Monterrey 64700, Mexico; Tomohiko Shimatani, Professor, Lab of Adult Nursing and Internal Medicine, Hiroshima International University, 5-1-1 Hirokoshingai, Kure 7370112, Japan

Lee BE, Kim GH. Globus pharyngeus: A review of its etiology, diagnosis and treatment. *World J Gastroenterol* 2012; 18(20): 2462-2471 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2462.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2462

INTRODUCTION

Globus, a persistent or intermittent non-painful sensation of a lump or foreign body in the throat, is a well-defined clinical symptom that is usually long-lasting, difficult to treat, and has a tendency to recur. This symptom frequently improves with eating and is generally unaccompanied by dysphagia or odynophagia[1]. It is a common condition that accounts for approximately 4% of new referrals to ear, nose and throat (ENT) clinics, and it is reported by up to 46% of apparently healthy individuals, with a peak incidence in middle age[2,3]. This condition is equally prevalent in men and women, though the latter are more likely to seek health care for

this symptom[4].

Hippocrates first noted globus pharyngeus approximately 2500 years ago[5]. In 1707, Purcell[6] was the first to accurately describe the condition; he believed that globus resulted from pressure on the thyroid cartilage due to contraction of the strap muscles of the neck. In the past, globus was described as "globus hystericus" because of its frequent association with menopause or psychogenic factors. However, Malcomson[7] coined the more accurate term "globus pharyngeus" in 1968 after discovering that most patients experiencing globus did not have a hysterical personality. The etiology of globus is still unknown but appears to be multifactorial (Table 1). Although data are limited, recent studies have focused on gastroesophageal reflux disease (GERD), abnormalities of the upper esophageal sphincter (UES), psychological and psychiatric disorders, and stress as major factors contributing to the globus sensation. The variety of potential etiologies has made it difficult to establish standard investigation and treatment strategies for affected patients.

The aim of this review is to present the current literature on globus and to discuss its natural history and potential causes, current trends in its diagnosis, and methods for its treatment.

NATURAL HISTORY

Since few long-term follow-up studies have been conducted on patients with globus, the natural history of this condition has not been fully elucidated. In one study that followed 74 globus patients for an average of 7 years and 7 mo, 45% of the patients had persistent symptoms during the follow-up period[8]. An in-depth analysis of the features at clinical presentation failed to reveal any reliable prognostic indicators. In another long-term follow-up study, 60% of the patients had improved or resolved symptoms over a 5-year period, and the male patients with a history of globus less than 3 mo and who did not complain of any associated throat symptoms were reported to have the greatest chance of becoming asymptomatic or symptomatically improved[9].

POTENTIAL CAUSES OF GLOBUS

Gastroesophageal reflux disease

Although there is still considerable debate about the causative role of GERD in patients with globus, gastroesophageal reflux (GER) has been suggested to be a major etiology of this symptom, potentially accounting for 23%-68% of globus patients[10-18].

Malcomson[7] was the first to link GERD to the globus sensation through the use of barium swallow to uncover the presence of reflux in over 60% of patients with globus. Moreover, Koufman[16] found that 58% of patients with globus had abnormal pH results, and Cherry *et al*[19] demonstrated that 10 out of 12 subjects complained of globus when acid was infused into the distal esophagus. In a study that performed 24-h double-probe pH monitoring on 25 patients with globus and hoarseness, 72% of the patients exhibited pathologic reflux[20], and the globus symptom score was significantly higher in patients with GERD than in those without[21]. Additionally, globus sensation improved after 8 wk of proton pump inhibitor (PPI) therapy[22]. Several population-based surveys have supported such a potential link between GERD and the globus symptom by demonstrating an increased risk of globus among patients with GERD symptoms[23-25]. In a study by Dore *et al*[26], 38.7% of patients with GERD had the globus symptom, and the globus sensation was more prevalent in the non-erosive reflux disease group. Discordant data have also been reported[4,27-31]. However, it is clear that many patients with globus have concomitant GERD and that there is a true association between GERD and globus.

Table 1 Potential cause of globus

Gastroesophageal reflux disease
Abnormal upper esophageal sphincter function
Esophageal motor disorders
Pharyngeal inflammatory causes including: pharyngitis, tonsillitis and chronic sinusitis
Upper aerodigestive malignancy
Hypertrophy of the base of the tongue
Retroverted epiglottis
Thyroid diseases
Cervical heterotopic gastric mucosa
Rare laryngopharyngeal tumors
Psychological factors and stress

Two basic mechanisms have been proposed to explain the association between GERD and the globus sensation[14,32,33]: (1) Direct irritation and inflammation of the laryngopharynx by retrograde flow of gastric contents, also known as laryngopharyngeal reflux (LPR)[15,16,34]; (2) Vagovagal reflex hypertonicity of the UES triggered by acidification or distention of the distal esophagus[18].

Abnormal upper esophageal sphincter function

Abnormal UES function has also been suggested to be a cause of globus sensation[20,35-38]. Elevated UES pressure has been found to be much more frequent in patients with globus sensation than in controls (28% *vs* 3%), suggesting that hypertensive UES is a background factor for globus[30]. Additionally, injection of botulinum toxin into the cricopharyngeal muscle in a patient with both globus and extremely high UES pressure led to a resolution of the globus symptom and a decrease in UES pressure[35]. In a study of high-resolution manometry in patients with globus sensation, normal controls, and GERD patients without globus, hyperdynamic respiratory UES pressure changes were most prevalent in patients reporting globus[38]. However, other studies have reported contrary results[39-41].

Esophageal motor disorders

The prevalence of esophageal motor disorders has been

reported to be 6%-90% in patients with globus, suggesting that esophageal motor disorders are a possible cause of, or a contributing factor in the development of globus[27,28,31,42]. Esophageal manometry has revealed abnormalities in as many as 67% of globus patients, with nonspecific esophageal motility disorder being the most frequent finding[31]. Moser *et al*[43] noted that esophageal motor disorders might, before giving rise to dysphagia, be sensed more vaguely and induce the globus sensation. However, to infer an etiological significance of this disorder in globus, it must be shown that the sensation resolves after treatment for the motor disorder.

Pharyngeal inflammatory causes

Many conditions that cause irritation and inflammation of the pharynx, such as pharyngitis, tonsillitis, and chronic sinusitis with postnasal drip, can be the cause of globus sensation by producing increased local sensitivity[28,44].

Upper aerodigestive tract malignancy

The presence of pharyngolaryngeal or upper esophageal malignancy must be excluded in patients with globus sensation, particularly in cases with "high risk" symptoms, such as weight loss, dysphagia, throat pain, and lateralization of pathology[5,45].

Hypertrophy of the tongue base

Globus can be induced by severe hypertrophy of the tongue base, probably due to the follicles touching the posterior wall of the pharynx. Mamede *et al*[46] demonstrated that hypertrophied follicles were frequent in patients with signs and symptoms of GER and that the symptoms of hypertrophy of the tongue base could be confused with those of GER.

Retroverted epiglottis

Through contact with the tongue base or the posterior pharyngeal wall, retroverted epiglottis may cause globus sensation. Symptom relief has been observed after partial epiglottectomy[47,48].

Thyroid diseases

Impalpable, ultrasound-detectable abnormalities in the thyroid are known to be more common in patients with globus sensation than in controls[32]. Burns *et al*[49] noted that as many as one-third of patients with a thyroid mass complained of globus-like symptoms. Post-thyroidectomy patients may also complain of globus pattern symptoms, but these frequently diminish with time. Although the exact mechanism of the association between globus and thyroid diseases is poorly understood, some reports have concluded that a thyroidectomy could improve the globus symptom[49-51].

Cervical heterotopic gastric mucosa

Globus sensation has also been linked to the presence of cervical heterotopic gastric mucosa (CHGM)[52-54], and acid secretion from CHGM appears to cause symptoms similar to those of GERD, including globus sensation. Patients with CHGM who complained of globus sensation and/or sore throat experienced a significant decrease in their symptoms after argon plasma ablation of CHGM[55,56]. Recently, it has been suggested that the globus symptom may be related to *Helicobacter pylori* infection of the CHGM[57].

Rare tumors

Smooth muscle tumors of the pharynx and post cricoid lymphangioma, as well as oropharyngeal metastasis of Merkel cell carcinoma, have been reported in patients complaining of globus[58-60]. These cases illustrate that patients with persistent globus should be further investigated to exclude rare lesions[32].

Psychological factors and stress

Psychogenic problems have often been thought to cause or trigger the globus sensation. Personality studies have found higher levels of alexithymia, neuroticism, and psychological distress (including anxiety, low mood, and somatic concerns) and lower levels of extraversion in patients presenting with globus[61,62]. In addition, several studies have reported increased numbers of stressful life events preceding symptom onset, suggesting that life stress might be a cofactor in symptom genesis and in exacerbation. Indeed, up to 96% of patients with globus report symptom exacerbation during periods of high emotional intensity[63,64]. However, some reports have found no differences in the psychological states of patients with globus compared to normal controls[4,10,65]. In actuality, psychiatric diagnoses are prevalent in subjects seeking health care for globus, but an explanation distinct from ascertainment bias has not been established, causing the etiological significance of these psychological characteristics to remain uncertain[1,65]. Two recent studies reported that psychological status might be different between LPR-positive and LPR-negative patients with globus[15,66]. Globus patients with LPR exhibited weaker psychological symptoms than non-LPR globus patients[15], and globus patients who did not respond to PPI had significantly higher anxiety scores[66].

Others

There have been numerous isolated case reports that have suggested an association of globus with cervical osteophytes[67], temporomandibular joint disorders[68], hyperviscosity of the nasopharyngeal mucosa[69], Eagle's syndrome[70], excessive laryngeal and pharyngeal tension[61], and salivary hypofunction[33].

DIAGNOSIS

There has been no consensus regarding how best to diagnose and manage globus. A study of United Kingdom-based ENT specialists found that 14% performed no

tests on globus patients but rather simply prescribed antacid medication if clinically indicated[71]. The remaining 86% investigated globus symptoms in a variety of ways, including rigid endoscopy (61%), barium swallow (56%), or a combination of these methods (17.5%).

Since globus is essentially a benign disorder, investigation is primarily aimed at identifying those few cases with upper aerodigestive malignancy. Thus, the first step of an investigation of globus symptoms should be to take a detailed patient history, paying particular attention to the presence of "high risk" symptoms, associated reflux symptoms, and psychological problems. Additionally, physicians should perform a physical examination of the neck followed by nasolaryngoscopic examination of the laryngopharynx, although the routine use of nasolaryngoscopy in patients with typical globus symptoms remains controversial[1]. Patients with typical globus symptoms usually require no further investigation beyond an outpatient nasolaryngoscopy[5]. However, patients with "alarm signs", such as dysphagia, odynophagia, throat pain, weight loss, hoarseness, and lateralization of pathology, should undergo more extensive evaluation[1].

Reflux symptom index and reflux finding score

The symptoms and physical findings of LPR are nonspecific and can be confused with other laryngeal conditions caused by smoking, allergies, infections, vocal abuse, postnasal discharge, or neurogenic mechanisms as well as non-pathological variations[72]. Belafsky *et al*[73,74] proposed a useful self-administered tool, the reflux symptom index (RSI), for assessing the degree of LPR symptoms and developed the reflux finding score (RFS) based on 8 endolaryngeal signs for documenting the physical findings and severity of LPR. However, Park *et al*[15] demonstrated that RFS and RSI have low specificity in globus patients, suggesting that these may not be valid diagnostic tools for LPR in patients with globus.

Barium swallow

Barium swallow studies have been reported to identify benign lesions in up to one-third of patients with globus, and the most common findings include hiatal hernia and/or reflux (8%-18%), cervical osteophytes (0.4%-23%), and cricopharyngeal spasm (2.2%)[27,29,75,76]. However, given the prevalence of these findings in the general population, it is difficult to link these disorders to globus[45]. Two studies demonstrated that barium swallow did not identify any malignancy in typical globus patients[5,29]. Additionally, no pharyngeal or esophageal malignancy was found in a study that reviewed 1145 barium swallows in patients presenting with globus, prompting the authors to conclude that barium swallow should not be systematically requested for the exclusion of malignancies in patients with globus[77]. Thus, this test seems to have limited diagnostic value in the investigation of patients with globus.

Videofluoroscopy

Of 23 globus patients who received videofluoroscopy, 8 patients showed abnormal results; 5 had laryngeal aspiration, 2 had barium stasis in the vallecula and pyriform sinuses, and 4 had poor pharyngeal elevation[78]. Although it is unlikely that this indicates a causal relationship, videofluoroscopy may help to identify pharyngeal dysfunction in a substantial proportion of globus patients.

24-h dual-probe ambulatory pH monitoring

Whereas dual-probe ambulatory pH monitoring has been widely used in the clinical assessment of supraesophageal GERD, this technology is not yet standardized, and its usefulness in the definition of a clinically relevant association with GERD is under debate. This technique has been used to show abnormal esophageal acid exposure in some globus patients[4,11]. However, reflux symptoms such as acid regurgitation and/or heartburn were also noted in these study populations. In a study of globus patients without reflux-like symptoms, all 24 focal individuals had normal dual-probe pH results[78]. Therefore, ambulatory pH monitoring seems to be less helpful for the evaluation of globus without reflux-like symptoms.

24-h multichannel intraluminal impedance monitoring

The results from several trials indicate that the best way to detect GER in patients with extraesophageal manifestations of GERD is to conduct multichannel intraluminal impedance (MII)/pH monitoring. In patients experiencing persistent globus during PPI therapy, MII/pH monitoring increased the diagnostic yield of standard pH testing in the identification of positive symptom indices through the detection of nonacid reflux; furthermore, proximal reflux was a significant predictor of the globus symptom[79]. In studies investigating the utility of MII/pH monitoring in patients displaying atypical symptoms while "off PPI", MII/pH monitoring increased the diagnostic yield for objective detection of atypical manifestations of GERD[80-82]. Thus, this technique appears to be a more promising method of obtaining reliable data for the detection of LPR than 24-h dual probe monitoring, as it can monitor acid as well as nonacid reflux events and can distinguish between liquid and gaseous events. Therefore, MII/pH monitoring appears to be useful for ruling out GERD and for redirecting management of patients with suspected extraesophageal manifestations of GERD.

Flexible esophagogastroscopy

Endoscopy has been shown to be superior to barium swallow as a principal means of diagnosing upper aerodigestive tract malignancy[83]. Excellent views of the pyriform fossa and the postcricoid area can be achieved by insufflating air *via* flexible esophagogastroscopy[75]. Moreover, this procedure enables full esophageal evalu-

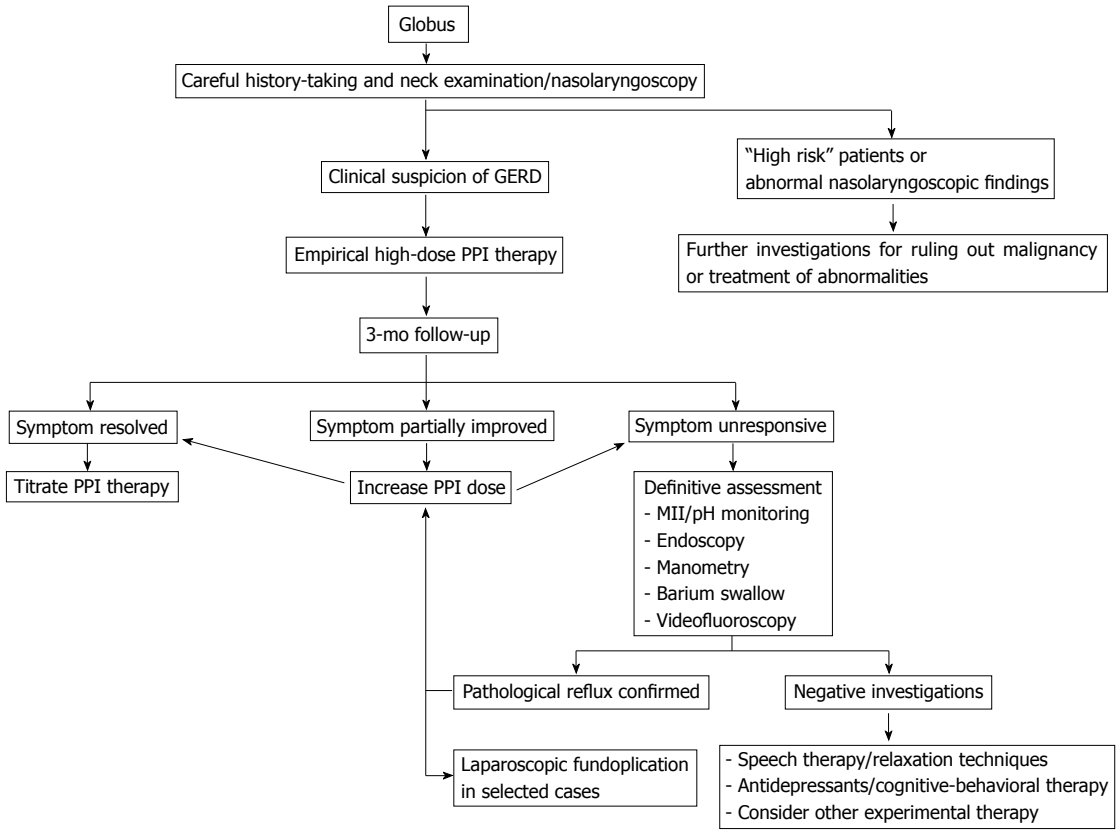


Figure 1 Algorithm for management of globus. GERD: Gastroesophageal reflux disease; PPI: Proton pump inhibitor; MII: Multichannel intraluminal impedance.

ation and diagnosis of reflux esophagitis and/or upper esophageal malignancy as a cause of globus. However, in general, endoscopy is known to have low sensitivity and to be of limited value for the diagnosis of extra-esophageal GERD. A study of 58 patients with pH-documented LPR found that only 19% had esophagitis or Barrett's metaplasia[84]. In another study of patients with suspected LPR symptoms, esophagitis was generally prevalent[85] but occurred least in patients with globus and throat symptoms. Due to the association between globus and CGHM, it is necessary to carefully evaluate the cervical esophagus[54].

Manometry

If abnormal UES function and esophageal motor disorder are suspected to be the potential cause of globus, manometry is a useful tool for assessing UES and lower esophageal sphincter pressure, esophageal body contraction amplitude, and peristaltic sequence. However, the etiological significance of such a disorder is difficult to define.

TREATMENT

A suggested algorithm for the management of globus patients based on available evidence is shown in Figure 1. Since there is a paucity of controlled studies on the treatment of globus, evidence-based treatment concepts are currently not available, and a review of the literature reveals that there is no single effective treatment. Given the benign nature of the condition, the likelihood of long-term symptom persistence, and the absence of highly effective pharmacotherapy, the mainstays of treatment are explanation and reassurance[1]. Other established treatment options include anti-reflux therapy, speech and language therapy, anti-depressants, and cognitive-behavioral therapy[86].

Anti-reflux treatment

Since GER has been suggested to be a major cause of globus[10-18], it seems practical that anti-reflux treatment should be the first attempted method for managing patients with globus. As diagnostic tests for GERD are somewhat invasive and costly and because a negative study result does not definitively rule out GER, it seems reasonable to use empirical PPI therapy as a combined method of diagnosis and treatment[87-92]. Although there are no controlled trials looking at the role of PPIs specifically for the treatment of globus, there is a variety of literature addressing the role of PPIs in LPR management[34,91,93-95]. Current evidence shows that the clinical response to PPIs in LPRD is variable[96,97] and that LPR symptoms improve more slowly than esophageal symptoms following acid-suppression therapy[98]. It is now widely accepted that extraesophageal GERD requires more aggressive and more prolonged therapy than typical GERD[32]. Empirical twice-daily therapy with PPIs for at least 3 mo is recommended; this can be extended

for a maximum period of 6 mo[87-92,99-102]. A PPI should be taken 30-60 min before meals so that it has reached its highest concentration by the time food intake stimulates the proton pumps. After 3-6 mo, responders can be weaned, whereas non-responders should undergo a definitive assessment, such as endoscopy, pH monitoring, or MII/pH monitoring. If available, MII/pH monitoring is preferable to simple pH monitoring because it facilitates the detection of nonacid reflux. Nocturnal acid breakthrough (NAB) may cause incomplete treatment response[103,104]. The addition of h.s. histamine-2 receptor antagonists to twice-daily PPI therapy has been suggested to control NAB[103], but it is currently unclear whether this method offers any additional benefit to the long-term control of LPRD[105,106]. Prokinetics are utilized when it is necessary to speed up esophageal and gastric emptying; they can be useful when the clinical response to PPIs is unsatisfactory[16]. Diet and behavioral modification can also decrease the amount of reflux. Recommended dietary modifications include a reduction in the intake of chocolate, fats, citrus fruits, carbonated beverages, spicy tomato-based products, red wines, caffeine, and late-night meals. Additionally, patients should make more general behavioral modifications, including exercising regularly, avoiding smoking and alcohol, elevating the head of the bed (10-15 cm), avoiding tight clothes around the waist, and losing weight. Sleeping on one's left-hand side also helps to decrease reflux. Steward *et al*[107] demonstrated that lifestyle modification was an independently significant variable in determining the response to pharmacological therapy. An alternative therapeutic strategy is anti-reflux surgery, with some authors having reported good improvement rates of LPR symptoms after laparoscopic nissen fundoplication[108-114]. To achieve a better patient outcome, a surgical approach must be taken into consideration in a carefully selected patient population, especially for patients who respond to treatment but are unable to tolerate PPIs due to side effects, those with confirmed pathological GER who do not respond to maximal medical treatment, and those in whom nonacid reflux has been demonstrated by a MII study[115]. However, if symptoms do not improve in the 4 mo following aggressive acid suppression, laparoscopic fundoplication may be unlikely to yield additional benefits[116]. Previous clinical responses to pharmacological acid suppression and abnormal pharyngeal pH results are preoperative predictors of relief from atypical symptoms[117].

Speech and language therapy/relaxation techniques

Speech therapy/relaxation techniques, including neck and shoulder exercises, general relaxation techniques, voice exercises, and voice hygiene to relieve vocal tract discomfort and tension, have successfully been used to treat patients with persistent globus symptoms[61]. In one uncontrolled study using these techniques on 25 globus patients, 92% experienced improvement following treatment. Khalil *et al*[118] randomly allocated 36 globus patients to either a speech therapy group or a reassurance group. Those in the speech therapy group used a number of exercises to relieve pharyngolaryngeal tension, including yawning, adopting a "giggle posture" (which helps retract the false vocal cords), and a "wet swallow" (as opposed to a "dry" or "check swallow," which patients often perform habitually and which tends to aggravate the globus symptom). Patients also attempted to eliminate throat clearing and promote adequate hydration by avoiding smoking, excess tea, and coffee. At the end of 3 mo, patients in the speech therapy group demonstrated significantly better globus symptom scores compared with those recorded prior to the intervention. Individuals in the speech therapy group also experienced significant improvements in globus symptoms when compared with controls. However, further research is needed to distinguish whether speech therapy has a specific effect or whether patients simply benefit from general attention and reassurance[119].

Cognitive-behavioral therapy/antidepressants

Globus is the fourth most common symptom of somatization disorder after vomiting, aphonia, and pain in the extremities[120]. Cognitive-behavioral therapy has emerged as the best treatment for a variety of somatoform disorders and medically unexplained symptoms[121]. Although there has not yet been a substantial trial of cognitive-behavioral therapy in globus patients, it is likely to be a promising treatment for repeat attenders whose symptoms remain refractory[76].

A small series of anti-depressants have been found to be beneficial for some globus patients with concomitant psychiatric disorders, such as panic, somatization, major depression, and agoraphobia[122,123].

Other treatment strategies

Thyroidectomy in patients with thyroid disorder[49-51] or partial epiglottectomy in selected cases whose retroverted epiglottis made contact with the tongue base[47,48], were both reported to significantly relieve the globus symptom. In addition, ablation of CHGM by argon plasma coagulation has shown some promise in improving chronic globus symptoms[55,56]. Although additional research of these techniques is needed, these approaches would provide some benefit to patients with unexplained chronic globus who are refractory to any medical treatments.

CONCLUSION

Although globus is a common clinical condition, its etiology remains uncertain, and there is no standard protocol for its diagnosis and management. The results of recent studies have strongly suggested that GERD is a major cause of globus, though this remains under considerable debate. Numerous other disorders, such as abnormal UES function, esophageal motility disorders, structural head and neck diseases, and psychological factors, have

been suggested as potential causes of globus. However, it has been rather difficult to establish a causal relationship between globus and these disorders because most of the reported studies were uncontrolled, had a small sample size, or were case reports. Currently, careful history taking and nasolaryngoscopy are essential as a first step in managing globus. Given the benign nature of the condition, patients with typical globus do not appear to need further investigation; rather, a 3-mo treatment with high-dose PPIs seems to be a reliable treatment option. If patients are nonresponsive to PPI therapy, they should undergo a definitive assessment, such as endoscopy, pH monitoring, or MII/pH monitoring; MII/pH monitoring in particular may increase the diagnostic yield of GER in globus patients. In cases with negative clinical investigations and consistent globus symptom, other treatment strategies, including speech therapy, antidepressants, and cognitive-behavioral therapy, should be considered. In the future, well-designed, randomized controlled studies are needed to definitively determine the effect of PPI treatment on globus. In addition, it is necessary to ascertain the etiology of globus *via* large-scale studies.

REFERENCES

1 **Galmiche JP**, Clouse RE, Bálint A, Cook IJ, Kahrilas PJ, Paterson WG, Smout AJ. Functional esophageal disorders. *Gastroenterology* 2006; **130**: 1459-1465
2 **Moloy PJ**, Charter R. The globus symptom. Incidence, therapeutic response, and age and sex relationships. *Arch Otolaryngol* 1982; **108**: 740-744
3 **Drossman DA**, Li Z, Andruzzi E, Temple RD, Talley NJ, Thompson WG, Whitehead WE, Janssens J, Funch-Jensen P, Corazziari E. U.S. householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact. *Dig Dis Sci* 1993; **38**: 1569-1580
4 **Batch AJ**. Globus pharyngeus (Part I). *J Laryngol Otol* 1988; **102**: 152-158
5 **Harar RP**, Kumar S, Saeed MA, Gatland DJ. Management of globus pharyngeus: review of 699 cases. *J Laryngol Otol* 2004; **118**: 522-527
6 **Purcell J**. A treatise of vapours or hysteric fits. 2nd ed. London: Edward Place, 1707: 72-74
7 **Malcomson KG**. Globus hystericus vel pharyngis (a recommaissance of proximal vagal modalities). *J Laryngol Otol* 1968; **82**: 219-230
8 **Rowley H**, O'Dwyer TP, Jones AS, Timon CI. The natural history of globus pharyngeus. *Laryngoscope* 1995; **105**: 1118-1121
9 **Timon C**, O'Dwyer T, Cagney D, Walsh M. Globus pharyngeus: long-term follow-up and prognostic factors. *Ann Otol Rhinol Laryngol* 1991; **100**: 351-354
10 **Hill J**, Stuart RC, Fung HK, Ng EK, Cheung FM, Chung CS, van Hasselt CA. Gastroesophageal reflux, motility disorders, and psychological profiles in the etiology of globus pharyngis. *Laryngoscope* 1997; **107**: 1373-1377
11 **Chevalier JM**, Brossard E, Monnier P. Globus sensation and gastroesophageal reflux. *Eur Arch Otorhinolaryngol* 2003; **260**: 273-276
12 **Wilson JA**, Pryde A, Piris J, Allan PL, Macintyre CC, Maran AG, Heading RC. Pharyngoesophageal dysmotility in globus sensation. *Arch Otolaryngol Head Neck Surg* 1989; **115**: 1086-1090
13 **Koufman JA**, Amin MR, Panetti M. Prevalence of reflux in 113 consecutive patients with laryngeal and voice disorders. *Otolaryngol Head Neck Surg* 2000; **123**: 385-388
14 **Oridate N**, Nishizawa N, Fukuda S. The diagnosis and management of globus: a perspective from Japan. *Curr Opin Otolaryngol Head Neck Surg* 2008; **16**: 498-502
15 **Park KH**, Choi SM, Kwon SU, Yoon SW, Kim SU. Diagnosis of laryngopharyngeal reflux among globus patients. *Otolaryngol Head Neck Surg* 2006; **134**: 81-85
16 **Koufman JA**. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope* 1991; **101**: 1-78
17 **Koufman J**, Sataloff RT, Toohill R. Laryngopharyngeal reflux: consensus conference report. *J Voice* 1996; **10**: 215-216
18 **Tokashiki R**, Funato N, Suzuki M. Globus sensation and increased upper esophageal sphincter pressure with distal esophageal acid perfusion. *Eur Arch Otorhinolaryngol* 2010; **267**: 737-741
19 **Cherry J**, Siegel CI, Margulies SI, Donner M. Pharyngeal localization of symptoms of gastroesophageal reflux. *Ann Otol Rhinol Laryngol* 1970; **79**: 912-914
20 **Smit CF**, van Leeuwen JA, Mathus-Vliegen LM, Devriese PP, Semin A, Tan J, Schouwenburg PF. Gastropharyngeal and gastroesophageal reflux in globus and hoarseness. *Arch Otolaryngol Head Neck Surg* 2000; **126**: 827-830
21 **Sinn DH**, Kim JH, Kim S, Son HJ, Kim JJ, Rhee JC, Rhee PL. Response rate and predictors of response in a short-term empirical trial of high-dose rabeprazole in patients with globus. *Aliment Pharmacol Ther* 2008; **27**: 1275-1281
22 **Tokashiki R**, Yamaguchi H, Nakamura K, Suzuki M. Globus sensation caused by gastroesophageal reflux disease. *Auris Nasus Larynx* 2002; **29**: 347-351
23 **Locke GR**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456
24 **Rey E**, Elola-Olaso CM, Rodríguez-Artalejo F, Locke GR, Díaz-Rubio M. Prevalence of atypical symptoms and their association with typical symptoms of gastroesophageal reflux in Spain. *Eur J Gastroenterol Hepatol* 2006; **18**: 969-975
25 **Cho YS**, Choi MG, Jeong JJ, Chung WC, Lee IS, Kim SW, Han SW, Choi KY, Chung IS. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Asan-si, Korea. *Am J Gastroenterol* 2005; **100**: 747-753
26 **Dore MP**, Pedroni A, Pes GM, Maragkoudakis E, Tadeu V, Pirina P, Realdi G, Delitala G, Malaty HM. Effect of antisecretory therapy on atypical symptoms in gastroesophageal reflux disease. *Dig Dis Sci* 2007; **52**: 463-468
27 **Wilson JA**, Heading RC, Maran AG, Pryde A, Piris J, Allan PL. Globus sensation is not due to gastro-oesophageal reflux. *Clin Otolaryngol Allied Sci* 1987; **12**: 271-275
28 **Batch AJ**. Globus pharyngeus: (Part II), Discussion. *J Laryngol Otol* 1988; **102**: 227-230
29 **Back GW**, Leong P, Kumar R, Corbridge R. Value of barium swallow in investigation of globus pharyngeus. *J Laryngol Otol* 2000; **114**: 951-954
30 **Corso MJ**, Pursnani KG, Mohiuddin MA, Gideon RM, Castell JA, Katzka DA, Katz PO, Castell DO. Globus sensation is associated with hypertensive upper esophageal sphincter but not with gastroesophageal reflux. *Dig Dis Sci* 1998; **43**: 1513-1517
31 **Färkkilä MA**, Ertama L, Katila H, Kuusi K, Paavolainen M, Varis K. Globus pharyngis, commonly associated with esophageal motility disorders. *Am J Gastroenterol* 1994; **89**: 503-508
32 **Remacle M**. The diagnosis and management of globus: a

perspective from Belgium. *Curr Opin Otolaryngol Head Neck Surg* 2008; **16**: 511-515
33 **Baek CH**, Chung MK, Choi JY, So YK, Son YI, Jeong HS. Role of salivary function in patients with globus pharyngeus. *Head Neck* 2010; **32**: 244-252
34 **Ford CN**. Evaluation and management of laryngopharyngeal reflux. *JAMA* 2005; **294**: 1534-1540
35 **Halum SL**, Butler SG, Koufman JA, Postma GN. Treatment of globus by upper esophageal sphincter injection with botulinum A toxin. *Ear Nose Throat J* 2005; **84**: 74
36 **Watson WC**, Sullivan SN. Hypertonicity of the cricopharyngeal sphincter: A cause of globus sensation. *Lancet* 1974; **2**: 1417-1419
37 **Hunt PS**, Connell AM, Smiley TB. The cricopharyngeal sphincter in gastric reflux. *Gut* 1970; **11**: 303-306
38 **Kwiatek MA**, Mirza F, Kahrilas PJ, Pandolfino JE. Hyperdynamic upper esophageal sphincter pressure: a manometric observation in patients reporting globus sensation. *Am J Gastroenterol* 2009; **104**: 289-298
39 **Caldarelli DD**, Andrews AH, Derbyshire AJ. Esophageal motility studies in globus sensation. *Ann Otol Rhinol Laryngol* 1970; **79**: 1098-1100
40 **Cook IJ**, Dent J, Collins SM. Upper esophageal sphincter tone and reactivity to stress in patients with a history of globus sensation. *Dig Dis Sci* 1989; **34**: 672-676
41 **Sun J**, Xu B, Yuan YZ, Xu JY. Study on the function of pharynx upper esophageal sphincter in globus hystericus. *World J Gastroenterol* 2002; **8**: 952-955
42 **Knight RE**, Wells JR, Parrish RS. Esophageal dysmotility as an important co-factor in extraesophageal manifestations of gastroesophageal reflux. *Laryngoscope* 2000; **110**: 1462-1466
43 **Moser G**, Vacariu-Granser GV, Schneider C, Abatzi TA, Pokieser P, Stacher-Janotta G, Gaupmann G, Weber U, Wenzel T, Roden M. High incidence of esophageal motor disorders in consecutive patients with globus sensation. *Gastroenterology* 1991; **101**: 1512-1521
44 **Lee JW**, Song CW, Kang CD, Hur BW, Jeen YT, Jeon HJ, Lee HS, Lee SW, Um SH, Choi JH, Kim CD, Ryu HS, Hyun JH. Pharyngoesophageal motility in patients with globus sensation. *Korean J Gastroenterol* 2000; **36**: 1-9
45 **Cathcart R**, Wilson JA. Lump in the throat. *Clin Otolaryngol* 2007; **32**: 108-110
46 **Mamede RC**, De Mello-Filho FV, Dantas RO. Severe hypertrophy of the base of the tongue in adults. *Otolaryngol Head Neck Surg* 2004; **131**: 378-382
47 **Agada FO**, Coatesworth AP, Grace AR. Retroverted epiglottis presenting as a variant of globus pharyngeus. *J Laryngol Otol* 2007; **121**: 390-392
48 **Quesada JL**, Lorente J, Quesada P. Partial epiglottectomy as a possible treatment for globus pharyngeus? *Eur Arch Otorhinolaryngol* 2000; **257**: 386-388
49 **Burns P**, Timon C. Thyroid pathology and the globus symptom: are they related? A two year prospective trial. *J Laryngol Otol* 2007; **121**: 242-245
50 **Marshall JN**, McGann G, Cook JA, Taub N. A prospective controlled study of high-resolution thyroid ultrasound in patients with globus pharyngeus. *Clin Otolaryngol Allied Sci* 1996; **21**: 228-231
51 **Maung KH**, Hayworth D, Nix PA, Atkin SL, England RJ. Thyroidectomy does not cause globus pattern symptoms. *J Laryngol Otol* 2005; **119**: 973-975
52 **von Rahden BH**, Stein HJ, Becker K, Liebermann-Meffert D, Siewert JR. Heterotopic gastric mucosa of the esophagus: literature-review and proposal of a clinicopathologic classification. *Am J Gastroenterol* 2004; **99**: 543-551
53 **Lancaster JL**, Gosh S, Sethi R, Tripathi S. Can heterotopic gastric mucosa present as globus pharyngeus? *J Laryngol Otol* 2006; **120**: 575-578
54 **Alaani A**, Jassar P, Warfield AT, Gouldesbrough DR, Smith I. Heterotopic gastric mucosa in the cervical oesophagus (inlet patch) and globus pharyngeus--an under-recognised association. *J Laryngol Otol* 2007; **121**: 885-888
55 **Meining A**, Bajbouj M, Preeg M, Reichenberger J, Kassem AM, Huber W, Brockmeyer SJ, Hannig C, Höfler H, Prinz C, Schmid RM. Argon plasma ablation of gastric inlet patches in the cervical esophagus may alleviate globus sensation: a pilot trial. *Endoscopy* 2006; **38**: 566-570
56 **Bajbouj M**, Becker V, Eckel F, Miehlke S, Pech O, Prinz C, Schmid RM, Meining A. Argon plasma coagulation of cervical heterotopic gastric mucosa as an alternative treatment for globus sensations. *Gastroenterology* 2009; **137**: 440-444
57 **Alagozlu H**, Simsek Z, Unal S, Cindoruk M, Dumlu S, Dursun A. Is there an association between Helicobacter pylori in the inlet patch and globus sensation? *World J Gastroenterol* 2010; **16**: 42-47
58 **Husamaldin Z**, Aung W, McFerran DJ. Smooth muscle tumour of the pharynx: a rare tumour presenting with globus pharyngeus symptoms. *J Laryngol Otol* 2004; **118**: 885-887
59 **Smith NM**, Stafford FW. Post cricoid lymphangioma. *J Laryngol Otol* 1991; **105**: 220-221
60 **Reichel OA**, Mayr D, Issing WJ. Oropharyngeal metastasis of a Merkel cell carcinoma of the skin. *Eur Arch Otorhinolaryngol* 2003; **260**: 258-260
61 **Wareing M**, Elias A, Mitchell D. Management of globus sensation by the speech therapist. *Logoped Phoniatr Vocol* 1997; **22**: 39-42
62 **Deary IJ**, Wilson JA, Kelly SW. Globus pharyngis, personality, and psychological distress in the general population. *Psychosomatics* 1995; **36**: 570-577
63 **Harris MB**, Deary IJ, Wilson JA. Life events and difficulties in relation to the onset of globus pharyngis. *J Psychosom Res* 1996; **40**: 603-615
64 **Thompson WG**, Heaton KW. Heartburn and globus in apparently healthy people. *Can Med Assoc J* 1982; **126**: 46-48
65 **Moser G**, Wenzel-Abatzi TA, Stelzeneder M, Wenzel T, Weber U, Wiesnagrotzki S, Schneider C, Schima W, Stacher-Janotta G, Vacariu-Granser GV, Pokieser P, Bergmann H, Stacher G. Globus sensation: pharyngoesophageal function, psychometric and psychiatric findings, and follow-up in 88 patients. *Arch Intern Med* 1998; **158**: 1365-1373
66 **Siupsinskiene N**, Adamonis K, Toohill RJ, Sereika R. Predictors of response to short-term proton pump inhibitor treatment in laryngopharyngeal reflux patients. *J Laryngol Otol* 2008; **122**: 1206-1212
67 **Maran A**, Jacobson I. Cervical osteophytes presenting with pharyngeal symptoms. *Laryngoscope* 1971; **81**: 412-417
68 **Kirveskari P**, Puhakka H. Effect of occlusal adjustment on globus symptom. *J Prosthet Dent* 1985; **54**: 832-835
69 **Shiomi Y**, Shiomi Y, Oda N, Hosoda S. Hyperviscoelasticity of epipharyngeal mucus may induce globus pharyngis. *Ann Otol Rhinol Laryngol* 2002; **111**: 1116-1119
70 **Beder E**, Ozgursoy OB, Karatayli Ozgursoy S, Anadolu Y. Three-dimensional computed tomography and surgical treatment for Eagle's syndrome. *Ear Nose Throat J* 2006; **85**: 443-445
71 **Webb CJ**, Makura ZG, Fenton JE, Jackson SR, McCormick MS, Jones AS. Globus pharyngeus: a postal questionnaire survey of UK ENT consultants. *Clin Otolaryngol Allied Sci* 2000; **25**: 566-569
72 **Vaezi MF**, Hicks DM, Abelson TI, Richter JE. Laryngeal signs and symptoms and gastroesophageal reflux disease (GERD): a critical assessment of cause and effect association. *Clin Gastroenterol Hepatol* 2003; **1**: 333-344
73 **Belafsky PC**, Postma GN, Koufman JA. Validity and reliability of the reflux symptom index (RSI). *J Voice* 2002; **16**: 274-277
74 **Belafsky PC**, Postma GN, Koufman JA. The validity and reliability of the reflux finding score (RFS). *Laryngoscope* 2001;

111: 1313-1317
75 **Takwoingi YM**, Kale US, Morgan DW. Rigid endoscopy in globus pharyngeus: how valuable is it? *J Laryngol Otol* 2006; **120**: 42-46
76 **Burns P**, O'Neill JP. The diagnosis and management of globus: a perspective from Ireland. *Curr Opin Otolaryngol Head Neck Surg* 2008; **16**: 503-506
77 **Alaani A**, Vengala S, Johnston MN. The role of barium swallow in the management of the globus pharyngeus. *Eur Arch Otorhinolaryngol* 2007; **264**: 1095-1097
78 **Chen CL**, Tsai CC, Chou AS, Chiou JH. Utility of ambulatory pH monitoring and videofluoroscopy for the evaluation of patients with globus pharyngeus. *Dysphagia* 2007; **22**: 16-19
79 **Anandasabapathy S**, Jaffin BW. Multichannel intraluminal impedance in the evaluation of patients with persistent globus on proton pump inhibitor therapy. *Ann Otol Rhinol Laryngol* 2006; **115**: 563-570
80 **Bajbouj M**, Becker V, Neuber M, Schmid RM, Meining A. Combined pH-metry/impedance monitoring increases the diagnostic yield in patients with atypical gastroesophageal reflux symptoms. *Digestion* 2007; **76**: 223-228
81 **Lee BE**, Kim GH, Ryu DY, Kim DU, Cheong JH, Lee DG, Song GA. Combined Dual Channel Impedance/pH-metry in Patients With Suspected Laryngopharyngeal Reflux. *J Neurogastroenterol Motil* 2010; **16**: 157-165
82 **Malhotra A**, Freston JW, Aziz K. Use of pH-impedance testing to evaluate patients with suspected extraesophageal manifestations of gastroesophageal reflux disease. *J Clin Gastroenterol* 2008; **42**: 271-278
83 **Levine B**, Nielsen EW. The justifications and controversies of panendoscopy--a review. *Ear Nose Throat J* 1992; **71**: 335-340, 343
84 **Koufman JA**, Belafsky PC, Bach KK, Daniel E, Postma GN. Prevalence of esophagitis in patients with pH-documented laryngopharyngeal reflux. *Laryngoscope* 2002; **112**: 1606-1609
85 **Poelmans J**, Feenstra L, Demedts I, Rutgeerts P, Tack J. The yield of upper gastrointestinal endoscopy in patients with suspected reflux-related chronic ear, nose, and throat symptoms. *Am J Gastroenterol* 2004; **99**: 1419-1426
86 **Karkos PD**, Wilson JA. The diagnosis and management of globus pharyngeus: our perspective from the United Kingdom. *Curr Opin Otolaryngol Head Neck Surg* 2008; **16**: 521-524
87 **Divi V**, Benninger MS. Diagnosis and management of laryngopharyngeal reflux disease. *Curr Opin Otolaryngol Head Neck Surg* 2006; **14**: 124-127
88 **Celik M**, Ercan I. Diagnosis and management of laryngopharyngeal reflux disease. *Curr Opin Otolaryngol Head Neck Surg* 2006; **14**: 150-155
89 **Remacle M**, Lawson G. Diagnosis and management of laryngopharyngeal reflux disease. *Curr Opin Otolaryngol Head Neck Surg* 2006; **14**: 143-149
90 **Pontes P**, Tiago R. Diagnosis and management of laryngopharyngeal reflux disease. *Curr Opin Otolaryngol Head Neck Surg* 2006; **14**: 138-142
91 **Mahieu HF**. Review article: The laryngological manifestations of reflux disease; why the scepticism? *Aliment Pharmacol Ther* 2007; **26** Suppl 2: 17-24
92 **Bove MJ**, Rosen C. Diagnosis and management of laryngopharyngeal reflux disease. *Curr Opin Otolaryngol Head Neck Surg* 2006; **14**: 116-123
93 **El-Serag HB**, Lee P, Buchner A, Inadomi JM, Gavin M, McCarthy DM. Lansoprazole treatment of patients with chronic idiopathic laryngitis: a placebo-controlled trial. *Am J Gastroenterol* 2001; **96**: 979-983
94 **Noordzij JP**, Khidr A, Evans BA, Desper E, Mittal RK, Reibel JF, Levine PA. Evaluation of omeprazole in the treatment of reflux laryngitis: a prospective, placebo-controlled, randomized, double-blind study. *Laryngoscope* 2001; **111**: 2147-2151
95 **Issing WJ**, Karkos PD, Perreas K, Folwaczny C, Reichel O. Dual-probe 24-hour ambulatory pH monitoring for diagnosis of laryngopharyngeal reflux. *J Laryngol Otol* 2004; **118**: 845-848
96 **Katz PO**, Castell DO. Medical therapy of supraesophageal gastroesophageal reflux disease. *Am J Med* 2000; **108** Suppl 4a: 170S-177S
97 **Vaezi MF**. Extraesophageal manifestations of gastroesophageal reflux disease. *Clin Cornerstone* 2003; **5**: 32-38; discussion 39-40
98 **Oridate N**, Takeda H, Asaka M, Nishizawa N, Mesuda Y, Mori M, Furuta Y, Fukuda S. Acid-suppression therapy offers varied laryngopharyngeal and esophageal symptom relief in laryngopharyngeal reflux patients. *Dig Dis Sci* 2008; **53**: 2033-2038
99 **Park W**, Hicks DM, Khandwala F, Richter JE, Abelson TI, Milstein C, Vaezi MF. Laryngopharyngeal reflux: prospective cohort study evaluating optimal dose of proton-pump inhibitor therapy and pretherapy predictors of response. *Laryngoscope* 2005; **115**: 1230-1238
100 **Williams RB**, Szczesniak MM, Maclean JC, Brake HM, Cole IE, Cook IJ. Predictors of outcome in an open label, therapeutic trial of high-dose omeprazole in laryngitis. *Am J Gastroenterol* 2004; **99**: 777-785
101 **Amin MR**, Postma GN, Johnson P, Digges N, Koufman JA. Proton pump inhibitor resistance in the treatment of laryngopharyngeal reflux. *Otolaryngol Head Neck Surg* 2001; **125**: 374-378
102 **Belafsky PC**, Postma GN, Koufman JA. Laryngopharyngeal reflux symptoms improve before changes in physical findings. *Laryngoscope* 2001; **111**: 979-981
103 **Peghini PL**, Katz PO, Bracy NA, Castell DO. Nocturnal recovery of gastric acid secretion with twice-daily dosing of proton pump inhibitors. *Am J Gastroenterol* 1998; **93**: 763-767
104 **Peghini PL**, Katz PO, Castell DO. Ranitidine controls nocturnal gastric acid breakthrough on omeprazole: a controlled study in normal subjects. *Gastroenterology* 1998; **115**: 1335-1339
105 **Fackler WK**, Ours TM, Vaezi MF, Richter JE. Long-term effect of H2RA therapy on nocturnal gastric acid breakthrough. *Gastroenterology* 2002; **122**: 625-632
106 **Ours TM**, Fackler WK, Richter JE, Vaezi MF. Nocturnal acid breakthrough: clinical significance and correlation with esophageal acid exposure. *Am J Gastroenterol* 2003; **98**: 545-550
107 **Steward DL**, Wilson KM, Kelly DH, Patil MS, Schwartzbauer HR, Long JD, Welge JA. Proton pump inhibitor therapy for chronic laryngo-pharyngitis: a randomized placebo-control trial. *Otolaryngol Head Neck Surg* 2004; **131**: 342-350
108 **Lindstrom DR**, Wallace J, Loehrl TA, Merati AL, Toohill RJ. Nissen fundoplication surgery for extraesophageal manifestations of gastroesophageal reflux (EER). *Laryngoscope* 2002; **112**: 1762-1765
109 **Oelschlager BK**, Eubanks TR, Oleynikov D, Pope C, Pellegrini CA. Symptomatic and physiologic outcomes after operative treatment for extraesophageal reflux. *Surg Endosc* 2002; **16**: 1032-1036
110 **Fernando HC**, El-Sherif A, Landreneau RJ, Gilbert S, Christie NA, Buenaventura PO, Close JM, Luketich JD. Efficacy of laparoscopic fundoplication in controlling pulmonary symptoms associated with gastroesophageal reflux disease. *Surgery* 2005; **138**: 612-616; discussion 612-616
111 **Westcott CJ**, Hopkins MB, Bach K, Postma GN, Belafsky PC, Koufman JA. Fundoplication for laryngopharyngeal reflux disease. *J Am Coll Surg* 2004; **199**: 23-30
112 **Catania RA**, Kavic SM, Roth JS, Lee TH, Meyer T, Fantry GT, Castellanos PF, Park A. Laparoscopic Nissen fundoplication effectively relieves symptoms in patients with laryn-

gopharyngeal reflux. *J Gastrointest Surg* 2007; **11**: 1579-1587; discussion 1579-1587
113 **Sala E**, Salminen P, Simberg S, Koskenvuo J, Ovaska J. Laryngopharyngeal reflux disease treated with laparoscopic fundoplication. *Dig Dis Sci* 2008; **53**: 2397-2404
114 **Antoniou SA**, Delivorias P, Antoniou GA, Natsiopoulos I, Kalambakas A, Dalenbäck J, Makridis C. Symptom-focused results after laparoscopic fundoplication for refractory gastroesophageal reflux disease--a prospective study. *Langenbecks Arch Surg* 2008; **393**: 979-984
115 **Hassall E**. Decisions in diagnosing and managing chronic gastroesophageal reflux disease in children. *J Pediatr* 2005; **146**: S3-12
116 **Qadeer MA**, Swoger J, Milstein C, Hicks DM, Ponsky J, Richter JE, Abelson TI, Vaezi MF. Correlation between symptoms and laryngeal signs in laryngopharyngeal reflux. *Laryngoscope* 2005; **115**: 1947-1952
117 **So JB**, Zeitels SM, Rattner DW. Outcomes of atypical symptoms attributed to gastroesophageal reflux treated by laparoscopic fundoplication. *Surgery* 1998; **124**: 28-32
118 **Khalil HS**, Bridger MW, Hilton-Pierce M, Vincent J. The use of speech therapy in the treatment of globus pharyngeus patients. A randomised controlled trial. *Rev Laryngol Otol Rhinol* (Bord) 2003; **124**: 187-190
119 **Millichap F**, Lee M, Pring T. A lump in the throat: Should speech and language therapists treat globus pharyngeus? *Disabil Rehabil* 2005; **27**: 124-130
120 **Othmer E**, DeSouza C. A screening test for somatization disorder (hysteria). *Am J Psychiatry* 1985; **142**: 1146-1149
121 **Kroenke K**. Efficacy of treatment for somatoform disorders: a review of randomized controlled trials. *Psychosom Med* 2007; **69**: 881-888
122 **Cybulska EM**. Globus hystericus--a somatic symptom of depression? The role of electroconvulsive therapy and antidepressants. *Psychosom Med* 1997; **59**: 67-69
123 **Brown SR**, Schwartz JM, Summergrad P, Jenike MA. Globus hystericus syndrome responsive to antidepressants. *Am J Psychiatry* 1986; **143**: 917-918

S- Editor Gou SX **L- Editor** Rutherford A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2472

World J Gastroenterol 2012 May 28; 18(20): 2472-2480
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Lactobacillus plantarum B7 inhibits *Helicobacter pylori* growth and attenuates gastric inflammation

Chompoonut Sunanliganon, Duangporn Thong-Ngam, Somying Tumwasorn, Naruemon Klaikeaw

Chompoonut Sunanliganon, Duangporn Thong-Ngam, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Somying Tumwasorn, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Naruemon Klaikeaw, Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Author contributions: Sunanliganon C performed the experiments, collected the data and wrote the manuscript; Thong-Ngam D designed the study, performed the experiments, analyzed the data and edited the manuscript; Tumwasorn S provided *Helicobacter pylori* and *Lactobacillus plantarum* B7; Klaikeaw N co-ordinated the pathological examination.
Supported by The 90th Anniversary of Chulalongkorn University Fund (Ratchada phiseksomphot Endowment Fund); and the grant of Ratchada phiseksomphot, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
Correspondence to: Duangporn Thong-Ngam, MD, Associate Professor, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. dr.duangporn@gmail.com
Telephone: +662-256-42672050 Fax: +662-256-42672823
Received: December 21, 2011 Revised: February 3, 2012
Accepted: March 9, 2012
Published online: May 28, 2012

Abstract

AIM: To determine the anti-*Helicobacter* property of *Lactobacillus plantarum* B7 (*L. plantarum*) B7 supernatants *in vitro* and the protective effects of *L. plantarum* B7 on serum tumor necrosis factor-alpha (TNF-α), gastric malondialdehyde (MDA) level, apoptosis, and histopathology in *Helicobacter pylori* (*H. pylori*)-induced gastric inflammation in rats.

METHODS: *In vitro*, the inhibition of *H. pylori* growth was examined using *L. plantarum* B7 supernatants at pH 4 and pH 7 and at the concentration of 1×, 5× and 10× on plates inoculated with *H. pylori*. The inhibitory effect of *H. pylori* was interpreted by the size of the inhibition zone. *In vitro*, male Sprague-Dawley rats were randomly divided into four groups including group 1 (control group), group 2 (*H. pylori* infected group), group 3 (*H. pylori* infected with *L. plantarum* B7 10^6 CFUs/mL treated group) and group 4 (*H. pylori* infected with *L. plantarum* B7 10^{10} CFUs/mL treated group). One week after *H. pylori* inoculation, *L. plantarum* B7 10^6 CFUs/mL or 10^{10} CFUs/mL were fed once daily to group 3 and group 4, respectively, for one week. Blood and gastric samples were collected at the end of the study.

RESULTS: *In vitro*, at intact pH 4, mean inhibitory zone diameters of 8.5 mm and 13 mm were noted at concentrations of 5× and 10× of *L. plantarum* B7 supernatant disks, respectively. At adjusted pH 7, *L. plantarum* B7 supernatants at concentrations of 5 × and 10× yielded mean inhibitory zone diameters of 6.5 mm and 11 mm, respectively. In the *in vitro* study, in group 2, stomach histopathology revealed mild to moderate *H. pylori* colonization and inflammation. The level of gastric MDA and epithelial cell apoptosis were significantly increased compared with group 1. The serum TNF-α level was significant decreased in group 3 compared with group 2 ($P < 0.05$). In addition, *L. plantarum* B7 treatments resulted in a significant improvement in stomach pathology, and decreased gastric MDA level and apoptotic epithelial cells.

CONCLUSION: *L. plantarum* B7 supernatant inhibits *H. pylori* growth. This inhibition was dose-dependent and greater at pH 4. Moreover, *L. plantarum* B7 attenuated *H. pylori*-induced gastric inflammation.

Key words: Apoptosis; Gastric inflammation; *Helicobacter pylori*; *Lactobacillus plantarum* B7; Lipid peroxidation

Peer reviewer: Francesco Luzza, Professor, Dipartimento di Medicina Sperimentale e Clini, Università di Catanzaro, Via Tommaso Campanella 115, 88100 Catanzaro, Italy

Sunanliganon C, Thong-Ngam D, Tumwasorn S, Klaikeaw N. *Lactobacillus plantarum* B7 inhibits *Helicobacter pylori* growth and attenuates gastric inflammation. *World J Gastroenterol* 2012; 18(20): 2472-2480 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2472.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2472

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral shaped bacterium that has the unique ability of being able to colonize the human gastric mucosa and infects more than half of the world's population. *H. pylori* causes chronic gastritis, plays an etiologic role in peptic ulcer disease and is considered a risk factor in the development of gastric cancer and gastric lymphoma[1]. In 1994, *H. pylori* was classified as a type I carcinogen by the World Health Organization[2].

H. pylori infection is characterized by enhanced production of proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-2, IL-6 and IL-8 and the infiltration of lamina propria with inflammatory cells. *H. pylori* lipopolysaccharides (LPS) and released surface proteins stimulate lamina propria mononuclear cells and macrophages to produce proinflammatory cytokines such as TNF-α, IL-1β, and the generation of reactive oxygen species (ROS)[3]. TNF-α and IL-1β are potent inducers of IL-8 expression in many cell types. Furthermore, *H. pylori* is capable of interacting with epithelial cell surfaces to produce IL-8. The release of these inflammatory mediators results in the expression of CD11b/CD18 on leukocytes and intercellular adhesion molecule-1 on endothelial cells, the migration of leukocytes to a site of inflammation, and finally the generation of ROS[4].

ROS can react with the double bonds of polyunsaturated fatty acids (PUFAs), present in the membranes of phospholipids, resulting in lipid peroxidation. One of the major secondary oxidation products of peroxidized PUFAs is malondialdehyde (MDA)[5]. *H. pylori* also induces gastric epithelial cell apoptosis both *in vitro*[6] and *in vivo*[7]. Studies have shown that the *H. pylori* colonized stomach contains more apoptotic epithelial cells than normal epithelial cells. Moreover, the increased numbers of apoptotic epithelial cells decrease to normal after eradication of *H. pylori*[7].

H. pylori eradication is suboptimal because current treatment regimens result in adverse side effects, poor compliance, and an increasing prevalence of antibiotic resistance[8]. Therefore, alternative treatments are of interest.

Lactobacilli are probiotics which, when administered in adequate amounts, may confer a benefit to the host[9]. The most commonly used organisms in probiotic products are *Lactobacillus sp.* and *Bifidobacterium sp.*[10]. *L. plantarum* is commonly found in the human gastrointestinal tract (GI-tract). It is important in the production of a variety of fermented foods such as sauerkraut, Korean kimchi, cheese, sausages and stockfish, and is also used as a probiotic. Moreover, there is increasing evidence that *L. plantarum* has anti-*Helicobacter* activity and shows modulatory effects on the immune system[11,12]. Importantly, *L. plantarum* is acid and bile tolerant, survives passage through the GI-tract, and is safe in humans and animals.

The aim of this study was to examine the *in vitro* anti-*Helicobacter* activity of *L. plantarum* B7 supernatants using the disk diffusion method and the effects of *L. plantarum* B7 on gastric histopathology, serum TNF-α, gastric MDA level, and cell apoptosis in *H. pylori* infection *in vivo*.

MATERIALS AND METHODS

In vitro study

The disk diffusion method was used to assess the anti-*H. pylori* activity of *L. plantarum* B7 supernatants at intact and neutralized pH and various concentrations of 1×, 5 × and 10× against *H. pylori*.

Bacterial strains and culture conditions: *H. pylori* ATCC 43504 was grown on Columbia agar (Oxoid, Basingstoke, United Kingdom) containing 7% sheep blood and 7% horse serum. Plates were incubated at 37 ℃ under microaerophilic conditions (10% CO_2, 5% O_2 and 85% N_2) produced by a gas generating system, Anaero-Pack (MGC, Japan), for 72 h in an anaerobic jar (Oxoid, Basingstoke, United Kingdom).

L. plantarum B7, isolated from Thai dyspeptic patients, was stored in de Man-Rogosa-Sharpe (MRS) broth (Oxoid, Basingstoke, United Kingdom) with 20% glycerol at -80 ℃. This strain was recovered from frozen stock and cultivated twice on MRS agar anaerobically (10% CO_2, 10% H_2 and 80% N_2) at 37 ℃ in an anaerobic jar for 48 h. A single colony of *L. plantarum* B7 was then inoculated into 10 mL of MRS broth and grown at 37 ℃ under anaerobic conditions for 24 h in a 15 mL conical centrifuge tube (Corning, New York, United States). The OD600 of the culture was determined using a spectrophotometer (Bio-Rad Smart SpecTM Plus), adjusted to OD600 with 0.1 in 10 mL of MRS broth and incubated for 48 h. After incubation, the culture supernatant was collected by centrifugation at 1000 × *g* for 10 min at 4 ℃ and then filtered using a 0.22 μm pore size filter unit (Minisart, Germany). The supernatant of *Lactobacillus* without the cell pellet was called the *Lactobacillus* condition media (LCM). The concentration and pH of LCM were adjusted to 1×, 5× and 10× by speed-vacuum drying (speed-vacuum, Savant Instruments, United States) and resuspending in an appropriate volume of intact pH 4 and adjusted pH 7 MRS broth. Sterile 6 mm-membrane disks (Whatman, Maidstone, United Kingdom) were then dipped into the resuspended LCM for at least 1 h at room temperature.

Disk diffusion method: The various concentrations of *L. plantarum* B7 supernatants were evaluated at two pH values, intact pH 4, and adjusted pH 7 with NaOH.

H. pylori was spread on Columbia blood agar plates, and *L. plantarum* B7 (LCM) disks were placed directly on the surface of the agar. The plates were incubated under microaerophilic conditions at 37 ℃ for 72 h, after which the diameters of the inhibition zones were measured in millimeters. In this study, the MRS broth was used as a negative control. The experiments were carried out in duplicate and mean values of the growth inhibition zones were measured.

In vivo study

Bacteria preparation: *H. pylori* was subcultured twice on Columbia blood agar. Plates were incubated at 37 ℃ under microaerophilic conditions for 72 h. *L. plantarum* B7 was originally obtained from Thai dyspeptic patients who visited King Chulalongkorn Memorial Hospital. This strain was cultivated twice on MRS agar anaerobically at 37 ℃ for 48 h.

Animal preparation: Thirty-two male Sprague-Dawley rats (Salaya Research Animal Center, Mahidol University, Bangkok, Thailand), weighing about 150-250 g at the beginning of the experiment, were used. The experimental protocol was approved by the Ethical Committee of Medicine Faculty, Chulalongkorn University, Thailand. The animals were housed in Macrolon cages (5 animals per cage), given food and tap water *ad libitum* at room temperature (18 ℃-22 ℃), humidity 55%, and a 12/12 h-light/dark cycle.

Experimental protocol: The rats were randomly divided into four experimental groups (eight rats each group) as follows. Group 1: Rats were fed phosphate buffered saline (PBS) 1 mL/rat by gavage twice a day at an interval of four hours for three consecutive days. Then, they were housed with free access to water and standard food for 1 wk. After that, the animals were treated with PBS 1 mL/rat by gavage once daily for 1 wk. Group 2: Rats were inoculated with *H. pylori* using the method of Thong-Ngam *et al*[13]. Briefly, the rats were pre-treated with streptomycin suspended in tap water (5 mg/mL) for three days before *H. pylori* inoculation. The *H. pylori* suspension (5×10^{10} CFUs/mL) in PBS was administered (1 mL/rat) by gavage twice daily at an interval of four hours for three consecutive days. One week after the inoculation, the animals were treated with PBS (1 mL/rat) by gavage once daily for one week. Group 3: One week after *H. pylori* inoculation, the rats were treated by gavage with *L. plantarum* B7 10^6 CFUs/mL suspended in PBS once daily for 1 wk. Group 4: One week after *H. pylori* inoculation, the rats were treated by gavage with *L. plantarum* B7 10^{10} CFUs/mL suspended in PBS once daily for 1 wk.

At the end of the experiment, animals were sacrificedby an overdose of intraperitoneal thiopental sodium. Blood samples were then collected for TNF-α determination using enzyme-linked immunosorbent assay (ELISA). The stomach was removed. One-half of the stomach was frozen in liquid nitrogen, and stored at -80 ℃ for MDA analysis. The remainder of the stomach was fixed in 4% paraformaldehyde in phosphate buffer solution to determine histopathology and epithelial cell apoptosis.

Determination of serum cytokine levels: Blood samples were taken by cardiac puncture, allowed to clot for two hours at room temperature before centrifuging for 20 min at approximately 1000 × *g*. Then, the serum was removed and stored at -80 ℃ for determination of TNF-α level using an ELISA kit (R and D Systems, United States).

Assessment of *H. pylori* infection and examination of histopathology: The presence of *H. pylori* infection in the rats was determined by the urease test and histopathological examination by a blinded pathologist. After completing the experiment, the rats were sacrificed. The stomach was removed and 2 mm^2 of gastric mucosa from the antrum was immediately dissected and placed in the urease tube to examine urease activity.

The remaining tissue from the gastric antrum biopsy was fixed in 4% paraformaldehyde in phosphate buffer solution at pH 7.4 and room temperature. The tissue was processed and stained with hematoxylin-eosin. The slides were observed by light microscopy and the presence of *H. pylori* was detected by Warthin-Starry staining in unclear cases. The level of bacterial colonization was evaluated using a grading system as follows. Score 0: No bacteria detected; Score 1: Mild colonization in some gastric crypts; Score 2: Mild colonization in most gastric crypts; Score 3: Moderate colonization in all gastric crypts. The results are presented as the bacterial colonization scores for each group. In addition to *H. pylori* colonization, the gastric inflammation level was estimated and scored following the updated Sydney System[14]. The infiltration of polymorphonuclear leukocytes in the gastric mucosa, defining the inflammatory scores, was recorded. Scores from 0 to 3 represented normal, mild, moderate and marked histopathological changes, respectively.

Determination of gastric malondialdehyde: Gastric MDA level was measured using the thiobarbituric acid (TBA) reactive substances assay kit (Cayman, United States). The principle is that the reaction of one molecule of MDA and two molecules of TBA form a red MDA-TBA complex under high temperature (90 ℃ -100 ℃) and acidic conditions, which can be quantitated using a spectrophotometer at 532 nm. The assay procedures were performed as described. The content of MDA was expressed in terms of nmol/mg protein.

Determination of gastric epithelial cell apoptosis: Apoptosis was measured by the identification of apoptotic nuclei in sections of stomachusing fragment end labeling of DNA (Apoptosis detectionkit, Chemicon, United States). In brief, the DNA fragments were al-

Table 1 Inhibition zone diameters (mm) of all *Lactobacillus plantarum* B7 supernatant concentrations at intact pH 4 and adjusted pH 7 (mean ± SD) (*n* = 2)

Concentration of *L. plantarum* B7 supernatant	Diameters of inhibition zone (mm)	
	Intact pH 4	Adjusted pH 7
MRS (negative control)	0	0
1×	0	0
5×	8.5 ± 0.7	6.5 ± 0.7
10×	13 ± 0	11 ± 1.4

L. plantarum: *Lactobacillus plantarum*; MRS: Man-Rogosa-Sharpe.

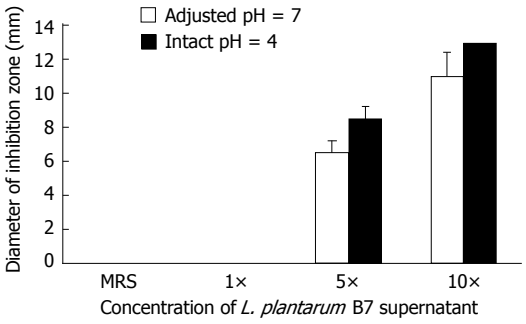


Figure 1 A bar graph shows the mean ± SD of inhibitory zone diameters (mm) of all *Lactobacillus plantarum* B7 supernatant concentrations at intact pH 4 and adjusted pH 7 (*n* = 2). *L. plantarum*: *Lactobacillus plantarum*; MRS: Man-Rogosa-Sharpe.

lowed to bind an antidigoxigenin antibody that was conjugated to a peroxidase. Diaminobenzidine was applied to develop a dark brown color and the slides were counterstained with hematoxylin. The positive stained cells showed dark brown nuclei under light microscopy. To verify the incidence of apoptosis, the dark brown-stained cells were counted. One thousand gastric epithelial cells were counted for each rat. The data were shown as a percentage (%) of apoptotic cells calculated as: the percentage of apoptotic cells (%) = (numbers of positive stained cells × 100)/1000.

Statistical analysis

All data are presented as mean ± SD. The means were compared by one-way analysis of variance followed by least significant different post hoc test. All statistical tests were performed using SPSS for Windows version 13.0 (SPSS Inc, Chicago, IL, United States). Differences were considered statistically significant at $P < 0.05$.

RESULTS

In vitro study

Disk diffusion method: At intact pH 4, mean inhibitory zone diameters of 8.5 ± 0.7 mm and 13 ± 0 mm were noted at the concentrations of 5× and 10× of *L. plantarum* B7 supernatant disks, respectively. At adjusted pH 7, mean inhibitory zone diameters of 6.5 ± 0.7 mm and 11 ± 1.4 mm were noted at the concentrations of 5× and 10× of *L. plantarum* B7 supernatant disks, respectively (Table 1). Both intact pH 4 and adjusted pH 7 of *L. plantarum* B7 supernatants showed dose-dependent anti-*H. pylori* activity. The supernatant of pH 4 *L. plantarum* B7 at the concentration of 10× showed the clearest inhibition (Figure 1).

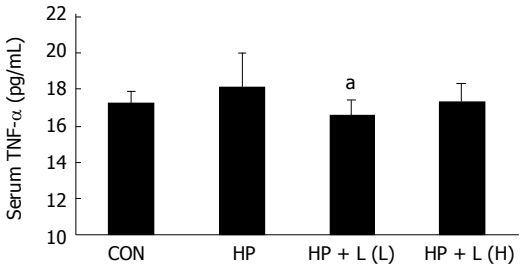


Figure 2 A bar graph shows the mean ± SD of serum tumor necrosis factor-alpha level (pg/mL) in all groups. CON: Control group; HP: *Helicobacter pylori* (*H. pylori*) infected group; HP + L (L): *Lactobacillus plantarum* (*L. plantarum*) B7 10^6 CFUs/mL treated group; HP + L (H): *L. plantarum* B7 10^{10} CFUs/mL treated group. Each group is represented by the mean of 8 rats. $^aP < 0.05$ *vs H. pylori* infected group. TNF-α: Tumor necrosis factor-alpha.

In vivo study

Changes in TNF-α level: The serum TNF-α level was not significantly different between the control group and *H. pylori* infected group. However, in the *L. plantarum* B7 10^6 CFUs/mL treated group, a significant decrease in serum TNF-α level was noted compared with the *H. pylori* infected group ($P = 0.019$). The average concentrations of serum TNF-α were 17.22 ± 0.63 pg/mL, 18.05 ± 1.94 pg/mL, and 16.52 ± 0.84 pg/mL in the control, *H. pylori* infected, and in the *L. plantarum* B7 10^6 CFUs/mL treated group, respectively. The average serum TNF-α levels in all groups are shown in Figure 2.

Histopathological examination: *H. pylori* infection in rats was determined by the urease test and histopathology. Histopathology in the control group was normal, while in the *H. pylori* infected group there was moderate *H. pylori* colonization and inflammation. The *L. plantarum* B7 10^6 CFUs/mL treated and *L. plantarum* B7 10^{10} CFUs/mL treated groups showed reduced *H. pylori* colonization and improved stomach inflammation (Figures 3 and 4). The histology scores for *H. pylori* colonization and gastric inflammation are summarized in Table 2.

Determination of gastric malondialdehyde: The level of gastric MDA increased significantly in the *H. pylori* infected compared with the control group (3.46 ± 1.25 nmol/mg *vs* 1.05 ± 0.41 nmol/mg protein, $P = 0.000$, respectively). After one week of 10^6 CFUs/mL or 10^{10} CFUs/mL of *L. plantarum* B7 suspension, there was a significant decrease in elevated gastric MDA level in both *L. plantarum* B7 treated groups compared with the *H. pylori* infected group (1.28 ± 0.69, 1.37 ± 0.66 nmol/mg *vs* 3.46 ± 1.25 nmol/mg protein, $P = 0.000$, respectively) (Figure 5).

Determination of gastric epithelial cell apoptosis: The percentage of apoptotic cells was significantly in-

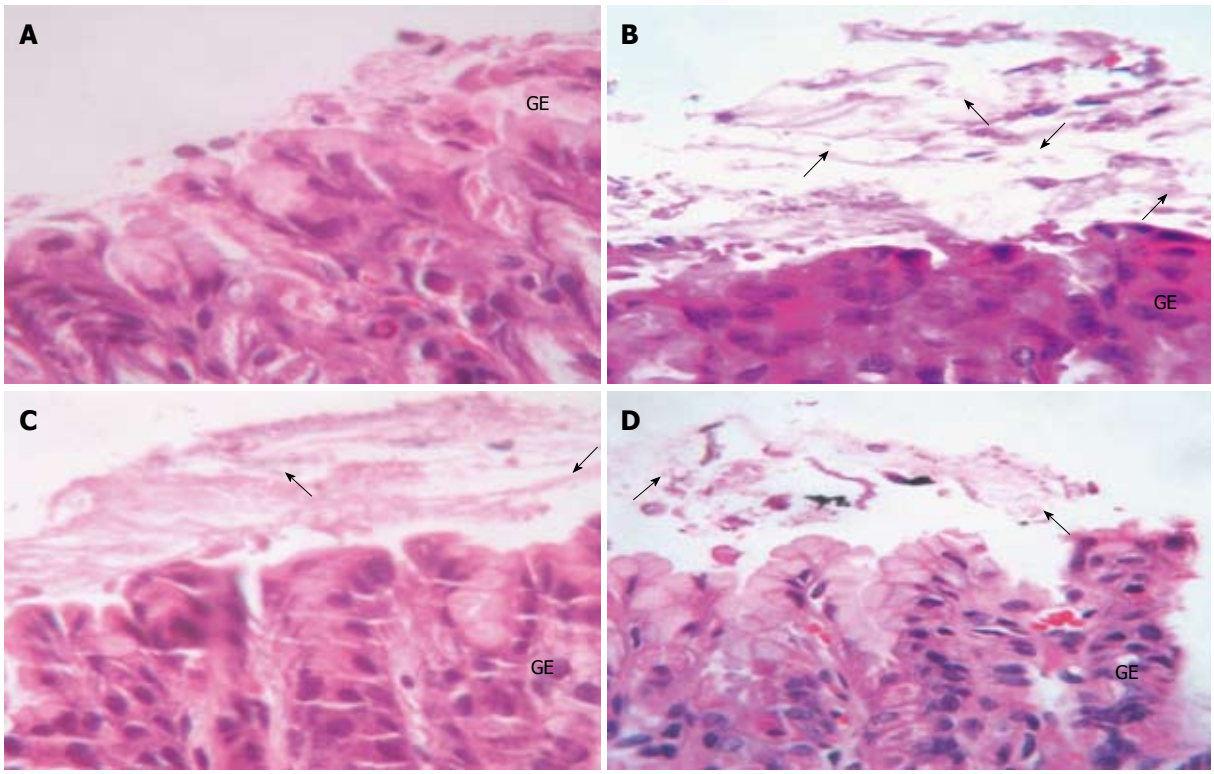


Figure 3 Hematoxylin-eosin stained gastric sections (×40). A: Control group showed no *Helicobacter pylori* (*H. pylori*); B: *H. pylori* infected group showed colonization (arrows) of *H. pylori*; C and D: *Lactobacillus plantarum* (*L. plantarum*) B7 10^6 CFUs/mL treated and *L. plantarum* B7 10^{10} CFUs/mL treated groups showed decreased *H. pylori* colonization. GE: Gastric epithelium.

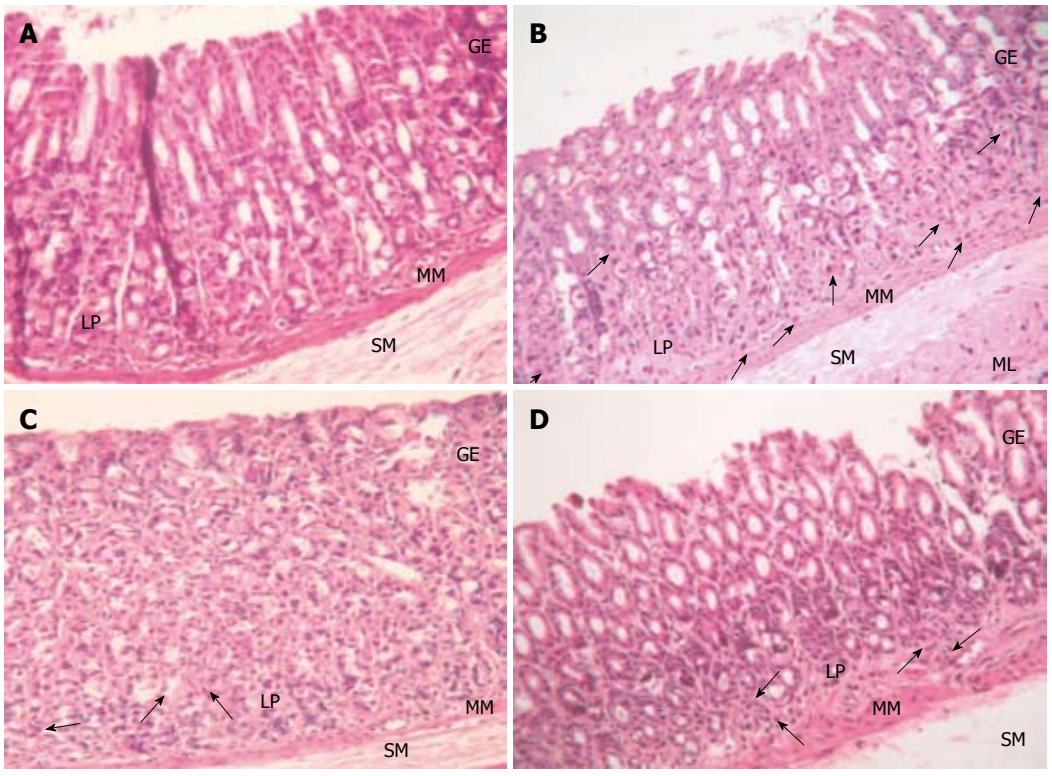


Figure 4 Hematoxylin-eosin stained gastric sections (×20). A: Control group showed normal gastric histopathology; B: *Helicobacter pylori* infected group showed infiltration of inflammatory cells (arrows); C and D: *Lactobacillus plantarum* (*L. plantarum*) B7 10^6 CFUs/mL treated and *L. plantarum* B7 10^{10} CFUs/mL treated groups showed improvements in gastric inflammation. GE: Gastric epithelium; LP: Lamina propria; MM: Muscularis mucosae; SM: Submucosa; ML: Muscularis.

Table 2 Summary of the scores for bacterial colonization levels and gastric inflammation in all groups

Group	Number	Level of *H. pylori* colonization[1]				Gastric inflammation[2]			
		0	1	2	3	0	1	2	3
Control group	8	8	-	-	-	8	-	-	-
H. pylori infected group	8	1	5	2	-	-	3	5	-
L. plantarum B7 10^6 CFUs/mL treated group	8	4	4	-	-	-	8	-	-
L. plantarum B7 10^{10} CFUs/mL treated group	8	3	5	-	-	-	8	-	-

[1]The stomach samples were evaluated for *Helicobacter pylori* (*H. pylori*) colonization by the pathologist using the following scoring system. Score 0: No bacteria detected; Score 1: Mild colonization in some gastric crypts; Score 2: Mild colonization in most gastric crypts; Score 3: Moderate colonization in all gastric crypts. [2]The gastric inflammation level was estimated and scored by the pathologist following the updated Sydney System[16]. The infiltration of polymorphonuclear leucocytes in the gastric mucosa defining the inflammatory scores was recorded. Scores from 0 to 3 represented normal, mild, moderate and marked histopathology changes, respectively. *L. plantarum*: *Lactobacillus plantarum*.

creased in the *H. pylori* infected group when compared with the control group (7.44 ± 2.65 *vs* 0.58 ± 0.13, P = 0.0001, respectively). After treatment with 10^6 CFUs/mL or 10^{10} CFUs/mL of *L. plantarum* B7 suspension, the percentage of apoptotic cells was significantly decreased at 10^6 CFUs/mL (P = 0.027) and 10^{10} CFUs/mL (P = 0.038) compared with the *H. pylori* infected group. The average percentages of apoptotic cells in all the groups are shown in Figure 6. Figure 7 shows gastric sections processed for apoptosis by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) reaction.

DISCUSSION

The *in vitro* study with intact pH 4 and adjusted pH 7 of *L. plantarum* B7 supernatants showed concentration-dependent anti-*H. pylori* activity, however, the culture supernatants of intact pH 4 *L. plantarum* B7 supernatant showed higher inhibition. This implied that low pH values are important for anti-*H. pylori* activity. In a study by Boyanova *et al*[15], the anti-*Helicobacter* activity of *L. delbrueckii subsp. bulgaricus* cultures was strain-dependent and better at their native pH.

It is known that *Lactobacillus* secretes metabolic products such as lactic acid which exerts activity against *H. pylori*[16]. Lactic acid inhibits the urease activity and viability of *H. pylori*. Several studies have reported that bacteriocin, peroxide, proteinase, exopolysaccharide and cell wall components, called *Lactobacillus*-inhibitory factors, have antibacterial effects[17,18]. In addition, Coconnier *et al*[19] showed that a heat-stable antimicrobial substance secreted by *L. acidophilus* LB was active against *H. pylori* infection.

In summary, our *in vitro* study found that *L. plantarum* B7 supernatant inhibited *H. pylori* growth in a dose-dependent manner and was better at intact pH 4 indicating that the amount of antimicrobial substance released by

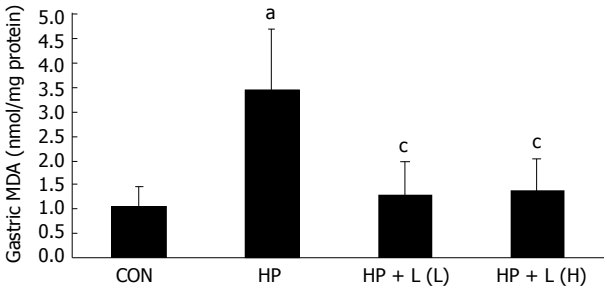


Figure 5 A bar graph shows the mean ± SD of gastric malondialdehyde levels (nmol/mg protein) in all groups. CON: Control group; HP: *Helicobacter pylori* (*H. pylori*) infected group; HP + L (L): *Lactobacillus plantarum* (*L. plantarum*) B7 10^6 CFUs/mL treated group; HP + L (H): *L. plantarum* B7 10^{10} CFUs/mL treated group. Each group is represented by the mean of 8 rats. $^aP < 0.05$ *vs* control group; $^cP < 0.05$ *vs H. pylori* infected group. MDA: Malondialdehyde.

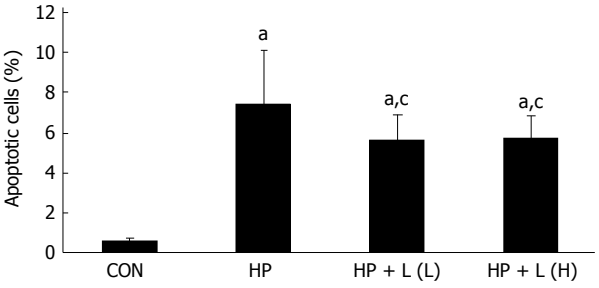


Figure 6 A bar graph shows the mean ± SD of apoptotic cells (%) in all groups. CON: Control group; HP: *Helicobacter pylori* (*H. pylori*) infected group; HP + L (L): *Lactobacillus plantarum* (*L. plantarum*) B7 10^6 CFUs/mL treated group; HP + L (H): *L. plantarum* B7 10^{10} CFUs/mL treated group. Each group is represented by the mean of 8 rats. $^aP < 0.05$ *vs* control group; $^cP < 0.05$ *vs H. pylori* infected group.

L. plantarum B7 correlated with the intensity of the inhibitory effect against *H. pylori*. Furthermore, the anti-*H. pylori* activity of this substance was supported by low pH values.

The present *in vivo* study showed that the gastric histopathology in the *H. pylori* infected group revealed mild to moderate *H. pylori* colonization and inflammation as well as increased gastric MDA and gastric epithelial cell apoptosis.

H. pylori induces a host inflammatory response including production of cytokines, resulting in mucosal damage. The produced cytokines lead to infiltration of inflammatory cells, namely polymorphonuclear neutrophils (PMNs), lymphocytes and macrophages, at the site of infection. These inflammatory cells then release large amounts of ROS, causing tissue injury. Wilson *et al*[20] showed that the gastric mucosal levels of proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8, were significantly higher in *H. pylori* positive patients than in *H. pylori* negative patients. Crabtree *et al*[21] showed that increased gastric mucosal production of TNF-α and IL-6 was associated with *H. pylori* gastritis. Moreover, they implied that inflammatory cytokines generated locally within the gastric mucosa can be relevant to the gastric physiology of *H. pylori* infection.

As mentioned above, infection with *H. pylori* in the

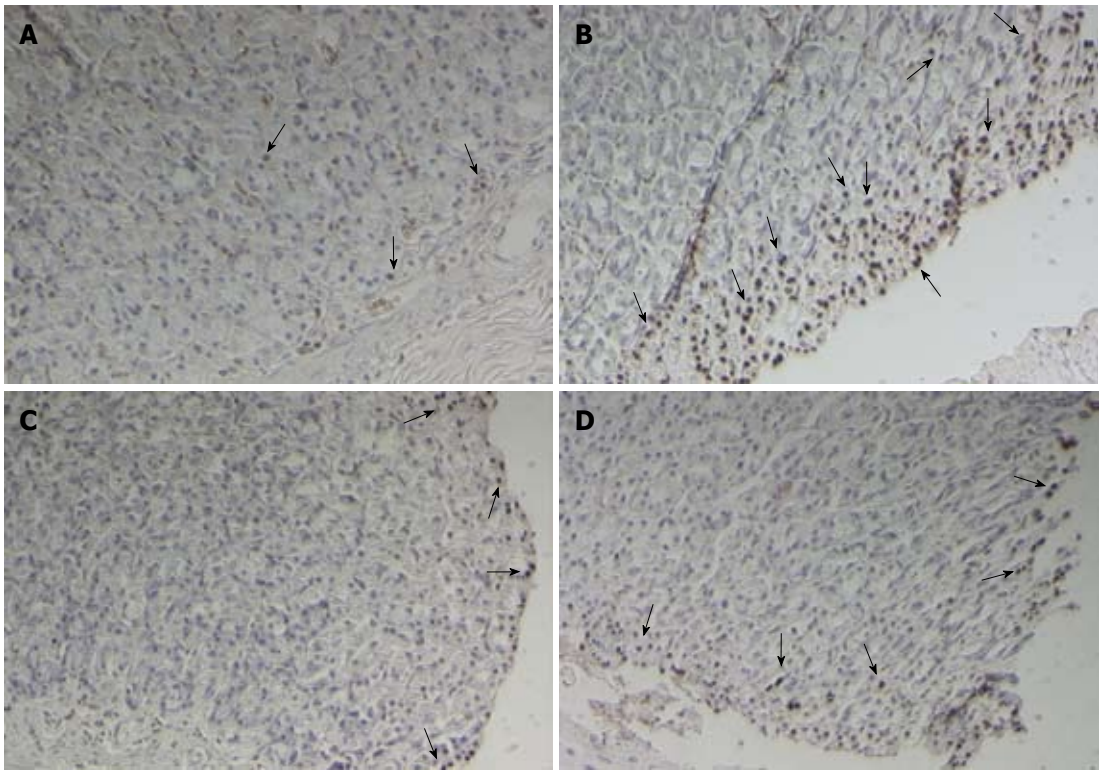


Figure 7 Representative gastric sections processed for the apoptosis assay by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling reaction (×20). A: Control group; B: *Helicobacter pylori* infected group; C and D: *Lactobacillus plantarum* (*L. plantarum*) B7 10^6 CFUs/mL treated and *L. plantarum* B7 10^{10} CFUs/mL treated groups showed a decrease in gastric epithelium apoptosis. The arrows indicate terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive gastric epithelial cell apoptosis.

gastric mucosa is known to activate the production of many proinflammatory cytokines including TNF-α, IL-1β, IL-6 and IL-8. The production of these proinflammatory cytokines is not limited to the local site of infection, as these cytokines are produced in numbers and contribute to the systemic circulation. In 2006, Prabjone *et al*[22] investigated the effects of chronic *H. pylori* infection on serum TNF-α level in rats. They found a significant increase in serum TNF-α in the *H. pylori* infected groups compared with the control groups. In the present study, no significant increase in serum TNF-α level was observed in the *H. pylori* infected group.

Several studies have shown that *H. pylori* strains with the *cagA*$^+$/*vacAs1* genotype are more virulent than strains with other genotypes[23]. Similarly, Azuma *et al*[24] reported that *H. pylori cagA*$^+$ strains were involved in more intense tissue responses than *cagA*$^-$ strains. Moreover, epidemiological studies have shown that colonization with *cagA*$^+$ *H. pylori* is associated with an increased risk for the development of both peptic ulcer disease and gastric cancer. In an *in vitro* study, Zhang *et al*[25] demonstrated that *H. pylori cagA*$^+$ strains induced an increased oxidative burst in PMNs with higher ROS production. Recently, studies have shown that ROS production in gastric mucosa is enhanced by infection with *cagA*$^+$ *H. pylori* species with an extensive accumulation of neutrophils in both patients with chronic gastritis and gastric ulcer[4,5]. In this study, rats infected with *H. pylori cagA*$^+$, *vacA*$^+$ strains were found to have significantly increased gastric MDA levels, suggesting that oxidative stress may be associated with the *cagA*$^+$ status of *H. pylori*.

Furthermore, we demonstrated that *H. pylori cagA*$^+$, *vacA*$^+$ strains can induce epithelial cell apoptosis in rats. The *cagA* gene or expression of VacA might be involved in gastroduodenal diseases by affecting apoptosis. The *cagA* gene is a marker of the presence of the pathogenicity island that encodes disease-associated virulence factors and is associated with the expression of VacA[26]. In 2006, Cabral *et al*[27] showed that the expression of pro-apoptotic proteins such as Bax and Bak was higher than anti-apoptotic proteins including Bcl-2 and Bcl-XL in most gastric biopsies from patients with *H. pylori* gastritis and was significantly higher in patients infected by *cagA*$^+$ strains than in those infected by *cagA*$^-$. Moreover, they found that Bak expression was higher at the lesser curvature (antrum and incisura) than in the other regions and was correlated with atrophy. These results suggest that in addition to *cagA*, *vacA* plays a crucial role in the induction of apoptosis. In the present study, our data also showed that infection with *H. pylori cagA*$^+$, *vacA*$^+$ strains leads to elevated gastric MDA levels, as previously mentioned. MDA, a major product of lipid peroxidation, can react with DNA to form MDA-DNA adducts, resulting in DNA damage.

Several previous investigations have shown the anti-inflammatory properties of *Lactobacillus*. A study by Johnson-Henry *et al*[28] found that the probiotic combination containing *L. rhamnosus* R0011 and *L. acidophilus*

R0052 decreased the effects of *H. pylori* infection in a C57BL/6 mouse model of infection by reducing *H. pylori* colonization and alleviating *H. pylori*-induced gastric mucosa inflammation. In 2003, Peña *et al*[29] showed that *L. rhamnosus* GG was able to antagonize *H. pylori* LPS-induced TNF-α production in murine macrophages *in vitro* by a contact-independent mechanism. Ko *et al*[12] reported that *L. plantarum* was capable of inhibiting epithelial barrier dysfunction and reducing IL-8 secretion induced by TNF-α. In addition to anti-inflammatory activity, several studies have shown that *Lactobacillus* also has effective antioxidative and anti-apoptotic properties. Truusalu *et al*[30] found that *L. fermentum* ME-3 suppressed excessive oxidative stress-associated inflammation induced by *S. typhimurium* infection in a mouse model. Using the same experimental typhoid fever model, they also showed that treatment with *L. fermentum* ME-3 alone or in combination with an antimicrobial quinolone (ofloxacin) leads to a significant decrease in lipid peroxidation and the glutathione redox ratio (GSSG/GSH). In 2010, Zhang *et al*[31] reported that oral *L. plantarum* treatment in rats with obstructive jaundice increased GSH levels in the liver and stimulated GSH biosynthesis, resulting in attenuated oxidative damage. Using the TUNEL assay, they also showed that treatment with *L. plantarum* significantly decreased hepatic apoptosis. In addition, Lam *et al*[32] showed that pre-treatment of rats with *L. rhamnosus* GG markedly reduced ethanol-induced mucosal lesion area and gastric cell apoptosis.

Interestingly, all of these studies were concordant with our results. In the current study, we found that *L. plantarum* B7 treatment resulted in improved stomach pathology, and decreased serum TNF-α level, gastric MDA level, and apoptotic epithelial cells. However, the mechanisms of action are unclear and require further investigation.

In conclusion, the present study showed that *H. pylori* infection induced gastric injury by increasing levels of *H. pylori* colonization and inflammation, gastric MDA and epithelial cell apoptosis. *L. plantarum* B7 may have anti-*H. pylori* activity *in vitro* and anti-inflammatory effects on *H. pylori* infection by improving stomach histopathology, and reducing serum TNF-α levels, gastric MDA and epithelial cell apoptosis.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection induces the production of proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-2, IL-6 and IL-8, and infiltration of the lamina propria with inflammatory cells as well as the generation of reactive oxygen species (ROS). However, these *H. pylori*-induced inflammatory responses do not appear to confer protective immunity, and may lead to the excess production of ROS, oxidative bursts caused by phagocytic cells, and gastric tissue damage. *Lactobacillus plantarum* (*L. plantarum*) B7 has anti-*H. pylori* activity *in vitro* and anti-inflammatory properties resulting in the alleviation of gastric injury in *H. pylori*-induced gastritis in rats.

Research frontiers

L. plantarum is a non-pathogenic gram-positive bacterium that exerts anti-*H. pylori* activity and immunomodulatory effects. *H. pylori* infection can cause gastric mucosal damage by increasing *H. pylori* colonization and inflammation levels, gastric malondialdehyde (MDA) and epithelial cell apoptosis. The hallmark of this study was the interesting results which showed an inhibitory effect of *L. plantarum* B7 supernatant on *H. pylori* growth *in vitro*, and an improvement in stomach pathology, reduction in serum TNF-α level, gastric MDA and epithelial cell apoptosis following treatment with *L. plantarum* B7.

Innovations and breakthroughs

A previous study showed that *L. plantarum* B7 has anti-inflammatory properties *in vitro*. However, it is not clear whether *L. plantarum* B7 has *in vivo* effects on *H. pylori*-induced gastric inflammation. Therefore, in this study, the authors examined the anti-inflammatory effect of *L. plantarum* B7 in rats and found that *L. plantarum* B7 ameliorated *H. pylori*-induced gastritis by improving stomach pathology, and decreasing TNF-α production, gastric MDA level and epithelial cell apoptosis. Moreover, supernatants of *L. plantarum* B7 showed anti-*H. pylori* activity *in vitro*.

Applications

L. plantarum B7 may be beneficial in clinical application, and can be used as an adjunct to antibiotics to decrease *H. pylori*-induced gastric inflammation and reduce side effects of triple therapy.

Peer review

This is an experimental study on the effect of *H. pylori* infection in gastric inflammation. This study shows the efficacy of *L. plantarum* B7 in treatment of *H. pylori*-induced gastritis reflects in attenuated levels of *H. pylori* colonization, gastric inflammation, cytokine production, gastric MDA, and apoptotic cells. Also, the results from *in vitro* study demonstrates the inhibitory effect of *L. plantarum* B7 supernatants on *H. pylori* growth.

REFERENCES

1 **Blaser MJ**. Helicobacter pylori and gastric diseases. *BMJ* 1998; **316**: 1507-1510
2 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131
3 **McGee DJ**, Mobley HL. Mechanisms of Helicobacter pylori infection: bacterial factors. *Curr Top Microbiol Immunol* 1999; **241**: 155-180
4 **Naito Y**, Yoshikawa T. Molecular and cellular mechanisms involved in Helicobacter pylori-induced inflammation and oxidative stress. *Free Radic Biol Med* 2002; **33**: 323-336
5 **Augusto AC**, Miguel F, Mendonça S, Pedrazzoli J, Gurgueira SA. Oxidative stress expression status associated to Helicobacter pylori virulence in gastric diseases. *Clin Biochem* 2007; **40**: 615-622
6 **Wagner S**, Beil W, Westermann J, Logan RP, Bock CT, Trautwein C, Bleck JS, Manns MP. Regulation of gastric epithelial cell growth by Helicobacter pylori: offdence for a major role of apoptosis. *Gastroenterology* 1997; **113**: 1836-1847
7 **Moss SF**, Calam J, Agarwal B, Wang S, Holt PR. Induction of gastric epithelial apoptosis by Helicobacter pylori. *Gut* 1996; **38**: 498-501
8 **Meyer JM**, Silliman NP, Wang W, Siepman NY, Sugg JE, Morris D, Zhang J, Bhattacharyya H, King EC, Hopkins RJ. Risk factors for Helicobacter pylori resistance in the United States: the surveillance of H. pylori antimicrobial resistance partnership (SHARP) study, 1993-1999. *Ann Intern Med* 2002; **136**: 13-24
9 **Fuller R**. Probiotics in man and animals. *J Appl Bacteriol* 1989; **66**: 365-378
10 **Saxelin M**, Tynkkynen S, Mattila-Sandholm T, de Vos WM. Probiotic and other functional microbes: from markets to mechanisms. *Curr Opin Biotechnol* 2005; **16**: 204-211
11 **Rokka S**, Pihlanto A, Korhonen H, Joutsjoki V. In vitro growth inhibition of Helicobacter pylori by lactobacilli belonging to the Lactobacillus plantarum group. *Lett Appl Microbiol* 2006; **43**: 508-513

12 **Ko JS**, Yang HR, Chang JY, Seo JK. Lactobacillus plantarum inhibits epithelial barrier dysfunction and interleukin-8 secretion induced by tumor necrosis factor-alpha. *World J Gastroenterol* 2007; **13**: 1962-1965

13 **Thong-Ngam D**, Prabjone R, Visedopas N, Chatsuwan T. A simple rat model of chronic Helicobacter pylori infection for research study. *Thai J Gastroenterol* 2005; **6**: 3-7

14 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181

15 **Boyanova L**, Stephanova-Kondratenko M, Mitov I. Anti-Helicobacter pylori activity of Lactobacillus delbrueckii subsp. bulgaricus strains: preliminary report. *Lett Appl Microbiol* 2009; **48**: 579-584

16 **Midolo PD**, Lambert JR, Hull R, Luo F, Grayson ML. In vitro inhibition of Helicobacter pylori NCTC 11637 by organic acids and lactic acid bacteria. *J Appl Bacteriol* 1995; **79**: 475-479

17 **Silva M**, Jacobus NV, Deneke C, Gorbach SL. Antimicrobial substance from a human Lactobacillus strain. *Antimicrob Agents Chemother* 1987; **31**: 1231-1233

18 **Pritchard GG**, Coolbear T. The physiology and biochemistry of the proteolytic system in lactic acid bacteria. *FEMS Microbiol Rev* 1993; **12**: 179-206

19 **Coconnier MH**, Lievin V, Hemery E, Servin AL. Antagonistic activity against Helicobacter infection in vitro and in vivo by the human Lactobacillus acidophilus strain LB. *Appl Environ Microbiol* 1998; **64**: 4573-4580

20 **Wilson M**, Seymour R, Henderson B. Bacterial perturbation of cytokine networks. *Infect Immun* 1998; **66**: 2401-2409

21 **Crabtree JE**, Lindley IJ. Mucosal interleukin-8 and Helicobacter pylori-associated gastroduodenal disease. *Eur J Gastroenterol Hepatol* 1994; **6** Suppl 1: S33-S38

22 **Prabjone R**, Thong-Ngam D, Wisedopas N, Chatsuwan T, Patumraj S. Anti-inflammatory effects of Aloe vera on leukocyte-endothelium interaction in the gastric microcirculation of Helicobacter pylori-infected rats. *Clin Hemorheol Microcirc* 2006; **35**: 359-366

23 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186

24 **Azuma T**, Ohtani M, Yamazaki Y, Higashi H, Hatakeyama M. Meta-analysis of the relationship between CagA seropositivity and gastric cancer. *Gastroenterology* 2004; **126**: 1926-1927; author reply 1926-1927

25 **Zhang Q**, Dawodu JB, Etolhi G, Husain A, Gemmell CG, Russell RI. Relationship between the mucosal production of reactive oxygen radicals and density of Helicobacter pylori in patients with duodenal ulcer. *Eur J Gastroenterol Hepatol* 1997; **9**: 261-265

26 **Cover TL**, Krishna US, Israel DA, Peek RM. Induction of gastric epithelial cell apoptosis by Helicobacter pylori vacuolating cytotoxin. *Cancer Res* 2003; **63**: 951-957

27 **Cabral MM**, Mendes CM, Castro LP, Cartelle CT, Guerra J, Queiroz DM, Nogueira AM. Apoptosis in Helicobacter pylori gastritis is related to cagA status. *Helicobacter* 2006; **11**: 469-476

28 **Johnson-Henry KC**, Mitchell DJ, Avitzur Y, Galindo-Mata E, Jones NL, Sherman PM. Probiotics reduce bacterial colonization and gastric inflammation in H. pylori-infected mice. *Dig Dis Sci* 2004; **49**: 1095-1102

29 **Peña JA**, Versalovic J. Lactobacillus rhamnosus GG decreases TNF-alpha production in lipopolysaccharide-activated murine macrophages by a contact-independent mechanism. *Cell Microbiol* 2003; **5**: 277-285

30 **Truusalu K**, Mikelsaar RH, Naaber P, Karki T, Kullisaar T, Zilmer M, Mikelsaar M. Eradication of Salmonella Typhimurium infection in a murine model of typhoid fever with the combination of probiotic Lactobacillus fermentum ME-3 and ofloxacin. *BMC Microbiol* 2008; **8**: 132

31 **Zhang L**, Li N, Caicedo R, Neu J. Alive and dead Lactobacillus rhamnosus GG decrease tumor necrosis factor-alpha-induced interleukin-8 production in Caco-2 cells. *J Nutr* 2005; **135**: 1752-1756

32 **Lam EK**, Yu L, Wong HP, Wu WK, Shin VY, Tai EK, So WH, Woo PC, Cho CH. Probiotic Lactobacillus rhamnosus GG enhances gastric ulcer healing in rats. *Eur J Pharmacol* 2007; **565**: 171-179

S- Editor Gou SX **L- Editor** Webster JR **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2481

World J Gastroenterol 2012 May 28; 18(20): 2481-2492
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Key factors in developing the trinitrobenzene sulfonic acid-induced post-inflammatory irritable bowel syndrome model in rats

Hong-Yan Qin, Hai-Tao Xiao, Justin CY Wu, Brian M Berman, Joseph JY Sung, Zhao-Xiang Bian

Hong-Yan Qin, Hai-Tao Xiao, Zhao-Xiang Bian, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong 999077, China
Justin CY Wu, Joseph JY Sung, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong 999077, China
Brian M Berman, Center for Integrative Medicine, University of Maryland, School of Medicine, Baltimore, MD 21201, United States
Author contributions: Qin HY performed the majority of experiments and data analysis, and wrote the manuscript; Xiao HT participated in the animal experiments; Wu JCY participated in data analysis; Berman BM participated in experimental design and reviewing of the manuscript; Sung JJY participated in the critical review of the manuscript; Bian ZX participated in the conception, experimental design and critical review and writing of the manuscript.
Supported by Hong Kong Jockey Club Institute of Chinese Medicine, No. JCICM-4-07
Correspondence to: Zhao-Xiang Bian, Professor, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong 999077, China. bzxiang@hkbu.edu.hk
Telephone: +852-34112905 Fax: +852-34112929
Received: October 22, 2011 Revised: December 15, 2011
Accepted: December 22, 2011
Published online: May 28, 2012

Abstract

AIM: To investigate the key factors in developing the trinitrobenzene sulfonic acid (TNBS)-induced post-inflammatory irritable bowel syndrome (PI-IBS) model in rats.

METHODS: TNBS was administered to rats at the following conditions: (1) with different doses (20, 10, 5 mg/0.8 mL per rat); (2) with same dose in different concentrations (20 mg/rat, 25, 50 mg/mL); (3) in different ethanol percentage (25%, 50%); and (4) at depth either 4 cm or 8 cm from anus. At 5 d and 4 wk after TNBS administration, inflammation severity and inflammation resolution were evaluated. At 4 and 8 wk after TNBS application, visceral hyperalgesia and enterochromaffin (EC) cell hyperplasia were assayed by abdominal withdrawal reflex test, silver staining and capillary electrophoresis.

RESULTS: Our results showed that: (1) TNBS induced dose-dependent acute inflammation and inflammation resolution. At 5 d post TNBS, the pathological score and myeloperoxidase (MPO) activity in all TNBS treated rats were significantly elevated compared to that of the control (9.48 ± 1.86, 8.18 ± 0.67, 5.78 ± 0.77 *vs* 0, and 3.55 ± 1.11, 1.80 ± 0.82, 0.97 ± 0.08 unit/mg *vs* 0.14 ± 0.01 unit/mg, $P < 0.05$). At 4 wk post TNBS, the pathological score in high and median dose TNBS-treated rats were still significantly higher than that of the control (1.52 ± 0.38 and 0.80 ± 0.35 *vs* 0, $P < 0.05$); (2) Intracolonic TNBS administration position affected the persistence of visceral hyperalgesia. At 4 wk post TNBS, abdominal withdrawal reflex (AWR) threshold pressure in all TNBS-treated groups were decreased compared to that of the control (21.52 ± 1.73 and 27.10 ± 1.94 mmHg *vs* 34.44 ± 1.89 mmHg, $P < 0.05$). At 8 wk post TNBS, AWR threshold pressure in 8 cm administration group was still significantly decreased (23.33 ± 1.33 mmHg *vs* 36.79 ± 2.29 mmHg, $P < 0.05$); (3) Ethanol percentage affected the TNBS-induced inflammation severity and visceral hyperalgesia. In TNBS-25% ethanol-treated group, the pathological score and MPO activity were significantly lowered compared to that of the TNBS-50% ethanol-treated group, while AWR threshold pressure were significantly elevated (36.33 ± 0.61 mmHg *vs* 23.33 ± 1.33 mmHg, $P < 0.05$); and (4) TNBS (5 mg/0.8 mL per rat, in 50% ethanol, 8 cm from anus)-treated rats recovered completely from the inflammation with acquired visceral hyperalgesia and EC cell hyperplasia at 4 wk after TNBS administration.

CONCLUSION: TNBS dosage, concentration, intraco-

lonic administration position, and ethanol percentage play important roles in developing visceral hyperalgesia and EC cell hyperplasia of TNBS-induced PI-IBS rats.

Key words: Post-inflammatory; Irritable bowel syndrome; Rat model; Trinitrobenzene sulfonic acid; Key factors

Peer reviewer: Kwang Jae Lee, Professor, Department of Gastroenterology, Ajou University Hospital, San5, Woncheondong, Yeongtongku, Suwon 443-721, South Korea

Qin HY, Xiao HT, Wu JCY, Berman BM, Sung JJY, Bian ZX. Key factors in developing the trinitrobenzene sulfonic acid-induced post-inflammatory irritable bowel syndrome model in rats. *World J Gastroenterol* 2012; 18(20): 2481-2492 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2481.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2481

INTRODUCTION

Post-infectious irritable bowel syndrome (PI-IBS) is a subgroup of IBS in which the IBS patients developed their symptoms after recovery from an acute gastrointestinal infection[1,2]. The features of PI-IBS, such as urgency, loose stool, and abdominal pain, are very similar to those of the diarrhea-predominant IBS[3,4]. Prospective studies indicate that 3%-36% of enteric infections may lead to the generation of new IBS symptoms; the precise incidence mainly depends on the infecting organism and the host responses[1]. Though the term "PI-IBS" was first coined by Chaudhary and Truelove in 1962[5], little attention has been paid to it until recently[1]. The causes and/or underlying mechanisms of PI-IBS are still not fully understood, although it is believed that altered gut flora, changed intestinal permeability, activated gut immunity, and functional or structural changes in enteric nervous system are important factors[1,6].

Validated animal models, which aim to mimic one or more features of human disease, play important roles in the studies of mechanisms and potential therapeutic agents for diseases. Based on the current understanding of the clinical features and underlying mechanisms of PI-IBS, the ideal model for PI-IBS should bear the character with one or more features of IBS, such as visceral hypersensitivity, motility dysfunction, alterations in permeability or secretion, and enterochromaffin (EC) cell hyperplasia, and complete recovery from initial infection/inflammation[7]. The chemical agent-induced post-inflammatory IBS animal models have been widely used in mechanistic studies of PI-IBS because the chemical agent-induced inflammatory response and host immune activation can, to some extent, mimic the characteristics of PI-IBS[8,9], and these alterations are consistent with the findings from clinical studies[10,11]. Our previous systematic review showed that the trinitrobenzene sulfonic acid (TNBS)-induced model is the most commonly used PI-IBS model[7], and the major features of IBS (i.e., visceral hypersensitivity, motility dysfunction, and alteration in permeability or secretion), can be developed in this model. However, there are large variations in the protocol of model development, such as TNBS dosage and concentration, position of intracolonic TNBS administration, ethanol percentage, and the optimal time after intracolonic TNBS administration for model application, *etc.*[7]. These variations make comparison between studies to be very difficult, if not impossible.

As a chemical hapten, TNBS is capable of binding to tissue proteins and stimulating T helper 1 cell mediated immunity; thus, it has been widely used to induce acute colitis by intracolonic administration[12]. It has been reported that TNBS-induced acute inflammation and damage becomes maximal from 3 d to 1 wk after instillation[9]. Previous studies have shown that TNBS had dose-dependent effects on mucosal inflammation[13,14], suggesting dosage is an important factor in TNBS-induced inflammation severity and colonic recovery from such inflammation. However, in previous studies on the TNBS-induced PI-IBS model, the dosage of TNBS varied from 20 mg per rat to 30 mg per rat[7]. Further, there is no consensus on how long it will take for TNBS-induced inflammation recovery after TNBS administration. Some studies, using TNBS (30 mg/rat), took 4 or 6 wk for inflammation recovery after intracolonic TNBS administration[15,16], while another study took at least 8 wk[17]. In addition, TNBS is generally administered into the colon lumen at either 4 cm or 8 cm from the anus[7]. Given the anatomical and physiological differences between these two sites (descending colon *vs* transverse colon), it is possible - but not known - whether the position of TNBS administration influences the results. Furthermore, ethanol is routinely used as a breaker to the mucosal barrier in TNBS-induced PI-IBS studies, and previous studies have shown that 30%-50% ethanol alone induced acute inflammation and hyperemia[18,19]. The commonly used ethanol percentage in developing TNBS-induced PI-IBS model are 25% and 50%[14,20]; and whether the ethanol percentage influences the development of TNBS-induced PI-IBS rat model is still not clear.

Concerning the above variations in developing protocol of the TNBS-induced PI-IBS rat model, the present study aimed to investigate the effects of some impact factors (i.e., TNBS dosage and concentration, position of TNBS administration, and ethanol percentage) on TNBS-induced acute inflammation, inflammation resolution, and later acquired features of IBS (i.e., visceral hyperalgesia and EC cell hyperplasia).

MATERIALS AND METHODS

Materials

TNBS (2,4,6-trinitrobenzenesulfonic acid solution), hexadecyltrimethylammonium bromide, o-dianisidine dihydrochloride, sodium hyposulfite, and silver nitrate were

Table 1 Study design and the treatments

Groups	Treatment (mg/mL per rat, i.col.)	Ethanol (%)	Position (cm to anus)	Time points for evaluation		
				5 d	4 wk	8 wk
1	TNBS, 20/0.4 per rat	50	8	IE	IE	-
2	TNBS, 20/0.8 per rat	50	8	IE	IE	-
3	TNBS, 10/0.8 per rat	50	8	IE	IE	-
4	TNBS, 5/0.8 per rat	50	8	IE	IE/PE	PE/EE
5	TNBS, 5/0.8 per rat	50	4	IE	IE/PE	PE
6	TNBS, 5/0.8 per rat	25	8	IE	IE/PE	PE
7	Saline, 0.8/rat	-	8	IE	IE/PE	PE/EE

TNBS: Trinitrobenzene sulfonic acid; IE: Inflammation evaluation; PE: Pain evaluation; EE: Enterochromaffin cell evaluation.

all purchased from Sigma-Aldrich (St. Louis, MO, United States). Chloral hydrate was purchased from Kou Hing Hong Scientific Supplies (Hong Kong, China).

Animals

Male Sprague-Dawley rats (aged 6 wk with body weight around 220 g) were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong. Rats were housed 5 per cage and maintained at 25 ℃ under 12 h-12 h alternating light-dark cycle with free access to food and water. Rats were maintained in laboratory conditions for 1 wk to adapt to the environment before each experiment. All animal studies were carried out in accordance with the guidelines of the Committee on Use of Human and Animal Subjects in Teaching and Research, Hong Kong Baptist University.

Experimental design

The rats were randomly divided into 7 groups. TNBS (20 mg/0.4 mL per rat) was given to rats in group 1 (n = 10). The rats in group 2 (n = 10), group 3 (n = 10), and group 4 (n = 14) were intracolonically administered with TNBS at a dose of 20, 10 and 5 mg per rat in 0.8 mL ethanol, respectively. In the above 4 groups, TNBS in 50% ethanol saline solution was administered at a depth of 8 cm from the anus. Rats in group 5 (n = 14) were given TNBS (5 mg/0.8 mL per rat in 50% ethanol) at a depth of 4 cm from the anus, while rats in group 6 (n = 14) were given TNBS (5 mg/0.8 mL per rat in 25% ethanol) at 8 cm from the anus. Group 7 (n = 14) was set as a control; rats in this group were given saline 0.8 mL at a depth of 8 cm from the anus (Table 1).

Five days after TNBS or saline administration, 4 rats in each group were selected randomly, and the colon tissues from these rats were harvested for inflammation evaluation. The remaining rats were allowed to recover until the pain threshold pressure was measured. At 4 wk after TNBS administration, five rats in each group were selected randomly for pain threshold pressure evaluation; then the colon tissues were collected for inflammation recovery examination. At 8 wk after TNBS administration, the rest of the rats in each group went through pain threshold pressure evaluation to test the stability of visceral hyperalgesia. In order to further investigate if the TNBS-induced PI-IBS model had EC cell hyperplasia in the colon tissue, a part of proximal and distal colon tissues were also collected for EC cell number counting and serotonin [5-hydroxytryptamine (5-HT)] content determination.

Induction of colitis

Colitis was induced according to previous reports, with little modification[16]. Briefly, rats were fasted for 24 h before experiments, and then deeply anesthetized with chloral hydrate (350 mg/kg, i.p.). A fine plastic catheter (external diameter = 0.96 mm) was gently inserted into the descending colon at a depth of 4 cm or 8 cm from anus. The rats were kept in a head-down vertical position, and then TNBS was instilled slowly into the colon lumen within 1 min. After TNBS instillation, the catheter was left in place for 1 min and then slowly removed. The TNBS-treated rats were left on a warm mound of bedding in head-down position to prevent drug leakage until they regained consciousness. The control rats were similarly administered with 0.8 mL saline instead of TNBS.

Tissue preparation

Rats were deeply anesthetized with chloral hydrate (350 mg/kg, i.p.); then an approximately 6 cm long piece of colon tissue with drug administration position in the middle was removed. After the photos were taken, 3 cm of the distal part (proximal to the anus) was fixed in 4% paraformaldehyde for histological evaluation., the remaining 3 cm (distal to the anus) was placed in liquid nitrogen and stored in a freezer at -80 ℃ for myeloperoxidase activity assay. In addition, approximate 3 cm of proximal colon (1-2 cm from cecum) and 3 cm of distal colon (1-2 cm from anus) tissue were also harvested from the PI-IBS model rats; the proximal part (about 1 cm) was fixed in 4% paraformaldehyde for EC cell number counting; the rest was placed in liquid nitrogen, and stored at -80 ℃ for 5-HT content determination.

Histological evaluation

The colon sections (5 μm thick) collected at 5 d and 4 wk after TNBS administration were all stained with hematoxylin and eosin (H and E). Masson trichrome staining[21] was performed on sections collected at 4 wk after TNBS administration for fibrosis evaluation. All sections were examined under a Nikon light microscope (Nikon Inc., Japan). The severity of the acute inflammation and the degree of inflammation resolution was graded using the macroscopic and histological scoring criteria (Tables 2 and 3), which were modified based on previous reports[22,23] according to the pathologist's suggestion. Five random fields were selected in each slide; images were captured with 100 × magnifications and analyzed using Image J NIH software. For fibroplasia evaluation, the area stained blue by Masson trichrome staining was measured and adjusted to reflect the total area of the colon tissue[21].

Myeloperoxidase activity assay

Myeloperoxidase (MPO) is an enzyme released by neu-

Table 2 Criteria for macroscopic scoring of colonic ulceration and inflammation

Score	Appearance
0	Normal appearance
1	Ulceration with inflammation at 1 or 2 sites
2	More sites of ulceration and inflammation
3	Major sites of damage extending > 1.5 cm along length of colon
4	Major sites of damage extending > 3 cm along length of colon

trophils in tissue under inflammatory conditions, and the level of MPO activity correlates directly with severity of inflammation[17]. In this study, MPO activity was measured by the modified method described by Krawisz *et al*[24] and Diop *et al*[18]. Briefly, the colon tissues were cut into small pieces and homogenized in 0.5% hexadecyltrimethylammonium bromide 1 mL per 100 mg of colon tissue. The homogenates were centrifuged at 19 000 *g* at 4 ℃ for 15 min. Aliquots of 80 mL supernatant were mixed with 120 μL potassium phosphate buffer (50 mmol, pH 6.0) with 0.0005% o-dianisidine dihydrochloride and 0.1% hydrogen peroxide. MPO activity was calculated from the rate of absorbance change during 1 min at 460 nm; one unit of MPO activity is equal to 1.13×10^{-2} changes in absorbance at 25 ℃. The results were normalized to the wet weight of colon tissue and expressed as MPO units/mg tissue.

Abdominal withdrawal reflex

Abdominal withdrawal reflex (AWR) test was performed as previously described to detect the pain threshold pressure[25]. Briefly, rats were lightly anesthetized with ether in order to place a 6 cm long flexible latex balloon into the descending colon and rectum through the anus. The end of the balloon was secured at least 1 cm proximal to the anal verge. Rats were then allowed to recover for at least 30 min. The tube of the balloon was connected *via* a Y-connector to a sphygmomanometer and colorectal distension was applied in increments of 5 mmHg until a visible contraction of the abdominal wall was observed by an investigator blinded to the treatment. The pain threshold pressure was defined as the intensity of colorectal distension that elicited an observable AWR, i.e., a sudden and persistent abdominal muscle contraction with abdomen lift off the platform (Score 3). The pain threshold pressure of all groups was recorded and repeated five times with intervals of at least 5 min for recovery.

Enterochromaffin cell counting

Tissue sections (5 μm thick) were deparaffinized in xylene, and rehydrated with graded ethanol for silver staining according to a method previously described, with little modification[26]. Sections were incubated with 5% ammoniacal silver solution for 4 h at room temperature, then 2 h in 56 ℃ and subsequently 12 h at room temperature in a dark humidified chamber. After rinsing with water, 5% sodium hyposulfite was added and sections were incubated for 5 min at room temperature. The brown to black silver precipitate in the cytoplasm of EC cells was considered as a positive reaction. Five random fields at 200× magnifications for each section were captured and saved in the same size and resolution by a researcher blinded to treatment. After calibration, the mucosal fields were circled, and the areas of mucosa were calculated using Image J NIH software. EC cell density was calculated and expressed as the number of EC cells per mm^2 of mucosal area.

Serotonin content assessment

5-HT content in the colonic tissue was assayed following the previously reported procedure[27]. Briefly, the colon segment was homogenized in 15% iced trichloroacetic acid; the supernatant of each sample was filtered using 0.22 μm filters and extracted with diethyl ether, then the prepared samples were added to derivatization solution and analyzed by capillary electrophoresis with laser-induced fluorescence detection.

Statistical analysis

Data are presented as mean ± SE. Differences between two groups were analyzed by Student *t* test. When multiple groups were compared, data were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test. Differences were considered significant when $P < 0.05$.

RESULTS

Effect of trinitrobenzene sulfonic acid concentration on body weight and mortality rate

Based on the findings from our previous systematic review, TNBS (20 mg/rat, in 50% ethanol) was selected and given to rats at 8 cm from anus in 0.4 or 0.8 mL volume. As shown in Table 4, compared to the control group, the body weight of TNBS-treated rats all decreased markedly with loose and bloody stools at 5 d post-TNBS ($P < 0.01$). Moreover, the body weight in high-concentration TNBS (50 mg/mL)-treated rats was significantly lowered when compared to that treated with low-concentration TNBS (25 mg/mL) ($P < 0.05$), and 3 rats in the high-concentration TNBS (50 mg/mL)-treated group were found dead. At 4 wk after TNBS administration, the average body weight of high-concentration TNBS-treated rats was still significantly decreased compared to that of rats treated with low-concentration TNBS and saline ($P < 0.05$); no significant difference was found in the body weight between low-concentration TNBS-treated rats and the saline-treated ones.

Effect of trinitrobenzene sulfonic acid dosage on the severity of acute inflammation and inflammation resolution

Five days after TNBS administration, the results from MPO activity assay and histopathological evaluation showed that, TNBS, when administered at the doses of

Table 3 Criteria of histological scoring in colon tissue

Variables	Severity and scoring			
	0	1	2	3
Ulceration	No ulcer	Ulcerations not exceeding lamina muscularis mucosae	Ulcerations not exceeding submucosa	Ulcerations exceeding submucosa
Edema	Normal thickness	Submucosal expansion < 30%	Submucosal expansion 30%-100%	Submucosal expansion > 100%
Inflammatory cells	No infiltration	Few scattered cells	Distributed but not dense	Dense
Fibroplasia	Normal collagen	Increase < 30%	Increase 30%-50%	Increase > 50%

Table 4 Effects of trinitrobenzene sulfonic acid concentration on body weight and mortality rate

Treatment	TNBS concentration	Body weight (g)		Mortality rate (%)
		5 d	4 wk	
Saline	-	249.6 ± 26.7	343.0 ± 28.9	0
TNBS 20 mg/0.8 mL per rat	25 mg/mL	214.5 ± 16.8[b]	324.5 ± 24.8	0
TNBS 20 mg/0.4 mL per rat	50 mg/mL	179.2 ± 21.4[d]	291.8 ± 42.6[a]	33

[a]$P < 0.05$, [b]$P < 0.01$ *vs* saline-treated group; [d]$P < 0.01$ *vs* TNBS (20 mg/0.8 mL)-treated group. TNBS: Trinitrobenzene sulfonic acid.

20, 10, and 5 mg/0.8 mL per rat in 50% ethanol, dose-dependently induced acute inflammation and damage in the colon tissue of rats (Figure 1). The results indicate that the higher the dose TNBS used, the more severe the inflammation and damage induced. As shown in macroscopic appearance and H and E sections (Figure 1A and B), high dose TNBS (20 mg/rat) induced multiple larger and deeper ulcerations, and dense inflammatory cell infiltration when compared to that treated with low dose TNBS.

As shown in Figure 2, four weeks after TNBS (5-20 mg/rat) administration, there was no significant difference in MPO activity between TNBS-treated rats and the control groups, suggesting no acute inflammation was left at this period. However, results from pathological evaluation showed that the histological scores in high dose (20 mg/rat) and median dose (10 mg/rat) TNBS-treated groups were still significantly higher compared to that of the control ($P < 0.05$), but no significant difference was found between low dose TNBS-treated rats and the control, suggesting low dose TNBS-treated rats had completely recovered from the initial inflammation at 4 wk post TNBS. As shown in Figure 2A and B, the colons from high and medium dose TNBS treated rats lost their normal appearance, and more collagen was found in the submucosa and smooth muscle in Masson staining sections, suggesting the occurrence of fibroplasia.

Effect of trinitrobenzene sulfonic acid administration position, and ethanol percentage on the severity of acute inflammation and inflammation resolution

Knowing that low dose TNBS (5 mg/0.8 mL per rat in 50% ethanol)-treated rats can completely recover from initial inflammation at 4 wk after TNBS administration, further studies were based on this dosage selection. As shown in Figure 3, compared to the control, MPO activity assay and histopathological evaluation all demonstrated that low dose TNBS (5 mg/0.8 mL per rat, in 50% ethanol), when given to rats at 8 cm or 4 cm from anus, induced marked acute inflammation and ulcers at 5 d post TNBS administration ($P < 0.01$), no difference was found between these two groups. However, the same dose TNBS (5 mg/0.8 mL per rat, 8 cm depth) in 25% ethanol induced significant mild non-ulcer inflammation compared to that treated with TNBS in 50% ethanol ($P < 0.01$). Four weeks after TNBS administration, there were no significant differences in MPO activity and histopathological score among all TNBS-treated groups and the control, suggesting that the rats treated with low dose TNBS (5 mg/0.8 mL per rat) had all completely recovered at 4 wk after TNBS administration.

Effect of trinitrobenzene sulfonic acid administration position, and ethanol percentage on the visceral hyperalgesia

To investigate whether the low dose TNBS (5 mg/0.8 mL per rat)-treated rats acquired long-lasting visceral hyperalgesia after inflammation resolution, AWR test was applied to all these TNBS-treated rats at 4 and 8 wk after TNBS administration. As shown in Figure 4, at 4 wk after TNBS administration, the pain threshold pressure in all TNBS-treated rats was decreased significantly compared to the control ($P < 0.05$), but no significant difference was found among these TNBS-treated rats. At 8 wk after TNBS administration, the pain threshold pressure in rats treated with TNBS at the depth of 8 cm was still significantly decreased compared to that of the controls ($P < 0.05$), but no significant difference was found in rats treated with TNBS at 4 cm from anus or in 25% ethanol.

Enterochromaffin cell hyperplasia in trinitrobenzene sulfonic acid-induced post-infectious irritable bowel syndrome rat model

To investigate whether the rats treated with low dose TNBS (5 mg/0.8 mL per rat, in 50% ethanol) at 8 cm from anus also acquired other features of PI-IBS, the EC cell number and 5-HT content in the proximal and distal colon tissues were further tested at 4 and 8 wk after TNBS administration. As shown in Figure 5, compared to the control, 5-HT content in the proximal colon, but not the distal colon, were significantly increased, with 32% ($P < 0.01$) and 23% ($P < 0.05$) increased at 4 and 8 wk after TNBS administration, respectively. A

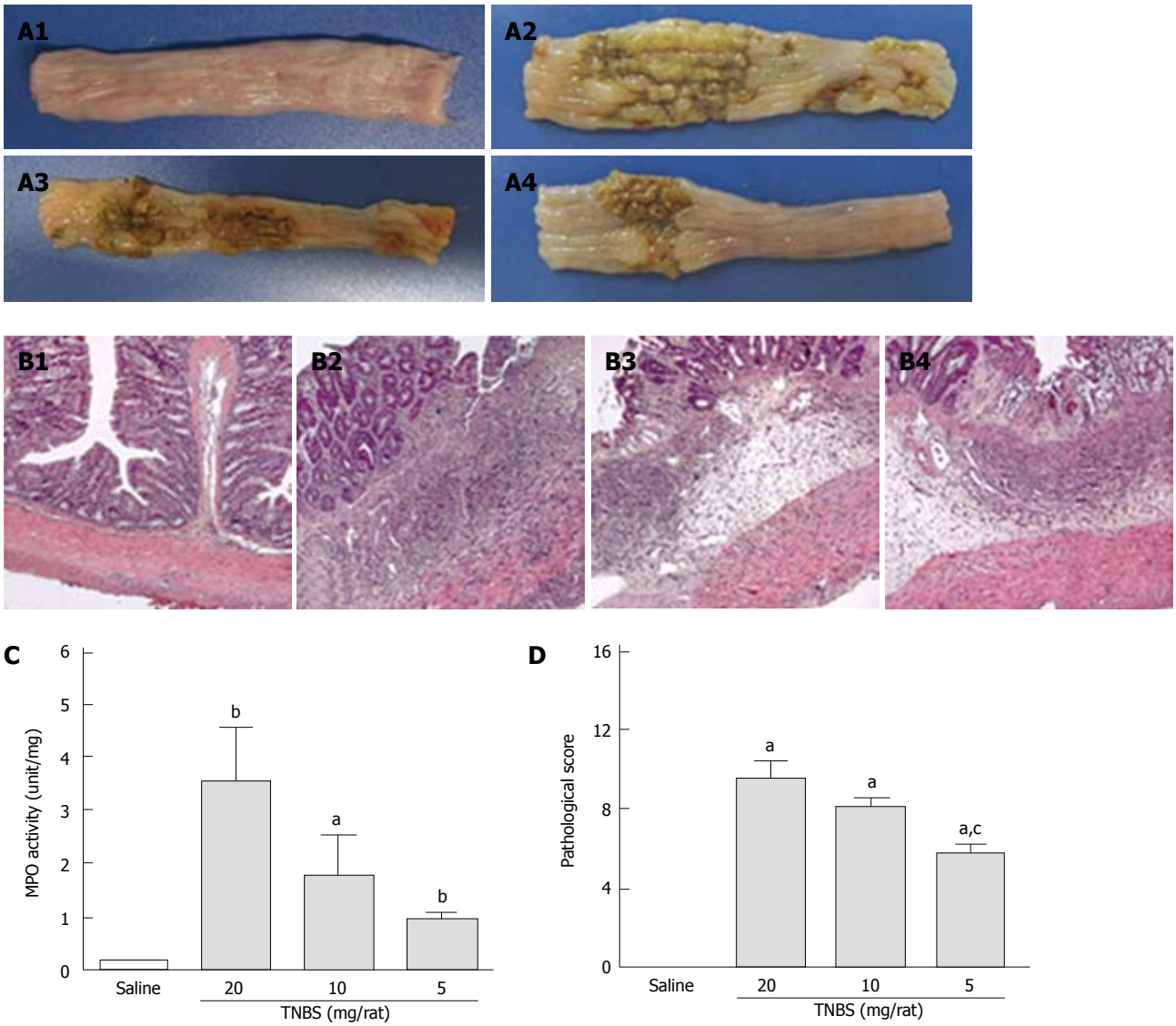


Figure 1 Acute colonic inflammation and damage induced by different doses of trinitrobenzene sulfonic acid at 5 d after trinitrobenzene sulfonic acid administration. Panel A depicts the appearance of colon tissue in saline (A1), high dose trinitrobenzene sulfonic acid (TNBS) (20 mg/rat, A2), medium dose TNBS (10 mg/rat, A3), and low dose TNBS (5 mg/rat, A4)-treated groups; Panel B depicts the representative histological changes of colon tissue in saline (B1), high dose TNBS (B2), median dose TNBS (B3), and low dose TNBS (B4)-treated groups (hematoxylin and eosin staining, 100×); Statistical analysis of myeloperoxidase (MPO) activity is shown in panel (C), and pathological score in panel (D). Data are shown as mean ± SE, $n = 4$ per group. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* saline-treated group; $^{c}P < 0.05$ *vs* high dose TNBS-treated group (one-way analysis of variance, Student-Newman-Keuls).

similar trend can also be found in the EC cell number; i.e., there were 68% and 60% increases at 4 and 8 wk after TNBS administration, respectively ($P < 0.01$), suggesting this TNBS-induced PI-IBS model also presents EC cell hyperplasia in the colon tissue.

DISCUSSION

TNBS is an agent commonly used in inducing post-inflammatory IBS model currently. Concerning the variations in developing a protocol of the TNBS-induced PI-IBS rat model and the three necessary features of PI-IBS animal model (i.e., initial inflammation/infection, inflammation/infection resolution, and acquired symptoms of IBS), the present study investigated the effects of TNBS dosage and concentration, intracolonic TNBS administration position, and ethanol percentage on the development of the TNBS-induced PI-IBS rat model. Our results showed that: (1) TNBS induced dose- and concentration-dependent acute inflammation and inflammation resolution at the dose range of 5-20 mg/rat; (2) Intracolonic TNBS administration position affected the persistence of later acquired visceral hyperalgesia, but not the initial inflammation severity; (3) Ethanol percentage affected the TNBS-induced inflammation severity and later acquired visceral hyperalgesia, and low ethanol percentage reduced the degree of TNBS-induced acute inflammation and shortened the persistence of acquired visceral hyperalgesia; and (4) The protocol with TNBS at 5 mg/0.8 mL per rat, dissolved in 50% ethanol, intracolonically administered 8 cm from anus may be a proper protocol in which rats can recover completely from the

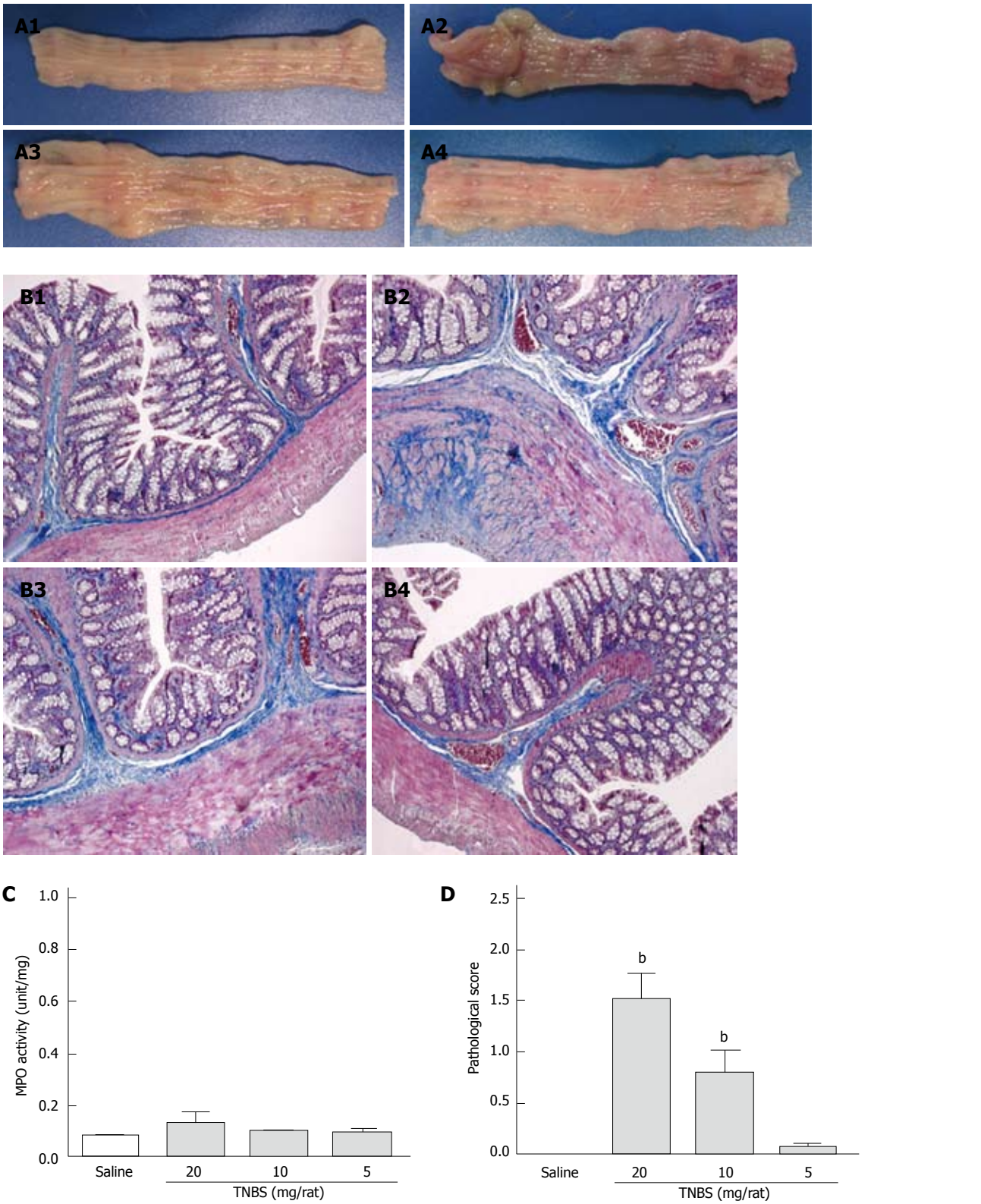


Figure 2 Effect of trinitrobenzene sulfonic acid dosage on severity of acute inflammation and inflammation resolution. The colon tissue was collected at 4 wk after trinitrobenzene sulfonic acid (TNBS) administration. Panel A depicts the appearance of colon tissue in saline (A1), high dose TNBS (20 mg/rat, A2), medium dose TNBS (10 mg/rat, A3), and low dose TNBS (5 mg/rat, A4)-treated rats; Panel B depicts the representative histological changes of colon tissue in saline (B1), high dose TNBS (B2), medium dose TNBS (B3), and low dose TNBS (B4)-treated rats (Masson trichrome staining, 100×); Statistical analysis of myeloperoxidase (MPO) activity is shown in panel (C), and pathological score in panel (D). Data are shown as mean ± SE, $n = 4$ per group. $^{b}P < 0.01$ *vs* saline-treated group (one-way analysis of variance, Student-Newman-Keuls).

initial inflammation but acquire persistent visceral hyperalgesia and EC cell hyperplasia at 4 wk after TNBS administration.

As shown in our results, in the dose range of 5-20

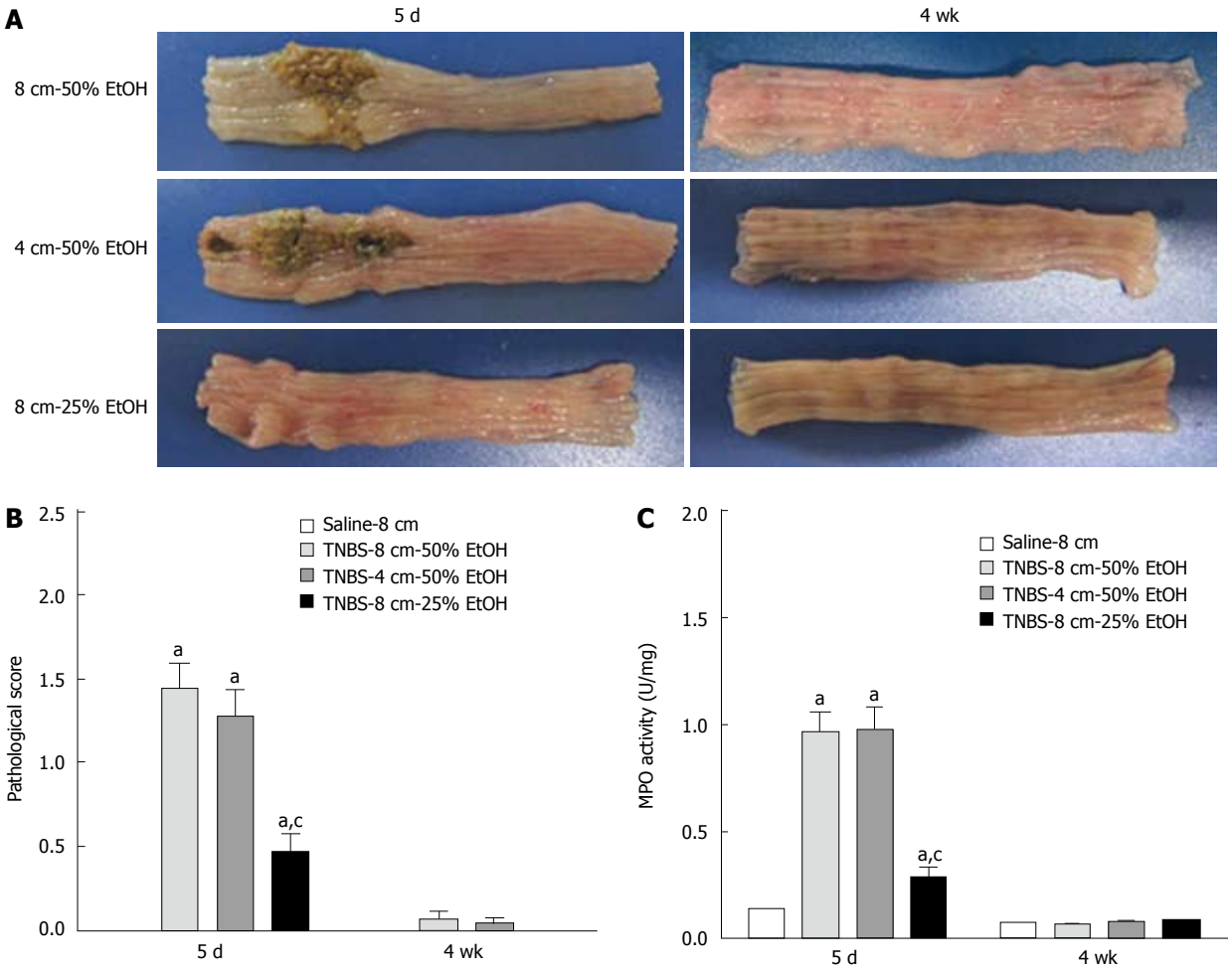


Figure 3 Effects of trinitrobenzene sulfonic acid administration position and ethanol percentage on severity of acute inflammation and inflammation resolution. Panel A depicts the appearance of colon tissue in rats treated with trinitrobenzene sulfonic acid (TNBS) at 4 cm or 8 cm from anus or in 25% ethanol. The colon tissues were collected at 5 d or 4 wk after TNBS administration, respectively; Statistical analysis of pathological score is shown in panel (B), and myeloperoxidase (MPO) activity in panel (C). Data are shown as mean ± SE, n = 4-5 per group. [a]$P < 0.05$ *vs* saline-treated rats, [c]$P < 0.05$ *vs* rats treated with TNBS at 8 cm from the anus in 50% ethanol (one-way analysis of variance, Student-Newman-Keuls).

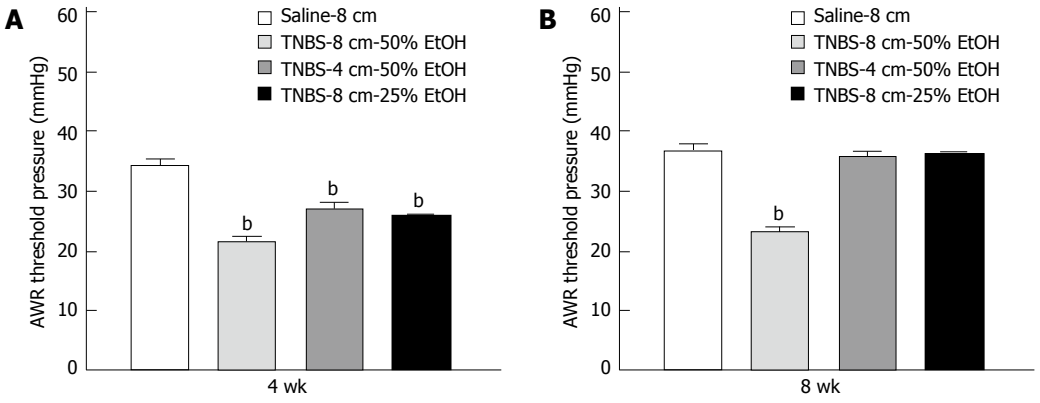


Figure 4 Effects of low dose trinitrobenzene sulfonic acid administration position and ethanol percentage on later acquired visceral hyperalgesia. Statistical analysis of pain threshold pressure at 4 wk and 8 wk after trinitrobenzene sulfonic acid (TNBS) administration are shown in panel (A) and (B), respectively. Data are shown as mean ± SE, n = 5 per group. [b]$P < 0.01$ *vs* saline-treated rats (one-way analysis of variance, Student-Newman-Keuls). AWR: Abdominal withdrawal reflex.

mg per rat, the higher the dose of TNBS, the more severe the acute inflammation and mucosa damage. This finding is consistent with previous reports that the severity and duration in TNBS-induced inflammation is dose-dependent[13,14,17]. It is notable that the same dose of TNBS (20 mg/rat) when given to rats in different vehicle volumes induced different degrees of damage; a higher TNBS concentration (50 mg/mL) induced

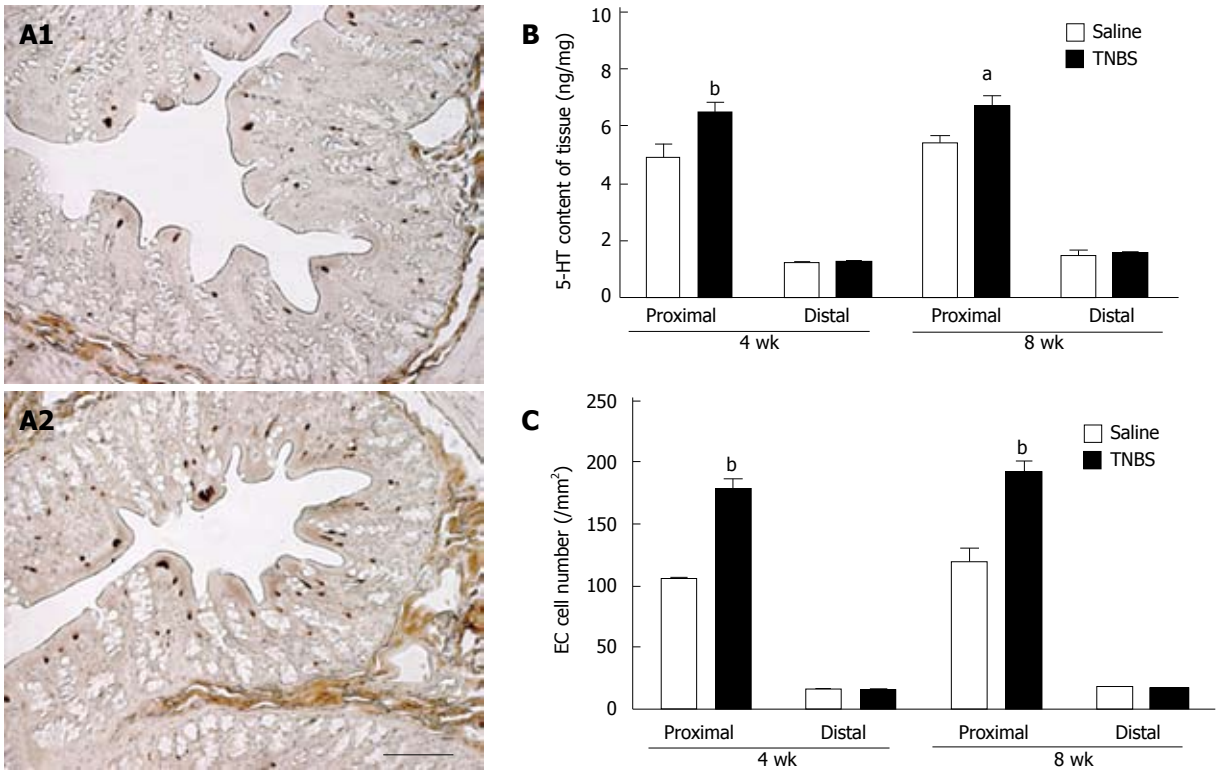


Figure 5 Persistent increases of enterochromaffin cell number and serotonin content in the colon tissue of trinitrobenzene sulfonic acid-induced post-infectious irritable bowel syndrome rat model. The colon tissue was collected at 4 wk and 8 wk after trinitrobenzene sulfonic acid (TNBS) administration. Panel A depicts the representative enterochromaffin (EC) cell staining in colon mucosa, the samples were from rats treated with saline (A1), or TNBS (A2) at 4 wk post TNBS (Scale bar, 100 μm). Statistical analysis of serotonin content is shown in panel (B), and EC cell number in panel (C). Data are shown as mean ± SE, n = 5 per group. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* saline-treated rats. 5-HT: 5-hydroxytryptamine.

more severe damage, even causing animal death in the acute phase. Our pathological results also showed that high-concentration TNBS-treated rats presented localized more severe inflammation and deeper ulcerations around the position of TNBS administration, which may be responsible for the occurrence of colitis complications, i.e., intestinal obstruction, intra-abdominal infection, and animal death. Concerning the fact that inflammation severity and duration have close correlation with TNBS dosage, the effects of TNBS dosage on inflammation resolution was assessed at 4 wk after TNBS administration. According to the pathologist's suggestion, fibroplasia evaluation index was added into the pathological evaluation criteria for the first time, so as to exactly evaluate inflammation resolution. Our results showed that the animals receiving low dose TNBS (5 mg/rat) recovered completely at 4 wk after TNBS administration, but those receiving high (20 mg/rat) and medium (10 mg/rat) dose were not recovered. This result seems to somewhat conflict with the finding from a previous study in which the rats receiving TNBS (5-20 mg/rat, in 25% ethanol) all recovered after 4 wk[14]. We speculate that this discrepancy may come from the protocol differences between these two studies, such as ethanol percentage, animal strain, or evaluation criteria. Consistent with our findings, the previous study showed that colonic inflammation induced by TNBS (30 mg/rat, in 50% ethanol) lasted for at least 8 wk[17], suggesting the inflammation resolution is also dose-dependent. Based on previous findings and our results, TNBS dosage plays a critical role in TNBS-induced inflammation severity and resolution, and low dose TNBS (5 mg/rat)-induced inflammation can recover completely at 4 wk after TNBS administration.

Currently, 25% and 50% ethanol are both used as the carrier of TNBS in establishing TNBS-induced PI-IBS model and TNBS is commonly given to rats at the depth of either 4 cm or 8 cm from the anus. Based on low dose TNBS (5 mg/0.8 mL per rat) selection, we further investigated the effects of ethanol percentage and intracolonic administration position on the severity and resolution of TNBS-induced inflammation. Our results showed that rats treated with low dose TNBS at a depth of 4 cm or 8 cm presented similar inflammation severity and resolution, while the rats treated with TNBS in 25% ethanol developed milder non-ulcer inflammation compared to those treated with TNBS in 50% ethanol. As the carrier of TNBS, ethanol is known as a breaker to the mucosal barrier, and 30%-50% ethanol alone has been proved to induce acute inflammation and hyperemia[18,28]. Moreover, previous study have shown that 30% ethanol alone induced small areas of hyperemia, while TNBS combined with 30% ethanol induced larger area of necrosis and hyperemia in the colon[18]. These findings indicate that ethanol alone can induce concentration-dependent inflammation, and can be considered

Table 5 Detailed protocols in developing trinitrobenzene sulfonic acid induced post-inflammatory irritable bowel syndrome model in Sprague-Dawley rats

Procedures	Cautions
1. 7-wk-old male Sprague-Dawley rats, fast 24 h before TNBS administration	
2. Mix 1 volume of 12.5 mg/mL TNBS-saline solution with 1 volume of absolute ethanol	Fresh preparation
3. Weight and anesthetized rats with chloral hydrate (350 mg/kg, i.p.)	Anesthetization may last for 3-4 h
4. Insert a catheter into the colon at 8 cm from anus	Proceed carefully to avoid damage the colon wall
5. Keep the rat with head-down vertical position, instill 0.8 mL TNBS solution slowly into the colon lumen within 1 min	Handle slowly to avoid TNBS leakage
6. Keep the rat in head-down vertical position for 1 min before gently removing the catheter	
7. Put the rats in a mound of bedding in head-down position until consciousness recovery	Ensure TNBS solution remains completely in the colon; Keep the rats warm
8. On day 3, 7, 14, 21 and 28, observe and weight the rats	On day 3, body weight decreased by 10%-20% with unformed bloody stool; On day 7, body weight regained and reached the original level, the stool become formed but still soft; From day 7 to day 28, body weight increased smoothly, and finally reached the control level

TNBS: Trinitrobenzene sulfonic acid.

as an invasive helper in enhancing the effects of TNBS. Therefore, it seems reasonable that milder inflammation was induced in rats treated with equal dose of TNBS (5 mg/rat) but in lower ethanol percentage (25%). These results can also explain why high dose or high concentration TNBS have also been used by some researchers in establishing a PI-IBS model: low concentration ethanol may ameliorate the effects of TNBS[13,14].

Visceral hyperalgesia, the major feature of IBS, was further tested in low dose TNBS (5 mg/0.8 mL per rat) treated rats. From 4 wk to 8 wk post TNBS intracolonic administration at a depth of 8 cm, significant and persistent decrease in visceral pain threshold pressure was found, while the intracolonic TNBS administration at a depth of 4 cm or in 25% ethanol presented short-term acquired visceral hyperalgesia. Nowadays, the duration and severity of inflammation are considered as important risk factors in the development of PI-IBS[29], and severe inflammation which results in deep impairment of the underlining nerve fibers has been proposed to play an important role in the pathogenesis of IBS[30]. Based on the above evidence, we believe the unstable visceral hyperalgesia (i.e., lasting for less than 4 wk) in rats treated with TNBS in 25% ethanol may have close correlation with the mild non-ulcer inflammation observed at the acute phase of inflammation. These results also provide us with the information that mild inflammation, even without ulceration, can induce later short-term visceral hyperalgesia. However, it seems interesting that the same dose of TNBS when given at the different positions of colon (transverse *vs* descending) induced different features of acquired visceral hyperalgesia (persistent *vs* short-term), even though the TNBS-induced acute inflammation and damage was similar at first. Considering the unclear and complex pathogenesis of visceral hypersensitivity in PI-IBS, it is not easy to provide a clear explanation for the current findings. However, during the acute phase of TNBS-induced inflammation, obvious colon dilation and a large amount of retained feces were found in the inflamed transverse colon, but not in the inflamed distal colon. We speculate that the persistent colonic inflammation and dilation may play important roles in enteric nerve system plasticity, and thus influence the feature of acquired visceral hypersensitivity. Currently, peripheral and central neuroplastic changes are considered to be associated with post-inflammatory persistent visceral hypersensitivity[1,31]. It is also well known that transient colorectal distension in the neonatal period can result in chronic visceral hypersensitivity and motility dysfunction in adulthood[25,32], and the underlying mechanism is thought to be associated with alterations in peripheral and central nerve systems[33]. Moreover, considering the different structure and functions of the transverse and descending colons, the regional differences in gut flora[34], mast cells[35] and EC cells[36] may also contribute to the different feature of acquired visceral hypersensitivity observed in our study. Further studies are needed to clarify these issues.

EC cell hyperplasia has been found in the colonic specimens of IBS and PI-IBS patients[37-39], and EC cell hyperplasia has been reported to play important role in the development of visceral hypersensitivity in IBS patients[40,41]. To identify whether visceral hyperalgesia is also accompanied with EC cell hyperplasia in the TNBS-induced PI-IBS rat model, EC cell number and 5-HT content were further investigated in the proximal and distal colon of PI-IBS rats. As shown in our study, the EC cell number and 5-HT content in the proximal colon, but not the distal colon, were significantly and persistently increased in this TNBS model, suggesting the occurrence of EC cell hyperplasia. However, this result differed from the finding from IBS patients, as EC cell hyperplasia in rectal mucosa was commonly reported[3]. This may be explained by the different distribution of

EC cells in the gut mucosa between human beings and rats: the vast majority of EC cells in humans mainly resides in the small intestine and rectum[42] while the major source of EC cells in rats largely locates in the cecum with a decline trend from the proximal to the distal colon[36]. Consistent with our findings, previous studies also showed that 5-HT content in the rat proximal colon tissue was significantly higher (about 5 fold) than that in the distal colon[43,44]. Therefore, it seems that EC cell hyperplasia in the proximal colon can be regarded as one of the features of the TNBS-induced PI-IBS rat model.

This present study, for the first time, showed that TNBS dosage and concentration, position of intracolonic TNBS administration, and ethanol percentage play important roles in developing the TNBS-induced PI-IBS model in Sprague-Dawley rats, suggesting more attention should be paid to these factors when developing a PI-IBS model with TNBS. Concerning the gene differences among animal strains, the protocol set up here may need some modulation when other rat strains except Sprague-Dawley rats, are used. Though this TNBS model presented persistent visceral hyperalgesia and EC cell hyperplasia for as long as 4 wk, more studies are still needed to observe the long-lasting visceral hyperalgesia and identify other features of PI-IBS in this model.

In the present study, we found out that the protocol with TNBS at 5 mg/0.8 mL per rat, dissolved in 50% ethanol, intracolonically administered at 8 cm from the anus resulted in persistent visceral hyperalgesia and colonic EC cell hyperplasia after complete recovery from the initial inflammation in rats. With this protocol, low-dose TNBS-induced mild mucosa damage and colonic inflammation is reproducible without any animal loss; visceral hyperalgesia and EC cell hyperplasia which last for at least 4 wk occur as early as 4 wk after TNBS administration. The details of this protocol are presented in Table 5. Ensuring the results from different laboratories would be comparable, we believe that widespread adoption of a recommended protocol will save a great quantity of time and resources.

ACKNOWLEDGMENTS

We are very grateful to Dr. Zhi Li for his kind suggestion in setting up pathological criteria.

COMMENTS

Background

Post-infectious irritable bowel syndrome (PI-IBS) is a subgroup of IBS in which the patients developed their symptoms after recovery from an acute gastrointestinal infection. Previous systematic review showed that the trinitrobenzene sulfonic acid (TNBS)-induced model is the most commonly used PI-IBS model, but there are large variations in the protocol of model development. These variations make comparison between studies to be very difficult, if not impossible.

Research frontiers

This study aimed to investigate the effects of TNBS dosage, concentration, intracolonic administration position, and ethanol percentage on TNBS-induced acute inflammation, inflammation resolution, and later acquired features of IBS (i.e., visceral hyperalgesia and enterochromaffin cell hyperplasia).

Innovations and breakthroughs

Recent studies have highlighted the important status of PI-IBS in functional diseases, as the clear onset and well defined pathophysiological changes of PI-IBS will help people understand not only the PI-IBS but also other subtypes of IBS. Moreover, the validated animal models, which aim to mimic one or more features of human disease, play important roles in the studies of mechanisms and potential therapeutic agents for diseases. This is the first study aimed to investigate the effects of key factors on developing TNBS-induced PI-IBS model in rats. The results and recommended protocol presented in this study will save a great deal of time and resources and ensure the results from different laboratories be comparable.

Applications

This study provides direct information about the effects of key factors on TNBS-induced acute inflammation, inflammation resolution, and later acquired features of IBS. More attention should be paid to these key factors when developing PI-IBS rat model with TNBS.

Peer review

This is a good descriptive study in which authors investigate the effects of TNBS dosage and concentration, intracolonic administration position, and ethanol percentage on the developing TNBS-induced PI-IBS rat model. The topic is interesting and useful for the study of pathophysiology of PI-IBS and the development of therapeutic agents.

REFERENCES

1 **Barbara G**, Cremon C, Pallotti F, De Giorgio R, Stanghellini V, Corinaldesi R. Postinfectious irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* 2009; **48** Suppl 2: S95-S97
2 **Neal KR**, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: a six year follow up study. *Gut* 2002; **51**: 410-413
3 **Dunlop SP**, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; **125**: 1651-1659
4 **Gwee KA**, Leong YL, Graham C, McKendrick MW, Collins SM, Walters SJ, Underwood JE, Read NW. The role of psychological and biological factors in postinfective gut dysfunction. *Gut* 1999; **44**: 400-406
5 **ChaudharyY NA**, Truelove SC. The irritable colon syndrome. A study of the clinical features, predisposing causes, and prognosis in 130 cases. *Q J Med* 1962; **31**: 307-322
6 **Thabane M**, Marshall JK. Post-infectious irritable bowel syndrome. *World J Gastroenterol* 2009; **15**: 3591-3596
7 **Qin HY**, Wu JC, Tong XD, Sung JJ, Xu HX, Bian ZX. Systematic review of animal models of post-infectious/post-inflammatory irritable bowel syndrome. *J Gastroenterol* 2011; **46**: 164-174
8 **Collins SM**, Piche T, Rampal P. The putative role of inflammation in the irritable bowel syndrome. *Gut* 2001; **49**: 743-745
9 **Lamb K**, Zhong F, Gebhart GF, Bielefeldt K. Experimental colitis in mice and sensitization of converging visceral and somatic afferent pathways. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G451-G457
10 **Bergin AJ**, Donnelly TC, McKendrick MW, Read NW. Changes in anorectal function in persistent bowel disturbance following salmonella gastroenteritis. *Eur J Gastroenterol Hepatol* 1993; **5**: 617-620
11 **Barbara G**, Cremon C, Gargano L, Giorgio RD, Cogliandro R, Stanghellini V, Corinaldesi R. T1394 Mesalazine Treatment for Intestinal Immune Activation in Patient with Irritable Bowel Syndrome: A Randomized Controlled Pilot Trial. *Gastroenterology* 2008; **134**: A-546
12 **Kim HS**, Berstad A. Experimental colitis in animal models. *Scand J Gastroenterol* 1992; **27**: 529-537
13 **Menozzi A**, Pozzoli C, Poli E, Lazzaretti M, Grandi D,

Coruzzi G. Long-term study of TNBS-induced colitis in rats: focus on mast cells. *Inflamm Res* 2006; **55**: 416-422
14 **Adam B**, Liebregts T, Gschossmann JM, Krippner C, Scholl F, Ruwe M, Holtmann G. Severity of mucosal inflammation as a predictor for alterations of visceral sensory function in a rat model. *Pain* 2006; **123**: 179-186
15 **Winchester WJ**, Johnson A, Hicks GA, Gebhart GF, Greenwood-van Meerveld B, McLean PG. Inhibition of endothelial cell adhesion molecule expression improves colonic hyperalgaesia. *Neurogastroenterol Motil* 2009; **21**: 189-196
16 **Collins SM**, McHugh K, Jacobson K, Khan I, Riddell R, Murase K, Weingarten HP. Previous inflammation alters the response of the rat colon to stress. *Gastroenterology* 1996; **111**: 1509-1515
17 **Morris GP**, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989; **96**: 795-803
18 **Diop L**, Raymond F, Fargeau H, Petoux F, Chovet M, Doherty AM. Pregabalin (CI-1008) inhibits the trinitrobenzene sulfonic acid-induced chronic colonic allodynia in the rat. *J Pharmacol Exp Ther* 2002; **302**: 1013-1022
19 **Asfaha S**, MacNaughton WK, Appleyard CB, Chadee K, Wallace JL. Persistent epithelial dysfunction and bacterial translocation after resolution of intestinal inflammation. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G635-G644
20 **Greenwood-Van Meerveld B**, Johnson AC, Foreman RD, Linderoth B. Spinal cord stimulation attenuates visceromotor reflexes in a rat model of post-inflammatory colonic hypersensitivity. *Auton Neurosci* 2005; **122**: 69-76
21 **Ferrini MG**, Kovanecz I, Sanchez S, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Med* 2009; **6**: 415-428
22 **Gué M**, Bonbonne C, Fioramonti J, Moré J, Del Rio-Lachèze C, Coméra C, Buéno L. Stress-induced enhancement of colitis in rats: CRF and arginine vasopressin are not involved. *Am J Physiol* 1997; **272**: G84-G91
23 **Fabia R**, Ar'Rajab A, Johansson ML, Willén R, Andersson R, Molin G, Bengmark S. The effect of exogenous administration of Lactobacillus reuteri R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993; **28**: 155-162
24 **Krawisz JE**, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984; **87**: 1344-1350
25 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285
26 **Grimelius L**. Silver stains demonstrating neuroendocrine cells. *Biotech Histochem* 2004; **79**: 37-44
27 **Qi SD**, Tian SL, Xu HX, Sung JJ, Bian ZX. Quantification of luminally released serotonin in rat proximal colon by capillary electrophoresis with laser-induced fluorescence detection. *Anal Bioanal Chem* 2009; **393**: 2059-2066
28 **Asfaha S**, Bell CJ, Wallace JL, MacNaughton WK. Prolonged colonic epithelial hyporesponsiveness after colitis: role of inducible nitric oxide synthase. *Am J Physiol* 1999; **276**: G703-G710
29 **Neal KR**, Hebden J, Spiller R. Prevalence of gastrointestinal symptoms six months after bacterial gastroenteritis and risk factors for development of the irritable bowel syndrome: postal survey of patients. *BMJ* 1997; **314**: 779-782
30 **Wang LH**, Fang XC, Pan GZ. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. *Gut* 2004; **53**: 1096-1101
31 **Hughes PA**, Brierley SM, Blackshaw LA. Post-inflammatory modification of colonic afferent mechanosensitivity. *Clin Exp Pharmacol Physiol* 2009; **36**: 1034-1040
32 **Chaloner A**, Rao A, Al-Chaer ED, Greenwood-Van Meerveld B. Importance of neural mechanisms in colonic mucosal and muscular dysfunction in adult rats following neonatal colonic irritation. *Int J Dev Neurosci* 2010; **28**: 99-103
33 **Lin C**, Al-Chaer ED. Long-term sensitization of primary afferents in adult rats exposed to neonatal colon pain. *Brain Res* 2003; **971**: 73-82
34 **Meslin JC**, Fontaine N, Andrieux C. Variation of mucin distribution in the rat intestine, caecum and colon: effect of the bacterial flora. *Comp Biochem Physiol A Mol Integr Physiol* 1999; **123**: 235-239
35 **Furgał A**, Litwin JA. Distribution of mast cells along and across successive segments of the rat digestive tract: a quantitative study. *Folia Histochem Cytobiol* 1998; **36**: 19-27
36 **Portela-Gomes GM**, Grimelius L, Petersson R, Bergström R. Enterochromaffin cells in the rat gastrointestinal tract. Aspects of factors influencing quantification. *Ups J Med Sci* 1984; **89**: 189-203
37 **Lee KJ**, Kim YB, Kim JH, Kwon HC, Kim DK, Cho SW. The alteration of enterochromaffin cell, mast cell, and lamina propria T lymphocyte numbers in irritable bowel syndrome and its relationship with psychological factors. *J Gastroenterol Hepatol* 2008; **23**: 1689-1694
38 **Dunlop SP**, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; **125**: 1651-1659
39 **Spiller RC**, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. *Gut* 2000; **47**: 804-811
40 **Mawe GM**, Coates MD, Moses PL. Review article: intestinal serotonin signalling in irritable bowel syndrome. *Aliment Pharmacol Ther* 2006; **23**: 1067-1076
41 **Park JH**, Rhee PL, Kim G, Lee JH, Kim YH, Kim JJ, Rhee JC, Song SY. Enteroendocrine cell counts correlate with visceral hypersensitivity in patients with diarrhoea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2006; **18**: 539-546
42 **Sjölund K**, Sandén G, Håkanson R, Sundler F. Endocrine cells in human intestine: an immunocytochemical study. *Gastroenterology* 1983; **85**: 1120-1130
43 **Tsukamoto K**, Ariga H, Mantyh C, Pappas TN, Yanagi H, Yamamura T, Takahashi T. Luminally released serotonin stimulates colonic motility and accelerates colonic transit in rats. *Am J Physiol Regul Integr Comp Physiol* 2007; **293**: R64-R69
44 **Bian ZX**, Qin HY, Tian SL, Qi SD. Combined effect of early life stress and acute stress on colonic sensory and motor responses through serotonin pathways: differences between proximal and distal colon in rats. *Stress* 2011; **14**: 448-458

S- Editor Gou SX **L- Editor** O'Neill M **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2493

World J Gastroenterol 2012 May 28; 18(20): 2493-2501
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Cost-benefit analysis of esophageal cancer endoscopic screening in high-risk areas of China

Juan Yang, Wen-Qiang Wei, Jin Niu, Zhi-Cai Liu, Chun-Xia Yang, You-Lin Qiao

Juan Yang, Jin Niu, Chun-Xia Yang, Department of Epidemiology, West China School of Public Health, Sichuan University, Chengdu 610041, Sichuan Province, China
Wen-Qiang Wei, You-Lin Qiao, Department of Epidemiology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China
Zhi-Cai Liu, Department of Thoracic Surgery, Linzhou Cancer Institute, Linzhou 456550, Henan Province, China
Author contributions: Wei WQ, Yang CX and Qiao YL designed the research and revised the manuscript; Yang J, Wei WQ, Niu J and Liu ZC performed the research; Yang J reviewed the literature, analyzed the data and prepared the paper.
Supported by The National Science and Technology Pillar Program of the 11th National Five-Year Plan of China, No. 2006BAI02A15
Correspondence to: Dr. Chun-Xia Yang, MD, PhD, Department of Epidemiology, West China School of Public Health, Sichuan University, Renming Nanlu 3-17, Chengdu 610041, Sichuan Province, China. chunxia815@yahoo.com.cn
Telephone: +86-28-85501604 Fax: +86-28-85501295
Received: June 21, 2011 Revised: December 2, 2011
Accepted: April 28, 2012
Published online: May 28, 2012

Abstract

AIM: To estimate the cost-benefit of endoscopic screening strategies of esophageal cancer (EC) in high-risk areas of China.

METHODS: Markov model-based analyses were conducted to compare the net present values (NPVs) and the benefit-cost ratios (BCRs) of 12 EC endoscopic screening strategies. Strategies varied according to the targeted screening age, screening frequencies, and follow-up intervals. Model parameters were collected from population-based studies in China, published literatures, and surveillance data.

RESULTS: Compared with non-screening outcomes, all strategies with hypothetical 100 000 subjects saved life years. Among five dominant strategies determined by the incremental cost-effectiveness analysis, screening once at age 50 years incurred the lowest NPV (international dollar-I$55 million) and BCR (2.52). Screening six times between 40-70 years at a 5-year interval [i.e., six times(40)f-strategy] yielded the highest NPV (I$99 million) and BCR (3.06). Compared with six times(40)f-strategy, screening thrice between 40-70 years at a 10-year interval resulted in relatively lower NPV, but the same BCR.

CONCLUSION: EC endoscopic screening is cost-beneficial in high-risk areas of China. Policy-makers should consider the cost-benefit, population acceptance, and local economic status when choosing suitable screening strategies.

Key words: Cost-benefit analysis; Esophageal cancer; Endoscopy; Screening; High-risk area

Peer reviewer: Julian Abrams, MD, MS, Assistant Professor of Clinical Medicine, Division of Digestive and Liver Diseases, Columbia University Medical Center, 622 W 168th Street, PH 20-303, New York, NY 10032, United States

Yang J, Wei WQ, Niu J, Liu ZC, Yang CX, Qiao YL. Cost-benefit analysis of esophageal cancer endoscopic screening in high-risk areas of China. *World J Gastroenterol* 2012; 18(20): 2493-2501 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2493.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2493

INTRODUCTION

Esophageal cancer (EC) is the eighth most common cancer and the sixth most common cause of cancer death worldwide[1]. Although the mortality of EC has

sharply reduced over the last three decades, EC remains the fourth leading cause of cancer death in China with a mortality of 15.21/100 000[2]. According to the "Third National Retrospective Sampling Survey of Death Causes Report in 2004-2005 of China", EC continues to be the major public health burden in some high-risk areas, where the mortality of EC was three times higher than the average of the country. EC is a fatal disease, with a 5-year survival rate of less than 20% even in developed countries[3,4].

To explore suitable control measures in high-risk areas of China, a great number of EC screening studies using endoscopic examinations (i.e., endoscopy with mucosal iodine staining and index biopsy as a screening technology, combined with pathological examination for confirming and staging the disease) have been conducted for several decades[5-10]. Through early detection and subsequent treatment, the 5-year survival rate of EC increased to 86%[10]. Furthermore, obvious reductions in incidence and mortality rates of EC were observed under endoscopic screening[11].

A national screening program for EC in high-risk areas has become available in 73 sites of 27 provinces of China based on evidence from previous studies. Nevertheless, due to lacking comprehensive health economic evaluations on such programs, two key public health questions remain to be answered: is the endoscopic screening cost-beneficial in the long run? Should we use the same screening strategy in both developed and developing high-risk areas of China?

The objective of this paper is to explore appropriate screening strategies for EC in high-risk areas of China from the health economic perspective by comparing the long-term cost-benefits of 12 endoscopic screening alternatives. It will provide valuable data for policy makers to make decisions on the current screening program.

MATERIALS AND METHODS

Decision analysis model

A Markov model was constructed to evaluate the cost-benefit of different screening strategies for EC. In each strategy simulation, a hypothetical cohort with 100 000 participants entered the model at age 40 years and were followed up until the age of 70 years. Costs and benefits were all discounted at an annual rate of 3%[12]. TreeAge Pro 2009 Suite by TreeAge Software Inc. was used for all analysis.

The natural history of EC was categorized as the following health status: normal, mild dysplasia (mD), moderate dysplasia (MD), severe dysplasia/carcinoma *in situ* (SD/CIS), intramucosal carcinoma (IC), submucosal carcinoma ($T_1N_0M_0$) (SC), invasive carcinoma (INC), and death. Figure 1 depicts the detailed transition processes of EC in the Markov model. Each rectangle represents a health state. During a Markov cycle (1 year), one could transit from his/her current health state to another (indicated by arrows between different states) or remain in the same state (indicated by half-circle arrows on the rectangles). Prior to the development of IC, the condition could spontaneously regress. Once IC developed, no regression could occur. We used the model to evaluate all screening alternatives and non-screening outcomes.

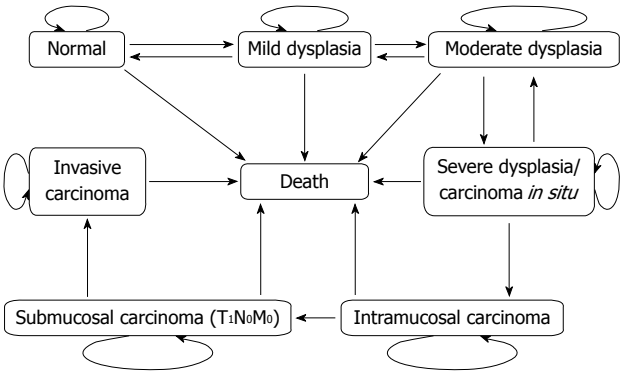


Figure 1 Bubble diagram representing the health states of esophageal cancer and transitions in natural-history Markov model.

Screening strategies

In the context of lacking guidelines for EC screening worldwide, we explored 12 screening strategies using endoscopic examinations. These strategies were performed at varying starting age for screening (40, 45 or 50 years), screening frequency (once, twice, thrice, or six times in the lifetime), and intervals of follow-up for mD and MD cases (e.g., 5 or 3 years). The strategies were listed as "t(y)nf/t(y)f", where t denotes the screening frequency, y represents the starting age of screening, nf means we do not follow up the mD and MD cases diagnosed by screening, and f means the mD and MD cases are followed up every 5 years and 3 years, respectively. For twice and thrice screening strategies in the lifetime, the screening intervals were 10 years; for six times screening strategy, the screening intervals were 5 years.

Screening, diagnosis, and treatment procedures for the strategies were all based on the current practice manuals. The participants were screened using endoscopy with iodine staining. If endoscopy revealed a suspected lesion (mD or worse), index biopsy combined with pathological examination were performed consecutively. The detailed procedures of endoscopy were the same with those in the literature[13]. For SD/CIS and IC cases detected by screening, Endoscopic Mucosal Resection and/or Argon Plasma Coagulation served as the standard treatment. For detected patients with SC or worse, therapies included esophagectomy, radiotherapy, and other routine treatments. Subjects who were not screened would be diagnosed and treated if they presented with symptomatic EC. All patients were followed up once by endoscopy in the first year after treatment.

Model parameters

The data used in the model were compiled from a variety of sources: (1) the results of our prospective cohort study based on the EC chemoprevention trial of selenomethionine and celecoxib in "early detection of EC" (EDEC) program; (2) the results of our population-based

Table 1 Non-age-specific parameters

Parameters	Value	Parameters	Value	Parameters	Value[1]
Initial probability[2]		Transition probability-continued		Compliance of screening[2]	67% (30%-100%)
Normal	0.8895	SD/CIS	see Table 2	Sensitivity of endoscopy[6]	96% (92%-99%)
mD	0.0820	IC	see Table 2	Specificity of endoscopy[6]	90% (59%-100%)
MD	0.0180	INC		Screening cost (I$ per capita)[2]	
SD/CIS	0.0090	Recovering to post-INC	0.7696	Direct cost	61.50 (37.00-119.00)
IC	0.0008	Relapsing to INC after treatment	0.2304	Indirect cost	8.31 (8.09-8.53)
SC	0.0005	After treatment[4]		Treatment cost (I$ per capita)[24][2]	
INC	0.0002	Post-SD/CIS		Direct cost	
Transition probability[9,16-21][2,3]		Recovering to normal	0.9950	SD/CIS	1292 (1114-1565)
Normal		Relapsing to SD/CIS	0.0050	IC	1292 (1114-1565)
Remaining normal	0.9760	Post-IC		SC	1818 (1519-2799)
Progression to mD	0.0240	Remaining post-IC	0.9450	INC-screening group	2767 (2332-4031)
mD		Relapsing to IC	0.0500	INC-control group	4888 (4333-6396)
Regression to normal	0.0500	Relapsing and progression to SC	0.0050	Indirect cost	
Remaining mD	0.9000	Post-SC		SD/CIS	1654 (1341-1968)
Progression to MD	0.0500	Remaining post-SC	0.8500	IC	1654 (1341-1968)
MD		Relapsing to SC	0.0500	SC	3369 (2872-3866)
Regression to mD	0.0800	Relapsing and progression to INC	0.1000	INC-screening group	3369 (2872-3866)
Remaining MD	0.8000	Post-INC (Same with "INC")		INC-control group	5526 (4584-6466)
Progression to SD/CIS	0.1200	Death probability	see Table 2	Discount rate[12][5]	3% (0%-6%)

mD: Mild dysplasia; MD: Moderate dysplasia; SD/CIS: Severe dysplasia/carcinoma *in situ*; IC: Intramucosal carcinoma; SC: Submucosal carcinoma ($T_1N_0M_0$); INC: Invasive carcinoma; I$: International dollar. [1]For compliance, sensitivity and specificity of endoscopy, screening and treatment costs, and discount rate, the numerals before and in the parenthesis denote the base case and ranges used in sensitivity analysis; [2]Data were calculated in terms of the project called "Early Detection and Early Treatment of Esophageal Cancer in Demonstration Centers in China"; [3]Data were collected from the death registry report of Linzhou County during 2004-2006; [4]Post-SD/CIS, post-IC, post-SC and post-INC represent the "health" condition after treatment of SD/CIS, IC, SC and INC; [5]Costs and life years were discounted during the Markov cycles.

screening project "Early Detection and Early Treatment of EC in Demonstration Centers in China" (EDETEC); (3) surveillance data; (4) published literatures; and (5) unpublished data.

In the chemoprevention trial of EDEC program, 2213 asymptomatic adults from Linzhou County, Henan Province of China, underwent an endoscopic screening in 1999[14]. Among them, 2189 participants who had histological diagnoses at the baseline evaluations were surveilled until 2007. The primary end-point was the occurrence of EC, confirmed by village doctors through checking the histological diagnoses in medical records. The project EDETEC covering 11 high-risk areas of EC was launched by the Chinese central government in 2005. The purpose was to increase the early detection and treatment rate as well as the 5-year survival rate of EC, and to improve the screening, early detection and treatment program and so forth[15].

Probabilities

At the initial model cycle, a hypothetical cohort of 100 000 participants was distributed among various health states based on the proportion of each pathologic stage of EC in the 40-44 years age group. The proportions were calculated in terms of the screening results of Linzhou County in the project EDETEC between 2005 and 2008. Among the 8267 asymptomatic participants aged 40-69 years, 8.2% cases were identified as mD in the 40-44 years age group. Full details are presented in Tables 1 and 2.

In each cycle of a Markov process, the transitions among health states occurred with annual transition probabilities. They were estimated in terms of: (1) published literatures[9,16-21]; and (2) the results of both EDEC and EDETEC projects in Linzhou County (Tables 1 and 2).

It is believed that persons with SD/CIS or lesser abnormality may not die from EC and that patients with IC or SC may die from all causes including EC. In patients with INC, we assumed that they may mainly die from EC. Therefore, in our model, the corresponding death probabilities for three different populations above were converted from non-esophageal-cancer mortality, all-cause mortality, and case fatality rate of EC, respectively. All age-specific mortality rates were obtained from the death registry reports of Linzhou County between 2004 and 2006. And they were converted to probabilities by the formula: Probability = 1 - Exp (-rt), where "r" represents the rate and 't' denotes the time (Tables 1 and 2).

Screening compliance

The compliance of EC screening in different settings varied from 33.4% to 77.1%[22]. In the EDETEC program, the screening compliance of EC in Linzhou County during 2005-2008 was 67% (8267/12 294), which was used as a baseline in this analysis (Table 1).

Screening and treatment cost

In our model, both screening and treatment costs included direct and indirect costs, which were calculated from a societal perspective. Direct costs referred to those associated with drugs, disposable supplies, equipment and facilities, staff, *etc*. In this study, we used costs rather than charges. And they were collected using Micro-costing methods in

Table 2 Age-specific parameters

Parameters	Value					
	40-yr	45-yr	50-yr	55-yr	60-yr	65-69-yr
Transition probability						
SD/CIS						
Regression to MD	0.17	0.15	0.14	0.12	0.11	0.09
Remaining SD/CIS	0.75	0.75	0.74	0.74	0.73	0.73
Progression to IC	0.08	0.10	0.12	0.14	0.16	0.18
IC						
Remaining IC	0.60	0.50	0.40	0.20	0.15	0.13
Progression to SC	0.40	0.50	0.60	0.80	0.85	0.87
SC						
Remaining SC	0.80	0.70	0.55	0.20	0.17	0.15
Progression to INC	0.20	0.30	0.45	0.80	0.83	0.85
Death probability						
Non-esophageal-cancer mortality	0.002270	0.003073	0.007054	0.017061	0.019744	0.024105
All-cause mortality	0.002438	0.003383	0.007967	0.019559	0.021985	0.027370
Case fatality rate of esophageal cancer	0.581700	0.581700	0.581700	0.581700	0.581700	0.581700

MD: Moderate dysplasia; SD/CIS: Severe dysplasia/carcinoma *in situ*; IC: Intramucosal carcinoma; SC: Submucosal carcinoma ($T_1N_0M_0$); INC: Invasive carcinoma.

the EDETEC program[23]. Indirect cost was also estimated from our EDETEC program, including those related to transportation, accommodation, and the productivity losses of both patients and their caregivers[24,25]. Considering differences in purchasing power, costs were presented in 2008 international dollars (I$).

Screening cost per capita using endoscopic examination was I$69.81. In screening group, the treatment costs for patients with SD/CIS or worse ranged from I$2964 to I$6136. In control group, the treatment cost for INC cases was I$10 414, much higher than that in screening group (Table 1).

Other variables and assumptions

According to a previous study in Linzhou County, the sensitivity and specificity of endoscopic examination were 96% and 90%, respectively[6] (Table 1). For individuals diagnosed as having precancerous lesions or EC (i.e., SD/CIS or worse), we assumed that they would complete the entire treatment procedures.

Health economic evaluation

The basic outcomes of the model were total costs (including screening costs and treatment costs) and expected life years. Then the net present values (NPVs) and the benefit-cost ratios (BCRs) were calculated under each of the strategies (for a hypothetical cohort of 100 000 subjects followed up from 40 years to 70 years of age).

For each screening cohort, the benefit consisted of the treatment cost averted and productivity gains from screening programs[25], and counted by the formula: benefit = GDP per capita of Linzhou in 2008 (I$6542) × (life years of screening cohort - life years of "non-screening" group) + treatment cost of "non-screening" group. The NPV was the benefit minus the total cost of the screening group; the BCR equaled to the benefit divided by the total cost. The strategies with a NPV > 0 and a BCR > 1 were considered cost-beneficial.

In addition, the screening alternatives were compared using an incremental cost-effectiveness analysis. The strategies that were more expensive and gained fewer life years (dominated), or less costly and less cost-effective (extended dominated) than an alternative were excluded.

Sensitivity analysis

Given the uncertainty about some parameters, univariate sensitivity analyses were used to assess the robustness of the model results by varying the values of screening compliance, discount rate, screening cost, treatment cost, sensitivity and specificity of endoscopy within reasonable ranges (Table 1).

Model validation

Based on the established natural-history model, the validity of the Markov model was assessed by comparing the model-predicted age-specific incidence and the age-specific proportion of each stage of EC with the observed data in real-world conditions.

RESULTS

Baseline results

Compared with non-screening outcomes, the screening strategies could save life years of 2539-15 384 for a hypothetical population of 100 000, with NPVs of I$24 million-I$99 million and BCRs of 1.61-3.06. Strategies with higher screening frequencies were more cost-beneficial than those with lower screening frequencies (Table 3).

When compared with each other, it indicated that the once(50)f-, twice(40)f-, twice(45)f-, thrice(40)f-, and six times(40)f-strategies were cost-effective, dominating or extended dominating others. In other words, other

Table 3 Estimated epidemiological and economic effects for each strategy with 100 000 people under baseline assumptions

Screening strategies t(y)nf/ t(y)f[1]	Life years	Life years saved (LYS)	Costs (I$)	ICER (I$/LYS)	Benefit (I$)	NPV (I$)	BCR
Non-screening	1 811 125	-	46 354 958	D	-	-	-
Once(40)nf	1 813 664	2539	39 133 890	D	62 964 854	23 830 964	1.61
Once(45)nf	1 814 180	3055	38 213 022	D	66 340 477	28 127 455	1.74
Once(50)nf	1 814 634	3509	36 989 316	D	69 310 502	32 321 186	1.87
Once(40)f	1 817 922	6797	38 007 700	D	90 820 285	52 812 585	2.39
Once(45)f	1 818 783	7658	36 792 906	ED	96 452 865	59 659 959	2.62
Once(50)f	1 817 966	6841	36 117 125	/	91 108 128	54 991 003	2.52
Twice(40)f	1 822 516	11 391	39 532 080	940	120 873 795	81 341 715	3.06
Twice(45)f	1 821 595	10 470	38 665 956	702	114 848 701	76 182 745	2.97
Twice(50)f	1 819 124	7999	38 261 433	ED	98 683 654	60 422 221	2.58
Thrice(40)f	1 823 528	12 403	41 665 346	2108	127 494 203	85 828 857	3.06
Thrice(45)f	1 821 827	10 702	40 775 616	D	116 366 423	75 590 807	2.85
Six times(40)f	1 826 509	15 384	48 042 566	2139	146 995 621	98 953 055	3.06

I$: International dollar. ICER means incremental cost-effectiveness ratio, which is defined as the additional cost of a specific strategy divided by its additional life years, as compared with the next-less-expensive strategy. D means dominated, e.g., the screening strategy is more expensive and less effective than another strategy. ED means extended dominated, e.g., if a screening strategy has a higher ICER than the next more costly, more effective strategy, it is extendedly dominated by that more cost-effective strategy. NPV: Net present value; BCR: Benefit-cost ratio. [1]t(y)nf/t(y)f: t denotes the frequencies of screening, y represents the starting age of screening, nf means we do not follow up the mild dysplasia and moderate dysplasia cases diagnosed by screening, and f means the mild dysplasia and moderate dysplasia cases diagnosed by screening are followed up every five and three years, respectively. For twice and thrice screening strategies in the lifetime, the screening intervals were 10 years; for the six times screening strategy in the lifetime, the screening intervals were five years. "-": Life years saved, benefits, NPVs and BCRs of screening strategies were all calculated by comparing with non-screening group, accordingly, those are null for non-screening group; "/": As the cheapest strategy, the ICER is null for the once(50)f-strategy.

strategies cost more and saved fewer lives, and were excluded. Among the cost-effective screening alternatives, the once(50)f-strategy saved the lowest life years of 6841, and resulted in the fewest NPV of I$55 million and BCR of 2.52. The highest life years saved were observed in the six times(40)f-strategy, with the maximum NPV of I$99 million and BCR of 3.06. Compared with six times(40)f-strategy, the thrice(40)f-strategy saved fewer life years and yielded lower NPV, but had the same BCR.

Sensitivity analysis

When the sensitivity and specificity of endoscopy, screening and treatment costs, discount rate, and screening compliance were changed once at a time (Table 1), once(50)f-, twice(40)f-, twice(45)f-, thrice(40)f-, and six times(40)f-strategies kept dominant. Uncertainty in those parameters had little effect on the choice of cost-effective strategies.

NPVs and BCRs changed obviously with screening cost, compliance, and discount rate under all cost-effective strategies. Both NPVs and BCRs were relatively less affected by the treatment cost, sensitivity and specificity of endoscopic examination. No matter how these parameters varied within the ranges, the results showed that screening was cost-beneficial with positive NPVs and BCRs > 1. In general, our results were robust (Figure 2).

Model validation

Comparison of incidence: The cancer registry report in Linzhou County during 2004-2006 showed that the age-specific incidence rates of EC were 47.44 per 100 000, 247.77 per 100 000, and 398.00 per 100 000 for the age groups of 40-49, 50-59, and 60-69 years, respectively. The corresponding model-predicted rates were 46.19/100 000, 248.14/100 000, and 424.78/100 000, respectively. The modeled estimates were about 94%-103% of the observed rates.

Comparison of proportions: First and most important, in any age group, the proportion of each histological grade of EC predicted by model was quite close to the screening results of the EDETEC program in Linzhou County during 2005-2008. And the estimated proportions were within the 99% confidence intervals of the observed data. Secondly, in each age group, the proportions decreased with the severity of the disease. And the proportions of mD ranked first. Last but not the least, for each pathologic grade of EC, the proportions increased with age, and reached the top in the 65-69 year-old group. Such tendency fit the characteristics of natural history of EC, and was also in agreement with the previous reports in other high-risk areas of China[26,27]. In summary, the validity of the model was satisfactory (Figure 3).

DISCUSSION

It was the first comprehensive cost-benefit assessment for the EC screening using endoscopic examination in China. Compared with no screening, all 12 screening strategies covering a hypothetical population of 100 000 resulted in substantial NPVs and high BCRs. However, when compared with each other, only five strategies were cost-effective based on the incremental cost-effectiveness analysis. Among all cost-effective strategies, screening once at age 50 yielded the lowest NPV (I$55 million) and BCR (2.52). Screening six times for those between 40-70 years of age at a 5-year interval yielded the highest NPV (I$99 million) and BCR (3.06). Compared with the six times(40)f-

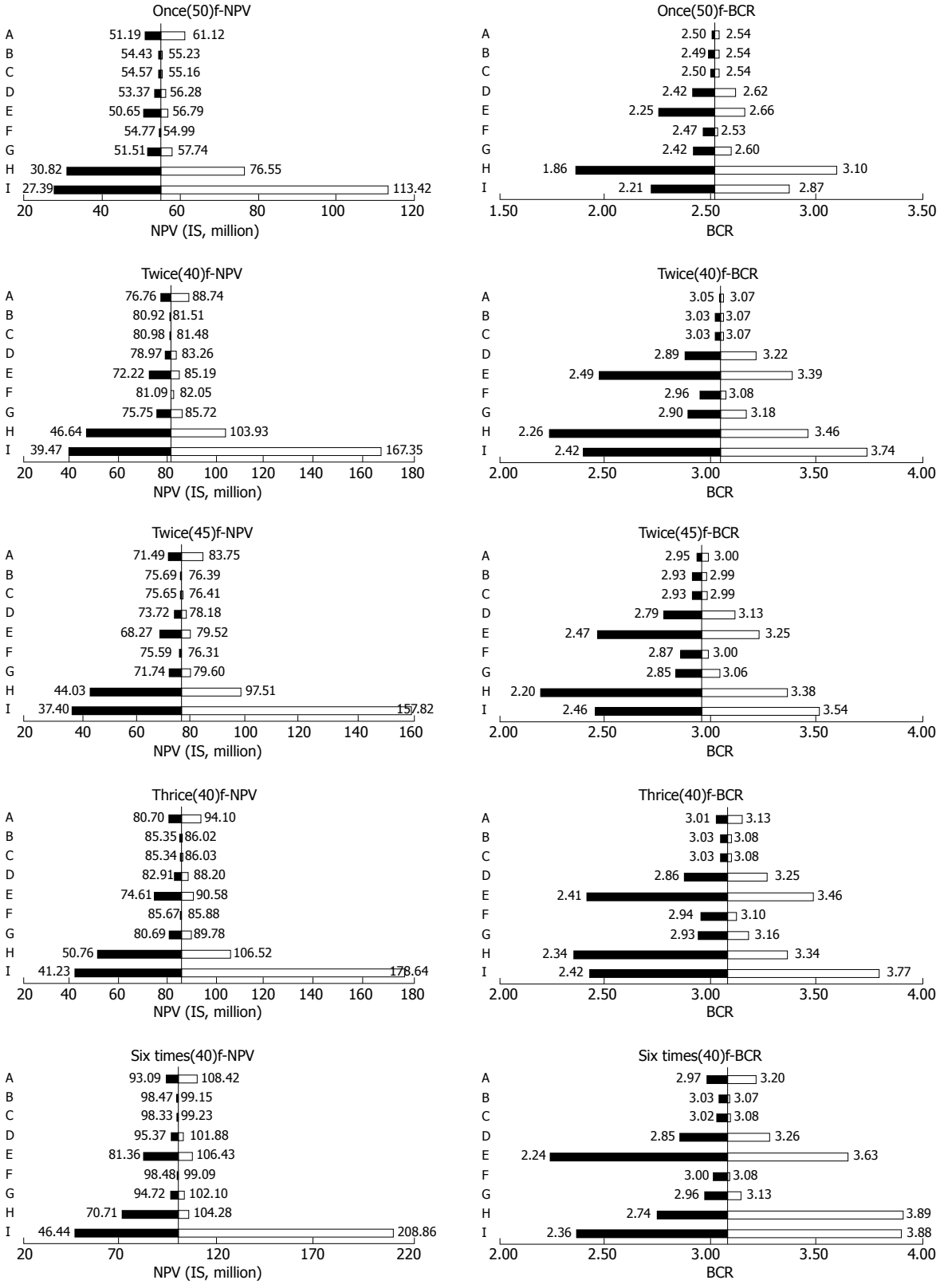


Figure 2 One-way sensitivity analyses for each cost-effective screening strategy. Strategies are expressed as t(y)f: t denotes the frequencies of screening; y denotes the starting age of screening; f means the mild dysplasia and moderate dysplasia diagnosed by screening were followed up every five and three years. For twice and thrice screening strategies in the lifetime, the screening intervals were 10 years; for the six times screening strategy in the lifetime, the screening intervals were 5 years. A: Treatment costs for invasive carcinoma of "non-screening" group; B: Treatment costs for invasive carcinoma of screening group; C: Treatment costs for submucosal carcinoma ($T_1N_0M_0$); D: Treatment costs for severe dysplasia/carcinoma *in situ*/intramucosal carcinoma; E: Screening costs; F: Specificity; G: Sensitivity; H: Screening compliance; I: Discount rate. Solid vertical lines represent the base cases of net present value (NPV)s/benefit-cost ratio (BCR)s. For B, C, D, E and I, the left of each bar, the lowest bound of NPVs/BCRs range, was counted on the basis of the maximum values of related parameters; and the right of each bar, the highest bound of NPVs/BCRs range, was counted according to the minimum values of related parameters. For other parameters, the left/right of each bar was calculated based on their minimum/maximum values.

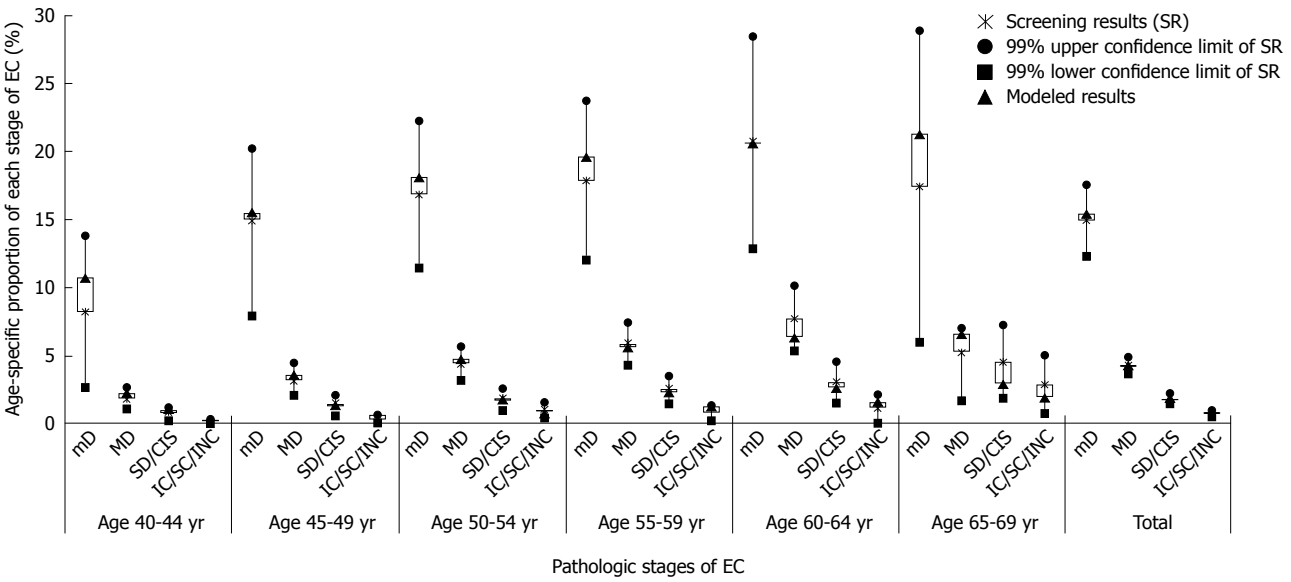


Figure 3 Comparison of modeled age-specific proportion of each pathologic stage of esophageal cancer with screening results. EC: Esophageal cancer; mD: Mild dysplasia; MD: Moderate dysplasia; SD/CIS: Severe dysplasia/carcinoma *in situ*; IC: Intramucosal carcinoma; SC: Submucosal carcinoma ($T_1N_0M_0$); INC: Invasive carcinoma.

strategy, screening thrice between 40-70 years of age at a 10-year interval saved fewer life years and produced lower NPV, but had the same BCR. Under these strategies, the mD and MD cases diagnosed by screening were followed up every 5 years and 3 years, respectively; all patients with SD/CIS or worse found by screening were treated, and followed up once by endoscopy in the first year after treatment. The validation assessment and the sensitivity analysis showed that our results were reliable.

Previously, two similar investigations presented BCRs of 4 and 4-12 for EC screening in China, which were higher than those in our analysis[25,28]. Explanations of the discrepancy from our estimates were: (1) Liu *et al*[28] and Wei *et al*[25] investigated 40-69 year old asymptomatic persons using cross sectional analyses, while we conducted a hypothetic birth cohort analysis, and followed up the cohort from 40 years to 70 years of age. Previous studies did not consider that some "normal/mild/moderate" cases defined by screening would progress and suffer from EC in the following life years[9,19-20,29]. A prospective study found that 23.7% mD and 50% MD cases developed EC during the 13-year follow-up[19]. The treatment costs may be very high for these EC patients. Neglecting them would overestimate BCRs; (2) Compared with non-screening, most of the EC patients in the screening group were diagnosed at earlier stages (87% *vs* 8%)[24]. As a result, the treatment cost per capita for EC patients in the screening group was lower than that in "non-screening" group. According to the formula of BCR, we found that BCR was positively associated with the difference of treatment cost per person between screening and control groups. Unlike Wei *et al*[25], we estimated the costs from the perspective of resource expenditure other than hospital charges. The difference of treatment costs between the groups in our study was much smaller than that in prior studies. That could account for the difference of BCRs to some extent; and (3) The costs and benefits were not discounted in previous studies[25,28]. Our sensitivity analysis showed that the discount rates were inversely associated with BCRs. And the BCRs of almost all strategies increased to nearly 4 when the discount rate declined to zero.

As the most widely used summary measures in health economic evaluations, the NPV and BCR are used to determine the return on any investment. Our study demonstrated that an investment of I$ 36 117 125 would result in a return of I$ 54 991 003 under once(50)f-strategy. These economic benefits resulted from a reduction in the incidence and mortality of EC, and the productivity gains of the prolonged life years through early detection and subsequent treatment. Our results revealed that the return increased with the screening frequency, and the six times 40)f-strategy resulted in the highest NPV. Although thrice(40)f-strategy yielded lower benefits, it was much less costly than the six times(40)f-strategy. It means that the thrice(40)f-strategy was a suitable alternative for the six times(40)f-strategy if there was an emphasis on capital constraints.

In addition to cost-benefit outcomes, some other factors should be considered when choosing reasonable screening strategy in different settings. First of all, endoscopy is an invasive examination. Concerns related to the high frequency of screening (e.g., six times in the lifetime) can lead to the great deduction of the compliance if it is not appropriately addressed, especially in the areas with low compliance at the time of initial screening, such as some villages of Ci County (33.7%)[22]. Moreover, the total costs of the screening strategy, life years saved, local economic level, and health resource status should also be weighed and balanced by policy makers. In summary, we recommended that once(50)f-strategy which was the cheapest would be suitable in underdeveloped settings with inadequate health resources, and that thrice(40)f-

strategy which could save more life years would be preferable in developed settings with adequate health resources.

One issue needed to be emphasized in our analysis was that most data used in our model were calculated from specific epidemiological data of Linzhou, the highest incidence area of EC worldwide. A great number of endoscopic screenings in this area have been performed since the 1980s, and systematic cancer incidence and death registration have been established. Therefore, the related data from Linzhou County were available and reliable. Our sensitivity analyses displayed that variation in some important parameters within wide ranges did not have a significant effect on our results. This further confirmed that our evaluation results mainly based on data from Linzhou were objective and applicable for other similar high-risk areas in China.

It is known that the cost-benefit of screening for EC (or any other cancer) is highly dependent on the incidence (and subsequent mortality) of that particular cancer. Based on our model prediction and area-specific incidence of EC in Cancer Registry Annual Report of China in 2004, we preliminarily and roughly estimated the cost-benefit of screening program in moderate-(around the national average level of EC incidence, 15.22/100 000), and low-risk areas (less than half of the national average level for EC incidence, 7.61/100 000). In moderate-risk areas, the BCRs ranged from 1.09-1.59, and screening once at age 50 incurred the highest BCR. In low-risk areas, only the strategy of screening once at age 50 remained cost-beneficial (with a highest BCR of 1.09). The results revealed that in moderate- or low-risk areas, screening program was not so cost-beneficial as that in high-risk areas. The screening once at age 50 was relatively preferable. Therefore, our results should be prudential to be used in moderate- or low-risk areas. However, more researches are needed in the future.

Our analysis had several limitations. First, the screening and treatment costs did not include program costs, which might account for a large part of the total costs[12]. The underestimation of costs may result in overrating the benefits of screening strategies, whereas the one-way sensitivity analysis of costs found that even when the costs were increased by over 20%, the screening was still considered as cost-beneficial. Second, in this study, the transition probabilities of all health states should change with age. However, those of normal, mD and MD states were fixed due to the unavailability of the data, which could affect the models' results to some extent. Hence, further studies on the natural history of EC appear warranted. Finally, although we performed one-way sensitivity analyses to evaluate the impact of each uncertainty on the results, we could not quantify the total impact of combinations of the parameter values. We did not conduct a multivariate probabilistic sensitivity analysis, since data on the probability distributions of variables were unavailable. This may more or less influence the outcomes of the sensitivity analysis.

In conclusion, EC endoscopic screening is cost-beneficial in high-risk areas of China. The strategy with once screening at age 50 years in the lifetime is the cheapest but saves fewer life years. If decision makers wish to save more life years and get more benefit, the strategy of thrice screening from 40 years of age at an interval of 10 years would be preferable. In different high-risk areas of EC, policy makers should consider the cost-benefit of screening, acceptability in the population, local health resources and economic level when choosing appropriate screening strategies.

ACKNOWLEDGMENTS

The authors thank Professor Guo-Qing Wang for providing suggestions for the transition probabilities and the natural history of EC.

COMMENTS

Background

Esophageal cancer (EC) remains the fourth-leading cause of cancer death in China, and continues to be the major public health burden in some high-risk areas. Previous studies found that EC screening program using endoscopic examination (i.e., endoscopy with mucosal iodine staining and index biopsy as a screening technology, combined with pathological examination for confirming and staging the disease) could increase the 5-year survival rate, decrease the incidence and mortality of EC. A national screening program for EC in high-risk areas has become available in 73 sites of 27 provinces of China. Nevertheless, the health economic effects in the long run on such programs remain unknown. And whether screening strategy is suitable in regions with different health resources and economic level is not clear.

Research frontiers

To assess the cost-benefit of screening program in the long run, large-population-based perspective studies are difficult and expensive to conduct, and results would be obtained in decades. Instead, in the area of health economic evaluation for secondary prevention of cancer, the research hotspot is to use Markov model to explore suitable strategies which are cost-effective and cost-beneficial.

Innovations and breakthroughs

Previous researches with regard to cost-benefit analyses of EC screening program in China were cross-sectional studies without follow-up, and only evaluated the health economic effects of one screening strategy which is used currently. The authors conducted a hypothetic birth cohort analysis and followed up the cohort from 40 years to 70 years of age on the basis of Markov model, and compared 12 hypothetic screening strategies (different at starting age of screening, screening intervals, *etc*.) so as to explore preferable screening strategies in different areas.

Applications

The study results suggest that EC endoscopic screening is cost-beneficial in high-risk areas of China. The strategy, screening once at age 50 years in the lifetime, is the cheapest but saves fewer life years. If decision makers wish to save more life years and get more benefit, the strategy, screening thrice from 40 years of age at an interval of 10 years, would be preferable. The results will provide policy makers important information on updating such screening program in high-risk areas.

Terminology

Markov model: Markov model is considered as a powerful tool for simulating the development process of chronic diseases. In Markov models, health states passed through by patients are defined separately; and then through modeling on the basis of a system of transitional probability among states within a cycle (usually 1 year), the development of diseases and the medical resources used in population could be estimated; Cost-benefit analysis: Cost-benefit analysis is a systematic process for calculating and comparing benefits and costs of a project to see whether the benefits outweigh the costs for two purposes: (1) to determine if it is a sound investment; and (2) to see how it compares with alternate projects.

Peer review

The authors present the results of a decision analysis of endoscopic screening for esophageal squamous cell cancer for a high-risk region in China. They conclude that endoscopic screening, compared to no screening, is cost-effective, with several different screening schedules that could be used. Overall, this is a nicely done study and is well-written.

REFERENCES

1 **World Health Organization**. GLOBOCAN 2008-Oesophageal Cancer Incidence and Mortality Worldwide in 2008 summary. Available from: URL: http://globocan.iarc.fr/factsheets/cancers/oesophagus.asp

2 **Wei WQ**, Yang J, Zhang SW, Chen WQ, Qiao YL. [Analysis of the esophageal cancer mortality in 2004 - 2005 and its trends during last 30 years in China]. *Zhonghua Yufang Yixue Zazhi* 2010; **44**: 398-402

3 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96

4 **Sant M**, Aareleid T, Berrino F, Bielska Lasota M, Carli PM, Faivre J, Grosclaude P, Hédelin G, Matsuda T, Møller H, Möller T, Verdecchia A, Capocaccia R, Gatta G, Micheli A, Santaquilani M, Roazzi P, Lisi D. EUROCARE-3: survival of cancer patients diagnosed 1990-94--results and commentary. *Ann Oncol* 2003; **14** Suppl 5: v61-118

5 **Guanrei Y**, Songliang Q. Endoscopic surveys in high-risk and low-risk populations for esophageal cancer in China with special reference to precursors of esophageal cancer. *Endoscopy* 1987; **19**: 91-95

6 **Dawsey SM**, Fleischer DE, Wang GQ, Zhou B, Kidwell JA, Lu N, Lewin KJ, Roth MJ, Tio TL, Taylor PR. Mucosal iodine staining improves endoscopic visualization of squamous dysplasia and squamous cell carcinoma of the esophagus in Linxian, China. *Cancer* 1998; **83**: 220-231

7 **Wang GQ**. [30-year experiences on early detection and treatment of esophageal cancer in high risk areas]. *Zhongguo Yixue Kexueyuan Xuebao* 2001; **23**: 69-72

8 **Lu XJ**, Chen ZF, Guo CL, Li SS, Bai WL, Jin GL, Wang YX, Meng FS, Gao F, Hou J. Endoscopic survey of esophageal cancer in a high-risk area of China. *World J Gastroenterol* 2004; **10**: 2931-2935

9 **Wang SJ**, Zhang LW, Wen DG, Li YS, Yu WF, Wang XL, Wang JH, Li SP, Ma CF, Li YW, Wang SP, Er LM. [Analysis of endoscopic screening for the natural history of precancerous lesions in esophagus and cardia]. *Zhongguo Zhongliu Linchuang* 2007; **34**: 370-373

10 **Wang GQ**, Jiao GG, Chang FB, Fang WH, Song JX, Lu N, Lin DM, Xie YQ, Yang L. Long-term results of operation for 420 patients with early squamous cell esophageal carcinoma discovered by screening. *Ann Thorac Surg* 2004; **77**: 1740-1744

11 **Qiao YL**, Hou J, Yang L, He YT, Liu YY, Li LD, Li SS, Lian SY, Dong ZW. [The trends and preventive strategies of esophageal cancer in high-risk areas of Taihang Mountains, China]. *Zhongguo Yixue Kexueyuan Xuebao* 2001; **23**: 10-14

12 **World Health Organization**. WHO-CHOICE. Available from: URL: http://www.who.int/choice/publications/p_2003_generalised_cea.pdf

13 **Roth MJ**, Liu SF, Dawsey SM, Zhou B, Copeland C, Wang GQ, Solomon D, Baker SG, Giffen CA, Taylor PR. Cytologic detection of esophageal squamous cell carcinoma and precursor lesions using balloon and sponge samplers in asymptomatic adults in Linxian, China. *Cancer* 1997; **80**: 2047-2059

14 **Limburg PJ**, Wei W, Ahnen DJ, Qiao Y, Hawk ET, Wang G, Giffen CA, Wang G, Roth MJ, Lu N, Korn EL, Ma Y, Caldwell KL, Dong Z, Taylor PR, Dawsey SM. Randomized, placebo-controlled, esophageal squamous cell cancer chemoprevention trial of selenomethionine and celecoxib. *Gastroenterology* 2005; **129**: 863-873

15 **Wei WQ**, Wang JB, Yang J, Qiao YL. Esophageal cancer. In: Tuncer AM, Moore M, Qiao YL, Yoo KY, Tajima K, Ozgul N, Gultekin M, editors. Asian Pacific Organization for Cancer Prevention. Cancer report 2010: The 5th International APOCP Conference; 2010 Apr 3-7; Istanbul, Turkey. Ankara: MN Medical and Nobel Publishing Company, 2010: 209-214

16 **Wen DG**, Zhang LW, Wang SJ, Li YS, Yu WF, Wang XL, Wang JH, Li SP, Li YW, Wang SP, Er LM, Cong QW, Ma CF. [Sojourn time observation of esophageal and cardia precancerous lesions by periodic endoscopic screening of 301 subjects in Shexian]. *Zhengzhou Daxue Xuebao* 2007; **42**: 62-66

17 **Jacob P**, Kahrilas PJ, Desai T, Hidvegi D, Walloch J, Yokoo H, Gurley AM, Ostrow JD. Natural history and significance of esophageal squamous cell dysplasia. *Cancer* 1990; **65**: 2731-2739

18 **Benedetti G**, Sablich R, Vitalba A, Lacchin T, Guido E, Del Bianco T. Intraepithelial carcinoma of the oesophagus: report of 25 cases from north-east Italy. *Eur J Surg* 2000; **166**: 622-627

19 **Wang GQ**, Abnet CC, Shen Q, Lewin KJ, Sun XD, Roth MJ, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Histological precursors of oesophageal squamous cell carcinoma: results from a 13 year prospective follow up study in a high risk population. *Gut* 2005; **54**: 187-192

20 **Dawsey SM**, Lewin KJ, Wang GQ, Liu FS, Nieberg RK, Yu Y, Li JY, Blot WJ, Li B, Taylor PR. Squamous esophageal histology and subsequent risk of squamous cell carcinoma of the esophagus. A prospective follow-up study from Linxian, China. *Cancer* 1994; **74**: 1686-1692

21 **Wang LD**, Yang HH, Fan ZM, Lü XD, Wang JK, Liu XL, Sun Z, Jiang YN, He X, Zhou Q. Cytological screening and 15 years' follow-up (1986-2001) for early esophageal squamous cell carcinoma and precancerous lesions in a high-risk population in Anyang County, Henan Province, Northern China. *Cancer Detect Prev* 2005; **29**: 317-322

22 **Song GH**, Meng FS, Chen C, Chen ZF. [Analysis on the factors influencing the compliance to endoscopic screening for early diagnosis and treatment in high-risk area of esophageal cancer]. *Zhonghua Liuxingbingxue Zazhi* 2009; **30**: 977-978

23 **Yang J**, Wei WQ, Niu J, He YT, Liu ZC, Song GH, Zhao de L, Qiao YL, Yang CX. Estimating the costs of esophageal cancer screening, early diagnosis and treatment in three high risk areas in China. *Asian Pac J Cancer Prev* 2011; **12**: 1245-1250

24 **Lv SH**, Li BY, Wei WQ, Shao Y, Niu J, Yang J, Lian SY, Qiao YL, Yang CX. [Cost analysis on esophageal cancer treatment among screening residents in Linzhou of Henan province]. *Xiandai Yufang Yixue* 2010; **19**: 3667-3669

25 **Wei WQ**, Yang CX, Lu SH, Yang J, Li BY, Lian SY, Qiao YL. Cost-benefit analysis of screening for esophageal and gastric cardiac cancer. *Chin J Cancer* 2011; **30**: 213-218

26 **Wang GQ**, Wei WQ, Lu N, Hao CQ, Lin DM, Zhang HT, Sun YT, Qiao YL, Wang GQ, Dong ZW. [Significance of screening by iodine staining of endoscopic examination in the area of high incidence of esophageal carcinoma]. *Ai Zheng* 2003; **22**: 175-177

27 **He Z**, Zhao Y, Guo C, Liu Y, Sun M, Liu F, Wang X, Guo F, Chen K, Gao L, Ning T, Pan Y, Li Y, Zhang S, Lu C, Wang Z, Cai H, Ke Y. Prevalence and risk factors for esophageal squamous cell cancer and precursor lesions in Anyang, China: a population-based endoscopic survey. *Br J Cancer* 2010; **103**: 1085-1088

28 **Liu ZR**, Wei WQ, Huang YQ, Qiao YL, Wu M, Dong ZW. [Economic evaluation of "early detection and treatment of esophageal cancer"]. *Ai Zheng* 2006; **25**: 200-203

29 **Chen ZF**, Wang GQ, Hou J, Zhang JH, Song GH, Qiao CY, Li SS, Chen C. [Follow-up results of esophageal squamous severe dysplasia in 158 cases]. *Zhongguo Zhongliu Linchuang* 2004; **31**: 306-308

S- Editor Shi ZF **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2502

World J Gastroenterol 2012 May 28; 18(20): 2502-2510
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Cervical inlet patch-optical coherence tomography imaging and clinical significance

Chao Zhou, Tejas Kirtane, Tsung-Han Tsai, Hsiang-Chieh Lee, Desmond C Adler, Joseph M Schmitt, Qin Huang, James G Fujimoto, Hiroshi Mashimo

Chao Zhou, Tsung-Han Tsai, Hsiang-Chieh Lee, James G Fujimoto, Department of Electrical Engineering and Computer Science, Research Laboratory of Electronics, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4307, United States
Tejas Kirtane, Qin Huang, Hiroshi Mashimo, Gastroenterology Section, VA Boston Healthcare System/Harvard School of Medicine, 150 S. Huntington Avenue, Boston, MA 02131, United States
Desmond C Adler, Joseph M Schmitt, Light Lab Imaging-St Jude Medical, Inc., Westford, MN 55117-9983, United States
Author contributions: Zhou C, Kirtane T and Mashimo H designed the study; Zhou C, Tsai TH, Lee HC, Adler DC and Schmitt JM developed the optical coherence tomography imaging system; Zhou C, Tsai TH, Lee HC and Mashimo H collected the data; Zhou C, Kirtane T, Tsai TH, Huang Q and Mashimo H analyzed the data; Fujimoto JG and Mashimo H obtained funding for the study; Zhou C, Kirtane T, Fujimoto JG and Mashimo H wrote the manuscript; Fujimoto JG and Mashimo H are principal investigators for this study.
Supported by The VA Boston Healthcare System and NIH grants R01-CA75289-14, to Fujimoto JG and Mashimo H; and K99-EB010071-01A1, to Zhou C; Air Force Office of Scientific Research contract FA9550-10-1-0063, to Fujimoto JG; Medical Free Electron Laser Program contract FA9550-10-1-0551, to Fujimoto JG; and the MIT/CIMIT Medical Engineering Fellowship, to Tsai TH
Correspondence to: Hiroshi Mashimo, MD, PhD, Gastroenterology Section, VA Boston Healthcare System/Harvard School of Medicine, 150 S. Huntington Avenue, Boston, MA 02131, United States. hmashimo@hms.harvard.edu
Telephone: +1-857-2035640 Fax: +1-857-2035666
Received: June 24, 2011 Revised: January 9, 2012
Accepted: February 8, 2012
Published online: May 28, 2012

Abstract

AIM: To demonstrate the feasibility of optical coherence tomography (OCT) imaging in differentiating cervical inlet patch (CIP) from normal esophagus, Barrett's esophagus (BE), normal stomach and duodenum.

METHODS: This study was conducted at the Veterans Affairs Boston Healthcare System (VABHS). Patients undergoing standard esophagogastroduodenoscopy at VABHS, including one patient with CIP, one representative patient with BE and three representative normal subjects were included. White light video endoscopy was performed and endoscopic 3D-OCT images were obtained in each patient using a prototype OCT system. The OCT imaging probe passes through the working channel of the endoscope to enable simultaneous video endoscopy and 3D-OCT examination of the human gastrointestinal (GI) tract. Standard hematoxylin and eosin (H and E) histology was performed on biopsy or endoscopic mucosal resection specimens in order to compare and validate the 3D-OCT data.

RESULTS: CIP was observed from a 68-year old male with gastroesophageal reflux disease. The CIP region appeared as a pink circular lesion in the upper esophagus under white light endoscopy. OCT imaging over the CIP region showed columnar epithelium structure, which clearly contrasted the squamous epithelium structure from adjacent normal esophagus. 3D-OCT images obtained from other representative patients demonstrated distinctive patterns of the normal esophagus, BE, normal stomach, and normal duodenum bulb. Microstructures, such as squamous epithelium, lamina propria, muscularis mucosa, muscularis propria, esophageal glands, Barrett's glands, gastric mucosa, gastric glands, and intestinal mucosal villi were clearly observed with OCT and matched with H and E histology. These results demonstrated the feasibility of using OCT to evaluate GI tissue morphology *in situ* and in real-time.

CONCLUSION: We demonstrate *in situ* evaluation of CIP microstructures using 3D-OCT, which may be a useful tool for future diagnosis and follow-up of patients with CIP.

Key words: Cervical inlet patch; Heterotopic gastric mucosa; Optical coherence tomography; Optical biopsy; Barrett's esophagus

Peer reviewers: Kevin M Reavis, The Oregon Clinic, 1040 NW 22nd Ave, Suite 560, Portland, OR 97210, United States; Jeff Butterworth, Shrewsbury and Telford Hospitals NHS Trust, 102 The Mount, Shrewsbury SY3 8PH, United Kingdom; John B Marshall, MD, Professor, Division of Gastroenterology, University of Missouri School of Medicine, One Hospital Drive, Columbia, MO 65212, United States

Zhou C, Kirtane T, Tsai TH, Lee HC, Adler DC, Schmitt JM, Huang Q, Fujimoto JG, Mashimo H. Cervical inlet patch-optical coherence tomography imaging and clinical significance. *World J Gastroenterol* 2012; 18(20): 2502-2510 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2502.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2502

INTRODUCTION

Cervical inlet patch (CIP) is characterized by the presence of heterotopic columnar gastric mucosa in the upper esophagus, most commonly located just below the upper esophageal sphincter (UES). Other sites for heterotopic gastric mucosa have been reported in the duodenum, jejunum, cystic duct, ampulla of vater, gallbladder, rectum and the anus[1-7], but their etiology and pathological significance remain unclear. The incidence of CIP has been reported from as low as 1%, to as much as 10% of endoscopic cases in different adult studies[8,9]. A large autopsy series of 1000 children demonstrated a prevalence of 4.5%[10]. During esophagogastroduodenoscopy (EGD), the region just below the UES is often quickly traversed after overcoming the initial resistance. CIP is usually best seen at the end of an EGD exam while withdrawing back through the esophagus and specifically looking for the condition. One study found almost a 6-8 fold increase in the incidence, from 0.3% to 2.3%, depending upon the endoscopist's awareness of this entity and thoroughness of examination[11]. Although generally asymptomatic, CIP can present with dysphagia[12], stricture[13], ulcers[14], bleeding[15] or fistula[16]. It is unclear whether CIP is congenital or acquired. One postulate is that CIP originates from incomplete embryonic replacement of the stratified epithelium, which normally starts at the 4th month of gestation. The greater incidence of CIP seen in pediatric populations and in the upper esophageal pouch of children with tracheoesophageal fistula supports this hypothesis[10,17,18]. Also, immunohistochemical studies suggest an embryologic origin for CIP on account of differences in endocrine markers such as serotonin, glucagon, pancreatic polypeptide, somatostatin and neurotensin in histologic specimens of CIP and Barrett's esophagus (BE)[19].

A second postulate is that CIP, especially as noted in adults, is an acquired metaplastic change occurring in the squamous mucosa of the esophagus and is associated with predisposing factors for gastroesophageal reflux disease (GERD), such as sliding hiatal hernia[20]. Its incidence is up to four-fold higher in patients having BE[21] and CIP was found in almost a third of patients having dysplastic BE or adenocarcinoma[22]. Thus, long-standing acid reflux is thought to lead to columnar metaplasia in the upper esophagus, similar to BE. Several reports suggest that CIP may progress to adenocarcinoma[23-26].

In this study, we evaluate whether optical coherence tomography (OCT) can assess epithelial differences in CIP compared to normal esophagus, BE, normal stomach and duodenum. OCT is an emerging medical imaging technology that enables micron-scale, cross-sectional, and three-dimensional (3D) imaging of biological tissues *in situ* and in real-time[27,28]. OCT is similar to ultrasound B-mode imaging, except that echoes of light, instead of sound, are used to achieve micron-scale image resolutions. *In vivo* endoscopic OCT imaging was first demonstrated in rabbit gastrointestinal (GI) and respiratory tracts in 1997[29], and was quickly adopted by multiple groups for investigations in the human GI tract[30-36]. A prospective study involving 121 patients demonstrated 97% sensitivity and 92% specificity for diagnosing BE[33]. Our group has developed a portable, catheter-based prototype OCT system, where the OCT probe can be passed through the accessory channel of a standard endoscope, and achieves imaging speeds of up to 100 000 axial scans per second with axial resolutions of 5 μm to 7 μm in tissues[37]. Real-time cross-sectional OCT image display and 3D capture capabilities were demonstrated in animals[37], and humans[36,38,39]. 3D-OCT volumetric imaging enables the synthesis of *en face* views (similar to magnification endoscopy images), the generation of virtual cross-sectional images with arbitrary orientation, the average of multiple frames to reduce speckle and improve contrast, and quantitative measurements of tissue morphology. To our knowledge, this is the first description of *in vivo* CIP microstructure using OCT.

MATERIALS AND METHODS

Imaging protocol

This study was conducted at the Veterans Affairs Boston Healthcare System (VABHS), in compliance with an approved protocol by the institutional review boards at VABHS, Harvard Medical School and Massachusetts Institute of Technology. Five male Caucasian patients undergoing regular EGD at VABHS from August 2009 to April 2011 were enrolled in this study. This includes one patient with CIP, one representative patient with BE and three representative normal subjects. The representative patient with BE and normal subjects were selected from a large cohort of subjects who were imaged using OCT for another study. White light video endoscopy was performed using the Evis Extra Ⅲ high definition system (Olympus America, Center Valley, PA), and endoscopic

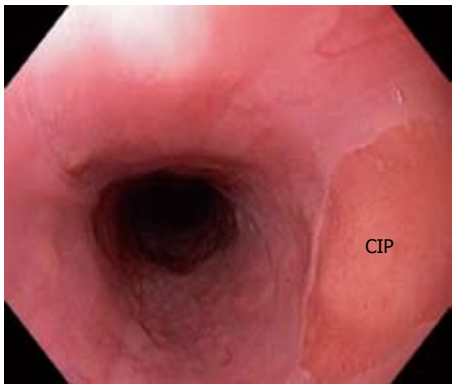


Figure 1 Endoscopic view of cervical inlet patch.

3D-OCT images were obtained using the system described below.

Optical coherence tomography system

The 3D-OCT endomicroscopy system was developed in collaboration with LightLab Imaging - St Jude Medical, Inc. and is similar to the system previously described by our group[37]. A Fourier Domain Mode Locking swept laser with a center wavelength of 1310 nm and average output power of 42 mW at a sweep repetition rate of 59 kHz was used as the light source. The full-width-half-maximum bandwidth of the laser sweep was about 120 nm, which supports about 5 μm axial resolution in tissue. The system sensitivity was 103 dB with 13 mW of incident power. The imaging probe, with an outer diameter of 2.5 mm, was introduced through a standard working channel of a high-definition endoscope (Olympus GIF-Q180) to enable simultaneous video endoscopy and 3D-OCT imaging examination. The output beam from the probe was focused to a 15 μm spot and was emitted at an angle of about 80 ℃ from the probe axis by a prism. The internal optics in the probe was rotated rapidly for radial scanning at 60 (or 70) frames per second (fps). Each image frame had about 512 × 1000 pixels at 60 fps (or about 512 × 900 pixels at 70 fps). To acquire a spirally scanned, volumetric OCT data set of the GI tract, the probe was pulled back at 1.0 mm/s along the sheath, which corresponds to a frame-to-frame spacing of 14-17 μm. At this image acquisition speed, a 20 mm × 8 mm × 2 mm 3D-OCT data set was acquired in 20 s.

Individual 2D-OCT frames were displayed on screen for real-time preview. The volumetric data sets were acquired and streamed to a hard drive. During post-processing, each 2D radial frame was unwrapped to create a rectangular frame. A custom program was written to detect the surface of the plastic probe sheath in each frame, which is used to flatten the image. The flattened 3D-OCT data sets were then loaded into Amira (ResolveRT, Mercury Computer Systems) for 3D rendering and visualization in different orthogonal imaging planes.

Histology analysis

Standard hematoxylin and eosin (H and E) histology was performed by the pathology service at VABHS on biopsy or endoscopic mucosal resection (EMR) specimens in order to compare and validate the 3D-OCT data. Photomicrographs of the H and E slides were taken under a standard Olympus BX40 microscope using a 4× objective.

RESULTS

The endoscopic view of a CIP in a 68-year old patient referred for endoscopic treatment for long-segment BE is shown in Figure 1. During retraction of the endoscope, a pink circular lesion was observed under white light endoscopy in the upper esophagus (about 20 cm from the tooth). The histology of the biopsies taken from the lesion later confirmed the finding of CIP (Figure 2C). Endoscopic OCT imaging was performed over the CIP under direct simultaneous visualization with a white light endoscope. From cross-sectional OCT images shown in Figure 2A and B, regions with CIP and the adjacent squamous epithelium can be identified. In addition, the CIP region clearly shows shallower light penetration compared with the adjacent normal esophagus. This is similar to typical images from normal gastric mucosa of representative other subjects. Zoomed views shown in Figure 2D and E clearly demonstrate columnar and squamous epithelium in the CIP and the adjacent normal esophagus, respectively. The columnar features observed in the CIP are consistent with the corresponding H and E histology shown in Figure 2C.

For comparison, OCT images of a normal gastro-esophageal junction (GEJ) obtained from a representative patient with chronic heartburn symptom (Figure 3). The *en face* OCT projection image at 350 μm underneath the tissue surface clearly shows the GEJ. The OCT imaging probe scans a large field (20 mm × 8 mm) on the tissue, which is about 100× larger compared to the region sampled by a standard biopsy (1-2 mm^2). Regions with gastric glandular mucosa (left) and esophageal squamous mucosa (right) exhibit clearly different patterns. Cross-sectional OCT images in Figure 3B-D show the GEJ and esophageal squamous epithelium along the probe pullback and rotation directions, respectively. The GEJ, squamous epithelium, lamina propria/muscularis mucosa, and esophageal glands underneath the squamous epithelium are clearly observed. Features observed in OCT images also match the representative histology of a normal GEJ shown in Figure 3E.

3D-OCT images from a representative patient with a long segment BE confirmed with histology (Figure 4). The *en face* projection OCT image at 200 μm underneath the tissue surface shows a similar angulated pattern compared with the *en face* image shown in the gastric mucosa (Figure 4A). Cross-sectional OCT images (Figure 4B and D) clearly show layered structures, where the original squamous mucosa in the esophagus is replaced by the columnar BE mucosa. Two hyper-scattering layers are observed underneath the BE mucosa, where the top layer corresponds to the newly formed muscularis mucosa layer which replaces the lamina propria, and the bottom

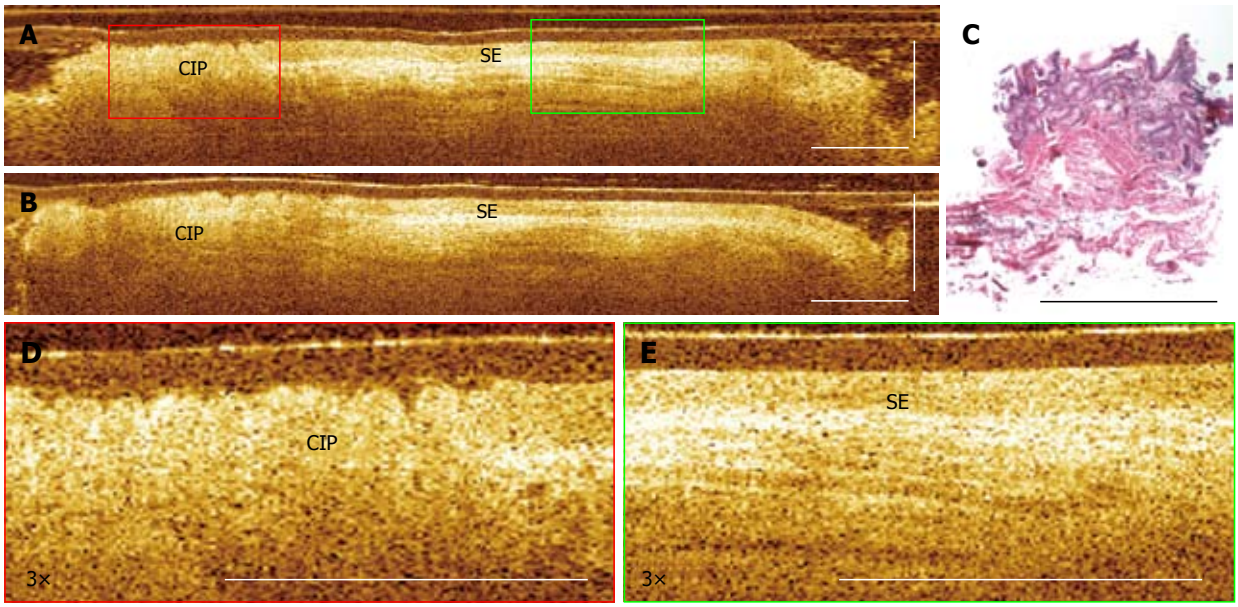


Figure 2 Endoscopic optical coherence tomography imaging of cervical inlet patch. A: Cross-sectional optical coherence tomography images of cervical inlet patch (CIP); B: Adjacent squamous epithelium, respectively; C: Corresponding hematoxylin and eosin histology obtained from a biopsy at the CIP site; D: 3× magnification of the CIP; E: Squamous epithelium (SE) region marked in (A). Scale bars: 1 mm.

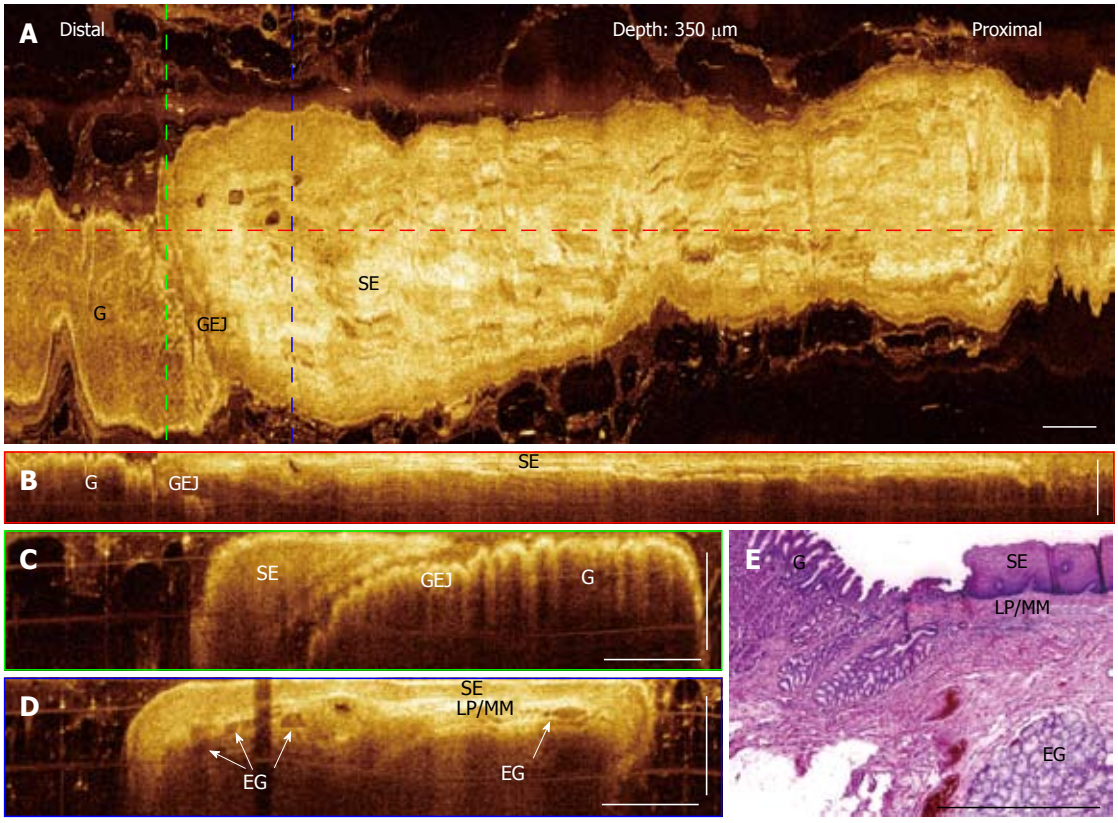


Figure 3 Three-dimensional-optical coherence tomography images of a normal gastro-esophageal junction. A: *En face* projection optical coherence tomography (OCT) image at a depth of 350 μm; B: Regions with gastric mucosa and squamous mucosa show distinct features; Cross-sectional OCT image along the probe pullback direction showing the gastro-esophageal junction (GEJ) and normal squamous epithelium (SE) clearly; C, D: Cross-sectional images of the GEJ and SE, corresponding to the green and blue dashed lines marked in (A), respectively. Structures, such as SE, lamina propria (LP)/muscularis mucosa (MM), esophageal glands (arrows) (EG), and gastric mucosa, can be clearly identified; E: Representative histology at the GEJ. Scale bars: 1 mm.

layer corresponds to the muscularis propria. These OCT features are confirmed with corresponding histology of an EMR specimen obtained at the imaging area in the same patient.

Representative OCT images of normal stomach from a patient with chronic heart burn symptom (Figure 5).

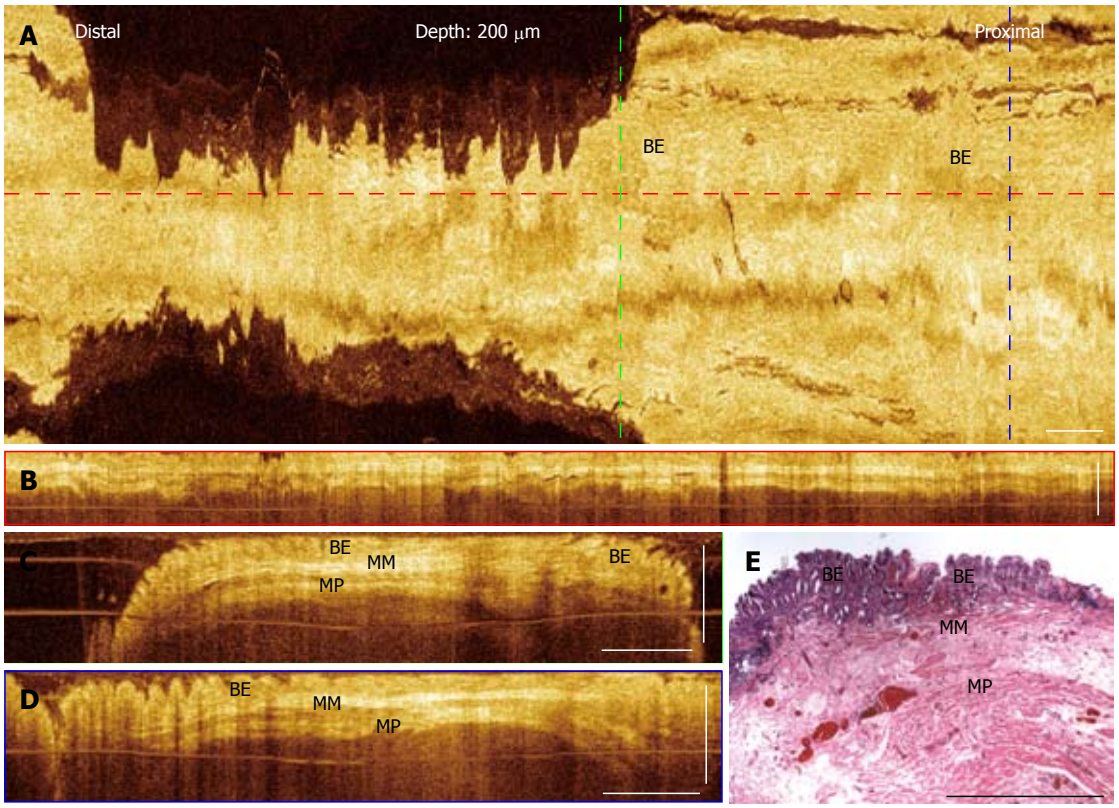


Figure 4 Three-dimensional-optical coherence tomography images of a long segment Barrett's esophagus. A: *En face* projection optical coherence tomography (OCT) image at a depth of 200 μm; B: Cross-sectional OCT images of the long segment Barrett's esophagus (BE) along the probe pullback direction; C, D: Cross-sectional OCT images, corresponding to the green and blue dashed lines marked in (A). BE glands, the muscularis mucosa (MM), and the muscularis propria (MP) layers are clearly seen; D: Histology of an endoscopic mucosal resection specimen obtained from the same subject shows corresponding features observed in the OCT images. Scale bars: 1 mm.

The *en face* projection image (Figure 5A) at a depth of 250 μm under the tissue surface represents the typical angulated gastric glandular mucosa pattern. Cross-sectional OCT images (Figure 5B and C) clearly show the gastric glandular mucosa. Gastric pits and gastric glands can be observed from cross-sectional OCT images and the image features match the representative histology of gastric mucosa shown in Figure 5D. Light penetration in normal gastric tissues is also shallower compared with normal esophagus and BE.

Furthermore, 3D-OCT images of normal duodenum from a patient with chronic heart burn symptom are shown in Figure 6. Distinctive features of the intestinal mucosal villi are observed in the *en face* OCT projection image (Figure 6A), as well as in the cross-sectional OCT images (Figure 6B and C). The length of individual villi, measured to be around 300-600 μm, matches the corresponding histology shown in Figure 6E. These results demonstrate the feasibility of using OCT to evaluate GI tissue morphology *in situ* and in real-time.

DISCUSSION

CIP is an under-appreciated entity in general gastroenterologist's practice. In this study, we present imaging results from OCT, a relatively new imaging technology, to describe the gastric type of epithelial patterns in CIP, as clearly distinct from normal esophageal squamous epithelium, BE, or from normal duodenum. Under OCT, CIP exhibits similar columnar structures compared with normal gastric mucosa, and the imaging depth in both CIP and gastric tissues are low. In practice, obtaining biopsies from CIP in patients with troublesome supraesophageal or laryngeal symptoms may be difficult owing to poor view just below the UES. OCT may allow "optical biopsy" of the CIP epithelium without the need for obtaining tissue specimens, and may be used to assess changes suspicious for malignancy in the future. Given its small diameter (2.5 mm) and flexibility, the OCT probes may be introduced orally or nasally without an endoscope, and with better tolerance and potentially less motion artifacts. This may further negate the need for sedation, nursing, or use of the endoscopy unit which has implications beyond endoscopy costs.

There are a number of case reports of adenocarcinoma arising from heterotopic gastric mucosa in the upper esophagus[23-26,40]. To our knowledge, 31 cases have been reported in the literature where esophageal adenocarcinoma was found arising from an inlet patch[41-46] and two cases where laryngeal squamous cell carcinoma was found associated with or bordering inlet patches[47]. The pathogenetic link between BE and CIP raises concerns of dysplastic transformation in CIP. Using immunohistochemical markers to address potential cellular origins,

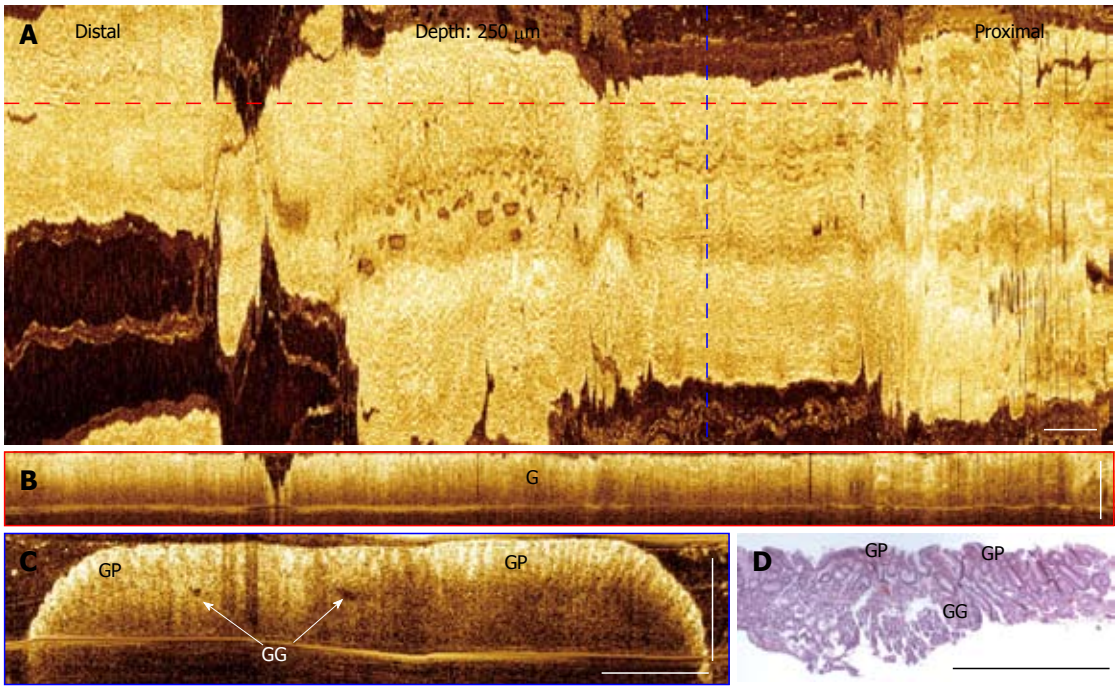


Figure 5 Three-dimensional-optical coherence tomography images of a normal stomach. A: *En face* projection optical coherence tomography (OCT) image at a depth of 250 μm; B: Cross-sectional OCT image along the probe pullback direction, corresponding to the red dashed line marked in (A); C: Cross-sectional images of the gastric mucosa, corresponding to the blue dashed line marked in (A). Gastric pits (GP) and gastric glands (arrows) (GG) can be identified; D: Representative histology of a gastric mucosa. Scale bars: 1 mm.

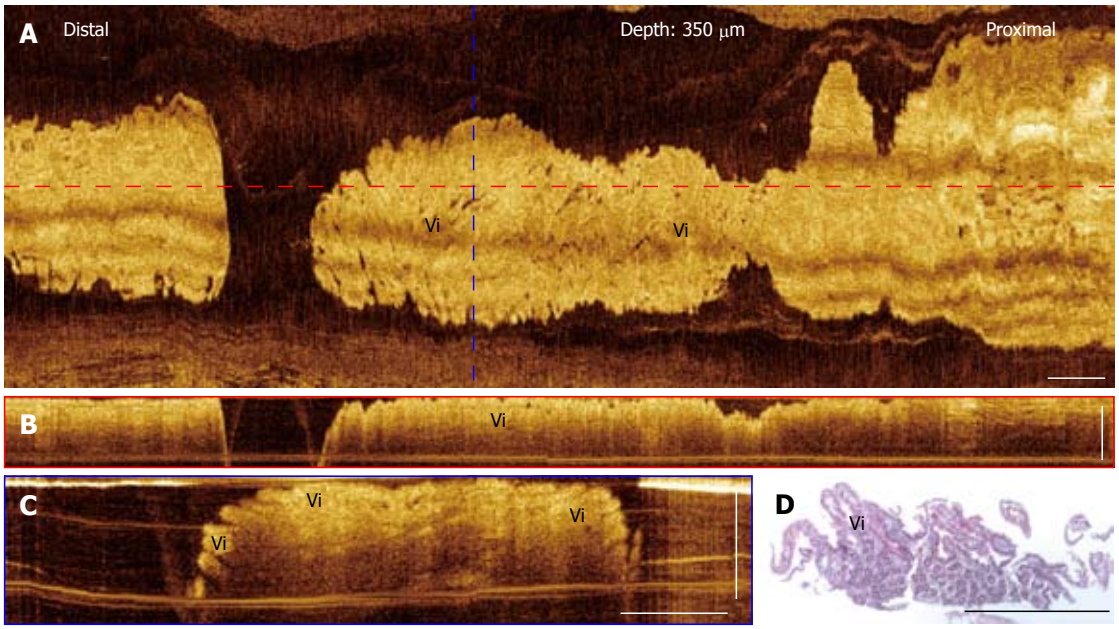


Figure 6 Three-dimensional-optical coherence tomography images of a normal duodenum. A: *En face* projection optical coherence tomography (OCT) image at a depth of 350 μm; B: Cross-sectional OCT images of the duodenum along the probe pullback direction; C: Cross-sectional OCT image, corresponding to the blue dashed line marked in (A); Mucosal villous structures (Vi) in the duodenum are clearly seen; D: Corresponding histology of the duodenum showing the villi. Scale bars:

Lauwers *et al*[48] demonstrated similar mechanisms of pathogenesis for CIP and BE on the basis of similarity between immunohistochemical staining patterns between the two entities. Furthermore, the similarity in the expression pattern for cytokeratins 7 and 20 and MUC6 mucin protein were not influenced by the presence or absence of GERD in these CIP patients[48-50]. Based on these findings, Lauwers *et al*[48] suggested that CIP may arise as a metaplastic change occurring in the esophageal epithelium. In light of these findings and the pathogenetic similarity between CIP and BE based on the cytokeratin expression study by Lauwers, the suspicion that the CIP, at least in adults, arises from local stem cells within the esophagus at or near this "heterotopic" patch

is strong. However, unlike BE, a consensus guideline for surveillance of CIP has not been established on account of its relatively low incidence and lack of information on its natural history for dysplastic changes. In this study, we present an alternative approach to evaluate CIP based on OCT imaging. Recently, balloon based OCT probes have been developed in order to allow imaging over the entire esophageal lumen for screening purposes[35,51-53]. The balloon design also helps stabilize the imaging probe to minimize motion artifacts. The advantages of OCT, such as real-time imaging, large area of coverage, and depth resolved imaging, *etc*., suggest that it may be a useful tool for detection of various GI diseases, including CIP and Barrett's esophagus.

In addition, OCT enables visualization of the deeper esophageal glands underneath squamous mucosa, which may not be accessible with standard or jumbo biopsy forceps. As suggested by Lauwers *et al*[48] CIP may arise from submucosal esophageal mucous glands. If these glands are the origin of dysplasia or malignant transformation, 3D-OCT may be uniquely suited for identifying these early dysplastic changes and following them up. Recently, novel methods have been developed to perform OCT imaging with contrast agents, such as gold nanoparticles[54-57], and therefore enable molecular targeted imaging for early cancer detection. In the future, OCT may also be combined with biomarkers, e.g., the superficial expression of Lgr5 in BE. Localization with depth resolution of such markers may help with directed biopsies or targeted ablation.

Presently, there are no commercially available OCT systems for endoscopic applications. The OCT probe used in this study passes through the working channel of a standard white light endoscope, and the entire OCT system is portable and could fit in a standard endoscopy suite to provide complementary real-time information on tissue microstructures during endoscopy.

One limitation of the current study is the small sample size. Multiple normal subjects and patients with BE were imaged and representative results were shown from a large cohort, but only one subject with CIP was available. The objective of this pilot study is to demonstrate the feasibility of *in situ* imaging of CIP using 3D-OCT and identify characteristic imaging features of CIP compared to other organs in human upper GI tract. One set of representative OCT images from each organ was demonstrated. However, it is not possible to reach any statistical conclusion from this feasibility study.

In conclusion, we demonstrate *in situ* evaluation of CIP microstructure using endoscopic 3D-OCT. OCT imaging visualized columnar epithelial structures within the CIP region, which clearly contrasted with the squamous epithelium from adjacent normal esophagus, gastric mucosa in the stomach and villous structure in the duodenum. The microstructural features observed with OCT also matched those from H and E histological sections. These results demonstrated the feasibility of using OCT to evaluate GI tissue morphology *in situ* and in real-time. Since OCT imaging can be performed with small diameter probes introduced orally or nasally, this emerging technology might be used to screen patients with troublesome upper esophageal symptoms for CIP, BE and other changes in the epithelium, even without endoscopy or the need for conscious sedation.

ACKNOWLEDGMENTS

The authors acknowledge the facility support from VA Boston Healthcare System and administrative assistance of Marisa Figueiredo.

COMMENTS

Background

Cervical inlet patch (CIP) is an under-appreciated entity encountered by gastroenterologists in general practice. Although rare, several reports suggest that CIP may progress to adenocarcinoma. Optical coherence tomography (OCT) is an emerging medical imaging technology that enables micron-scale, cross-sectional, and 3D imaging of biological tissues *in situ* and in real-time.

Research frontiers

This study evaluates whether OCT can optically assess epithelial differences in upper human gastrointestinal (GI) tract *in situ*.

Innovations and breakthroughs

OCT imaging over the CIP region showed columnar epithelium structure, which clearly contrasted the squamous epithelium structure from adjacent normal esophagus. OCT images obtained from other patients demonstrated distinctive patterns of the normal esophagus, Barrett's esophagus, normal stomach, and normal duodenum. Microstructures, such as squamous epithelium, lamina propria, muscularis mucosa, muscularis propria, esophageal glands, Barrett's glands, gastric mucosa, gastric glands, and intestinal mucosal villi were clearly observed with OCT and matched with hematoxylin and eosin histology. OCT may allow real-time "optical biopsy" of the CIP epithelium without the need for obtaining tissue specimens, and may be used to assess suspicious changes of malignancy in the future.

Applications

Given its small diameter and flexibility, the OCT probe may be introduced orally or nasally without an endoscope or need for moderate sedation, and with better tolerance and potentially less motion artifacts compared to endoscopy. In addition, the OCT imaging probe scans an approximately 100× larger field compared to biopsy, and therefore, might be useful in the future to screen for CIP.

Peer review

The paper is a very interesting piece of work exploring the utility of OCT primarily in characterizing cervical inlet patch but also in other esophageal disorders. It provides a reader with the future directions of and technological advances in upper GI endoscopy in an era when a number of non-white light endoscopic contrast techniques are competing for prime time use to more accurately delineate and diagnose pathology.

REFERENCES

1 **Rifat Mannan AA**, Kahvic M, Bharadwaj S, Grover VK. Gastric heterotopia of the anus: report of two rare cases and review of the literature. *Indian J Pathol Microbiol* 2008; **51**: 240-241
2 **Orizio P**, Villanacci V, Bassotti G, Falchetti D, Torri F, Ekema G. Heterotopic gastric mucosa in the cystic duct. *Int J Surg Pathol* 2011; **19**: 364-365
3 **Mann NS**, Mann SK, Rachut E. Heterotopic gastric tissue in the duodenal bulb. *J Clin Gastroenterol* 2000; **30**: 303-306
4 **Jarry J**, Rault A, Sa Cuhna A, Collet D, Masson B. Acute recurrent pancreatitis by heterotopic fundic mucosa at the ampulla of vater. *Pancreas* 2009; **38**: 351-353
5 **Debas HT**, Chaun H, Thomson FB, Soon-Shiong P. Func-

tioning heterotopic oxyntic mucosa in the rectum. *Gastroenterology* 1980; **79**: 1300-1302
6 **Boybeyi O**, Karnak I, Güçer S, Orhan D, Senocak ME. Common characteristics of jejunal heterotopic gastric tissue in children: a case report with review of the literature. *J Pediatr Surg* 2008; **43**: e19-e22
7 **Xeropotamos N**, Skopelitou AS, Batsis C, Kappas AM. Heterotopic gastric mucosa together with intestinal metaplasia and moderate dysplasia in the gall bladder: report of two clinically unusual cases with literature review. *Gut* 2001; **48**: 719-723
8 **Tang P**, McKinley MJ, Sporrer M, Kahn E. Inlet patch: prevalence, histologic type, and association with esophagitis, Barrett esophagus, and antritis. *Arch Pathol Lab Med* 2004; **128**: 444-447
9 **Borhan-Manesh F**, Farnum JB. Incidence of heterotopic gastric mucosa in the upper oesophagus. *Gut* 1991; **32**: 968-972
10 **Rector LE**, Connerly ML. Abberant mucosa in the esophagus in infants and in children. *Arch Pathol* 1941; **31**: 285-294
11 **Azar C**, Jamali F, Tamim H, Abdul-Baki H, Soweid A. Prevalence of endoscopically identified heterotopic gastric mucosa in the proximal esophagus: endoscopist dependent? *J Clin Gastroenterol* 2007; **41**: 468-471
12 **Akbayir N**, Alkim C, Erdem L, Sökmen HM, Sungun A, Başak T, Turgut S, Mungan Z. Heterotopic gastric mucosa in the cervical esophagus (inlet patch): endoscopic prevalence, histological and clinical characteristics. *J Gastroenterol Hepatol* 2004; **19**: 891-896
13 **Yarborough CS**, McLane RC. Stricture related to an inlet patch of the esophagus. *Am J Gastroenterol* 1993; **88**: 275-276
14 **Byrne M**, Sheehan K, Kay E, Patchett S. Symptomatic ulceration of an acid-producing oesophageal inlet patch colonized by helicobacter pylori. *Endoscopy* 2002; **34**: 514
15 **Bataller R**, Bordas JM, Ordi J, Llach J, Elizalde JI, Mondelo F. Upper gastrointestinal bleeding: a complication of "inlet patch mucosa" in the upper esophagus. *Endoscopy* 1995; **27**: 282
16 **Kohler B**, Köhler G, Riemann JF. Spontaneous esophagotracheal fistula resulting from ulcer in heterotopic gastric mucosa. *Gastroenterology* 1988; **95**: 828-830
17 **Skandalakis JE**, Gray SW, Ricketts R. The Esophagus. Embryology for Surgeons The Embryological Basis for the Treatment of Congenital Defects. Baltimore: Williams and Wilkins, 1972: 63-90
18 **Liebermann-Meffert D**, Duranceau A, Stein HJ. Anatomy and Embryology. In: Orringer MB, Heitliller R, editors. Shackelford's Surgery of the Alimentary Tract. 5th ed. Philadelphia: WB Saunders, 2002: 3-29
19 **Feurle GE**, Helmstaedter V, Buehring A, Bettendorf U, Eckardt VF. Distinct immunohistochemical findings in columnar epithelium of esophageal inlet patch and of Barrett's esophagus. *Dig Dis Sci* 1990; **35**: 86-92
20 **Yüksel I**, Usküdar O, Köklü S, Başar O, Gültuna S, Unverdi S, Oztürk ZA, Sengül D, Arikök AT, Yüksel O, Coban S. Inlet patch: associations with endoscopic findings in the upper gastrointestinal system. *Scand J Gastroenterol* 2008; **43**: 910-914
21 **Avidan B**, Sonnenberg A, Chejfec G, Schnell TG, Sontag SJ. Is there a link between cervical inlet patch and Barrett's esophagus? *Gastrointest Endosc* 2001; **53**: 717-721
22 **Malhi-Chowla N**, Ringley RK, Wolfsen HC. Gastric metaplasia of the proximal esophagus associated with esophageal adenocarcinoma and Barrett's esophagus: what is the connection? Inlet patch revisited. *Dig Dis* 2000; **18**: 183-185
23 **Sperling RM**, Grendell JH. Adenocarcinoma arising in an inlet patch of the esophagus. *Am J Gastroenterol* 1995; **90**: 150-152
24 **Mion F**, Lambert R, Partensky C, Cherkaoui M, Berger F. High-grade dysplasia in an adenoma of the upper esophagus developing on heterotopic gastric mucosa. *Endoscopy* 1996; **28**: 633-635
25 **Klaase JM**, Lemaire LC, Rauws EA, Offerhaus GJ, van Lanschot JJ. Heterotopic gastric mucosa of the cervical esophagus: a case of high-grade dysplasia treated with argon plasma coagulation and a case of adenocarcinoma. *Gastrointest Endosc* 2001; **53**: 101-104
26 **Berkelhammer C**, Bhagavan M, Templeton A, Raines R, Walloch J. Gastric inlet patch containing submucosally infiltrating adenocarcinoma. *J Clin Gastroenterol* 1997; **25**: 678-681
27 **Huang D**, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA. Optical coherence tomography. *Science* 1991; **254**: 1178-1181
28 **Fujimoto JG**, Pitris C, Boppart SA, Brezinski ME. Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. *Neoplasia* 2000; **2**: 9-25
29 **Tearney GJ**, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, Fujimoto JG. In vivo endoscopic optical biopsy with optical coherence tomography. *Science* 1997; **276**: 2037-2039
30 **Bouma BE**, Tearney GJ, Compton CC, Nishioka NS. High-resolution imaging of the human esophagus and stomach in vivo using optical coherence tomography. *Gastrointest Endosc* 2000; **51**: 467-474
31 **Pitris C**, Jesser C, Boppart SA, Stamper D, Brezinski ME, Fujimoto JG. Feasibility of optical coherence tomography for high-resolution imaging of human gastrointestinal tract malignancies. *J Gastroenterol* 2000; **35**: 87-92
32 **Sivak MV**, Kobayashi K, Izatt JA, Rollins AM, Ung-Runyawee R, Chak A, Wong RC, Isenberg GA, Willis J. High-resolution endoscopic imaging of the GI tract using optical coherence tomography. *Gastrointest Endosc* 2000; **51**: 474-479
33 **Poneros JM**, Brand S, Bouma BE, Tearney GJ, Compton CC, Nishioka NS. Diagnosis of specialized intestinal metaplasia by optical coherence tomography. *Gastroenterology* 2001; **120**: 7-12
34 **Yang VX**, Tang SJ, Gordon ML, Qi B, Gardiner G, Cirocco M, Kortan P, Haber GB, Kandel G, Vitkin IA, Wilson BC, Marcon NE. Endoscopic Doppler optical coherence tomography in the human GI tract: initial experience. *Gastrointest Endosc* 2005; **61**: 879-890
35 **Suter MJ**, Vakoc BJ, Yachimski PS, Shishkov M, Lauwers GY, Mino-Kenudson M, Bouma BE, Nishioka NS, Tearney GJ. Comprehensive microscopy of the esophagus in human patients with optical frequency domain imaging. *Gastrointest Endosc* 2008; **68**: 745-753
36 **Adler DC**, Zhou C, Tsai TH, Lee HC, Becker L, Schmitt JM, Huang Q, Fujimoto JG, Mashimo H. Three-dimensional optical coherence tomography of Barrett's esophagus and buried glands beneath neosquamous epithelium following radiofrequency ablation. *Endoscopy* 2009; **41**: 773-776
37 **Adler DC**, Chen Y, Huber R, Schmitt J, Connolly J, Fujimoto JG. Three-dimensional endomicroscopy using optical coherence tomography. *Nature Photonics* 2007; **1**: 709-716
38 **Adler DC**, Zhou C, Tsai TH, Schmitt J, Huang Q, Mashimo H, Fujimoto JG. Three-dimensional endomicroscopy of the human colon using optical coherence tomography. *Opt Express* 2009; **17**: 784-796
39 **Zhou C**, Adler DC, Becker L, Chen Y, Tsai TH, Figueiredo M, Schmitt JM, Fujimoto JG, Mashimo H. Effective treatment of chronic radiation proctitis using radiofrequency ablation. *Therap Adv Gastroenterol* 2009; **2**: 149-156
40 **Chennat J**, Ross AS, Konda VJ, Lin S, Noffsinger A, Hart J, Waxman I. Advanced pathology under squamous epithelium on initial EMR specimens in patients with Barrett's esophagus and high-grade dysplasia or intramucosal carcinoma: implications for surveillance and endotherapy management. *Gastrointest Endosc* 2009; **70**: 417-421
41 **von Rahden BH**, Stein HJ, Becker K, Liebermann-Meffert D, Siewert JR. Heterotopic gastric mucosa of the esophagus: literature-review and proposal of a clinicopathologic clas-

sification. *Am J Gastroenterol* 2004; **99**: 543-551
42 **von Rahden BH**, Stein HJ, Becker K, Siewert RJ. Esophageal adenocarcinomas in heterotopic gastric mucosa: review and report of a case with complete response to neoadjuvant radiochemotherapy. *Dig Surg* 2005; **22**: 107-112
43 **Balon JM**, Mariette C, Fabre S, Tiret E, Triboulet JP. [Primary adenocarcinoma of the cervical esophagus arising from heterotopic gastric mucosa]. *Gastroenterol Clin Biol* 2003; **27**: 836-838
44 **Alrawi SJ**, Winston J, Tan D, Gibbs J, Loree TR, Hicks W, Rigual N, Lorè JM. Primary adenocarcinoma of cervical esophagus. *J Exp Clin Cancer Res* 2005; **24**: 325-330
45 **Alagozlu H**, Ergun M, Cindoruk M, Unal S, Dumlu S, Poyraz A, Dursun A. The rare presentations of a large polyp and an esophageal carcinoma in heterotropic gastric mucosa: a case series. *J Med Case Reports* 2007; **1**: 127
46 **Abe T**, Hosokawa M, Kusumi T, Kusano M, Hokari K, Kagaya H, Watanabe A, Fujita M, Sasaki S. Adenocarcinoma arising from ectopic gastric mucosa in the cervical esophagus. *Am J Clin Oncol* 2004; **27**: 644-645
47 **Satoh S**, Nakashima T, Watanabe K, Toda S, Kuratomi Y, Sugihara H, Inokuchi A. Hypopharyngeal squamous cell carcinoma bordering ectopic gastric mucosa "inlet patch" of the cervical esophagus. *Auris Nasus Larynx* 2007; **34**: 135-139
48 **Lauwers GY**, Mino M, Ban S, Forcione D, Eatherton DE, Shimizu M, Sevestre H. Cytokeratins 7 and 20 and mucin core protein expression in esophageal cervical inlet patch. *Am J Surg Pathol* 2005; **29**: 437-442
49 **Chatelain D**, de Lajarte-Thirouard AS, Tiret E, Flejou JF. Adenocarcinoma of the upper esophagus arising in heterotopic gastric mucosa: common pathogenesis with Barrett's adenocarcinoma? *Virchows Arch* 2002; **441**: 406-411
50 **Bogomoletz WV**, Geboes K, Feydy P, Nasca S, Ectors N, Rigaud C. Mucin histochemistry of heterotopic gastric mucosa of the upper esophagus in adults: possible pathogenic implications. *Hum Pathol* 1988; **19**: 1301-1306
51 **Li XD**, Boppart SA, Van Dam J, Mashimo H, Mutinga M, Drexler W, Klein M, Pitris C, Krinsky ML, Brezinski ME, Fujimoto JG. Optical coherence tomography: advanced technology for the endoscopic imaging of Barrett's esophagus. *Endoscopy* 2000; **32**: 921-930
52 **Xi J**, Huo L, Wu Y, Cobb MJ, Hwang JH, Li X. High-resolution OCT balloon imaging catheter with astigmatism correction. *Opt Lett* 2009; **34**: 1943-1945
53 **Kang W**, Wang H, Pan Y, Jenkins MW, Isenberg GA, Chak A, Atkinson M, Agrawal D, Hu Z, Rollins AM. Endoscopically guided spectral-domain OCT with double-balloon catheters. *Opt Express* 2010; **18**: 17364-17372
54 **Loo C**, Lin A, Hirsch L, Lee MH, Barton J, Halas N, West J, Drezek R. Nanoshell-enabled photonics-based imaging and therapy of cancer. *Technol Cancer Res Treat* 2004; **3**: 33-40
55 **Adler DC**, Huang SW, Huber R, Fujimoto JG. Photothermal detection of gold nanoparticles using phase-sensitive optical coherence tomography. *Opt Express* 2008; **16**: 4376-4393
56 **Skala MC**, Crow MJ, Wax A, Izatt JA. Photothermal optical coherence tomography of epidermal growth factor receptor in live cells using immunotargeted gold nanospheres. *Nano Lett* 2008; **8**: 3461-3467
57 **Zhou C**, Tsai TH, Adler DC, Lee HC, Cohen DW, Mondelblatt A, Wang Y, Connolly JL, Fujimoto JG. Photothermal optical coherence tomography in ex vivo human breast tissues using gold nanoshells. *Opt Lett* 2010; **35**: 700-702

S- Editor Gou SX **L- Editor** O'Neill M **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2511

World J Gastroenterol 2012 May 28; 18(20): 2511-2516
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Endomysial antibodies predict celiac disease irrespective of the titers or clinical presentation

Kalle Kurppa, Tiia Räsänen, Pekka Collin, Sari Iltanen, Heini Huhtala, Merja Ashorn, Päivi Saavalainen, Katri Haimila, Jukka Partanen, Markku Mäki, Katri Kaukinen

Kalle Kurppa, Tiia Räsänen, Sari Iltanen, Merja Ashorn, Markku Mäki, Pediatric Research Centre, University of Tampere and Tampere University Hospital, Fin-33014, Tampere, Finland
Pekka Collin, Katri Kaukinen, Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital and School of Medicine, University of Tampere, Fin-33014, Tampere, Finland
Heini Huhtala, Tampere School of Public Health, University of Tampere, Fin-33014, Tampere, Finland
Päivi Saavalainen, Department of Medical Genetics and Research Program for Molecular Medicine, University of Helsinki, Fin-00014, Helsinki, Finland
Katri Haimila, Jukka Partanen, Research and Development, Finnish Red Cross Blood Service, Fin-00310, Helsinki, Finland
Author contributions: Kurppa K, Räsänen T, Collin P, Iltanen S, Huhtala H, Ashorn M, Saavalainen P, Haimila K, Partanen J, Mäki M and Kaukinen K designed the research, contributed to the acquisition of the data and performed critical revision of the manuscript; Kurppa K, Huhtala H and Kaukinen K analyzed the data; Kurppa K and Kaukinen K wrote the paper.
Supported by The Academy of Finland Research Council for Health; the Competitive Research Funding of the Pirkanmaa Hospital District; the Sigrid Juselius Foundation; the Foundation for Paediatric Research; the National Graduate School of Clinical Investigation; the Ehrnrooth Foundation; the Finnish Gastroenterology Society; the Finnish Pediatric Society and the Finnish Celiac Society
Correspondence to: Katri Kaukinen, MD, PhD, Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital and School of Medicine, University of Tampere, Fin-33014, Tampere, Finland. katri.kaukinen@uta.fi
Telephone: +358-3-35518403 Fax: +358-3-35518402
Received: July 28, 2011 Revised: November 2, 2011
Accepted: March 10, 2012
Published online: May 28, 2012

Abstract

AIM: To investigate the association between serum antibody levels and a subsequent celiac disease diagnosis in a large series of children and adults.

METHODS: Besides subjects with classical gastrointestinal presentation of celiac disease, the study cohort included a substantial number of individuals with extraintestinal symptoms and those found by screening in at-risk groups. Altogether 405 patients underwent clinical, serological and histological evaluations. After collection of data, the antibody values were further graded as low [endomysial (EmA) 1:5-200, transglutaminase 2 antibodies (TG2-ab) 5.0-30.0 U/L] and high (EmA 1: $\geqslant$ 500, TG2-ab $\geqslant$ 30.0 U/L), and the serological results were compared with the small intestinal mucosal histology and clinical presentation.

RESULTS: In total, 79% of the subjects with low and 94% of those with high serum EmA titers showed small-bowel mucosal villous atrophy. Furthermore, 96% of the 47 EmA positive subjects who had normal mucosal villi and remained on follow-up either subsequently developed mucosal atrophy while on a gluten-containing diet, or responded positively to a gluten-free diet.

CONCLUSION: Irrespective of the initial serum titers or clinical presentation, EmA positivity as such is a very strong predictor of a subsequent celiac disease diagnosis.

Key words: Celiac disease; Diagnosis; Endomysial antibodies; Transglutaminase 2 antibodies; Clinical presentations

Peer reviewers: Khaled Jadallah, MD, Assistant Professor of Medicine, Gastroenterologist and Hepatologist, Department of Internal Medicine, King Abdullah University Hospital, Jordan University of Science and Technology, Irbid 22110, Jordan; Alyssa M Krasinskas, MD, Assistant Professor, Department of Pathology, University of Pittsburgh Medical Center, Presbyterian

Hospital, A610, 200 Lothrop Street, Pittsburgh, PA 15213-2546, United States

Kurppa K, Räsänen T, Collin P, Iltanen S, Huhtala H, Ashorn M, Saavalainen P, Haimila K, Partanen J, Mäki M, Kaukinen K. Endomysial antibodies predict celiac disease irrespective of the titers or clinical presentation. *World J Gastroenterol* 2012; 18(20): 2511-2516 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2511.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2511

INTRODUCTION

Recent serological screening studies have revealed that up to 1%-2% of the Western population might be affected by celiac disease[1,2]. However, due to its heterogeneous clinical picture the disease remains markedly underdiagnosed. Sensitive serum endomysial (EmA) and transglutaminase 2 antibodies (TG2-ab) are widely used as a method to select subjects for further investigations, but the diagnosis is based on the presence of small-bowel mucosal villous atrophy and crypt hyperplasia[3,4]. Unfortunately, the histological definition of the disease involves several problems. First, invasive studies are needed to acquire the mucosal specimens. In addition, biopsy samples may be of poor quality or wrongly orientated, increasing the risk of false positive or negative results[5]. The mucosal damage may be patchy and missed even if several samples are taken[6,7]. Finally, the histological lesion develops gradually and interpretation of borderline cases can be challenging. Since particularly EmA and high values of TG2-ab seem to predict celiac disease with a high specificity, it has been advocated that in seropositive subjects endoscopic studies might not always be needed to establish the diagnosis[8-15]. However, most studies so far have been carried out in tertiary centers with high-risk patients, and the results might not be applicable in everyday clinical practice.

In our local health-care district active celiac disease case-finding has been carried out since the 1980s. As a result, a substantial part of the patients are detected because of atypical symptoms or by active risk-group screening, and currently about 0.7% of the population have a biopsy-proven diagnosis[16]. Hence, we now sought to establish whether the serum antibodies could predict subsequent celiac disease also in subjects with mild or atypical clinical presentation. Because of the high specificity, EmA has traditionally been considered the gold standard for celiac disease serology, and was thus chosen as the primary inclusion criterion[17,18]. In addition, the results were compared to the widely used serum TG2-ab.

MATERIALS AND METHODS

The study cohort comprised consecutive EmA positive children and adults investigated at the Departments of Pediatrics and Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital. Primary care physicians were encouraged to refer individuals with celiac disease suspicion for further investigations applying a low index of suspicion. In addition, subjects who participated in population-based research studies were accepted. In the hospital demographic data, a family history of celiac disease and symptoms leading to the disease suspicion were recorded, and all subjects underwent extensive clinical, serological and histological evaluations. Thereafter, voluntary EmA positive children and adults continued in the trial. Participants who showed small-bowel mucosal villous atrophy and crypt hyperplasia (Marsh Ⅲ) received a celiac disease diagnosis and were placed on a gluten-free diet. Subjects who had normal villi continued on a gluten-containing diet and were placed on regular serological and histological follow-up. In addition, the possibility to start an experimental trial with a gluten-free diet was offered to EmA positive individuals with normal villous structure (Marsh 0-Ⅱ). Those who consented were re-evaluated after one year, and if a positive clinical, serological and histological response was observed, celiac disease diagnosis was established. Finally, serum TG2-ab were used for comparison in all from whom they were available.

Serum immunoglobulin A (IgA)-class EmA were measured by an indirect immunofluorescence method using human umbilical cord as antigen[19]. A dilution of 1:5 was considered positive, and positive sera were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000 and 1:4000. The antibody titers were further graded as low (1:5-1:200) and high (1:500-1:4000). Serum IgA-class TG2-ab were measured by enzyme-linked immunosorbent assay (ELISA) (Celikey, Phadia GmbH, Freiburg, Germany) according to the manufacturers' instructions. Serum TG2-ab values ≥ 5.0 U were considered positive, and the values were further graded as low (5.0-29.9 U/L) and high (≥ 30 U/L)[13]. Total IgA values were tested in all subjects negative to the IgA class serological tests. In case of IgA deficiency the corresponding antibodies were measured in immunoglobulin G (IgG) class.

Upon upper gastrointestinal endoscopy a minimum of three forceps specimens were taken from the distal duodenum, and small-bowel mucosal morphology was determined from several well-oriented biopsy sections as previously described[17]. The degree of mucosal damage was further graded according to the Marsh-Oberhuber classification, where Marsh 0 represents normal mucosa, Marsh Ⅰ-Ⅱ represents increased intraepithelial lymphocytosis without (Ⅰ) or with (Ⅱ) hyperplastic crypts and Marsh Ⅲ partial (a), subtotal (b) or total (c) villous atrophy[20,21]. A patchy mucosal lesion was graded according to the most severe histological damage.

Genotyping of the participants for celiac disease-associated human leukocyte antigen (HLA)-DQB1*02 and DQB1*0302 alleles (DQ2 and DQ8) was performed using the DELFIA® Celiac Disease Hybridization Assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) or the SSP™ DQB1 low resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden) according to the

Table 1 Demographic data on the study participants and primary reason for celiac disease suspicion *n* (%)

Female	265 (67)
Age below 18 yr	92 (23)
Age (yr), median (range)	40 (1-79)
Main reason for disease suspicion	
Gastrointestinal symptoms[1]	166 (43)
Anemia or malabsorption	38 (10)
Extraintestinal symptoms[2]	50 (13)
Screening in at-risk groups[3]	97 (24)
Screening in the population[4]	39 (10)
Unknown	5 (1)

[1]Diarrhea, abdominal pain, flatulence, constipation, dyspepsia, and heartburn; [2]Osteoporosis, infertility, aphthous stomatitis, short stature, delayed puberty, arthralgia, ataxia, epilepsy, fatigue, and alopecia; [3]Family history of celiac disease, type 1 diabetes, thyroid disorders, Sjögren´s syndrome, and immunoglobulin A nephropathy; [4]Population-based research studies that included serological screening.

Table 2 Serum endomysial and transglutaminase 2 antibody values, divided according to the clinical presentation

	EmA, titer		TG2-ab, U/L	
	Low 1:5-200 *n* = 224, %	High 1:≥ 500 *n* = 154, %	Low 5.0-29.9 *n* = 116, %	High ≥ 30 *n* = 166, %
Abdominal symptoms	45	40	45	45
Anemia or malabsorption	8	12	5	13
Extraintestinal symptoms	9	16	5	13
Screen-detected subjects	38	31	45	28
	$P = 0.061$		$P = 0.002$	

EmA: Endomysial; TG2-ab: Transglutaminase 2 antibody.

manufacturer's instructions.

χ^2 with cross-tabulation was used for statistical analysis. A P value less than 0.05 was considered statistically significant.

The study protocol was approved by the Ethics Committee of Tampere University Hospital. All subjects or their parents gave written informed consent.

RESULTS

In total, 405 EmA positive children and adults participated in the study. In 10 subjects the quality of the small-bowel biopsies was insufficient, in 14 EmA was determined as positive (1:5) without further dilution and in three subjects the clinical data were ambiguous. These cases were excluded from further statistical analyses. One patient had selective IgA deficiency and the corresponding antibodies were measured in the IgG class. Gastrointestinal symptoms remained the primary reason for celiac disease suspicion, but almost half of the patients were detected on the basis of extraintestinal symptoms or by screening of at-risk groups and the population (Table 1).

By definition, all participants were positive for EmA.

Table 3 Association between high and low serum endomysial and transglutaminase 2 antibody values and small-bowel mucosal morphology

	EmA (L)		TG2-ab(U/L)	
	Low 1:5-200 *n* = 227, %	High 1:≥ 500 *n* = 156, %	Low 5.0-29.9 *n* = 146, %	High ≥ 30 *n* = 169, %
Marsh 0	5	1	4	1
Marsh I - II	16	5	16	5
Marsh IIIa	22	13	24	12
Marsh IIIb	28	29	31	28
Marsh IIIc	29	52	25	53
	$P < 0.001$		$P < 0.001$	

EmA: Endomysial; TG2-ab: Transglutaminase 2 antibody.

Table 4 Association between endomysial antibody titers and small-bowel mucosal damage

EmA titer	Subjects	Marsh 0-II, *n* (%)	Marsh III, *n* (%)
1: ≥ 5	372	57 (15)	315 (85)
1: ≥ 50	323	39 (12)	284 (88)
1: ≥ 100	282	26 (9)	256 (91)
1: ≥ 200	243	22 (9)	221 (91)
1: ≥ 500	155	9 (6)	146 (94)
1: ≥ 1000	96	3 (3)	93 (97)
1: ≥ 2000	48	2 (4)	46 (96)
1:4000	20	0 (0)	20 (100)

EmA: Endomysial.

Serum TG2-ab were measured in 316 EmA positive subjects and proved positive in 286 (91%) of them. Altogether 41% of the participants had high EmA and 54% high TG2-ab value defined at baseline. There was a significant association between serum TG2-ab level and clinical presentation, low antibody values being more common in the screen- than symptom-detected subjects (Table 2). A similar trend was observed with EmA, but the results were not statistically significant ($P = 0.061$).

Small-bowel mucosal villous atrophy and crypt hyperplasia (Marsh III) were found in altogether 85% of the EmA-positive subjects. There was a significant association between high antibody values and more severe small-bowel mucosal deterioration; in total 94% of those with high EmA titer evinced villous atrophy (Table 3). There was in this respect no significant difference between children and adults. The percentage of subjects evincing severe small-bowel mucosal damage increased progressively with higher EmA titers, but only the highest titer 1:4000 was 100% predictive of subsequent villous atrophy and crypt hyperplasia (Table 4).

In total, 40 patients had low and 17 high serum antibody values without simultaneous villous atrophy (Table 5). Irrespective of the baseline titers, 45 (79%) of these subjects (96% of those who remained on follow-up) either subsequently developed villous atrophy while on a gluten-containing diet, or experienced a positive clinical and serological response and disappearance of early mucosal changes on a gluten-free diet (Table 5). The pres-

Table 5 Baseline and follow-up data on subjects with positive endomysial antibodies but normal small-bowel mucosal villous structure

	Low[1] EmA and TG2-ab, *n* = 40	High EmA or TG2-ab, *n* = 17
Baseline		
Age, median (range), yr	39 (5-68)	39 (6-70)
Females, *n* (%)	30 (75)	11 (65)
Age below 18 yr	7 (18)	7 (41)
Gastrointestinal symptoms	28 (70)	12 (71)
Extraintestinal symptoms	2 (5)	4 (23)
Screen-detected subjects	10 (25)	1 (6)
EmA, median (range), titer	1:50 (1:5-1:200)	1:500 (1:5-1:2000)
TG2-ab, median (range), U/L	6.3 (0-24.8)	45.5 (13.9-> 100)
HLA DQ2 or DQ8, *n* (%)	33/33 (100)	16/16 (100)
Marsh 0	9 (23)	3 (18)
Marsh I - II	31 (77)	14 (82)
Follow-up		
Celiac disease diagnosis	29 (73)	16 (94)
Villous atrophy later[2]	12 (30)	8 (47)
Positive response to GFD	17 (43)	8 (47)
Gluten, no villous atrophy	2 (5)	0
Lost to follow-up	9 (22)	1 (6)

[1]EmA titer 1: < 500, TG2-ab value < 30.0 U/L; [2]Up to 10 yr of follow-up. EmA: Endomysial; TG2-ab: Transglutaminase 2 antibody; HLA: Human leukocyte antigen; GFD: Gluten-free diet.

ence of the celiac disease-associated HLA-DQ2 or DQ8 genotype was assessed in 299 EmA positive subjects and was found in all of them.

DISCUSSION

In our large series consisting of both children and adults, approximately half of the participants evinced high serum EmA levels, which was indicative of subsequent small-bowel mucosal villous damage in up to 94% of them. The results showed a high antibody titer to be an excellent predictor of villous atrophy and celiac disease also in high disease prevalence areas and in subjects with subtle or atypical symptoms. In the past few decades it has been observed that besides the classical gastrointestinal presentation, celiac disease patients may have a wide range of different extraintestinal symptoms. The patients may suffer for example from arthralgia or arthritis, osteoporosis, infertility and different neurological symptoms. In addition, screen-detected celiac patients may show only minor laboratory abnormalities or have no symptoms at all[3]. It was essential to investigate the performance of the celiac autoantibodies also in these atypical patients, as they are frequently seen in clinical practice, and may in fact represent the most common clinical presentation of celiac disease[16].

In patients with classical gastrointestinal celiac disease, Valdimarsson *et al*[8] observed a 100% positive predictive value of EmA for celiac disease in 19 adults, and suggested that histological confirmation might not be necessary in all such seropositive patients. Recently, similar results have been obtained with high values of TG2-ab. In a study by Barker *et al*[10], 48 out of 49 children having TG2-ab more than five times the upper limit of normal (ULN) had diagnostic small bowel mucosal damage. Likewise, Donaldson *et al*[11] observed a 100% positive predictive value for celiac disease by using the same cut-off level. In adults, Hill *et al*[13] suggested that TG2-ab levels more than ten times ULN would be exclusively indicative for celiac disease, and some other authors have presented comparable results[12].

Our findings thus largely accord with those in earlier studies carried out in specialized centers with high-risk patients having classical gastrointestinal presentation of celiac disease. Nevertheless, in the present study there was still a subpopulation of individuals in whom the current histological criteria were not fulfilled. Approximately 6 % of the participants with high and up to 21% of those with low antibody values had normal small-bowel mucosal villous structure, and in total this was seen in 15% of the EmA-positive subjects. It could thus be argued that EmA are not sufficiently specific for a definite diagnosis of celiac disease as such. Interestingly, however, there is an increasing body of data showing that EmA positivity is a very strong predictor of forthcoming celiac disease also in subjects with initially normal villi[17,22-26]. In line with this conception, almost all of our EmA positive patients who had no structural villous damage either evinced a positive serological, clinical and histological response to a gluten-free diet, or subsequently developed villous atrophy while on a normal diet. The existence of a celiac-type disorder in these individuals was further supported by the presence of the relevant HLA type in all in whom it was measured. There is thus strong evidence that, irrespective of the initially normal villous morphology, these EmA positive subjects are truly suffering from celiac disease.

Our study is subject to some limitations. First, although a high percentage of the participants had mild or atypical clinical presentation, the number of those found by population-based serological screening was rather low. Consequently, the results cannot be generalized to this patient group, and further studies are needed[27]. Secondly, the mucosal biopsies were taken from the distal duodenum as previously recommended[28]. Judging from recent evidence, however, villous atrophy can occasionally be detected only in the bulb area of the small intestine, and in theory celiac disease might in such cases already have been confirmed at the time of the first biopsy[7]. Nevertheless, interpretation of bulb specimens may be biased on Brunner glands or peptic inflammation, and their role in the diagnostics remains controversial. In addition, a patchy small-bowel mucosal lesion is always possible in celiac disease, which further highlights the importance of serology in the diagnosis.

Although EmA shows excellent specificity for an untreated celiac disease, it has certain limitations. The immunofluerescence method is laborious, time-consuming and always somewhat subjective. Since TG2-abs can be easily measured using a practical ELISA method, it would be tempting to use it instead of EmA. Neverthe-

less, TG2-ab are measured by commercial tests which use different epitopes of TG2 as antigen, and thus the specificity figures for the method have been somewhat inconsistent. Consequently, the positive predictive value of TG2-ab has sometimes been rather low, particularly in low-risk populations[29]. TG2-ab can also be positive in some conditions such as in liver diseases[30]. For these reasons, we decided to use the more laborious and time-consuming but celiac disease-specific EmA as the primary inclusion criterion in our series. As a consequence, the results should not be applied to TG2-ab positive EmA negative subjects. Finally, since antibody-negative subjects were not included in our study, the overall sensitivity of the serological tests could not be obtained.

To conclude, EmA positivity as such is a very strong predictor of a subsequent celiac disease diagnosis also in patients with low serum antibody titers and subtle or atypical clinical presentation. Judging from the findings here, invasive endoscopic studies might not be obligatory in all such seropositive patients.

COMMENTS

Background

The diagnosis of celiac disease is based on the presence of small-bowel mucosal villous atrophy and crypt hyperplasia, but this histological definition involves several problems. Since particularly endomysial (EmA) and high values of transglutaminase 2 antibodies (TG2-ab) seem to predict celiac disease with high specificity, it has been advocated that in seropositive subjects with gastrointestinal symptoms endoscopic studies might not be obligatory to establish the diagnosis.

Research frontiers

New diagnostic criteria of celiac disease based to serological tests might make the burdensome and invasive endoscopic investigations unnecessary. The authors aimed to investigate the association between serum antibody levels and a subsequent celiac disease diagnosis in children and adults with a heterogeneous clinical presentation.

Innovations and breakthroughs

The results showed that high positive values of serological tests are excellent predictors of subsequent villous atrophy and celiac disease also in subjects with atypical or subtle clinical presentation. In addition, positivity of EmA is a very strong indicator of celiac disease diagnosis irrespective of the baseline titers.

Applications

These results indicate that gastrointestinal endoscopy might be omitted and celiac disease diagnosis established without further histological confirmation in children and adults with positive EmA.

Terminology

Celiac disease is a chronic autoimmune-based triggered by ingested gluten in genetically susceptible individuals. EmA and TG2-ab are serological test with high accuracy for an untreated celiac disease.

Peer review

This is a nice study that highlights the significance of positive EmA results in a large series of children and adults. The paper is well-written and adds pertinent information to the literature.

REFERENCES

1 **Fasano A**, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, Elitsur Y, Green PH, Guandalini S, Hill ID, Pietzak M, Ventura A, Thorpe M, Kryszak D, Fornaroli F, Wasserman SS, Murray JA, Horvath K. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med* 2003; **163**: 286-292

2 **Lohi S**, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, Lohi O, Bravi E, Gasparin M, Reunanen A, Mäki M. Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther* 2007; **26**: 1217-1225

3 **Green PH**, Cellier C. Celiac disease. *N Engl J Med* 2007; **357**: 1731-1743

4 Revised criteria for diagnosis of coeliac disease. Report of Working Group of European Society of Paediatric Gastroenterology and Nutrition. *Arch Dis Child* 1990; **65**: 909-911

5 **Collin P**, Kaukinen K, Vogelsang H, Korponay-Szabó I, Sommer R, Schreier E, Volta U, Granito A, Veronesi L, Mascart F, Ocmant A, Ivarsson A, Lagerqvist C, Bürgin-Wolff A, Hadziselimovic F, Furlano RI, Sidler MA, Mulder CJ, Goerres MS, Mearin ML, Ninaber MK, Gudmand-Høyer E, Fabiani E, Catassi C, Tidlund H, Alainentalo L, Mäki M. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. *Eur J Gastroenterol Hepatol* 2005; **17**: 85-91

6 **Scott BB**, Losowsky MS. Patchiness and duodenal-jejunal variation of the mucosal abnormality in coeliac disease and dermatitis herpetiformis. *Gut* 1976; **17**: 984-992

7 **Gonzalez S**, Gupta A, Cheng J, Tennyson C, Lewis SK, Bhagat G, Green PH. Prospective study of the role of duodenal bulb biopsies in the diagnosis of celiac disease. *Gastrointest Endosc* 2010; **72**: 758-765

8 **Valdimarsson T**, Franzen L, Grodzinsky E, Skogh T, Ström M. Is small bowel biopsy necessary in adults with suspected celiac disease and IgA anti-endomysium antibodies? 100% positive predictive value for celiac disease in adults. *Dig Dis Sci* 1996; **41**: 83-87

9 **Scoglio R**, Di Pasquale G, Pagano G, Lucanto MC, Magazzù G, Sferlazzas C. Is intestinal biopsy always needed for diagnosis of celiac disease? *Am J Gastroenterol* 2003; **98**: 1325-1331

10 **Barker CC**, Mitton C, Jevon G, Mock T. Can tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations? *Pediatrics* 2005; **115**: 1341-1346

11 **Donaldson MR**, Firth SD, Wimpee H, Leiferman KM, Zone JJ, Horsley W, O'Gorman MA, Jackson WD, Neuhausen SL, Hull CM, Book LS. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. *Clin Gastroenterol Hepatol* 2007; **5**: 567-573

12 **Donaldson MR**, Book LS, Leiferman KM, Zone JJ, Neuhausen SL. Strongly positive tissue transglutaminase antibodies are associated with Marsh 3 histopathology in adult and pediatric celiac disease. *J Clin Gastroenterol* 2008; **42**: 256-260

13 **Hill PG**, Holmes GK. Coeliac disease: a biopsy is not always necessary for diagnosis. *Aliment Pharmacol Ther* 2008; **27**: 572-577

14 **Vivas S**, Ruiz de Morales JG, Riestra S, Arias L, Fuentes D, Alvarez N, Calleja S, Hernando M, Herrero B, Casqueiro J, Rodrigo L. Duodenal biopsy may be avoided when high transglutaminase antibody titers are present. *World J Gastroenterol* 2009; **15**: 4775-4780

15 **Sugai E**, Moreno ML, Hwang HJ, Cabanne A, Crivelli A, Nachman F, Vázquez H, Niveloni S, Argonz J, Mazure R, La Motta G, Caniggia ME, Smecuol E, Chopita N, Gómez JC, Mauriño E, Bai JC. Celiac disease serology in patients with different pretest probabilities: is biopsy avoidable? *World J Gastroenterol* 2010; **16**: 3144-3152

16 **Collin P**, Huhtala H, Virta L, Kekkonen L, Reunala T. Diagnosis of celiac disease in clinical practice: physician's alertness to the condition essential. *J Clin Gastroenterol* 2007; **41**: 152-156

17 **Kurppa K**, Collin P, Viljamaa M, Haimila K, Saavalainen P, Partanen J, Laurila K, Huhtala H, Paasikivi K, Mäki M, Kaukinen K. Diagnosing mild enteropathy celiac disease: a randomized, controlled clinical study. *Gastroenterology* 2009; **136**: 816-823

18 **Tosco A**, Salvati VM, Auricchio R, Maglio M, Borrelli M, Coruzzo A, Paparo F, Boffardi M, Esposito A, D'Adamo G, Malamisura B, Greco L, Troncone R. Natural history of potential celiac disease in children. *Clin Gastroenterol Hepatol* 2011; **9**: 320-335; quiz e336

19 **Ladinser B**, Rossipal E, Pittschieler K. Endomysium antibodies in coeliac disease: an improved method. *Gut* 1994; **35**: 776-778

20 **Marsh MN**. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ("celiac sprue"). *Gastroenterology* 1992; **102**: 330-354

21 **Oberhuber G**, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**: 1185-1194

22 **Mäki M**, Holm K, Koskimies S, Hällström O, Visakorpi JK. Normal small bowel biopsy followed by coeliac disease. *Arch Dis Child* 1990; **65**: 1137-1141

23 **Troncone R**. Latent coeliac disease in Italy. The SIGEP Working Group on Latent Coeliac Disease. Italian Society for Paediatric Gastroenterology and Hepatology. *Acta Paediatr* 1995; **84**: 1252-1257

24 **Kaukinen K**, Mäki M, Partanen J, Sievänen H, Collin P. Celiac disease without villous atrophy: revision of criteria called for. *Dig Dis Sci* 2001; **46**: 879-887

25 **Paparo F**, Petrone E, Tosco A, Maglio M, Borrelli M, Salvati VM, Miele E, Greco L, Auricchio S, Troncone R. Clinical, HLA, and small bowel immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa. *Am J Gastroenterol* 2005; **100**: 2294-2298

26 **Kurppa K**, Ashorn M, Iltanen S, Koskinen LL, Saavalainen P, Koskinen O, Mäki M, Kaukinen K. Celiac disease without villous atrophy in children: a prospective study. *J Pediatr* 2010; **157**: 373-380, 380.e1

27 **van Koppen EJ**, Schweizer JJ, Csizmadia CG, Krom Y, Hylkema HB, van Geel AM, Koopman HM, Verloove-Vanhorick SP, Mearin ML. Long-term health and quality-of-life consequences of mass screening for childhood celiac disease: a 10-year follow-up study. *Pediatrics* 2009; **123**: e582-e588

28 **Hill ID**, Dirks MH, Liptak GS, Colletti RB, Fasano A, Guandalini S, Hoffenberg EJ, Horvath K, Murray JA, Pivor M, Seidman EG. Guideline for the diagnosis and treatment of celiac disease in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2005; **40**: 1-19

29 **Hopper AD**, Cross SS, Hurlstone DP, McAlindon ME, Lobo AJ, Hadjivassiliou M, Sloan ME, Dixon S, Sanders DS. Pre-endoscopy serological testing for coeliac disease: evaluation of a clinical decision tool. *BMJ* 2007; **334**: 729

30 **Villalta D**, Crovatto M, Stella S, Tonutti E, Tozzoli R, Bizzaro N. False positive reactions for IgA and IgG anti-tissue transglutaminase antibodies in liver cirrhosis are common and method-dependent. *Clin Chim Acta* 2005; **356**: 102-109

S- Editor Shi ZF **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2517

World J Gastroenterol 2012 May 28; 18(20): 2517-2525
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Efficacy of mosapride citrate with polyethylene glycol solution for colonoscopy preparation

Masahiro Tajika, Yasumasa Niwa, Vikram Bhatia, Hiroki Kawai, Shinya Kondo, Akira Sawaki, Nobumasa Mizuno, Kazuo Hara, Susumu Hijioka, Kazuya Matsumoto, Yuji Kobayashi, Akira Saeki, Asana Akabane, Koji Komori, Kenji Yamao

Masahiro Tajika, Yasumasa Niwa, Hiroki Kawai, Shinya Kondo, Department of Endoscopy, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan
Vikram Bhatia, Department of Medical Hepatology, Institute of Liver and Biliary Sciences, New Delhi 110070, India
Akira Sawaki, Nobumasa Mizuno, Kazuo Hara, Susumu Hijioka, Kazuya Matsumoto, Yuji Kobayashi, Akira Saeki, Asana Akabane, Kenji Yamao, Department of Gastroenterology, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan
Koji Komori, Department of Gastroenterological Surgery, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan
Author contributions: Tajika M contributed to study conception and design, analysis, and interpretation of the data, and drafting of the article; Niwa Y and Yamao K provided critical revision of the article for important intellectual content and final approval of the article; Bhatia V drafted the article and provided critical revision; Tajika M, Kawai H, Kondo S, Sawaki A, Mizuno N, Hara K, Hijioka S, Matsumoto K, Kobayashi Y, Saeki A and Akabane A performed the research and collected data; Komori K performed the data analysis.
Correspondence to: Masahiro Tajika, MD, PhD, Department of Endoscopy, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. mtajika@aichi-cc.jp
Telephone: +81-52-7626111 Fax: +81-52-7635233
Received: July 8, 2011 Revised: September 22, 2011
Accepted: February 27, 2012
Published online: May 28, 2012

Abstract

AIM: To evaluate the efficacy and safety of adjunctive mosapride citrate for bowel preparation before colonoscopy.

METHODS: We conducted a randomized, double-blind, placebo-controlled study with mosapride in addition to polyethylene glycol (PEG)-electrolyte solution. Of 250 patients undergoing colonoscopy, 124 were randomized to receive 2 L PEG plus 15 mg of mosapride citrate (mosapride group), and 126 received 2 L PEG plus placebo (placebo group). Patients completed a questionnaire reporting the acceptability and tolerability of the bowel preparation process. The efficacy of bowel preparation was assessed by colonoscopists using a 5-point scale based on Aronchick's criteria. The primary end point was optimal bowel preparation rates (scores of excellent/good/fair *vs* poor/inadequate).

RESULTS: A total of 249 patients were included in the analysis. In the mosapride group, optimal bowel preparation rates were significantly higher in the left colon compared with the placebo group (78.2% *vs* 65.6%, $P < 0.05$), but not in the right colon (76.5% *vs* 66.4%, $P = 0.08$). After excluding patients with severe constipation, there was a significant difference in bowel preparation in both the left and right colon (82.4% *vs* 66.7%, 80.8% *vs* 67.5%, $P < 0.05$, $P < 0.01$). The incidence of adverse events was similar in both groups. Among the subgroup who had previous colonoscopy experience, a significantly higher number of patients in the mosapride group felt that the current preparation was easier compared with patients in the placebo group (34/72 patients *vs* 24/74 patients, $P < 0.05$).

CONCLUSION: Mosapride citrate may be an effective and safe adjunct to PEG-electrolyte solution that leads to improved quality of bowel preparation, especially in patients without severe constipation.

Key words: Mosapride citrate; Bowel preparation; Polyethylene glycol-electrolyte solution; Colonoscopy; Prokinetics

Peer reviewers: Vito Annese, MD, Associate Professor, Department of Surgical and Medical Sciences, Unit of Gastroenterology, University Hospital Careggi, Pad. S. Luca Nuovo 16c, Largo Brambilla, 3, 50134 Florence, Italy; Luis Bujanda, PhD, Profes-

sor, Departament of Gastroenterology, CIBEREHD, University of Country Basque, Donostia Hospital, Paseo Dr. Beguiristain s/n, 20014 San Sebastián, Spain; Dr. Mitsuhiro Fujishiro, Department of Gastroenterology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

Tajika M, Niwa Y, Bhatia V, Kawai H, Kondo S, Sawaki A, Mizuno N, Hara K, Hijioka S, Matsumoto K, Kobayashi Y, Saeki A, Akabane A, Komori K, Yamao K. Efficacy of mosapride citrate with polyethylene glycol solution for colonoscopy preparation. *World J Gastroenterol* 2012; 18(20): 2517-2525 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2517.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2517

INTRODUCTION

Polyethylene glycol (PEG)-electrolyte solution is used worldwide for bowel cleansing. Approximately 2 L of this oral solution, along with a laxative, is usually required for adequate bowel preparation in Japan[1]. However, the need to drink such large volumes of liquid with an unpalatable taste has a negative impact on patient compliance[2]. A thorough bowel preparation is required for safe and effective colonoscopy, and inadequate preparation not only decreases the sensitivity, but also increases the difficulty of the procedure[3-5]. Therefore, more effective bowel preparation regimens for colonoscopy are required to improve the acceptability and tolerability of the procedure. Prokinetics such as domperidone, metoclopramide, and cisapride have been used in combination with PEG-electrolyte solution to improve the quality of bowel preparation[6-12]. However, the addition of prokinetic agents to PEG-electrolyte solution has not been proven to improve patient tolerance or colonic cleansing[10-12] and is sometimes associated with serious adverse effects. For example, domperidone and metoclopramide may cause extrapyramidal symptoms with long-term use[13]. Cisapride was withdrawn from the market because of severe cardiac side effects, including QT-interval prolongation and ventricular arrhythmias[14]. Thus, safer and more effective prokinetic agents are needed.

Mosapride citrate (mosapride) is a selective 5-hydroxytryptamine-4 (5-HT4) receptor agonist. Mosapride enhances gastric emptying and motility by facilitating acetylcholine release from the enteric cholinergic neurons, without blocking dopaminergic D2 receptors[15]. It is known to be effective in gastroesophageal reflex disease[16], functional gastrointestinal disorders, such as functional dyspepsia[17], chronic gastritis with delayed gastric emptying, and diabetic gastroparesis[18]. As 5-HT4 receptors are also located in the human colon and rectum[19,20], mosapride is also expected to have a prokinetic effect on the colo-rectum. A few clinical studies have reported that mosapride in combination with PEG may enhance bowel cleansing and improve patient acceptability and tolerability[21,22]. However, the efficacy and tolerability of a PEG-electrolyte solution with or without mosapride has not been studied in a double-blind, randomized trial.

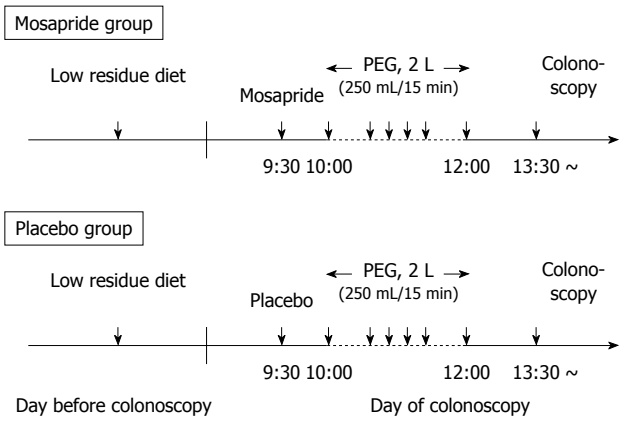


Figure 1 Steps of preparation for colonoscopy.

We conducted this study to evaluate the efficacy, acceptability, and tolerability of mosapride as an adjuvant to PEG-electrolyte solution for colonoscopy preparation.

MATERIALS AND METHODS

This was a prospective, double-blind, randomized, controlled study that included patients who underwent colonoscopy at Aichi Cancer Center Hospital (ACCH), Nagoya, from January 2009 to October 2009. This study was reviewed and approved by the ethics committee of ACCH.

Study population

All consecutive outpatients aged 20-80 years who were scheduled for colonoscopy at ACCH were evaluated for study inclusion. Patients with the following clinical features were excluded: presence of significant cardiac, renal, hepatic, or metabolic comorbidities; presence of ascites or bowel obstruction; known allergy to PEG-electrolyte solution; history of gastric stapling or bypass procedure; or a history of prior colonic or rectal surgery. A gastroenterologist assessed patient eligibility, and written informed consent was obtained from each patient prior to inclusion.

Randomization and blinding

Patients were randomly allocated to receive one of two bowel preparation regimens using a computer-generated random-number list. Patients were randomized in block sizes of two, with serially numbered, sealed, opaque envelopes. Concealed allocation was accomplished through non-research personnel who were not involved in this study. Comparisons between subjects who received 2 L PEG plus mosapride (mosapride group) and 2 L PEG plus placebo (placebo group) were made in a double-blind fashion.

Bowel preparation methods

The colonoscopy preparation steps used in this study are shown in Figure 1. The day before colonoscopy, all patients were instructed to eat a pre-packaged, low-residue diet (Enimaclin CS; Horii Pharmaceutical Ind., Ltd., Osaka, Japan) that consisted of a lunch, snack, and dinner, and were asked to drink more than 2 L of clear

liquid. On the day of the colonoscopy, all participants reported to the endoscopy room at 9:00 am, and received in-hospital bowel preparation. In-hospital preparation is important to ensure the uniformity of procedures within the study, and to remove any confounding caused by poor patient adherence. More than 10 toilet facilities were made available in the endoscopy unit for patient comfort. Six mosapride tablets (15 mg) (Gasmotin; Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan) or six identical-looking placebo tablets were administered orally with water at 09:30. The timing of administration of the mosapride tablets was based on its pharmacokinetics[23]. After 30 min, both groups were instructed to drink 0.25 L of PEG-electrolyte solution (Niflec; Ajinomoto Pharmaceticals Co., Ltd., Tokyo, Japan) every 15 min.

Evaluation of bowel preparation

The efficacy of bowel preparation was assessed using Aronchick's criteria[24], as follows: (1) Excellent (small volume of clear liquid, or greater than 95% of colonic surface seen); (2) Good (large volume of clear liquid covering 5% to 25% of colonic surface, but greater than 90% of surface seen); (3) Fair (some semisolid stool that could be suctioned or washed away, but greater than 90% of surface seen); (4) Poor (some semisolid stool that could not be suctioned or washed away, and less than 90% of surface seen); and (5) Inadequate (repeat preparation and colonoscopy needed). Participating endoscopists were trained to use Aronchick's criteria to achieve a good level of agreement. Investigators performed calibration exercises involving more than 20 colonoscopies prior to study commencement, based on their interpretation of scale anchors, to ensure that their findings agreed. The final assessment of bowel preparation was divided into two categories: optimal and non-optimal. Bowel preparations rated as fair, good, or excellent, based on Aronchick's criteria, were considered optimal; poor or inadequate ratings were considered non-optimal. After colonoscopy, two observers, including the endoscopist performing the procedure, determined the score by mutual agreement. They scored the quality of the preparation in the right colon (proximal to the splenic flexure), and the left colon (distal to the splenic flexure) and rectum separately. If the decision was discordant, a third expert reviewer graded and scored the recorded images, and this evaluation was used in the final analysis.

During or immediately following the colonoscopy, the investigator completed a physician questionnaire regarding the assessment of bowel preparation, amount of irrigation fluid used, time to reach the cecum, and ease of insertion into the cecum and visualization of the colonic lumen regardless of peristalses.

Patient tolerance and other measurements

The nursing staff recorded the time required to drink the indicated volume of lavage solution. They also recorded the time and number of motions from start of ingestion to the appearance of clear excretions. The nursing staff checked excretions until 1 h after patients finished the PEG + mosapride solution. If there was a solid stool with muddy excretions or no excretion at that time, we gave the patient an additional preparation, such as additional PEG or enema. A warm water enema of 500 mL volume was given until the excretions were clear. Patients who received an additional preparation were defined by Aronchick's criteria as inadequate. The patient questionnaire, which was administered before bowel cleansing, consisted of 20 questions pertaining to patient characteristics, tolerability, and acceptability of study medication. It also included questions about the following: age; height; body weight; average number of bowel movements per week for the last year; number of previous colonoscopies; compliance with ingestion of PEG-electrolyte solution; willingness to repeat the same preparation regimen again, if required; ease/difficulty of taking the preparation compared with previous experiences; and presence of subjective symptoms while drinking PEG-electrolyte solution, such as nausea, vomiting, fullness, abdominal pain, and circulatory reactions such as palpitations or chest discomfort. We defined patients who suffered from constipation (defined as < 2 bowel movements per week) for > 1 year as having severe constipation. Patients completed the questionnaire before undergoing the colonoscopy and submitted the form to the nursing staff.

Endpoints

The primary endpoint was the difference in optimal rate of colon cleansing in the mosapride and placebo groups. Secondary endpoints included differences in patients' acceptability and tolerance of solutions, time to first defecation, frequency of defecation, complete time for colonic preparation, time needed to reach the cecum, amount of irrigation fluid used, and subjective difficulty in colonoscopy insertion to the cecum and in observing the lumen of the colo-rectum because of peristalses.

Statistical analysis

The study was designed to detect an inter-group difference of 11% in the percentage of patients with optimal bowel preparation, with an α error of 5% and a power of 80%. This difference was based on a previous study[21]. The number of patients needed to demonstrate an 11% difference was 125 per treatment group, assuming a drop-out rate of 10%.

The primary efficacy analysis was based on an intent-to-treat analysis and included patients who were randomized and received any treatment. The preparation of patients in this group was considered optimal or non-optimal based on the colonoscopist's score regarding cleansing. Patients who did not undergo colonoscopy because of preparation-related adverse events, or preparation failure, or in whom the right colon could not be reached because of bowel obstruction or for technical reasons were excluded. The rates of optimal preparation were compared between the groups by the Chi-square test or Fisher's exact test for categorical variables.

Table 1 Baseline characteristics

Variable	Overall		Excluding patients with severe constipation		*P* value	
	A (mosapride)	B (placebo)	C (mosapride)	D (placebo)	A *vs* B	C *vs* D
No. of patients	124	125	108	120		
Age (yr, mean ± SD)	67.3 ± 8.6	67.8 ± 10.1	67.3 ± 8.5	67.5 ± 10.2	NS	NS
< 60	21	21	18	21		
60-69	42	44	37	42	NS	NS
≥ 70	61	60	53	57		
Male	69	83	64	80	NS	NS
Female	55	42	44	40		
Body mass index (kg/m^2, mean ± SD)	22.5 ± 2.9	22.6 ± 2.7	22.7 ± 2.9	22.7 ± 2.6	NS	NS
Bowel movements per week						
< 2	16	5	0	0	< 0.05	NS
≥ 2	108	120	108	120		
Previous colonoscopy						
None (first time)	38	27	32	26	NS	NS
≥ 2	86	98	76	94		

P value by the χ^2 test. NS: Not significant.

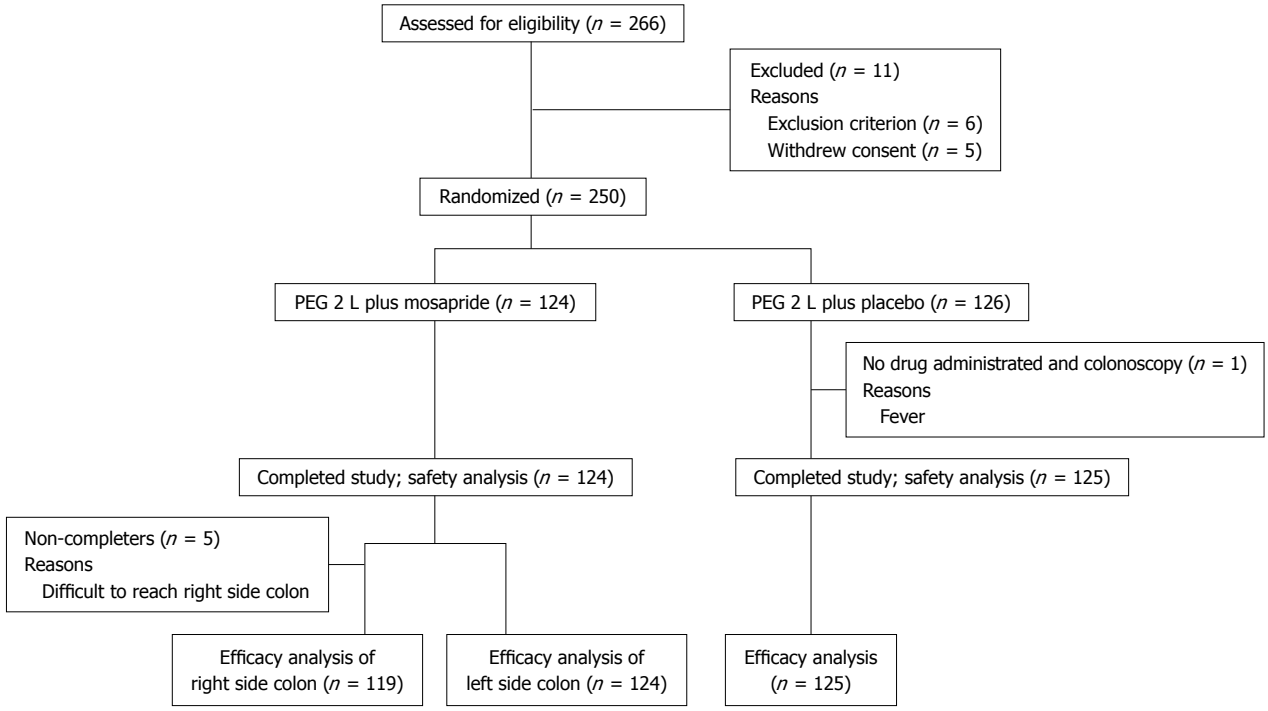


Figure 2 Patient disposition flow chart. PEG: Polyethylene glycol.

For secondary endpoints, the Mann-Whitney *U* test was used for comparison between continuous variables. Categorical variables were analyzed using the corrected χ^2 or two-sided Fisher's exact tests, where appropriate. The criterion for statistical significance was $P < 0.05$.

All statistical analyses were performed using Statistical Analysis Software (SPSS, version 12.0J for the PC, SPSS Japan, Inc., Tokyo, Japan).

RESULTS

Patient characteristics

A total of 250 patients were randomized into two groups (Figure 2). Of those randomized to treatment, only one patient did not receive any treatment or undergo colonoscopy because he felt chilled and had a fever before treatment. Although 249 patients were analyzed, insertion of the colonoscope into the right colon failed in five (4%) patients in the mosapride group (advanced stenosing cancer in three and patient refusal in two), because of pain on colonoscopic advancement to the proximal colon. These five patients were excluded from the efficacy analysis of the right colon. Baseline characteristics of the patients are shown in Table 1. Differences in age, gender, body mass index, and the number of previous colonoscopies between the mosapride and placebo groups were not significant. However, significantly more patients suffering from severe constipation (defined as < 2 bowel

Table 2 Results of the preparation and endoscopic findings

Variable	Overall		Excluding patients with severe constipation		*P* value	
	A (mosapride)	B (placebo)	C (mosapride)	D (placebo)	A *vs* B	C *vs* D
No. of patients	124	125	108	120		
Time to first defecation (min, mean ± SD)	55.4 ± 27.3	71.2 ± 28.6	52.9 ± 26.2	70.4 ± 28.9	< 0.001	< 0.001
Frequency of defecation (times, median, quartile)	8.3 (4-18)	8.6 (4-18)	8.3 (4-18)	8.0 (4-18)	NS	NS
Time of bowel preparation (min, mean ± SD)	185.1 ± 63.8	198.0 ± 76.5	178.6 ± 58.2	198.0 ± 76.6	0.11	< 0.05
Elapsed time from last fluid intake to colonoscopy (min, mean ± SD)	154.6 ± 48.9	154.1 ± 48.2	157.1 ± 63.2	155.5 ± 62.1	NS	NS
Cecal intubation rate, *n* (%)	119 (96.0)	124 (99.2)	108 (100)	120 (100)	NS	NS
Insertion time[1] (min, median, quartiles)	7.8 (2-55)	8.5 (2-38)	7.4 (2-55)	8.5 (2-38)	NS	NS
Feel of peristalsis, *n* (%)	20 (16.1)	22 (17.6)	14 (13.0)	22 (18.3)	NS	NS
Amount of irrigation fluid						
None	67	65	57	62		
< 50 mL	40	47	33	46	NS	NS
50-100 mL	15	11	12	11		
> 100 mL	2	1	2	1		
Endoscopic findings						
Cancer	5	5	3	5		
Polyps	70	82	61	79	NS	NS
Diverticulosis	32	39	29	38		

P value by the Mann-Whitney *U* test. [1]Insertion time was based on patients in whom the cecal portion of the colon examined. NS: Not significant.

Table 3 Efficacy of overall colon-cleansing

Variable	Right colon		Left colon and rectum		*P* value	
	Mosapride	Placebo	Mosapride	Placebo	Right	Left
No. of patients	119	125	124	125		
Overall score						
Excellent	39	24	48	33	< 0.05	< 0.05
Good	34	38	37	39	NS	NS
Fair	18	21	12	10	NS	NS
Poor	3	3	2	4	NS	NS
Inadequate	25	39	25	39	0.07	0.06
Optimal ratings, *n* (%)	91 (76.5)	83 (66.4)	97 (78.2)	82 (65.6)	0.08	< 0.05

P value by the χ^2 test. NS: Not significant.

movements per week for > 1 year) were included in the mosapride group compared with the placebo group ($P < 0.05$). Therefore, we compared the efficacy, acceptability, and tolerability of the bowel preparation solution in subgroups of patients with or without severe constipation.

Bowel cleansing efficacy

As shown in Table 2, time to first defecation was significantly shorter in the mosapride group compared with the placebo group ($P < 0.001$). After excluding patients with severe constipation, the completion time for bowel preparation was significantly shorter in the mosapride group compared with the placebo group ($P < 0.05$). There were no differences in frequency of defecation, the elapsed time from last fluid intake to colonoscopy, time needed to reach the cecum, amount of irrigation fluid used, subjective difficulties in insertion to the cecum, and in observing the lumen of the colo-rectum between groups, or in the frequency of positive endoscopic findings.

The efficacy of bowel preparation is shown in Table 3. Twenty-five (20.2%) patients required additional preparation in the mosapride group (mean 0.6 L additional PEG in 22 patients, 0.5 L enema in one patient, and both in two patients). Thirty-eight (30.4%) patients required additional preparation in the placebo group (mean 0.75 L PEG in 35 patients, 0.5 L enema in one patient, and both in two patients).

In the right colon, the number of bowel preparations rated as excellent was significantly higher in the mosapride group than in the placebo group ($P < 0.05$). However, the rate of optimal preparations did not differ significantly between groups ($P = 0.08$) (Table 3). After excluding patients with severe constipation, there were significant differences in the number of bowel preparations rated as excellent and the rate of optimal preparation in the mosapride group ($P < 0.01$ and $P < 0.05$ in the right colon and $P < 0.05$ and $P < 0.01$ in the left colon, respectively) (Table 4). In the left colon and rectum, the number of bowel preparations rated as excellent and the rate of optimal preparation were significantly higher

Table 4 Results of colon-cleansing efficacy excluding patients with severe constipation

Variable	Right colon		Left colon and rectum		*P* value	
	Mosapride	Placebo	Mosapride	Placebo	Right	Left
No. of patients	104	120	108	120		
Overall score						
Excellent	37	24	46	33	< 0.01	< 0.05
Good	31	37	33	38	NS	NS
Fair	16	20	10	9	NS	NS
Poor	2	3	1	4	NS	NS
Inadequate	18	36	18	36	< 0.05	< 0.05
Optimal ratings, *n* (%)	84 (80.8)	81 (67.5)	89 (82.4)	80 (66.7)	< 0.05	< 0.01

P value by the χ^2 test. NS: Not significant.

Table 5 Results of patient questionnaire *n* (%)

Variable	Overall		Excluding patients with severe constipation		*P* value	
	A (mosapride)	B (placebo)	C (mosapride)	D (placebo)	A *vs* B	C *vs* D
No. of patients	124	125	108	120		
Compliance > 80%	120 (96.8)	119 (95.2)	105 (97.2)	114 (95.0)	NS	NS
100% intake	112 (90.3)	115 (92.0)	97 (89.8)	110 (91.7)	NS	NS
Any symptom						
Nausea	5 (4.0)	6 (4.8)	3 (2.7)	4 (3.3)	NS	NS
Vomiting	0	1 (0.8)	0	0	NS	NS
Distension	40 (32.3)	31 (24.8)	31 (28.7)	28 (23.3)	NS	NS
Abdominal pain	4 (3.2)	2 (1.6)	3 (2.8)	2 (1.7)	NS	NS
Circulatory reactions	0	0	0	0	NS	NS
Willingness to repeat the same regimen	77/115 (66.9)	82/112 (73.2)	67/100 (67.0)	79/108 (73.1)	NS	NS
How easy/difficult to take preparation compared with previous one (easy/invariable/difficult)	34/42/10	24/67/7	32/37/8	23/66/5	< 0.05	< 0.05

P value by the χ^2 test. NS: Not significant.

in the mosapride group than in the placebo group ($P < 0.05$ and $P < 0.05$, respectively). These significant differences were maintained even after excluding patients with severe constipation ($P < 0.05$ and $P < 0.05$, respectively).

Patient tolerability and safety

There were no significant differences in compliance, as defined by > 80% and 100% intake of the PEG solution between the two groups (Table 5). Frequencies of symptoms such as nausea, vomiting, distention, abdominal pain, and circulatory reactions were similar in both groups. The proportion of patients who were willing to repeat the same preparation regimen was also similar in the two groups. However, among the subgroup of patients who had undergone a colonoscopy more than twice in the past, a significantly higher number of patients in the mosapride group felt that the current preparation was easier compared with patients in the placebo group ($P < 0.05$). This significant difference was maintained even after excluding patients with severe constipation ($P < 0.05$).

DISCUSSION

This is the first prospective, randomized, double-blind, placebo-controlled study to evaluate the efficacy, acceptability, and tolerance of mosapride as an adjuvant to PEG-electrolyte solution for colonoscopy preparation. Mosapride is a benzisoxazole derivative prokinetic drug that is used for the treatment of gastrointestinal symptoms associated with chronic gastritis and functional dyspepsia[16,17,25-28]. It facilitates acetylcholine release from the enteric cholinergic neurons by its selective 5-HT4 receptor agonistic action[26]. It is also active through its main metabolite M1, which is a 5-HT3 agonist. The action of mosapride resembles that of a previously used 5-HT4 agonist, cisapride, which had been reported to be useful for bowel preparation[29,30]. Cisapride had additional effects of blocking K channels and D2 dopaminergic receptors and was withdrawn after its K channel blocking properties led to reports of QT interval prolongation and cardiac arrhythmias. In contrast to cisapride, mosapride does not block K channels or D2 dopaminergic receptors and is believed to have less cardiac toxicity[31].

5-HT4 receptors are present in the myenteric plexus and the muscle of stomach and colon, and mosapride has high affinity for these receptors[20,29,32]. In human studies, mosapride has been found effective for slow transit constipation, outlet obstruction-type constipation, constipation in Parkinson's disease, and constipation associated with irritable bowel syndrome[33-35]. Recently, in guinea

pigs, it was reported that mosapride enhanced the colon cleansing action of PEG *via* an increase in colonic transit, reducing not only fecal residue but also excessive fluid in the colonic lumen[36]. However, it is unclear whether mosapride would have additive beneficial effects on bowel cleansing before colonoscopy in humans.

We found that the rate of optimal preparation was significantly higher in the mosapride group compared with the placebo group in the left colon and rectum, but not in the right colon. The number of patients with bowel preparations rated as excellent was significantly higher for the mosapride group compared with the placebo group, especially for the right colon. Kim *et al*[37] have also described this differential efficacy of mosapride between the right and left colon in guinea pigs, and ascribed this finding to the differential distribution of colonic 5-HT4 receptors. Although the rates of optimal preparation were not significantly different in the right colon, the number of bowel preparations rated as excellent was significantly higher with the use of mosapride. Furthermore, after excluding patients with severe constipation, the rate of optimal preparation was significantly higher in the mosapride group compared with the placebo group. These findings support the efficacy of mosapride for bowel preparation.

In this study, many patients required additional bowel preparation. One possible reason may be that the nursing staff checked excretions 1 h after finishing the preparation, which may have been too short an interval for the PEG solution to adequately cleanse the colon. However, the rate of inadequate cleansing was significantly lower in the mosapride group compared with the placebo group. Furthermore, the time to first defecation was also significantly shorter in the mosapride group. The beneficial effect of mosapride on gastric emptying was expected to ameliorate nausea, vomiting, and fullness of the abdomen during bowel preparation. Mishima *et al*[22] showed that administration of mosapride prior to PEG solution significantly decreased the incidence of uncomfortable abdominal symptoms. However, there were no significant differences in the frequencies of these symptoms between the mosapride group and the placebo group in this study, and more patients were willing to repeat the same preparation regimen in the placebo group. This finding may be due to more abdominal distension and pain in the mosapride group due to its prokinetic effects. According to a postmarketing surveillance study, the most common adverse events associated with mosapride are abdominal pain and loose stools (both 0.35%)[38]. On the other hand, a larger proportion of patients in the mosapride group than in the placebo group felt that the preparation was easier to complete. These findings may support the efficacy of mosapride in terms of patient tolerance and acceptability. It is possible that 2 L of PEG solution is so large that these symptoms are unavoidable; in addition, it is also possible that the dose of mosapride was not sufficient to alleviate these symptoms.

Co-administration of laxatives such as sennoside and bisacodyl with lavage solution has been shown to improve colonic cleansing during colonoscopy. Addition of these adjunctive therapies has also allowed for lower volume PEG solutions to be administered with equivalent or increased efficacy. However, the adjunctive therapies have to be taken the day before the procedure. This may lead to sleep disturbances and inconvenience due to frequent defecation. If it is possible to begin the bowel preparation using mosapride on the same day as the colonoscopy, patient tolerability may improve.

Recently, it has been suggested that co-administration of mosapride and PEG-electrolyte solution is useful in preparing the colon for barium enema examination as it allows good evacuation of remaining feces[39]. As a result, mosapride is now approved in Japan for preparation for a barium enema examination, and a total dose of 40 mg mosapride is used. Major side effects have not been reported in Japan with this dose. In the present study, we administered 15 mg of mosapride for colonoscopy preparation, which is the recommended usual daily dosage of mosapride for adult patients with chronic gastritis. However because the effects of mosapride are reported to be dose-dependent[35,40], additional studies that address optimal dosage and timing of administration are required to clarify the best regimen for colonoscopy.

One of the limitations of this study was that there was a significant difference in the number of patients with severe constipation between the two groups. However, even with the inclusion of patients with severe constipation, there was a non-significant trend for improved preparation in the mosapride group, and this difference became significant after the exclusion of this subgroup. It is possible that for patients with severe constipation, the dose of 15 mg mosapride may be insufficient. The second limitation of this study was its single center location. Finally, we did not evaluate laboratory abnormalities, as co-administration of mosapride and PEG-electrolyte solution is already common in Japan for preparation for barium enema examination[39]. No serious laboratory abnormalities have been reported in Japan with a 40-mg dose of mosapride.

In conclusion, we demonstrated that co-administration of mosapride with PEG-electrolyte solution improves the quality of bowel preparation for colonoscopy in the left colon. Mosapride may be an effective and safe adjunct to PEG that leads to improved quality of bowel preparation, especially in patients without severe constipation.

COMMENTS

Background

Although prokinetics have been used in combination with polyethylene glycol (PEG)-electrolyte solution to improve patient acceptability and tolerance, as well as improve bowel cleansing, the efficacy and safety of these agents remain unproven. Prokinetics such as domperidone, metoclopramide, and cisapride have been used in combination with PEG-electrolyte solution to improve the quality of bowel preparation. However, the addition of prokinetic agents to PEG-electrolyte solution has not been proven to improve patient tolerance or colonic cleansing and can be sometimes associated with serious adverse effects. Thus, safer and more effective prokinetic agents are needed.

Research frontiers

Mosapride citrate (mosapride) is a selective 5-hydroxytryptamine-4 (5-HT4) receptor agonist. Mosapride enhances gastric emptying and motility by facilitating acetylcholine release from the enteric cholinergic neurons, without blocking dopaminergic D2 receptors. It is known to be effective in gastroesophageal reflex disease, functional gastrointestinal disorders, such as functional dyspepsia, chronic gastritis with delayed gastric emptying, and diabetic gastroparesis. As 5-HT4 receptors are also located in the human colon and rectum, mosapride is also expected to have a prokinetic effect on the colo-rectum. A few clinical studies have reported that mosapride in combination with PEG may enhance bowel cleansing and improve patient acceptability and tolerability.

Innovations and breakthroughs

This is the first prospective, randomized, double-blind, placebo-controlled study to evaluate the efficacy, acceptability, and tolerance of mosapride as an adjuvant in PEG-electrolyte solution for colonoscopy preparation. This study demonstrated that co-administration of mosapride with PEG-electrolyte solution improves the quality of bowel preparation for colonoscopy in the left colon.

Applications

The study results suggest that mosapride may be an effective and safe adjunct to PEG, leading to an improved quality of bowel preparation, especially in patients without severe constipation. Additional studies that address optimal dosage and timing of administration are required to clarify the best bowel preparation method for colonoscopy.

Terminology

PEG-electrolyte solution: PEG-electrolyte solution is used worldwide for bowel cleansing. Approximately 2 L of this oral solution with some laxatives is usually required for adequate bowel preparation in Japan. However, the need to drink such large volumes of liquid with an unpalatable taste has a negative impact on patient compliance.

Peer review

This is an interesting and well written study. The methodology and evaluation of data is correct. The conclusion sounds good and useful for the general practice.

REFERENCES

1 **Iida Y**, Miura S, Asada Y, Fukuoka K, Toya D, Tanaka N, Fujisawa M. Bowel preparation for the total colonoscopy by 2,000 ml of balanced lavage solution (Golytely) and sennoside. *Gastroenterol Jpn* 1992; **27**: 728-733

2 **Harewood GC**, Wiersema MJ, Melton LJ. A prospective, controlled assessment of factors influencing acceptance of screening colonoscopy. *Am J Gastroenterol* 2002; **97**: 3186-3194

3 **Regula J**, Rupinski M, Kraszewska E, Polkowski M, Pachlewski J, Orlowska J, Nowacki MP, Butruk E. Colonoscopy in colorectal-cancer screening for detection of advanced neoplasia. *N Engl J Med* 2006; **355**: 1863-1872

4 **Wexner SD**, Beck DE, Baron TH, Fanelli RD, Hyman N, Shen B, Wasco KE. A consensus document on bowel preparation before colonoscopy: prepared by a task force from the American Society of Colon and Rectal Surgeons (ASCRS), the American Society for Gastrointestinal Endoscopy (ASGE), and the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES). *Gastrointest Endosc* 2006; **63**: 894-909

5 **Ness RM**, Manam R, Hoen H, Chalasani N. Predictors of inadequate bowel preparation for colonoscopy. *Am J Gastroenterol* 2001; **96**: 1797-1802

6 **Ziegenhagen DJ**, Zehnter E, Tacke W, Gheorghiu T, Kruis W. Senna vs. bisacodyl in addition to Golytely lavage for colonoscopy preparation--a prospective randomized trial. *Z Gastroenterol* 1992; **30**: 17-19

7 **Lazarczyk DA**, Stein AD, Courval JM, Desai D. Controlled study of cisapride-assisted lavage preparatory to colonoscopy. *Gastrointest Endosc* 1998; **48**: 44-48

8 **Reiser JR**, Rosman AS, Rajendran SK, Berner JS, Korsten MA. The effects of cisapride on the quality and tolerance of colonic lavage: a double-blind randomized study. *Gastrointest Endosc* 1995; **41**: 481-484

9 **Katsinelos P**, Pilpilidis I, Paroutoglou G, Xiarchos P, Tsolkas P, Papagiannis A, Giouleme O, Kapelidis P, Papageorgiou A, Dimiropoulos S, Eugenidis N. The administration of cisapride as an adjuvant to PEG-electrolyte solution for colonic cleansing: a double-blind randomized study. *Hepatogastroenterology* 2005; **52**: 441-443

10 **Martínek J**, Hess J, Delarive J, Jornod P, Blum A, Pantoflickova D, Fischer M, Dorta G. Cisapride does not improve precolonoscopy bowel preparation with either sodium phosphate or polyethylene glycol electrolyte lavage. *Gastrointest Endosc* 2001; **54**: 180-185

11 **Brady CE**, DiPalma JA, Pierson WP. Golytely lavage--is metoclopramide necessary? *Am J Gastroenterol* 1985; **80**: 180-184

12 **Rhodes JB**, Engstrom J, Stone KF. Metoclopramide reduces the distress associated with colon cleansing by an oral electrolyte overload. *Gastrointest Endosc* 1978; **24**: 162-163

13 **Brogden RN**, Carmine AA, Heel RC, Speight TM, Avery GS. Domperidone. A review of its pharmacological activity, pharmacokinetics and therapeutic efficacy in the symptomatic treatment of chronic dyspepsia and as an antiemetic. *Drugs* 1982; **24**: 360-400

14 **Tonini M**, De Ponti F, Di Nucci A, Crema F. Review article: cardiac adverse effects of gastrointestinal prokinetics. *Aliment Pharmacol Ther* 1999; **13**: 1585-1591

15 **Yoshida N**, Omoya H, Oka M, Furukawa K, Ito T, Karasawa T. AS-4370, a novel gastrokinetic agent free of dopamine D2 receptor antagonist properties. *Arch Int Pharmacodyn Ther* 1989; **300**: 51-67

16 **Ruth M**, Finizia C, Cange L, Lundell L. The effect of mosapride on oesophageal motor function and acid reflux in patients with gastro-oesophageal reflux disease. *Eur J Gastroenterol Hepatol* 2003; **15**: 1115-1121

17 **Mizuta Y**, Shikuwa S, Isomoto H, Mishima R, Akazawa Y, Masuda J, Omagari K, Takeshima F, Kohno S. Recent insights into digestive motility in functional dyspepsia. *J Gastroenterol* 2006; **41**: 1025-1040

18 **Asakawa H**, Hayashi I, Fukui T, Tokunaga K. Effect of mosapride on glycemic control and gastric emptying in type 2 diabetes mellitus patients with gastropathy. *Diabetes Res Clin Pract* 2003; **61**: 175-182

19 **McLean PG**, Coupar IM. Stimulation of cyclic AMP formation in the circular smooth muscle of human colon by activation of 5-HT4-like receptors. *Br J Pharmacol* 1996; **117**: 238-239

20 **Sakurai-Yamashita Y**, Yamashita K, Kanematsu T, Taniyama K. Localization of the 5-HT(4) receptor in the human and the guinea pig colon. *Eur J Pharmacol* 1999; **383**: 281-285

21 **Nagashima M**, Okamura S, Iizuka H, Ohmae Y, Sagawa T, Kudo T, Masuo T, Kobayashi R, Marubashi K, Ishikawa T, Oshimoto H, Yoshida M, Motegi K, Sakamoto T, Iesaki K, Mori M. Mosapride citrate for colonoscopy preparation with lavage. *Kitakanto Med J* 2002; **52**: 111–115

22 **Mishima Y**, Amano Y, Okita K, Takahashi Y, Moriyama N, Ishimura N, Furuta K, Ishihara S, Adachi K, Kinoshita Y. Efficacy of prokinetic agents in improving bowel preparation for colonoscopy. *Digestion* 2008; **77**: 166-172

23 **Sakashita M**, Yamaguchi T, Miyazaki H, Sekine Y, Nomiyama T, Tanaka S, Miwa T, Harasawa S. Pharmacokinetics of the gastrokinetic agent mosapride citrate after single and multiple oral administrations in healthy subjects. *Arzneimittelforschung* 1993; **43**: 867-872

24 **Aronchick CA**, Lipshutz WH, Wright SH, Dufrayne F, Bergman G. A novel tableted purgative for colonoscopic preparation: efficacy and safety comparisons with Colyte and Fleet Phospho-Soda. *Gastrointest Endosc* 2000; **52**: 346-352

25 **Hunt RH**, Tougas G. Evolving concepts in functional gastrointestinal disorders: promising directions for novel pharmaceutical treatments. *Best Pract Res Clin Gastroenterol* 2002; **16**: 869-883

26 **Yoshida N**, Kato S, Ito T. Mosapride Citrate. *Drugs Future* 1993; **18**: 513-515

27 **Hongo M**. Initial approach and pharmacotherapy for functional dyspepsia-a large clinical trial in Japan. *Gastroenterology* 2006; **130**: A-506

28 **Kanaizumi T**, Nakano H, Matsui Y, Ishikawa H, Shimizu R, Park S, Kuriya N. Prokinetic effect of AS-4370 on gastric emptying in healthy adults. *Eur J Clin Pharmacol* 1991; **41**: 335-337

29 **Inui A**, Yoshikawa T, Nagai R, Yoshida N, Ito T. Effects of mosapride citrate, a 5-HT4 receptor agonist, on colonic motility in conscious guinea pigs. *Jpn J Pharmacol* 2002; **90**: 313-320

30 **Jost WH**, Schimrigk K. Long-term results with cisapride in Parkinson's disease. *Mov Disord* 1997; **12**: 423-425

31 **Kii Y**, Nakatsuji K, Nose I, Yabuuchi M, Mizuki Y, Ito T. Effects of 5-HT(4) receptor agonists, cisapride and mosapride citrate on electrocardiogram in anaesthetized rats and guinea-pigs and conscious cats. *Pharmacol Toxicol* 2001; **89**: 96-103

32 **Taniyama K**, Makimoto N, Furuichi A, Sakurai-Yamashita Y, Nagase Y, Kaibara M, Kanematsu T. Functions of peripheral 5-hydroxytryptamine receptors, especially 5-hydroxytryptamine4 receptor, in gastrointestinal motility. *J Gastroenterol* 2000; **35**: 575-582

33 **Odaka T**, Suzuki T, Seza A, Yamaguchi T, Saisho H. [Serotonin 5- HT4 receptor agonist (mosapride citrate)]. *Nihon Rinsho* 2006; **64**: 1491-1494

34 **Liu Z**, Sakakibara R, Odaka T, Uchiyama T, Uchiyama T, Yamamoto T, Ito T, Asahina M, Yamaguchi K, Yamaguchi T, Hattori T. Mosapride citrate, a novel 5-HT4 agonist and partial 5-HT3 antagonist, ameliorates constipation in parkinsonian patients. *Mov Disord* 2005; **20**: 680-686

35 **Shimatani H**, Kojima Y, Kadowaki M, Nakagawa T, Fujii H, Nakajima Y, Takaki M. A 5-HT4 agonist mosapride enhances rectorectal and rectoanal reflexes in guinea pigs. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G389-G395

36 **Mine Y**, Morikage K, Oku S, Yoshikawa T, Shimizu I, Yoshida N. Effect of mosapride citrate hydrate on the colon cleansing action of polyethylene glycol electrolyte lavage solution (PEG-ELS) in guinea pigs. *J Pharmacol Sci* 2009; **110**: 415-423

37 **Kim HS**, Choi EJ, Park H. The effect of mosapride citrate on proximal and distal colonic motor function in the guinea-pig in vitro. *Neurogastroenterol Motil* 2008; **20**: 169-176

38 **Oikawa T**, Takemoto Y, Haramu K. Post-marketing surveillance of mosapride citrate (Gasmotin) in patients with non-ulcer dyspepsia on long-term administration [in Japanese]. *Rinsho Iyaku* 2005; **21**: 831-837

39 **Futei S**, Sugino Y, Kuribayashi S, Imai Y, Ueno F, Hibi T, Mitsushima T. [New preparation method for barium enema: efficacy and administration of oral intestinal lavage solution with gastrointestinal prokinetic agent]. *Nihon Igaku Hoshasen Gakkai Zasshi* 2004; **64**: 22-30

40 **Miyoshi A**, Miwa T, Harasawa S, Yoshida Y, Masamune O, Kimura K, Niwa H, Mori H, Asakura H, Hayakawa T, Kamata T, Kajiyama G, Hayakawa A, Nakashima M. Dose-finding study of AS-4370 (mosapride citrate) in patients with chronic gastritis [in Japanese]. *Rinsho Iyaku* 1998; **14**: 1069-1090

S- Editor Cheng JX **L- Editor** Cant MR **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2526

World J Gastroenterol 2012 May 28; 18(20): 2526-2532
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Endoscopic ultrasound-guided biliary drainage with placement of a fully covered metal stent for malignant biliary obstruction

Tae Hyeon Kim, Seong Hun Kim, Hyo Jeong Oh, Young Woo Sohn, Seung Ok Lee

Tae Hyeon Kim, Department of Internal Medicine, School of Medicine, Wonkwang University, Iksan, Chonbuk 570-749, South Korea
Seong Hun Kim, Seung Ok Lee, Department of Internal Medicine, Chonbuk National University Medical School and Hospital, Jeonju, Chonbuk 561-712, South Korea
Hyo Jeong Oh, Young Woo Sohn, Department of Internal Medicine, School of Medicine, Wonkwang University, Sanbon, Gyounggi 570-749, South Korea
Author contributions: Kim TH and Kim SH made substantial contributions to conception and design, drafting the article revising it critically for important intellectual content; these authors contributed equally to this paper; Oh HJ and Sohn YW made the analysis of data; Kim TH, Kim SH and Lee SO contributed to collect patients, endoscopic procedures; and all authors approved the version to be published.
Supported by Institute of Wonkwang Medical Science in 2011
Correspondence to: Tae Hyeon Kim, MD, Associate Professor, Department of Internal Medicine, School of Medicine, Wonkwang University, Iksan, Chonbuk 570-749, South Korea. kth@wonkwang.ac.kr
Telephone: +82-63-8592564 Fax: +82-63-8502205
Received: September 27, 2011 Revised: February 6, 2012
Accepted: February 16, 2012
Published online: May 28, 2012

Abstract

AIM: To determine the utility of endoscopic ultrasound-guided biliary drainage (EUS-BD) with a fully covered self-expandable metal stent for managing malignant biliary stricture.

METHODS: We collected data from 13 patients who presented with malignant biliary obstruction and underwent EUS-BD with a nitinol fully covered self-expandable metal stent when endoscopic retrograde cholangiopancreatography (ERCP) fails. EUS-guided choledochoduodenostomy (EUS-CD) and EUS-guided hepaticogastrostomy (EUS-HG) was performed in 9 patients and 4 patients, respectively.

RESULTS: The technical and functional success rate was 92.3% (12/13) and 91.7% (11/12), respectively. Using an intrahepatic approach (EUS-HG, *n* = 4), there was mild peritonitis (*n* = 1) and migration of the metal stent to the stomach (*n* = 1). With an extrahepatic approach (EUS-CD, *n* = 10), there was pneumoperitoneum (*n* = 2), migration (*n* = 2), and mild peritonitis (*n* = 1). All patients were managed conservatively with antibiotics. During follow-up (range, 1-12 mo), there was re-intervention (4/13 cases, 30.7%) necessitated by stent migration (*n* = 2) and stent occlusion (*n* = 2).

CONCLUSION: EUS-BD with a nitinol fully covered self-expandable metal stent may be a feasible and effective treatment option in patients with malignant biliary obstruction when ERCP fails.

Key words: Endoscopic ultrasound-guided; Biliary drainage; Metal stent; Biliary obstruction

Peer reviewer: Yucel Ustundag, Professor, Department of Gastroenterology, Zongudak Karaelmas University Hospital, Kozlu Zonguldak Turkey, 67600 Zonguldak, Turkey

Kim TH, Kim SH, Oh HJ, Sohn YW, Lee SO. Endoscopic ultrasound-guided biliary drainage with placement of a fully covered metal stent for malignant biliary obstruction. *World J Gastroenterol* 2012; 18(20): 2526-2532 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2526.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2526

INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP)

is a well-established technique for providing biliary decompression in patients with bile duct obstruction. The success rate for effective biliary decompression with ERCP ranges from 90% to 95%[1]. However, there are patients in whom ERCP fails because of unsuccessful biliary cannulation, or inaccessible papilla due to duodenal stenosis caused by tumor invasion. In these cases, percutaneous biliary drainage (PTBD) is required. However, PTBD can lead to significant complications, including biliary peritonitis, hemobilia, pneumothorax, hematoma, liver abscesses, and patient discomfort related to the catheter[2].

Therefore, endoscopic ultrasound-guided biliary drainage (EUS-BD) using plastic stents has been introduced as an alternative to PTBD in cases of biliary obstruction when ERCP fails[3-6]. However, plastic stent malfunction due to stent clogging after EUS-BD is not uncommon[3,7,8]. Fully covered self-expandable metal stent (FCSEMS) with a large bore diameter may have advantages over plastic stents. Recently, EUS-BD with a FCSEMS was introduced in a few cases of malignant biliary obstruction after a failed ERCP[9]. The current study was conducted to determine the feasibility, outcomes, and risks of EUS-guided hepaticogastrostomy (EUS-HG) and EUS-guided choledochoduodenostomy (EUS-CD) with an FCSEMS as a biliary diversion technique in patients with malignant biliary obstruction for whom interventional ERCP was unsuccessful.

MATERIALS AND METHODS

Study population

We collected data on all patients who presented with obstructive jaundice and who underwent EUS-BD after a failed ERCP during a 20-mo period from February 2009 to September 2010. Failed ERCP was defined as the inability to relieve jaundice or failed biliary cannulation. A total of 2209 ERCPs at Wonkwang University Hospital (n = 780) and Jeonbuk National University Hospital (n = 1429) were performed during the study period, and 366 required biliary decompression. Of the 22 (6%) patients who underwent alternative methods for biliary decompression as a result of failed ERCP, 13 had EUS-BD. Study eligibility was determined as follows: (1) Initial biliary cannulation or bile duct decompression by ERCP failed because of accompanying duodenal obstruction, periampullary tumor infiltration, and difficult cannulation (n = 11); (2) A high-grade left-sided hilar stricture as a result of segmental tumor progression with an occluded biliary metal stent was unable to be crossed by a guidewire (n = 2); (3) The patient refused PTBD (n = 13).

ERCP was performed by 3 experienced endoscopists (Kim TH, Kim SH and Lee SO). Each endoscopist performs 400 to 450 ERCPs annually. EUS-BD was performed by 2 experienced endoscopists (Kim TH and Kim SH) who perform more than 250 EUS procedures for pancreaticobiliary diseases annually.

In this study, stent occlusion was classified as predominantly tumor ingrowth (seen on fluoroscopy as a narrowing within the stent), tumor overgrowth (seen radiographically or endoscopically as a new narrowing at the proximal or distal margin of the stent), or sludge/debris (demonstrated as echoendoscopic findings or multiple radiographic filling defects that disappeared after extraction with a biliary balloon along with endoscopic visualization of the extracted sludge)[10]. The Institutional Review Boards at Wonkwang University Hospital and Jeonbuk National University Hospital, South Korea approved this study. All patients provided written informed consent for participation in this study.

Techniques

Antibiotics were permitted in all cases for 3 d to 5 d before and after the intervention. ERCP was initially attempted in each patient by using a therapeutic duodenoscope (TJF-240; Olympus Optical Co, Tokyo, Japan). When the ERCP was unsuccessful, an EUS was performed using a GF-UCT 240 linear-array echoendoscope (Olympus Corp., Tokyo, Japan) in a second session (on the same or next day). These patients underwent an EUS-HG or EUS-CD. Using the transgastric approach, the echoendoscope was placed in the cardia or the lesser curvature of the stomach to view the dilated segment 3 of the liver (Figure 1). Using the transduodenal approach, the echoendoscope was placed in the duodenal bulb to image the extrahepatic bile duct (Figure 2). Color Doppler ultrasonography was used to identify the regional vasculature, and a bile duct puncture was performed with a 19-gauge needle (EUSN-19-T; Cook Endoscopy, Winston-Salem, NC, United States). To confirm successful biliary access, contrast medium was injected under fluoroscopy to demonstrate biliary opacification. A 0.035-inch guidewire was introduced through the EUS needle and advanced in an antegrade or retrograde fashion. Afterwards, 6F and 7F tapered, biliary, bougie catheters (catheter tip, 4F; Cook Endoscopy) were inserted and removed, over the guidewire to dilate the tract. If there was resistance when advancing the 6F bougie catheter, a needle-knife (Microtome; Boston Scientific, Natick, MA, United States) with a 7F shaft diameter or tapered MTW catheter (WTW, Endoscopie, Wesel, Germany) was inserted over the guidewire to dilate the tract. To accomplish this, the tip of a needle-knife (Microtome) with a pure current was gently inserted over the guidewire into the biliary system. The needle was then withdrawn, and the needle-knife was pushed in to dilate the tract. A commercially available, fully silicon-covered metal stent with an 8F deployment system and an olive tip (BONASTENT, a nitinol stent with a 10-mm diameter and 6-cm length; Standard Sci Tech Inc., Seoul, South Korea) was placed under echoendoscopic and fluoroscopic view.

Definition of events

Technical success was defined as the deployment of the metal stent across the stomach or duodenum, along with the flow of contrast medium and/or bile through the stent. Functional success was defined as a reduction in bilirubin to less than 50% of the pretreatment value

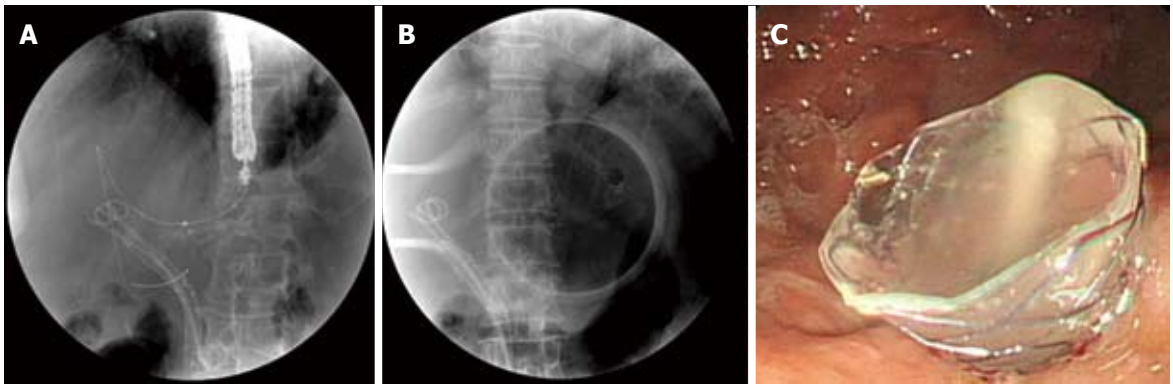


Figure 1 Endoscopic ultrasound-guided hepaticogastrostomy. A: In the intrahepatic approach, the linear array echoendoscope was placed in the lesser curvature of the stomach for viewing the left intrahepatic system. A 0.035-inch guidewire was introduced through the endoscopic ultrasonography-needle and advanced in an antegrade manner; B and C: A fully covered metal stent was placed under echoendoscopic and fluoroscopic view.

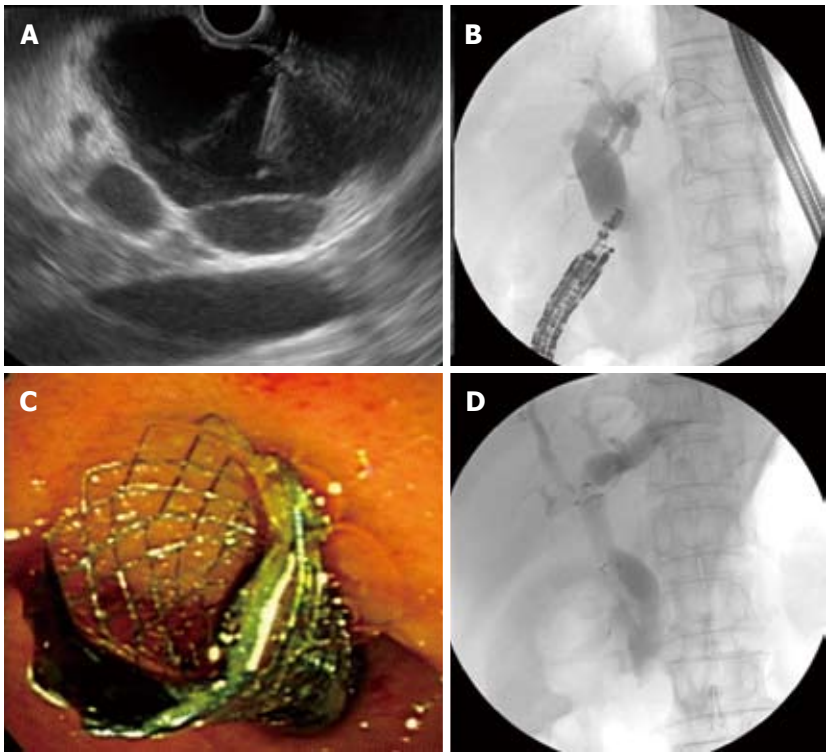


Figure 2 Endoscopic ultrasound-guided choledochoduodenostomy. A: The extrahepatic approach was carried out using an echoendoscope in the duodenal bulb, permitting imaging of the choledochus. Bile duct puncture was carried out with a 19-gauge needle; B: A 0.035-inch guidewire was introduced through the endoscopic ultrasonography-needle and advanced in a retrograde fashion; C and D: A fully covered metal stent was placed under echoendoscopic and fluoroscopic view.

within 2 wk[11]. An early complication was defined as any stent-related complication within 30 d, including complications of bile leakage, pneumoperitoneum, bleeding, and stent migration. A late complication was defined as any stent-related complication, such as stent migration and stent occlusion, occurring 30 d after stent placement. Biliary reintervention was defined as any type of endoscopic, percutaneous, or surgical procedure that was required to improve biliary drainage after placement of the stent. Stent occlusion was defined as the recurrence of jaundice and cholestasis and/or evidence of a dilated biliary system on ultrasound (US) or computed tomography (CT) with a direct view of the upper endoscope, which in all cases would require biliary intervention. Procedure time was defined as the time between puncture of the biliary tract with a 19-gauge needle and placement of an FCSEMS.

Follow-up

Follow-up continued from stent insertion until the death of the patient or to the end of the study. Biochemical parameters and simple abdominal films were assessed at 2 d, 1 wk and 1 mo after stent placement and every 2 mo thereafter. Patient follow-up was based on outpatient examination findings. Imaging (US or CT and simple abdominal films) was routinely checked every 3 mo.

Statistical analysis

Procedure time (intrahepatic and extrahepatic approaches) and liver function test before and after the EUS-BD using a metal stent were compared using a 2-sample Wilcoxon signed-rank test. Statistical analysis was carried out using

Table 1 Patients' clinical characteristics and results of endoscopic ultrasound-guided biliary drainage (n = 13)

No. of patient	Age/sex	Diagnosis	Reason for failed ERCP	Biliary drainage route	Device for puncture/ dilatation	Diameter length of stent (mm/cm)	Technical success	Functional success	Complication	Reintervention	Duration (mo) of 1st stent placement	Status
1	60/M	CBD cancer	Duodenal stenosis due to duodenal ulcer	Transduodenal	19G FN/cystostome	10/6	Success	Success	No	No	Whipple's operation	Alive
2	83/F	CBD cancer	Failed to access the bile duct	Transduodenal	19G FN/cystostome	10/6	Success	Success	Obstruction	Stent reinsertion	6	Dead
3	76/F	CBD cancer	Failed to access the bile duct	Transduodenal	19G FN/cystostome	10/4	Success	Success	Migration	Stent reinsertion	2	Dead
4	68/F	CBD cancer	Duodenal obstruction due to mass	Transduodenal	19G FN/cystostome	10/6	Success	Success	No	No	7	Alive
5	66/M	Pancreatic cancer	Periampullary tumor infiltration	Transduodenal	19G FN/tapered ERCP cannula and cystostome	10/4	Success	Success	Pneumoperitoneum/ peritonitis	No	3	Dead
6	68/M	Pancreatic cancer	Periampullary tumor infiltration	Transduodenal	19G FN/bougie dilatator	10/6	Success	Success	No	No	1	Dead
7	67/F	Pancreatic cancer	Periampullary tumor infiltration	Transduodenal	19G FN/NK and bougie dilatator	10/6	Success	Success	No	No	3	Alive
8	59/M	Pancreatic cancer	Periampullary tumor infiltration	Transduodenal	19G FN/tapered ERCP cannula and cystostome	10/6	Success	Success	No	No	4	Alive
9	80/M	Pancreatic cancer	Periampullary tumor infiltration	Transduodenal	19G FN/tapered ERCP cannula and cystostome	10/6	Success	Success	No	No	3	Alive
10	81/M	Klatskin's tumor	Stricture could not be crossed (hilar)	Transgastric	19G FN/cystostome	10/6	Success	Fail	Migration/pneumo-peritoneum/peritonitis	PTBD	5	Dead
11	55/M	Intrahepatic cholangiocar-cinoma	Stricture could not be crossed (hilar)	Transgastric	19G FN/cystostome	10/6	Success	Success	No	No	3	Dead
12	62/M	Pancreatic cancer	Stricture could not be crossed (distal CBD)	Transgastric	19G FN	10/6	Failed					Dead
13	70/M	CBD cancer	Stricture could not be crossed (distal CBD)	Transgastric	19G FN/bougie dilatator	10/6	Success	Success	Migration	Stent reinsertion	2	Alive

F: Female; M: Male; CBD: Common bile duct; G: Gauge; NK: Needel knife; FN: Fine needle; PTBD: Percutaneous biliary drainage; ERCP: Endoscopic retrograde cholangiopancreatography.

SPSS 12.0 (SPSS, Chicago, IL, United States), and a 2-tailed P value of < 0.05 was considered statistically significant. Findings are expressed as the median and range.

RESULTS

Patient characteristics, procedural data, and follow-up results are presented in Table 1. A total of 13 patients (9 men and 4 women) with malignant biliary obstruction underwent EUS-BD with a fully covered metal stent after a failed ERCP. The etiology of biliary obstruction was pancreatic cancer in 5 patients, distal common bile duct cancer in 6 patients, Klatskin's tumor in 1 patient, and peripheral cholangiocarcinoma in 1 patient.

Reasons for failed ERCP were duodenal stenosis due to a previous duodenal ulcer (n = 1), tumor obstruction of the duodenum (n = 1), periampullary tumor infiltration (n = 5),

Table 2 Summary of published studies and current study on endoscopic ultrasound-guided biliary drainage with metal stent

Study	No. of EUS-BD-metal biliary stents	Technical success %	Clinical success %	Type of metal stent	Procedure-related complications (No. cases)
Will *et al*[6]	6	100	83.3	Partially covered (3), uncovered (3)	Cholangitis (1)
Artifon *et al*[27]	1	100	100	Partially covered	None
Bories *et al*[3]	11	91	100	Partially covered metal stent (3)	Biloma (1), cholangitis (1)
Park *et al*[9]	9	100	100	Fully covered	Pneumoperitoneum (2)
Park *et al*[28]	5	100	100	Fully covered	None
Siddiqui *et al*[29]	8	100	100	Fully covered	Duodenal perforation (1)
This study	13	92.3	91.7	Fully covered	Mild peritonitis (2)

EUS-BD: Endoscopic ultrasound-guided biliary drainage.

failure to access the common bile duct (n = 2), high-grade stricture of the distal common bile duct with an occluded biliary plastic stent (n = 2), and high-grade left-sided hilar stricture from segmental tumor progression with an occluded biliary metal stent (n = 2). Patients with obstruction of the duodenum received a concomitant duodenal stent at time of the procedure.

Technical and functional success

Technical success was 92.3% (12/13); there was 1 failure of guidewire insertion after puncture through the transgastric approach, because the diameter of intrahepatic bile duct was so small and had sharp angulation. During EUS-BD, no wire passage for rendezvous was attempted in any of the patients. For biliary access, a cystostome only was used in 6 of the 12 patients (4 extrahepatic approach and 2 intrahepatic approach), a tapered MTW catheter and a cystostome together were used 3 of 12 patients, and just bougie dilatators were used in 3 patients, respectively. Median procedure time was 19.5 min (range, 14-35 min). Nine patients were treated using an extrahepatic approach (all transduodenal). Four patients were treated using an intrahepatic approach (all transgastric). The median diameter of the left intrahepatic bile duct as determined by EUS was 7.3 mm (range, 5.3-9.4 mm).

Functional success was 91.7% (11/12). After the placement of a EUS-BD, the median bilirubin level decreased significantly from 11.6 mg/dL to 1.7 mg/dL (P = 0.001). The median alkaline phosphatase level also decreased significantly from 1629.0 IU/L to 113.0 IU/L (P = 0.001). Migration of a metal stent to stomach occurred in 1 patient treated with the transgastric approach.

Early complications after endoscopic ultrasound-guided biliary drainage

With the intrahepatic approach (n = 4), there was stent migration to the stomach with mild peritonitis (n = 1). With the extrahepatic approach (n = 9), there was pneumoperitoneum with mild peritonitis (n = 1). All patients with peritonitis were managed conservatively with antibiotics. Bleeding and cholangitis were not observed in any of the enrolled patients after the procedure. In addition, there was no significant pain after the procedure. Two patients with duodenal obstruction also received duodenal metal stents.

Follow-up

One patient with distal common bile duct (CBD) cancer underwent curative resection (Whipple's operation), and this procedure did not disturb the operative procedure. None of the patients, except the patients that underwent the operation, were lost during the follow-up period (median, 5 mo; range, 1-12 mo). With regard to re-intervention, there was re-intervention in 4 of the 13 cases (30.7%), which was necessitated by stent migration (n = 3) and stent occlusion (n = 1). Stent migration occurred 2 d later (EUS-HG) and 2 mo later (EUS-CD and EUS-HG), respectively. In the patients with stent migration, the stents passed spontaneously without lodgment in the bowel. A second FCSEMS was placed through the previous choledochoduodenostomy site in 1 patient. In 2 patients with stent migration, PTBD was inserted into the obstructed left hepatic duct due to his severe general weakness. In 1 patient with stent occlusion by tumor ingrowth into the metal stent (choledochoduodenostomy), an additional FCSEMS was placed through the previous choledochoduodenostomy site.

DISCUSSION

EUS-BD may be an alternative method in some patients when endoscopic biliary drainage is unsuccessful because of failed biliary cannulation or tumor infiltration, which limits the endoscopic approach to the major papilla. At present, there are 2 approaches for EUS-BD, the transgastric route and the transduodenal route. A plastic or metal stent can be placed by this method. However, most papers reported EUS-BD with a plastic stent[4,5,7,8,12-14]. We thought that FCMEMS with a large bore diameter may have advantages over the plastic stent. There are a few papers describing EUS-BD with a metal stent[6,9,15] (Table 2), however, there are no reports regarding preoperative drainage of biliary obstruction by EUS-BD. The current study demonstrated that transduodenal EUS-BD using a nitinol FCSEMS may be safe and feasible after a failed ERCP. The reasons for failed ERCP were duodenal stenosis by a duodenal ulcer, periampullary tumor invasion, and difficult biliary cannulation. In one patient with distal CBD cancer who had duodenal stenosis due to a previous duodenal ulcer, initially EUS-BD was performed to resolve cholangitis and bile drainage, and Whipple's operation was performed. But EUS-guided metal insertion in this patient did not disturb the operation procedure.

A significant number of self-expandable metal stent

(SEMS) and plastic stents placed to palliate malignant obstructions will be occluded. Repeat transpapillary stent placement may be unsuccessful for an occluded biliary metal or plastic stent after the placement of a hilar metal stent or combined duodenal and biliary metal stent placement. In such circumstances, PTBD may be uncomfortable for patients and has a 10%-30% complication rate, with complications such as cholangitis, bile leak, peritonitis, and stent occlusion[16]. EUS-HG with a FCSEMS may be recommended in such circumstances. In our 2 patients with a hilar metal stent, dilation of the left main duct caused by segmental tumor progression was shown to be aggravated. In another 2 patients with periampullary cancer drained by a pigtail plastic stent, this stent was occluded by tumor ingrowth and bile plug. Because these patients had jaundice and cholangitis, EUS-HG with a FCSEMS was performed. Some experts have recommended the intrahepatic approach because it seems to be safer[7,15]. In the intrahepatic approach, one-step placement of a partially covered wall stent may have been limited in earlier studies because there was 1 bile peritonitis and 1 cholangitis. However, we found the transduodenal or extrahepatic approach to be safer and more effective and it is probably less technically difficult compared with the intrahepatic approach. The advantage is that the duodenum is very close to the extrahepatic bile duct and the duodenal wall is thin without major vascular structures.

There were complications with EUS-BD, including stent migration, pneumoperito-neum, and cholangitis[17,18]. In this study, during follow-up (median, 3 mo), there was re-intervention (4/12, 33.3%) necessitated by stent migration (n = 3) and stent occlusion (n = 1). The 3 stent migrations occurred 2 d later (EUS-HG), 2 mo later (EUS-CD), and 2 mo later (EUS-CD). The stent migration rate (3/12, 25.0%) was higher than that reported (7%) in another paper[9]. Although a flare at both ends and minimal shortening of this newly designed nitinol stent may contribute to the prevention of stent migration[19], a slippery covered metal stent with low axial force may lead to migration. In addition, marked CBD dilatation may have affected FCSEMS floating resulting in the minimization of the anchoring effect of the proximal flared end of the FCSEMS.

Studies with uncovered SEMS reported stent occlusion rates ranging between 18% and 46%[20,21] with the main cause of obstruction being tumor ingrowth. Although partially or fully covered SEMS were designed to prevent this complication, these stents can not completely protect tumor ingrowth in the biliary metal stent[22]. Occasional authors have reported no tumor ingrowth with covered SEMSs. However, these series often include few patients and frequently have a relatively high prevalence of tumor overgrowth or sludge formation as a cause of stent failure[23-25]. Our study shows tumor overgrowth was observed in one case with distal CBD cancer.

During the EUS-BD procedure, the leakage of bile into the peritoneum with peritonitis can occur because the gap between the stent and the fistula is likely to occur. The shortening of metal stent can lead to failure of correct stent placement, and stent migration and dislocation after placement. Experience with partially coverd SEMS is limited and associated with a higher complication rates due to stent shortening[3,6]. To prevent bile leakage, we placed a FCSEMS that is made of nitinol. This stent, with both ends flared, was designed to prevent distal or proximal migration. The nitinol stent has less shortening compared with a partially covered Wall stent, and a fully covered metal stent may prevent bile leakage[19]. Therefore, due to the characteristics of this stent, it may prevent bile peritonitis. Because the diameter of the working channel of the linear array echoendoscope is only 3.7 mm, it is possible to quickly perform one-step placement of FCSEMS with an 8F-diameter delivery device. In this study, although bile leakage occurred during the procedure, all patients had only mild peritonitis and were treated with conservative management, such as antibiotics and nothing by mouth.

If the gap between the bile duct and the GI tract grows farther after EUS guided biliary drainage with stent, early migration of stent into the abdominal cavity may occur, particularly in the case of a transgastric approach. A fatal complication due to sepsis after development of large fistula related to stent migration into the abdominal cavity has recently been reported[26]. However, bile leakage *via* the relatively large fistula can make bile peritonitis and the bile duct collapse. Therefore, it may be difficult or impossible to carry out EUS-BD in these patients. In such a case, PTBD or emergency surgery must also be considered. To prevent severe complications, an endoscopist must have considerable knowledge about periduodenal and hepatobiliary anatomy in linear array EUS. Also, the procedure must be performed quickly and efficiently with appropriately sized stents. When selecting a stent, stent structure, axial force and shortening of stent should be considered.

In conclusion, EUS-guided transgastric or transduodenal biliary drainage with one-step placement of an FCSEMS may be a promising alternative method to PTBD. It may be a reasonable, feasible, and promising minimally invasive endoscopic approach in selected patients as indicated as above. However, several complications can occur, and special attention of the potential complications such as peritonitis and migration are needed. Therefore, additional experience and the development of new comfortable accessories for this procedure are needed. Also, a large case series and prospective trials are needed to further assess this technique.

COMMENTS

Background

For palliation of patients with malignant biliary obstruction, percutaneous biliary drainage (PTBD) is a classic method in some patients when endoscopic biliary drainage is unsuccessful because of failed biliary cannulation or tumor infiltration, which limits the endoscopic approach to the major papilla. Recently endoscopic ultrasonography-guided biliary drainage (EUS-BD) with plastic stent or uncovered self-expandable metal stent (SEMS) has been introduced as an alternative to PTBD in these cases of biliary obstruction.

Research frontiers

The authors thought that a nitinol fully covered SEMS (FCSEMS) with a large bore diameter may have advantages over the plastic stent and other types of metal stent.

Innovations and breakthroughs

Technical and functional success with FCSEMS for palliation of patients with malignant biliary obstruction was respectively 92.3% (12/13) and 91.7% (11/12). Although there was early mild complication such as one case of immediate

stent migration and 2 cases of bile peritonitis, all complications improved with conservative treatment.

Applications

EUS-guided transgastric or transduodenal biliary drainage with one-step placement of a nitinol FCSEMS may be a promising alternative method to PTBD. Additional experience and the development of new comfortable accessories for this procedure are needed. Also, a large case series and prospective trials are needed to further assess this technique.

Terminology

There are 2 approaches for EUS-BD, the transgastric route and the transduodenal route. EUS-guided hepaticogastrostomy was performed through the transgastric route, and EUS-guided choledochoduodenostomy through the transduodenal route.

Peer review

The authors reported high success rate with this challenging procedure. They clearly demonstrated that extrahepatic approach is easier and safer than intrahepatic approach, Although technical expertise is important, the authors demonstrated more than 90% technical success with low major complication rates. This paper motivates talented endoscopists to solve biliary obstruction *via* EUS guided technics.

REFERENCES

1 **Fogel EL**, Sherman S, Devereaux BM, Lehman GA. Therapeutic biliary endoscopy. *Endoscopy* 2001; **33**: 31-38
2 **Yee AC**, Ho CS. Complications of percutaneous biliary drainage: benign vs malignant diseases. *AJR Am J Roentgenol* 1987; **148**: 1207-1209
3 **Bories E**, Pesenti C, Caillol F, Lopes C, Giovannini M. Transgastric endoscopic ultrasonography-guided biliary drainage: results of a pilot study. *Endoscopy* 2007; **39**: 287-291
4 **Burmester E**, Niehaus J, Leineweber T, Huetteroth T. EUS-cholangio-drainage of the bile duct: report of 4 cases. *Gastrointest Endosc* 2003; **57**: 246-251
5 **Giovannini M**, Pesenti C, Rolland AL, Moutardier V, Delpero JR. Endoscopic ultrasound-guided drainage of pancreatic pseudocysts or pancreatic abscesses using a therapeutic echo endoscope. *Endoscopy* 2001; **33**: 473-477
6 **Will U**, Thieme A, Fueldner F, Gerlach R, Wanzar I, Meyer F. Treatment of biliary obstruction in selected patients by endoscopic ultrasonography (EUS)-guided transluminal biliary drainage. *Endoscopy* 2007; **39**: 292-295
7 **Yamao K**, Bhatia V, Mizuno N, Sawaki A, Ishikawa H, Tajika M, Hoki N, Shimizu Y, Ashida R, Fukami N. EUS-guided choledochoduodenostomy for palliative biliary drainage in patients with malignant biliary obstruction: results of long-term follow-up. *Endoscopy* 2008; **40**: 340-342
8 **Yamao K**, Sawaki A, Takahashi K, Imaoka H, Ashida R, Mizuno N. EUS-guided choledochoduodenostomy for palliative biliary drainage in case of papillary obstruction: report of 2 cases. *Gastrointest Endosc* 2006; **64**: 663-667
9 **Park do H**, Koo JE, Oh J, Lee YH, Moon SH, Lee SS, Seo DW, Lee SK, Kim MH. EUS-guided biliary drainage with one-step placement of a fully covered metal stent for malignant biliary obstruction: a prospective feasibility study. *Am J Gastroenterol* 2009; **104**: 2168-2174
10 **Rogart JN**, Boghos A, Rossi F, Al-Hashem H, Siddiqui UD, Jamidar P, Aslanian H. Analysis of endoscopic management of occluded metal biliary stents at a single tertiary care center. *Gastrointest Endosc* 2008; **68**: 676-682
11 **van der Gaag NA**, Rauws EA, van Eijck CH, Bruno MJ, van der Harst E, Kubben FJ, Gerritsen JJ, Greve JW, Gerhards MF, de Hingh IH, Klinkenbijl JH, Nio CY, de Castro SM, Busch OR, van Gulik TM, Bossuyt PM, Gouma DJ. Preoperative biliary drainage for cancer of the head of the pancreas. *N Engl J Med* 2010; **362**: 129-137
12 **Itoi T**, Itokawa F, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Moriyasu F. Endoscopic ultrasound-guided choledochoduodenostomy in patients with failed endoscopic retrograde cholangiopancreatography. *World J Gastroenterol* 2008; **14**: 6078-6082
13 **Püspök A**, Lomoschitz F, Dejaco C, Hejna M, Sautner T, Gangl A. Endoscopic ultrasound guided therapy of benign and malignant biliary obstruction: a case series. *Am J Gastroenterol* 2005; **100**: 1743-1747
14 **Tarantino I**, Barresi L, Repici A, Traina M. EUS-guided biliary drainage: a case series. *Endoscopy* 2008; **40**: 336-339
15 **Kahaleh M**, Hernandez AJ, Tokar J, Adams RB, Shami VM, Yeaton P. Interventional EUS-guided cholangiography: evaluation of a technique in evolution. *Gastrointest Endosc* 2006; **64**: 52-59
16 **Pessa ME**, Hawkins IF, Vogel SB. The treatment of acute cholangitis. Percutaneous transhepatic biliary drainage before definitive therapy. *Ann Surg* 1987; **205**: 389-392
17 **Savides TJ**, Varadarajulu S, Palazzo L. EUS 2008 Working Group document: evaluation of EUS-guided hepaticogastrostomy. *Gastrointest Endosc* 2009; **69**: S3-S7
18 **Itoi T**, Yamao K. EUS 2008 Working Group document: evaluation of EUS-guided choledochoduodenostomy (with video). *Gastrointest Endosc* 2009; **69**: S8-12
19 **May A**, Ell C. A new self-expanding nitinol stent (JoStent SelfX) for palliation of malignant biliary obstruction: a pilot study. *Endoscopy* 2004; **36**: 329-333
20 **Davids PH**, Groen AK, Rauws EA, Tytgat GN, Huibregtse K. Randomised trial of self-expanding metal stents versus polyethylene stents for distal malignant biliary obstruction. *Lancet* 1992; **340**: 1488-1492
21 **Isayama H**, Komatsu Y, Tsujino T, Sasahira N, Hirano K, Toda N, Nakai Y, Yamamoto N, Tada M, Yoshida H, Shiratori Y, Kawabe T, Omata M. A prospective randomised study of "covered" versus "uncovered" diamond stents for the management of distal malignant biliary obstruction. *Gut* 2004; **53**: 729-734
22 **Kullman E**, Frozanpor F, Söderlund C, Linder S, Sandström P, Lindhoff-Larsson A, Toth E, Lindell G, Jonas E, Freedman J, Ljungman M, Rudberg C, Ohlin B, Zacharias R, Leijonmarck CE, Teder K, Ringman A, Persson G, Gözen M, Eriksson O. Covered versus uncovered self-expandable nitinol stents in the palliative treatment of malignant distal biliary obstruction: results from a randomized, multicenter study. *Gastrointest Endosc* 2010; **72**: 915-923
23 **Kahaleh M**, Tokar J, Conaway MR, Brock A, Le T, Adams RB, Yeaton P. Efficacy and complications of covered Wallstents in malignant distal biliary obstruction. *Gastrointest Endosc* 2005; **61**: 528-533
24 **Yang KY**, Ryu JK, Seo JK, Woo SM, Park JK, Kim YT, Yoon YB. A comparison of the Niti-D biliary uncovered stent and the uncovered Wallstent in malignant biliary obstruction. *Gastrointest Endosc* 2009; **70**: 45-51
25 **Nakai Y**, Isayama H, Komatsu Y, Tsujino T, Toda N, Sasahira N, Yamamoto N, Hirano K, Tada M, Yoshida H, Kawabe T, Omata M. Efficacy and safety of the covered Wallstent in patients with distal malignant biliary obstruction. *Gastrointest Endosc* 2005; **62**: 742-748
26 **Martins FP**, Rossini LG, Ferrari AP. Migration of a covered metallic stent following endoscopic ultrasound-guided hepaticogastrostomy: fatal complication. *Endoscopy* 2010; **42** Suppl 2: E126-E127
27 **Artifon EL**, Chaves DM, Ishioka S, Souza TF, Matuguma SE, Sakai P. Echoguided hepatico-gastrostomy: a case report. *Clinics* (Sao Paulo) 2007; **62**: 799-802
28 **Park do H**, Song TJ, Eum J, Moon SH, Lee SS, Seo DW, Lee SK, Kim MH. EUS-guided hepaticogastrostomy with a fully covered metal stent as the biliary diversion technique for an occluded biliary metal stent after a failed ERCP (with videos). *Gastrointest Endosc* 2010; **71**: 413-419
29 **Siddiqui AA**, Sreenarasimhaiah J, Lara LF, Harford W, Lee C, Eloubeidi MA. Endoscopic ultrasound-guided transduodenal placement of a fully covered metal stent for palliative biliary drainage in patients with malignant biliary obstruction. *Surg Endosc* 2011; **25**: 549-555

S- Editor Gou SX **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2533

World J Gastroenterol 2012 May 28; 18(20): 2533-2539
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Concomitant lung metastasis in patients with advanced hepatocellular carcinoma

Tian Yang, Jun-Hua Lu, Chuan Lin, Song Shi, Ting-Hao Chen, Rong-Hua Zhao, Yi Wang, Meng-Chao Wu

Tian Yang, Jun-Hua Lu, Chuan Lin, Song Shi, Yi Wang, Meng-Chao Wu, Department of Hepatic Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai 200438, China
Ting-Hao Chen, Department of Hepatobiliary Surgery, the First People's Hospital of Ziyang, Ziyang 641205, Sichuan Province, China
Rong-Hua Zhao, Department of Surgery, Royal University Hospital, College of Medicine University of Saskatchewan, Saskatoon, SK S7N0W8, Canada
Author contributions: Yang T, Lu JH, Lin C and Shi S contributed equally to this work; Yang T, Lu JH, Lin C and Wu MC designed the research; Yang T, Shi S and Wang Y performed the research; Yang T, Lin C and Chen TH analyzed the data; Yang T, Zhao RH and Wang Y wrote the paper.
Supported by Grants from National Natural Science Foundation of China, No. 81000166; Shanghai Science and Technology Development Commission, No. 10411963300; Shanghai Program for Excellent Talents in Health System, No. XYQ2011033; and Shanghai Health Bureau, No. ab8307000-2010-92
Correspondence to: Meng-Chao Wu, MD, Professor of Surgery, Director of Eastern Hepatobiliary Surgery Hospital, Department of Hepatic Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai 200438, China. mengchao_wu@sina.com
Telephone: +86-21-81875293 Fax: +86-21-65566085
Received: July 27, 2011 Revised: February 8, 2012
Accepted: February 26, 2012
Published online: May 28, 2012

Abstract

AIM: To investigate the clinical features and prognostic factors of advanced hepatocellular carcinoma (HCC) patients presenting with lung metastasis at initial diagnosis.

METHODS: Between 2001 and 2010, we recruited 76 consecutive HCC patients initially presenting with lung metastasis, without co-existing metastasis from other sites. These patients were divided into three groups: untreated group (n = 22), single treatment group (n = 19), and combined treatment group (n = 35).

RESULTS: Metastasis of bilateral lung lobes was common and noted in 35 patients (46.1%), and most of patients (59/76, 77.6%) presented with multiple lung metastatic nodules. Nineteen patients (25.0%) received single-method treatment, including hepatectomy in 4, transcatheter arterial chemoembolization in 6, radiotherapy in 5, and oral sorafenib in 4. Thirty-five patients (46.1%) received combined treatment modalities. The overall median survival of the all patients was 8.7 ± 0.6 mo; 4.1 ± 0.3, 6.3 ± 2.5 and 18.6 ± 3.9 mo, respectively in the untreated group, single treatment group and combined treatment group, respectively, with a significant difference (log-rank test, $P < 0.001$). Multivariate analysis revealed that Child-Pugh score, the absence or presence of portal vein tumor thrombus, and treatment modality were three independent prognostic factors affecting survival of patients with advanced HCC and concomitant lung metastasis.

CONCLUSION: Combined treatment modalities tend to result in a better survival as compared with the conservative treatment or single treatment modality for HCC patients initially presenting with lung metastasis.

Key words: Hepatocellular carcinoma; Lung metastasis; Prognosis; Survival; Prognostic factor

Peer reviewer: Dr. Alessandro Ferrero, Surgery, Ospedale Mauriziano, Largo Turati 62, 10128 Torino, Italy

Yang T, Lu JH, Lin C, Shi S, Chen TH, Zhao RH, Wang Y, Wu MC. Concomitant lung metastasis in patients with advanced hepatocellular carcinoma. *World J Gastroenterol* 2012; 18(20): 2533-2539 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2533.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2533

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. The number of new cases is estimated to be 500 000-1 000 000 per year[1]. Overall, 80% of HCC are attributed to chronic hepatitis B and C infection. Surgical resection with complete extirpation of tumor gives the best chance of a cure for patients with HCC[2]. However, a majority of HCC patients are considered as advanced or even at end-stage at their first hospital visit, with extensive tumor status, for example, macroscopic vascular invasion, extrahepatic metastasis, *etc*. Of various metastatic sites, the most common site is lung, followed by lymph node, bone and brain[3].

As one type of advanced HCC, HCC presenting with lung metastasis is not unusual, and its prognosis is very poor[4]. Nowadays, there are various treatment modalities for both intrahepatic tumor and extrahepatic metastatic foci of HCC, including surgical resection, transcatheter arterial chemoembolization (TACE), radiotherapy, chemotherapeutics, and recent molecular targeted therapeutic drugs[2]. However, the prognosis and treatment outcomes of advanced HCC presenting with lung metastasis remain poorly evaluated. We investigated the prognosis, treatment outcomes, and independent prognostic factors affecting the survival of a series of HCC patients presenting with lung metastasis at initial diagnosis.

MATERIALS AND METHODS

Patients

From January 2001 to December 2010, 103 consecutive patients were diagnosed as having lung metastasis at the first time of HCC diagnosis in the 5th Department of Hepatobiliary Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China. To evaluate the efficacy of anti-tumor treatment modality for patients with HCC and concomitant isolated lung metastasis, we excluded those patients who showed evidence of severe organ failure (renal, respiratory, or cardiologic problems), poor liver function (Child-Pugh class C) that could affect survival, or co-existing metastasis from other sites. Therefore, 76 of the 103 patients (59 males and 17 females) were recruited for the study. The last follow-up date was January 30, 2011. This study protocol was approved by the Institutional Review Board of the Eastern Hepatobiliary Hospital.

We used a prospectively maintained database and conducted a retrospective study among these patients. We focused on the prognosis and prognostic factors affecting the survival of HCC patients presenting with lung metastasis. To investigate whether local regional or systemic therapy could affect survival, we stratified these 76 patients into three groups: untreated group (n = 22), single treatment group (n = 19), and combined treatment group (n = 35). All of the patients in the untreated group refused any anti-tumor invasive treatment other than conservative treatment, including the support of liver function, after being informed of the expenses and the possible side effects of any anti-tumor invasive treatment. The single treatment method for these patients included hepatectomy, TACE, radiotherapy, chemotherapeutics, and oral sorafenib, while combined treatment modalities were defined as more than one of the above treatment methods or pulmonary metastasectomy.

Laboratory tests

Laboratory blood tests including hepatitis B virus (HBV) markers, anti-hepatitis C virus, serum α-fetoprotein (AFP), carcinoembryonic antigen, platelet, serum albumin, serum total bilirubin, alanine transaminase, aspartate aminotransferase, and prothrombin time were performed.

Diagnosis of HCC and lung metastasis

The diagnosis of HCC was based on concordance between two imaging examinations [ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI)] showing arterial hypervascularity in a focal lesion ⩾ 2 cm or with the combined criteria of an imaging examination and a serum AFP level greater than 400 ng/mL, according to the criteria of the Conference of the European Association for Study of the Liver[5]. In the present study, the diagnosis of HCC was also confirmed by pathohistology in 20 patients.

Lung metastases were diagnosed in all the 76 patients using imaging techniques, including chest CT (n = 62), positron emission tomography-CT (n = 11), and chest MRI (n = 3). In addition, one patient was further confirmed by pathohistology after a biopsy, and four patients after pulmonary metastasectomy.

Statistical analysis

Continuous data were expressed as mean ± SD or median (range). Categorical variables were compared by the χ^2 test or Fisher exact test, and continuous variables were compared by the Student t test or one-way analysis of variance. The survival rate was calculated using the Kaplan-Meier method, and the log-rank test was used to compare survival rates among three groups. Cox's proportional hazards model was used for multivariate analysis. All statistical analysis in this study were done using software package SPSS11.0 (SPSS Inc., Chicago, IL). A P value < 0.05 was defined to be statistically significant.

RESULTS

Patient characteristics

The baseline characteristics of these 76 patients with advanced HCC initially presenting with lung metastasis are summarized in Table 1. The mean age of patients was 52.4 ± 10.7 years. The most common etiology of the liver disease was HBV infection, which occurred in 63 patients (82.9%). There was no statistical difference in the baseline characteristics among the untreated, single treatment and combined treatment groups, including age, etiology of the liver disease, the presence of cirrhosis or ascites, AFP level, Eastern Cooperative Oncology Group (ECOG) scale, Child-Pugh score, and liver function.

Table 1 Baseline characteristics of hepatocellular carcinoma patients presenting with lung metastasis (mean ± SD) *n* (%)

	Total (*n* = 76)	Untreated group (*n* = 22)	Single treated group (*n* = 19)	Combined treated group (*n* = 35)	*P* value
Sex					
Male	59 (77.6)	16 (72.7)	15 (78.9)	28 (80.0)	0.804
Female	17 (22.4)	6 (27.3)	4 (21.1)	7 (20.0)	
Age (yr)	52.4 ± 10.7	52.8 ± 9.3	52.5 ± 11.6	52.1 ± 11.4	0.971
Etiology					
HBV	63 (82.9)	18 (82.9)	16 (84.2)	29 (82.9)	0.989
HCV	3 (3.9)	1 (4.5)	1 (5.3)	1 (2.9)	
Alcohol	5 (6.6)	1 (4.5)	1 (5.3)	3 (8.6)	
Non-B and non-C	5 (6.6)	2 (9.1)	1 (5.3)	2 (5.7)	
Cirrhosis	65 (85.5)	20 (90.9)	17 (89.5)	28 (80.0)	0.443
Platelet ($^{-3}$)	185.8 ± 75.5	180.9 ± 95.1	174.3 ± 74.0	195.1 ± 62.6	0.593
Albumin (g/L)	36.5 ± 4.3	36.3 ± 5.4	35.3 ± 3.5	37.3 ± 3.7	0.276
ALT (IU/L), median (range)	35 (10-352)	35 (15-352)	37 (15-124)	34 (10-210)	0.456
AST (IU/L), median (range)	35 (16-453)	41 (16-453)	44 (20-173)	33 (17-223)	0.364
Total bilirubin (μmol/L)	23.7 ± 13.3	28.1 ± 17.4	23.3 ± 8.1	21.2 ± 12.2	0.162
Prothrombin time (s)	12.9 ± 1.7	13.3 ± 2.3	13.0 ± 1.3	12.5 ± 1.3	0.241
AFP (ng/mL)					
< 400	36 (47.4)	10 (45.5)	7 (36.8)	19 (54.3)	0.461
⩾ 400	40 (52.6)	12 (54.5)	12 (63.2)	16 (52.6)	
ECOG score					
0	17 (22.4)	4 (18.2)	4 (21.1)	9 (25.7)	0.672
1	54 (71.1)	16 (72.7)	15 (78.9)	23 (65.7)	
2	5 (6.6)	2 (9.1)	0 (0)	3 (8.6)	
Ascites					
None	67 (88.2)	18 (81.8)	16 (84.2)	33 (94.3)	0.303
Mild–moderate	9 (11.8)	4 (18.2)	3 (15.8)	2 (5.7)	
Child-Pugh score					
A	61 (80.3)	16 (72.7)	14 (73.7)	31 (88.6)	0.243
B	15 (19.7)	6 (27.3)	5 (26.3)	4 (11.4)	

HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALT: Alanine transaminase; AST: Aspartate aminotransferase; AFP: Alpha-fetoprotein; ECOG: Eastern cooperative oncology group.

Characteristics of intrahepatic tumor and lung metastasis

For intrahepatic tumor status, there was no significant difference in tumor number, tumor location, and the probability of portal vein tumor thrombus or hepatic vein tumor thrombus among the three treatment groups, except for maximum tumor diameter ($P = 0.007$) (Table 2). As such, there is also no significant difference in number, location, and maximum diameter of lung metastatic nodules among these three groups (Table 2).

Treatment modalities

Among all the 76 patients, 22 patients did not receive any anti-tumor treatment but conservative treatment. We divided the remaining 54 patients into two groups according to their treatment schemes. Nineteen patients (25.0%) received single treatment modality (locoregional or systemic therapy), including hepatectomy in 4 patients, TACE in 6, radiotherapy for intrahepatic tumor and lung metastatic nodules in 5 (including γ knife radiosurgery in 3 and X-ray radiotherapy in 2), and oral sorafenib in 4. Thirty-five patients (46.1%) received combined treatment modalities (Table 3). The common treatment modality in the combined treatment group was radiotherapy (30/35, 85.7%), followed by hepatectomy (23/35, 65.7%), TACE (18/35, 51.4%), and oral sorafenib therapy (11/35, 31.4%). In the combined treatment group, radiotherapy (γ knife radiosurgery in 24 and X-ray radiotherapy in 4) was mainly used for lung metastatic nodules (27/28) rather than intrahepatic tumor nodules (8/28). In addition, pulmonary metastasectomies were carried out in 4 patients who underwent hepatectomy during the same operation.

Overall survival

After a median follow-up of 8.7 ± 0.6 mo (range, 1.1-68.7 mo), 59 patients died and 17 patients remained alive. The causes of mortality were disease progression, including hepatic failure in 31 patients (52.5%); cachexia in 12 (20.3%); upper gastrointestinal bleeding in four (6.8%); pneumonia in one (1.7%); and pulmonary thromboembolism in one (1.7%). The cause of mortality of 10 patients was not confirmed. Among 17 surviving patients, only 4 patients were disease-free, including 3 patients who underwent hepatectomy and pulmonary metastasectomy, and one patient who received hepatectomy and subsequent γ-knife radiosurgery. Among 13 patients who were not disease-free but alive, 6 patients still took medicine of sorafenib, and achieved complete or partial response on radiographic examination (Figure 1A and B). The 6-mo, 1-year and 2-year cumulative survival rates of all these patients were 64.5%, 40.1% and 18.5%, respectively.

Table 2 Characteristics of intrahepatic tumor and lung metastasis of hepatocellular carcinoma patients presenting with lung metastasis (mean ± SD) *n* (%)

	Total (*n* = 76)	Untreated group (*n* = 22)	Single treated group (*n* = 19)	Combined treated group (*n* = 35)	*P* value
Intrahepatic tumor					
Maximum tumor diameter (cm)	9.3 ± 3.0	10.7 ± 3.2	9.7 ± 2.8	8.2 ± 2.5	0.007
Tumor number					
Solitary	29 (38.2)	9 (40.9)	6 (31.6)	14 (40.0)	0.791
Multiple/diffuse	47 (61.8)	13 (59.1)	13 (68.4)	21 (60.0)	
Tumor location					
Right	39 (51.3)	13 (59.1)	8 (42.1)	18 (51.4)	0.834
Left	15 (19.7)	4 (18.2)	4 (21.1)	7 (20.0)	
Both	22 (28.9)	5 (22.7)	7 (36.8)	10 (28.6)	
Portal vein tumor thrombus					
Absence	54 (71.1)	14 (63.6)	12 (63.2)	28 (80.0)	0.283
Presence	22 (28.9)	8 (36.4)	7 (36.8)	7 (20.0)	
Hepatic vein tumor thrombus					
Absence	67 (88.2)	19 (86.4)	15 (78.9)	33 (94.2)	0.238
Presence	9 (11.8)	3 (13.6)	4 (21.1)	2 (5.7)	
Lung metastasis					
Number of metastasis					
Solitary	17 (22.4)	4 (18.2)	3 (15.8)	10 (28.6)	0.479
Multiple	59 (77.6)	18 (81.8)	16 (84.2)	25 (71.4)	
Location of metastasis					
Right lung lobe	28 (36.8)	6 (27.3)	8 (42.1)	14 (40.0)	0.721
Left lung lobe	13 (17.1)	4 (18.2)	2 (10.5)	7 (20.0)	
Bialteral lung lobes	35 (46.1)	12 (54.5)	9 (47.4)	14 (40.0)	
Maximum metastasis diameter (cm)	2.5 ± 0.9	2.6 ± 0.9	2.2 ± 1.2	2.5 ± 0.8	0.445

Table 3 Treatment modalities for intrahepatic tumor and/or metastatic lung nodule(s)

Treatment modalities	*n* (%)
Single treatment modality in treated patients (*n* = 19)	
Hepatectomy	4 (21.1)
Pulmonary metastasectomy	0 (0)
Transcatheter arterial chemoembolization	6 (31.6)
Radiotherapy	5 (26.3)
Oral sorafenib	4 (21.1)
Combined treatment modalities in treated patients (*n* = 35)	
Hepatectomy + pulmonary metastasectomy	3 (8.6)
Hepatectomy + pulmonary metastasectomy + oral sorafenib	1 (2.9)
Hepatectomy + transcatheter arterial chemoembolization + radiotherapy	6 (17.1)
Hepatectomy + radiotherapy	9 (25.7)
Hepatectomy + transcatheter arterial chemoembolization + radiotherapy + oral sorafenib	2 (5.7)
Hepatectomy + radiotherapy + oral sorafenib	2 (5.7)
Transcatheter arterial chemoembolization + radiotherapy	6 (17.1)
Transcatheter arterial chemoembolization + radiotherapy + oral sorafenib	3 (8.6)
Transcatheter arterial chemoembolization + oral sorafenib	1 (2.9)
Radiotherapy + oral sorafenib	2 (5.7)

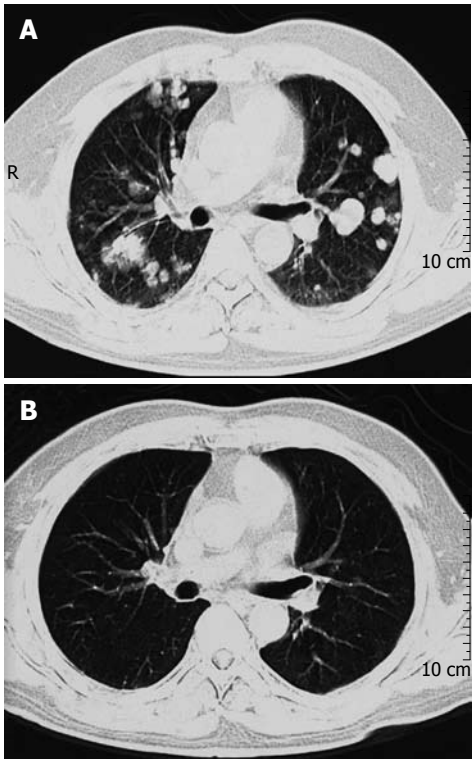


Figure 1 Overall survival. A: Computed tomography (CT) scan showing multiple metastatic nodules in both lung lobes before anti-tumor treatment in a 54-year-old male patient with lung metastasis due to hepatocellular carcinoma; B: CT scan showing the disappearance of previous lung metastatic nodules in 5 mo after γ-knife radiosurgery and oral sorafenib.

Comparison of survival among three treatment groups

The median survival time of combined treatment group was 18.6 ± 3.9 mo, which was longer than that of single treatment group (6.3 ± 2.5 mo) and untreatment group (4.1 ± 0.3 mo), and there was significant statistical differences among these groups (log-rank test, $P < 0.001$) (Figure 2). The 6-mo cumulative survival rate of combined treatment, single treatment and untreated group was 88.6%, 57.9% and 31.8%, respectively; and the 1-year survival rate of the three groups was 68.5%, 21.1% and 8.0%, respectively.

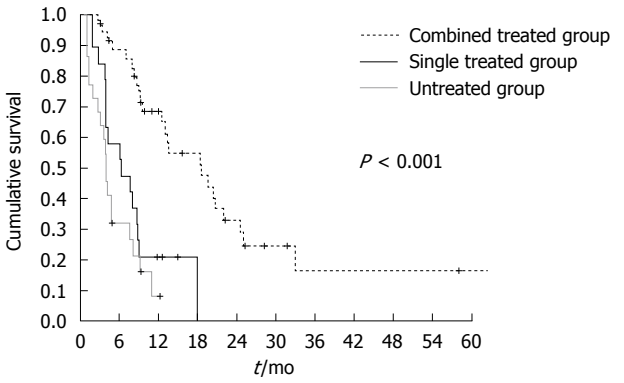
Table 4 Univariate and multivariate analyses to identify prognostic factors of overall survival for hepatocellular carcinoma patients presenting with lung metastasis

Variables	*P* value	
	Univariate analysis	Multivariate analysis
Sex		
Male/female	0.988	–
Age (yr)		
< 50/≥ 50	0.423	–
Etiology		
Non-hepatitis/hepatitis	0.111	–
Cirrhosis		
Absence/presence	0.36	–
ECOG score		
2000/1/2	0.012	0.3
Child-Pugh score		
A/B	< 0.001	< 0.001
Ascites		
No/yes	0.031	0.879
Platelet (^{3})		
< 100/≥ 100	0.198	–
Albumin (g/L)		
< 37.7/≥ 37.7	0.134	–
ALT (IU/L)		
< 40/≥ 40	0.734	–
AST (IU/L)		
< 40/≥ 40	0.356	–
Total bilirubin (μmol/L)		
< 17.1/≥ 17.1	0.319	–
Prothrombin time (s)		
< 14.0/≥ 14.0	0.251	–
AFP (ng/mL)		
< 400/≥ 400	0.03	0.384
Maximum intrahepatic tumor diameter (cm)		
< 8.0/≥ 8.0	0.039	0.214
Intrahepatic tumor number		
Solitary/multiple or diffuse	0.279	–
Intrahepatic tumor location		
Single lobe/both lobes	0.435	–
Portal vein tumor thrombus		
No/yes	< 0.001	< 0.001
Hepatic vein tumor thrombus		
No/yes	0.031	0.194
Lung metastatic tumor number		
Solitary/multiple	0.018	0.321
Lung metastatic tumor location		
Single lobe/both lobes	0.156	–
Maximum metastasis diameter (cm)		
< 2.5/≥ 2.5	0.129	–
Treatment modality		
No/yes	< 0.001	< 0.001

The cutoffs of continuous variable were set according to median value. ECOG: Eastern cooperative oncology group; ALT: Alanine transaminase; AST: Aspartate aminotransferase; AFP: α-fetoprotein.

Univariate and multivariate analyses

In investigation of prognostic factors of survival of HCC presenting with lung metastasis at initial diagnosis, univariate analysis showed that ECOG score, Child-Pugh score, ascites, AFP level, maximum intrahepatic tumor diameter, the absence or presence of portal vein tumor thrombus, the absence or presence of hepatic vein tumor thrombus, lung metastatic tumor number, and treatment modality had prognostic significance (Table 4).



Patients at risk	Total	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo	42 mo	48 mo	54 mo	60 mo
Combined treated	35	31	20	15	8	4	2	2	2	2	1
Single treated	19	11	3	0	0	0	0	0	0	0	0
Untreated	22	7	1	0	0	0	0	0	0	0	0

Figure 2 Comparison of survival among three treatment groups. Survival curves of untreated group (n = 22), single treatment group (n = 19), and combined treatment group (n = 35).

In the Cox proportional hazard model, Child-Pugh score, the absence or presence of portal vein tumor thrombus at the initial presentation, and treatment modality were three independent prognostic factors that affected the survival of HCC patients that presented with lung metastasis at initial diagnosis (Table 4).

DISCUSSION

To the best of our knowledge, this is the first study that investigates in detail the prognosis and prognostic factors of advanced HCC presenting with lung metastasis at initial diagnosis. In our study, the variables of tumor status and liver function status at initial diagnosis were almost comparable. However, the survival times from date of diagnosis until death or last visit among the three groups were significantly different, and patients underwent specific combined treatment modalities had longer survival than those treated with single method or those without anti-tumor treatment. However, it must be noted that there are many therapeutic modalities for tumor nodules, whether regional or systemic, which have their own indications and contraindications, and could not be applied altogether on a specific individual.

There are many case reports and clinical studies of patients with advanced HCC and lung metastasis who underwent radical hepatectomy and pulmonary metastasectomy[6-12]. In the present study, four patients also underwent combined surgical resection, three of whom were still alive at the end of follow-up. We think that the combined radical surgery should be positively considered if intrahepatic tumor and lung metastatic lesion were completely resectable, if the remaining volume of the liver was adequate, and the lung metastatic lesion was single[13]. In theory, removing all existing tumor lesions,

including primary and second lesions, is the only possible therapeutic approach till now.

Recently, with development in radiotherapy techniques, radiotherapy has been shown to play a potential role in a wide spectrum of HCC, therefore it is necessary to evaluate the effect of radiotherapy[14-16]. In our center, stereotactic radiotherapy, particularly γ knife radiosurgery, has become the major therapeutic mordality for lung and brain metastases of HCC. In the present study, there were 35 patients who underwent radiotherapy for intrahepatic HCC tumors and/or lung metastatic nodules, accounting for more than 50%, whether or not they are combined with other anti-tumor treatment modalities. Despite advances in radiotherapy delivery, liver toxicity following radiotherapy remains a dose-limiting factor, and investigations to better understand the pathophysiology of radiotherapy-induced liver toxicity are warranted. There is a particular interest in combining radiotherapy with anti-vascular endothelial growth factor targeting agents for their independent activity in HCC as well as their radiation sensitization properties[17].

Sorafenib is a multikinase inhibitor with effects against tumor proliferation and angiogenesis, and was recently approved for the treatment of advanced HCC[18,19]. Maintenance sorafenib would probably prevent or delay the intrahepatic and extrahepatic spread of HCC after radiotherapy, which provides the rationale for the combination of these treatment modalities[20]. In the present study, 15 patients received oral sorafenib. Although we did not find its significant efficacy in HCC presenting with lung metastasis due to few cases and less strict design, we believed that sorafenib could be used as one part of combined treatment modalities for HCC patients with lung metastasis. However, a large-scale randomized controlled trial is needed to confirm it. Combining surgical resection with sorafenib would be considered as the most optimal treatment modality for HCC patients with lung metastasis.. However, it remains to be confirmed by a randomized controlled clinical trial in the future.

Till now, we have not obtained enough evidences to confirm the role of locoregional hepatectomy in HCC patients with lung metastasis. In our opinion, hepatectomy can be performed when primary intrahepatic HCCs are completely resected, the number of lung metastases is less than 3, and the diameter of individual lung metastasis is less than 3 cm. In addition, any of treatment modalities, such as radiotherapy or TACE, should be used together for lung metastasis.

In the univariate and subsequent multivariate analyses, Child-Pugh score was an independent prognostic factor for HCC patients with lung metastasis. This indicates that although invasive treatment other than conservative treatment can prolong the survival in well-selected patients, one must be careful before applying invasive treatment to all patients.

This study has several limitations. First, it was designed retrospectively. Although the patients' status, including ECOG scale, Child-Pugh score, and liver function, was reviewed in the medical records and did not differ statistically among the three treatment groups, the clinical circumstances at the initial presentation might differ. Therefore, the decision for the treatment mordalities might be biased, and the subsequent patient stratification into various treatment groups might also be biased. Second, treatment modalities, i.e., radiotherapy, TACE, varied, and the number of patients treated was too small to confirm the effectiveness of each treatment modality. Third, the indications of specific treatment modality varied, and selective modalities were given to selective patients with advanced HCC and lung metastasis. It is very hard to build up a standard therapeutic regime for all patients, regardless of primary and secondary tumor site, size and number, liver functional reserve, and patients' general condition.

In conclusion, the present study showed that the prognosis of advanced HCC with concomitant lung metastasis at initial diagnosis is very poor, and combined comprehensive treatment modalities tended to significantly prolong the survival of the patients compared with conservative treatment or single treatment modality. Furthermore, further randomized trials might be required to investigate the optimal treatment modality in the near future.

COMMENTS

Background

The prognosis and treatment outcomes of advanced hepatocellular carcinoma (HCC) presenting with lung metastasis remain poorly evaluated.

Research frontiers

There are various treatment modalities for advanced HCC, including surgical resection, transcatheter arterial chemoembolization, radiotherapy, chemotherapeutics, and administration of molecular targeted therapeutic drugs. This study investigated the prognosis, treatment outcomes, and independent prognostic factors affecting the survival of a series of HCC patients presenting with lung metastasis at initial diagnosis.

Innovations and breakthroughs

In this study, the survival times from date of diagnosis until death or last visit between untreated group, single treatment group, and combined treatment group were significantly different, and patients underwent specific combined treatment modalities had longer survival than those treated with single method or those without anti-tumor treatment. Multivariate analysis revealed that Child-Pugh score, the absence or presence of portal vein tumor thrombus, and treatment modality were three independent prognostic factors affecting survival of patients with advanced HCC and concomitant lung metastasis.

Applications

As one type of advanced HCC, HCC presenting with lung metastasis is not unusual, and its prognosis is very poor. The findings in this study may contribute to its prognosis, and combined treatment modalities tend to result in a better survival for patients with advanced HCC initially presenting with lung metastasis.

Peer review

The authors firstly investigated that combined treatment modalities tended to yield better survival prolongation compared with conservative treatment or single treatment modality for HCC patients initially presenting with lung metastasis, which may be contribute to its prognosis.

REFERENCES

1 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576

2 **Rahbari NN**, Mehrabi A, Mollberg NM, Müller SA, Koch M, Büchler MW, Weitz J. Hepatocellular carcinoma: current management and perspectives for the future. *Ann Surg* 2011; **253**: 453-469

3 **Natsuizaka M**, Omura T, Akaike T, Kuwata Y, Yamazaki K, Sato T, Karino Y, Toyota J, Suga T, Asaka M. Clinical features of hepatocellular carcinoma with extrahepatic metastases. *J Gastroenterol Hepatol* 2005; **20**: 1781-1787

4 **Zhang SM**, Zeng ZC, Tang ZY, Sun J, Cheng JM, Liu R, Wang P, Zhang BH. Prognostic analysis of pulmonary metastases from hepatocellular carcinoma. *Hepatol Int* 2008; **2**: 237-243

5 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430

6 **Lam CM**, Lo CM, Yuen WK, Liu CL, Fan ST. Prolonged survival in selected patients following surgical resection for pulmonary metastasis from hepatocellular carcinoma. *Br J Surg* 1998; **85**: 1198-1200

7 **Nakagawa T**, Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Matsushita M, Todo S. Pulmonary resection for metastases from hepatocellular carcinoma: factors influencing prognosis. *J Thorac Cardiovasc Surg* 2006; **131**: 1248-1254

8 **Tomimaru Y**, Sasaki Y, Yamada T, Eguchi H, Takami K, Ohigashi H, Higashiyama M, Ishikawa O, Kodama K, Imaoka S. The significance of surgical resection for pulmonary metastasis from hepatocellular carcinoma. *Am J Surg* 2006; **192**: 46-51

9 **Kuo SW**, Chang YL, Huang PM, Hsu HH, Chen JS, Lee JM, Lee PH, Lee YC. Prognostic factors for pulmonary metastasectomy in hepatocellular carcinoma. *Ann Surg Oncol* 2007; **14**: 992-997

10 **Chen F**, Sato K, Fujinaga T, Sonobe M, Shoji T, Sakai H, Miyahara R, Bando T, Okubo K, Hirata T, Date H. Pulmonary resection for metastases from hepatocellular carcinoma. *World J Surg* 2008; **32**: 2213-2217

11 **Kawamura M**, Nakajima J, Matsuguma H, Horio H, Miyoshi S, Nakagawa K, Fujisawa T, Kobayashi K. Surgical outcomes for pulmonary metastases from hepatocellular carcinoma. *Eur J Cardiothorac Surg* 2008; **34**: 196-199

12 **Kwon JB**, Park K, Kim YD, Seo JH, Moon SW, Cho DG, Kim YW, Kim DG, Yoon SK, Lim HW. Clinical outcome after pulmonary metastasectomy from primary hepatocellular carcinoma: analysis of prognostic factors. *World J Gastroenterol* 2008; **14**: 5717-5722

13 **Yang T**, Zhang J, Lu JH, Yang LQ, Yang GS, Wu MC, Yu WF. A new staging system for resectable hepatocellular carcinoma: comparison with six existing staging systems in a large Chinese cohort. *J Cancer Res Clin Oncol* 2011; **137**: 739-750

14 **Dawson LA**. The evolving role of radiation therapy in hepatocellular carcinoma. *Cancer Radiother* 2008; **12**: 96-101

15 **Cárdenes HR**. Role of stereotactic body radiotherapy in the management of primary hepatocellular carcinoma. Rationale, technique and results. *Clin Transl Oncol* 2009; **11**: 276-283

16 **Ma S**, Jiao B, Liu X, Yi H, Kong D, Gao L, Zhao G, Yang Y, Liu X. Approach to radiation therapy in hepatocellular carcinoma. *Cancer Treat Rev* 2010; **36**: 157-163

17 **Tse RV**, Guha C, Dawson LA. Conformal radiotherapy for hepatocellular carcinoma. *Crit Rev Oncol Hematol* 2008; **67**: 113-123

18 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390

19 **Colombo M**. Sorafenib in advanced hepatocellular carcinoma: a further step toward personalized therapy of liver cancer. *Gastroenterology* 2009; **136**: 1832-1835

20 **Zhao JD**, Liu J, Ren ZG, Gu K, Zhou ZH, Li WT, Chen Z, Xu ZY, Liu LM, Jiang GL. Maintenance of Sorafenib following combined therapy of three-dimensional conformal radiation therapy/intensity-modulated radiation therapy and transcatheter arterial chemoembolization in patients with locally advanced hepatocellular carcinoma: a phase I/II study. *Radiat Oncol* 2010; **5**: 12

S- Editor Gou SX **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2540

World J Gastroenterol 2012 May 28; 18(20): 2540-2544
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Detection of eukaryotic translation initiation factor 4E and its clinical significance in hepatocellular carcinoma

Xiao-Lin Wang, Hong-Pei Cai, Jun-Hui Ge, Xiao-Feng Su

Xiao-Lin Wang, Hong-Pei Cai, Xiao-Feng Su, Gastroenterology Department, The Second Military Medical University, Changzheng Hospital, Shanghai 200003, China
Jun-Hui Ge, Pathology Department, The Second Military Medical University, Changzheng Hospital, Shanghai 200003, China
Author contributions: Wang XL and Cai HP designed the study; Ge JH performed the immunohistochemical staining and contributed the analytic tools; Su XF collected and analysed data; and Wang XL wrote the manuscript.
Correspondence to: Hong-Pei Cai, Professor, Gastroenterology Department, The Second Military Medical University, Changzheng Hospital, Shanghai 200003, China. hongpeic@163.com
Telephone: +86-21-81885344 Fax: +86-21-63520020
Received: November 6, 2011 Revised: February 20, 2012
Accepted: February 26, 2012
Published online: May 28, 2012

Abstract

AIM: To study the expression of eukaryotic translation initiation factor 4E (eIF4E), which is closely correlated with malignant tumors, and its relationship to prognosis in hepatocellular carcinoma.

METHODS: Western blotting was performed to quantify the elF4E protein expression in the normal human liver cell line L02 and the hepatoma cell lines Hep3B, HepG2, and Huh7. Forty-six hepatocellular carcinoma samples with complete clinical data were obtained from Changzheng Hospital during the period of December 2008 to July 2009. The expression of eIF4E in the tumor samples and their adjacent tissues were detected by immunohistochemistry. The relationship between the test results and hepatocellular carcinoma (HCC) prognosis was statistically analysed by using a COX proportional hazard model.

RESULTS: Western blotting analysis showed that there were distinct eIF4E protein bands in all three of the hepatoma cell lines. In particular, the HepG2 cell line had the highest level of eIF4E protein expression. The L02 cell group had a low eIF4E expression. Immunohistochemical assay showed that there were 32 cases in which the tumour tissue expression was higher than their adjacent tissues, accounting for 69.57%. There were also 14 cases in which the tumour tissue expression was lower or no significant difference was found, accounting for 30.43%. COX proportional hazards model analysis showed that HCC prognosis was related to the depth of invasion, the overexpression of eIF4E and p53, possibly as independent HCC prognostic predictors.

CONCLUSION: In summary, eIF4E expression is associated with liver cancer, and patients with high eIF4E expression levels have a higher risk of recurrence.

Key words: Hepatocellular carcinoma; Eukaryotic translation initiation factor 4E; Western blotting; Immunohistochemistry; Prognosis

Peer reviewer: Ezio Laconi, Professor, University of Cagliari, Via Porcell 4, 09125 Cagliari, Italy

Wang XL, Cai HP, Ge JH, Su XF. Detection of eukaryotic translation initiation factor 4E and its clinical significance in hepatocellular carcinoma. *World J Gastroenterol* 2012; 18(20): 2540-2544 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2540.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2540

INTRODUCTION

Eukaryotic translation initiation factor 4E (eIF4E) is a member of the eIF family. It can specifically bind to the cap structure located at the 5' end of mRNAs named the "m7GpppN cap", which is necessary for mRNA translation initiation, and affects mRNA metabolism, processing, transportation and translation[1]. It plays an important role

in regulating the initial stage protein synthesis[2,3]. eIF4E is highly expressed in a variety of human malignancies[4-7], which has been confirmed to be relevant to the occurrence, invasion and metastasis of carcinomas such as head and neck squamous cell carcinoma[8], laryngeal cancer, non-small cell lung cancer[9-11], breast cancer[12-18], thyroid cancer, oesophageal cancer, stomach cancer, cholangiocarcinoma, colon cancer[19], non-Hodgkin's lymphoma, acute or chronic myeloid leukaemia[20], and lymphoma[21,22]. Experiments have also confirmed that eIF4E is closely related to the prognosis of many carcinomas. However, eIF4E-related studies in the context of hepatocellular carcinoma (HCC) are still rare.

In this study, we separately compared the eIF4E expression levels in normal liver cells with liver cancer cell lines and liver cancer tissues with precancerous tissues. Additionally, we investigated the influence of eIF4E expression on the prognosis of liver cancer. This research may provide an experimental basis for exploring new ways to treat liver cancer.

MATERIALS AND METHODS

Study objects

We selected 46 patients with pathological evidence of HCC and complete clinical data from Shanghai Changzheng Hospital who had liver surgery from January 2007 to January 2009. In these 46 cases, there were 40 males and 6 females who ranged in age from 31 years to 77 years (median age: 52.26 years). With regards to histological grade, there were 42 cases of moderately differentiated HCC, and 4 cases were poorly differentiated. A total of 33 patients had a cancer embolus in the intrahepatic bile duct or vein or had an infiltrated peplos, and 13 patients had no cancer tissue in cutting edge and gallbladder and no infiltrated peplos. p53 pathological testing was positive in 39 patients and negative in 7 cases. None of the patients received preoperative radiotherapy or chemotherapy. The follow-up time was 24 mo, and no case was lost.

Major materials and reagents

The cell lines used for Western blotting were the human liver cancer cell lines Hep3B, HepG2, Huh7, and the normal human liver cell line L02, which was provided by Shanghai Cell Biology Institution of Academia Sinica.

eIF4E (P-2) is a mouse anti-human monoclonal antibody raised against full-length eIF4E (Santa Cruz Biotechnology, Inc.). It is recommended for the detection of eIF4E by Western blotting (dilution: 1:200; dilution range: 1:100-1:1000) and immunohistochemistry (including paraffin-embedded sections; dilution: 1:50; dilution range: 1:50-1:500). The streptavidin-perosidase (SP) kit was provided by Fuzhou Maixin Biotechnology, Inc.

Detection methods

Western blotting analysis: We tested the *eIF4E* gene expression level in normal liver cells and different liver cancer cell lines. The four of cell lines (i.e., HepG2, Hep3B, Huh7, and L02) were incubated with high glucose Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at 37.0 ℃ with 5% CO_2 until the cell concentration reached 5×10^6 cells/mL. Then, we sequentially performed the protein extraction, bicinchoninic acid protein quantification, sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoresis, protein transfer, membrane closure, antibody incubation, and Bio-Rad chemiluminescence.

Immunohistochemistry: We detected the eIF4E protein expression levels in HCC and their adjacent tissues (SP, a particular type of immunohistochemistry). The tumour and adjacent tissues from the same patient were fixed, dehydrated, sectioned, and made into paraffin biopsies. We made 46 paraffin sections. The steps of the SP kit included heating on a baking sheet, incubation, washing, sealing, staining, drying, dehydration, and mounting. To analyse the results, we used two scoring methods. The samples were placed in an electron microscope and were scored for staining intensity as follows. 0: No colour; 1: A yellow colour; 2: A claybank colour; and 3: A brown colour. We then graded the samples for the positivity rate as follows. 0: No positive tumour cell staining; 1: ⩽ 10% positive cells; 2: 11% to 50% positive cells; 3: 51% to 75% positive cells; and 4: > 75% positive cells. Finally, we added the two scores together, and the sum represented the immunohistochemical score as follows. -: 0; +: 1 to 4; ++: 5 to 8; and + + +: 9 to 12. Each cancer tissue section was compared with its adjacent tissue.

Follow up: We analysed the number of cases that had HCC recurrence and metastasis during the post-operative 24 mo. The liver cancer recurrence risk was measured using COX proportional hazards model for statistical analysis. The patient age, gender, histological grade, depth of invasion, eIF4E, p53 status and other prognostic indicators were used for the COX proportional hazards model analysis.

Statistical analysis

We used the SPSS 17.0 statistical software for the statistical analysis. A COX proportional hazards model testing level of $\alpha = 0.05$ and a $P < 0.05$ was considered statistically significant.

RESULTS

eIF4E protein expression in liver cancer cell lines by western blotting

We tested the eIF4E protein expression level in the liver cell line L02 and the liver cancer cell lines Huh7, HepG2, Hep3B. The eIF4E protein bands are shown in Figure 1. The bands were detected by Bio-Rad chemiluminescence to obtain the data shown in Table 1.

The liver cancer cell lines HepG2, Huh7, Hep3B significantly expressed the eIF4E protein, and in particular, the HepG2 cell line had the highest level of eIF4E protein expression. The normal liver cell L02 also expressed

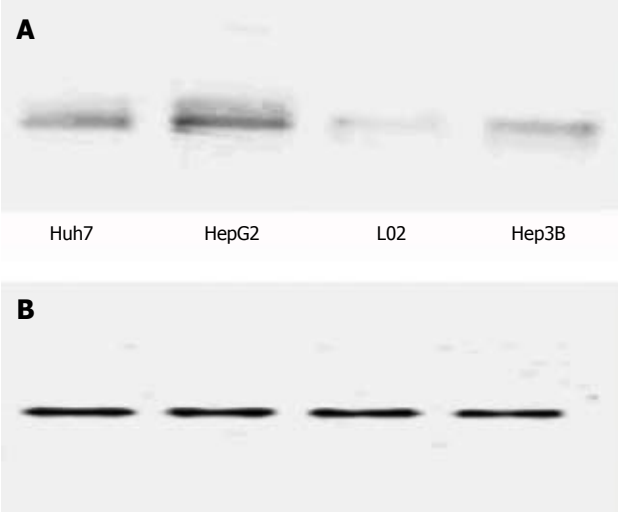


Figure 1 Eukaryotic translation initiation factor 4E protein and glyceraldehyde-3-phosphate dehydrogenase protein bands in a normal human liver cell line and three hepatoma carcinoma cell lines. A: Eukaryotic translation initiation factor 4E protein bands; B: Glyceraldehyde-3-phosphate dehydrogenase protein bands.

Table 1 Eukaryotic translation initiation factor 4E protein expression in the liver cells at different levels

	Huh7	HepG2	L02	Hep3B
GAPDH	827.165	884.682	885.437	848.552
eIF4E	3161.861	5651.885	775.440	4496.191

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; eIF4E: Eukaryotic translation initiation factor 4E.

eIF4E; however, its expression level was low. Glyceraldehyde-3-phosphate dehydrogenase, which was used as the internal reference had bands in each cell line, and no obvious differences were observed with this protein.

eIF4E protein expression in liver cancer and adjacent tissues by immunohistochemistry

We next detected the eIF4E protein expression level in HCC and adjacent tissues. There were 46 pathological tissue paraffin blocks in which 32 HCC tissue cases had higher eIF4E protein expression than their adjacent tissues, accounting for 69.57%. A total of 14 HCC tissue cases had lower expression or no significant difference compared with their adjacent tissues, accounting for 30.43%. The scores were weighted 154:97 = 1.59:1, meaning that, in general, HCC tissues had a higher eIF4E protein expression level than the adjacent tissues.

Figure 2 show that HCC tissues stained significantly stronger than adjacent tissues, indicating that tumour tissues had a higher eIF4E protein expression level. The lightly stained central area in Figure 2A represents necrotic tissue.

eIF4E may be an independent risk factor for liver cancer prognosis

Follow-up statistics showed recurrence in 33 cases and death in 12 cases. The patient age, gender, histological grade, depth of invasion, eIF4E overexpression, p53 positive status and other prognostic indicators were used for COX proportional hazards model for screening analysis. Ultimately, the depth of invasion, eIF4E, and p53 were included in the model with a Sig < 0.05 as shown in Table 2. The statistical significance suggests that these three factors are independent risk factors for liver cancer prognosis.

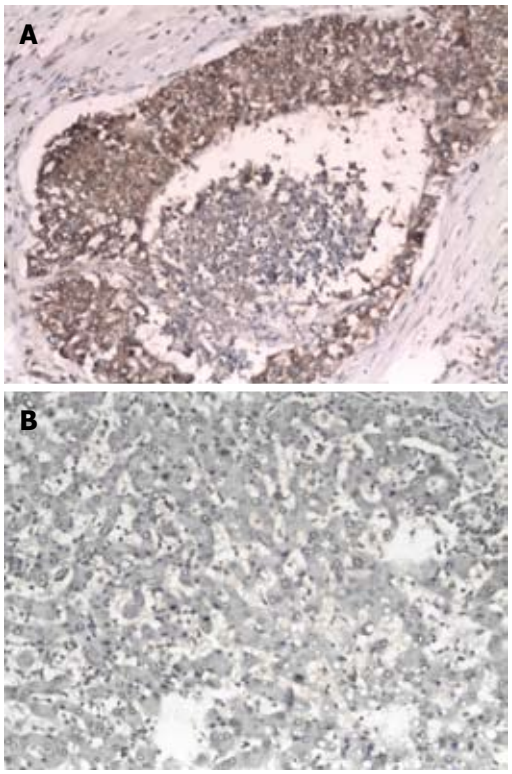


Figure 2 Photo of hepatocellular carcinoma tissue and adjacent tissue by electron microscopy. A: Hepatocellular carcinoma tissue and adjacent interstitial tissue; B: Adjacent tissue. (Hematoxylin and eosin stain, ×100).

DISCUSSION

In eukaryotic cells, translational regulation plays an important role in gene expression. eIF4E is involved in the regulation of the mRNA translation process. It can enhance the translation of some important growth factors and cell growth regulators and affect protein synthesis, the cell cycle, cancer gene activation, and apoptosis; it also plays an important role in malignant transformation and metastasis.

eIF4E regulates the translation of cancer-related mRNAs (i.e., it is involved in the activation of proto-oncogenes, angiogenesis, apoptosis, invasion and metastasis) that are involved in tumour occurrence and development. Normal tissues have a low eIF4E expression level. eIF4E was overexpressed, in a variety of malignant tumours including head and neck squamous cell carcinoma, laryngeal cancer, lung cancer, breast cancer, thyroid cancer and other cancer tissues[4,23]. Its high expression was correlated with tumour invasion and metastasis. However, studies of eIF4E in liver cancer are rare. At present, there are studies

Table 2 COX proportional hazards model analysis

	B	SE	Wald	df	Sig	Exp(B)	95% CI for Exp(B)	
							Lower	Upper
Eukaryotic translation initiation factor 4E	1.971	0.926	4.529	1	0.033	7.179	1.169	44.100
Depth of invasion	3.122	1.211	6.650	1	0.010	22.690	2.115	243.423
Histological grade	0.410	1.156	0.126	1	0.723	1.506	0.156	14.527
Gender	1.671	1.152	2.104	1	0.147	5.319	0.556	50.890
Age	-0.017	0.028	0.354	1	0.552	0.983	0.930	1.040
p53	-3.208	0.825	15.118	1	0.000	0.040	0.008	0.204

B: Coefficient of regression; SE.: Standard error; Wald: The index of regression effect; df: Degrees of freedom; Sig: *P* value; Exp(B): Odds ratio.

that involve the targeting eIF4E in head and neck squamous cell carcinoma[24,25], breast cancer[13-18], non-small cell lung cancer[26], blood malignancies[27-29] and other studies[6,30-32]. However, few studies have focused on targeting eIF4E in HCC.

In this study, we tested the expression of eIF4E protein in a normal human liver cell line and three different liver cancer cell lines. eIF4E protein expression was high in the three liver cancer cell lines and higher than in the normal liver cell L02. The HepG2 cell line had an especially high level of eIF4E protein expression. By comparing 46 cases of human liver cancer and adjacent tissues, we found that eIF4E protein expression was higher in most of the cancer tissues than in the adjacent tissues. COX proportional hazards model analysis showed that the depth of invasion, eIF4E, and p53 status were independent risk factors of liver cancer prognosis.

Based on these studies, we believe that eIF4E protein expression may be closely associated with the occurrence of human liver cancer development and prognosis. It has been confirmed *in vivo* and *in vitro* that sorafenib treatment can inhibit the RAF/MEK/ERK signal transduction pathway, reduce the eIF4E phosphorylation level, reduce Mcl-l protein, and induce hepatoma cell apoptosis[33,34]. Accordingly, we suggest that lower levels of *eIF4E* gene expression may inhibit liver cancer. Targeting and adjusting the eIF4E level and activity may inhibit cancer cell growth[6,30,31,35], which may become a new paradigm in the field of the biological treatment of liver cancer[36].

COMMENTS

Background

Hepatocellular carcinoma (HCC), which has a poor prognosis and a low five-year survival rate, is the most common malignant tumour in our country. At present, there are no effective therapies including radiotherapy, chemotherapy, and surgery. Eukaryotic translation initiation factor 4E (eIF4E) plays an important role in the translation initiation phase of a eukaryotic cell. It has been confirmed that eIF4E can specifically bind to the 5' mRNA cap (m7GpppN) and modulate its translation and expression. Its expression is closely associated with the generation, infiltration, and metastasis of many tumours such as head and neck, larynx, lung, mammary gland, thyroid gland, oesophagus, stomach, bile duct, colon.

Research frontiers

There are many researchers that are targeting eIF4E in head and neck squamous cell carcinoma, breast cancer, non-small cell lung cancer, blood malignancies and other carcinomas; however, studies that involve the targeting eIF4E in HCC are rare.

Innovations and breakthroughs

Research concerning the effects of eIF4E on HCC is limited. The authors tested the expression of the eIF4E protein in liver cancer cell lines and cancer tissues and used COX proportional hazards model analysis to show that eIF4E was an independent risk factor HCC prognosis.

Applications

The targeted regulation of the level and activity of eIF4E may inhibit cancer cell growth, which may become a new treatment paradigm in the liver cancer field.

Terminology

eIF4E is a member of the eIF family. It can specifically bind to the cap structure located at the 5' end of mRNAs named the "m7GpppN cap", which is necessary for mRNA translation initiation, and affects mRNA metabolism, processing, transportation and translation. It plays an important role in regulating the initial stage protein synthesis. eIF4E is highly expressed in a variety of human malignancies, which has been confirmed to be relevant to the occurrence, invasion and metastasis of carcinomas such as head and neck squamous cell carcinoma, laryngeal cancer, non-small cell lung cancer, breast, thyroid cancer, oesophageal cancer, stomach cancer, cholangiocarcinoma, colon cancer, non-Hodgkin's lymphoma, acute or chronic myeloid leukaemia, and lymphoma. Experiments have also confirmed that eIF4E is closely related to the prognosis of many carcinomas.

Peer review

This paper is interesting and worth being published if authors can satisfactorily address the concerns raised regarding immunohistochemical expression of eIF4E.

REFERENCES

1 **Rhoads RE**. eIF4E: new family members, new binding partners, new roles. *J Biol Chem* 2009; **284**: 16711-16715

2 **Sonenberg N**. eIF4E, the mRNA cap-binding protein: from basic discovery to translational research. *Biochem Cell Biol* 2008; **86**: 178-183

3 **Xu X**, Vatsyayan J, Gao C, Bakkenist CJ, Hu J. Sumoylation of eIF4E activates mRNA translation. *EMBO Rep* 2010; **11**: 299-304

4 **Furic L**, Rong L, Larsson O, Koumakpayi IH, Yoshida K, Brueschke A, Petroulakis E, Robichaud N, Pollak M, Gaboury LA, Pandolfi PP, Saad F, Sonenberg N. eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *Proc Natl Acad Sci USA* 2010; **107**: 14134-14139

5 **Fan S**, Ramalingam SS, Kauh J, Xu Z, Khuri FR, Sun SY. Phosphorylated eukaryotic translation initiation factor 4 (eIF4E) is elevated in human cancer tissues. *Cancer Biol Ther* 2009; **8**: 1463-1469

6 **Thumma SC**, Kratzke RA. Translational control: a target for cancer therapy. *Cancer Lett* 2007; **258**: 1-8

7 **Noske A**, Lindenberg JL, Darb-Esfahani S, Weichert W, Buckendahl AC, Röske A, Sehouli J, Dietel M, Denkert C. Activation of mTOR in a subgroup of ovarian carcinomas: correlation with p-eIF-4E and prognosis. *Oncol Rep* 2008; **20**: 1409-1417

8 **Sunavala-Dossabhoy G**, Palaniyandi S, Clark C, Nathan CO, Abreo FW, Caldito G. Analysis of eIF4E and 4EBP1 mRNAs in head and neck cancer. *Laryngoscope* 2011; **121**: 2136-2141

9 **Khoury T**, Alrawi S, Ramnath N, Li Q, Grimm M, Black J, Tan D. Eukaryotic initiation factor-4E and cyclin D1 expression associated with patient survival in lung cancer. *Clin Lung Cancer* 2009; **10**: 58-66

10 **Wang R**, Geng J, Wang JH, Chu XY, Geng HC, Chen LB. Overexpression of eukaryotic initiation factor 4E (eIF4E) and its clinical significance in lung adenocarcinoma. *Lung Cancer* 2009; **66**: 237-244

11 **Yoshizawa A**, Fukuoka J, Shimizu S, Shilo K, Franks TJ, Hewitt SM, Fujii T, Cordon-Cardo C, Jen J, Travis WD. Overexpression of phospho-eIF4E is associated with survival through AKT pathway in non-small cell lung cancer. *Clin Cancer Res* 2010; **16**: 240-248

12 **McClusky DR**, Chu Q, Yu H, Debenedetti A, Johnson LW, Meschonat C, Turnage R, McDonald JC, Abreo F, Li BD. A prospective trial on initiation factor 4E (eIF4E) overexpression and cancer recurrence in node-positive breast cancer. *Ann Surg* 2005; **242**: 584-590; discussion 590-592

13 **Holm N**, Byrnes K, Johnson L, Abreo F, Sehon K, Alley J, Meschonat C, Md QC, Li BD. A prospective trial on initiation factor 4E (eIF4E) overexpression and cancer recurrence in node-negative breast cancer. *Ann Surg Oncol* 2008; **15**: 3207-3215

14 **Zhou S**, Wang GP, Liu C, Zhou M. Eukaryotic initiation factor 4E (eIF4E) and angiogenesis: prognostic markers for breast cancer. *BMC Cancer* 2006; **6**: 231

15 **Wolfort R**, de Benedetti A, Nuthalapaty S, Yu H, Chu QD, Li BD. Up-regulation of TLK1B by eIF4E overexpression predicts cancer recurrence in irradiated patients with breast cancer. *Surgery* 2006; **140**: 161-169

16 **Flowers A**, Chu QD, Panu L, Meschonat C, Caldito G, Lowery-Nordberg M, Li BD. Eukaryotic initiation factor 4E overexpression in triple-negative breast cancer predicts a worse outcome. *Surgery* 2009; **146**: 220-226

17 **Coleman LJ**, Peter MB, Teall TJ, Brannan RA, Hanby AM, Honarpisheh H, Shaaban AM, Smith L, Speirs V, Verghese ET, McElwaine JN, Hughes TA. Combined analysis of eIF4E and 4E-binding protein expression predicts breast cancer survival and estimates eIF4E activity. *Br J Cancer* 2009; **100**: 1393-1399

18 **Hiller DJ**, Chu Q, Meschonat C, Panu L, Burton G, Li BD. Predictive value of eIF4E reduction after neoadjuvant therapy in breast cancer. *J Surg Res* 2009; **156**: 265-269

19 **Rosenwald IB**, Chen JJ, Wang S, Savas L, London IM, Pullman J. Upregulation of protein synthesis initiation factor eIF-4E is an early event during colon carcinogenesis. *Oncogene* 1999; **18**: 2507-2517

20 **Topisirovic I**, Guzman ML, McConnell MJ, Licht JD, Culjkovic B, Neering SJ, Jordan CT, Borden KL. Aberrant eukaryotic translation initiation factor 4E-dependent mRNA transport impedes hematopoietic differentiation and contributes to leukemogenesis. *Mol Cell Biol* 2003; **23**: 8992-9002

21 **Inamdar KV**, Romaguera JE, Drakos E, Knoblock RJ, Garcia M, Leventaki V, Medeiros LJ, Rassidakis GZ. Expression of eukaryotic initiation factor 4E predicts clinical outcome in patients with mantle cell lymphoma treated with hyper-CVAD and rituximab, alternating with rituximab, high-dose methotrexate, and cytarabine. *Cancer* 2009; **115**: 4727-4736

22 **Ruggero D**, Montanaro L, Ma L, Xu W, Londei P, Cordon-Cardo C, Pandolfi PP. The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. *Nat Med* 2004; **10**: 484-486

23 **Hagner PR**, Schneider A, Gartenhaus RB. Targeting the translational machinery as a novel treatment strategy for hematologic malignancies. *Blood* 2010; **115**: 2127-2135

24 **Nathan CO**, Liu L, Li BD, Abreo FW, Nandy I, De Benedetti A. Detection of the proto-oncogene eIF4E in surgical margins may predict recurrence in head and neck cancer. *Oncogene* 1997; **15**: 579-584

25 **Culjkovic B**, Borden KL. Understanding and Targeting the Eukaryotic Translation Initiation Factor eIF4E in Head and Neck Cancer. *J Oncol* 2009; **2009**: 981679

26 **Zhang B**, Zhu C, Chen B, Zhang X, Ye M, Lin A. [Expression and its clinical significance of eIF4E in non-small cell lung cancer]. *Zhongguo Feiai Zazhi* 2010; **13**: 1132-1135

27 **Borden KL**, Culjkovic-Kraljacic B. Ribavirin as an anti-cancer therapy: acute myeloid leukemia and beyond? *Leuk Lymphoma* 2010; **51**: 1805-1815

28 **Muta D**, Makino K, Nakamura H, Yano S, Kudo M, Kuratsu J. Inhibition of eIF4E phosphorylation reduces cell growth and proliferation in primary central nervous system lymphoma cells. *J Neurooncol* 2011; **101**: 33-39

29 **Assouline S**, Culjkovic B, Cocolakis E, Rousseau C, Beslu N, Amri A, Caplan S, Leber B, Roy DC, Miller WH, Borden KL. Molecular targeting of the oncogene eIF4E in acute myeloid leukemia (AML): a proof-of-principle clinical trial with ribavirin. *Blood* 2009; **114**: 257-260

30 **Graff JR**, Konicek BW, Carter JH, Marcusson EG. Targeting the eukaryotic translation initiation factor 4E for cancer therapy. *Cancer Res* 2008; **68**: 631-634

31 **Hsieh AC**, Ruggero D. Targeting eukaryotic translation initiation factor 4E (eIF4E) in cancer. *Clin Cancer Res* 2010; **16**: 4914-4920

32 **Choi CH**, Lee JS, Kim SR, Lee YY, Kim CJ, Lee JW, Kim TJ, Lee JH, Kim BG, Bae DS. Direct inhibition of eIF4E reduced cell growth in endometrial adenocarcinoma. *J Cancer Res Clin Oncol* 2011; **137**: 463-469

33 **Liu L**, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilhelm S, Lynch M, Carter C. Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. *Cancer Res* 2006; **66**: 11851-11858

34 **Huynh H**, Ngo VC, Koong HN, Poon D, Choo SP, Thng CH, Chow P, Ong HS, Chung A, Soo KC. Sorafenib and rapamycin induce growth suppression in mouse models of hepatocellular carcinoma. *J Cell Mol Med* 2009; **13**: 2673-2683

35 **Graff JR**, Konicek BW, Vincent TM, Lynch RL, Monteith D, Weir SN, Schwier P, Capen A, Goode RL, Dowless MS, Chen Y, Zhang H, Sissons S, Cox K, McNulty AM, Parsons SH, Wang T, Sams L, Geeganage S, Douglass LE, Neubauer BL, Dean NM, Blanchard K, Shou J, Stancato LF, Carter JH, Marcusson EG. Therapeutic suppression of translation initiation factor eIF4E expression reduces tumor growth without toxicity. *J Clin Invest* 2007; **117**: 2638-2648

36 **Jiang Y**, Zhang SH, Han GQ, Qin CY. Interaction of Pdcd4 with eIF4E inhibits the metastatic potential of hepatocellular carcinoma. *Biomed Pharmacother* 2010; **64**: 424-429

S- Editor Gou SX **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2545

World J Gastroenterol 2012 May 28; 18(20): 2545-2553
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Association between body mass index and erosive esophagitis: A meta-analysis

Nan Cai, Guo-Zhong Ji, Zhi-Ning Fan, Yan-Feng Wu, Fa-Ming Zhang, Zhi-Fei Zhao, Wei Xu, Zheng Liu

Nan Cai, Guo-Zhong Ji, Zhi-Ning Fan, Fa-Ming Zhang, Zhi-Fei Zhao, Wei Xu, Zheng Liu, Department of Digestive Endoscopy and Medical Center for Digestive Diseases, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan Road, Nanjing 210011, Jiangsu Province, China
Yan-Feng Wu, Department of Neurology, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan Road, Nanjing 210011, Jiangsu Province, China
Author contributions: Cai N designed the study, wrote the paper and reviewed all articles for inclusion with Wu YF; Liu Z performed the final consensus review and the final data analysis; and all authors contribute to this study and approved the final version to be published.
Correspondence to: Zheng Liu, MD, PhD, Department of Digestive Endoscopy and Medical Center for Digestive Diseases, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiangjiayuan Road, Nanjing 210011, Jiangsu Province, China. liuzheng117@126.com
Telephone: +86-25-58509883 Fax: +86-25-58509883
Received: August 6, 2011 Revised: March 2, 2012
Accepted: April 28, 2012
Published online: May 28, 2012

Abstract

AIM: To conduct a meta-analysis to estimate the determinants of the association between erosive esophagitis (EE) and body mass index (BMI).

METHODS: We identified the studies using PubMed. Studies were selected for analysis based on certain inclusion and exclusion criteria. Data were extracted from each study on the basis of predefined items. Meta-analyses were performed to verify the risk factors, such as obesity and gender.

RESULTS: Twenty-one studies were included in this systematic review. These studies demonstrated an association between increasing BMI and the presence of EE [95% confidence interval (CI): 1.35-1.88, overweight, odds ratio (OR) = 1.60, *P* value homogeneity = 0.003, 95% CI: 1.65-2.55, obese, OR = 2.05, *P* < 0.01]. The heterogeneity disappeared by stratifying for gender. No publication bias was observed in this meta-analysis by the Egger method.

CONCLUSION: This analysis demonstrates a positive association between BMI and the presence of EE, especially in males. The risk seems to progressively increase with increasing weight.

Key words: Erosive esophagitis; Gastroesophageal reflux disease; Obesity; Body mass index; Meta-analysis

Peer reviewers: Michele Cicala, Professor, MD, Department of Gastroenterology, University Campus Bio Medico, Via Longoni, 83, 00155 Rome, Italy; Wojciech Blonski, MD, PhD, University of Pennsylvania, GI Research-Ground Centrex, 3400 Spruce St, Philadelphia, PA 19104, United States; Kwang Jae Lee, MD, PhD, Professor, Department of Gastroenterology, Ajou University School of Medicine, San5, Woncheondong, Yeongtongku, Suwon 443-721, South Korea

Cai N, Ji GZ, Fan ZN, Wu YF, Zhang FM, Zhao ZF, Xu W, Liu Z. Association between body mass index and erosive esophagitis: A meta-analysis. *World J Gastroenterol* 2012; 18(20): 2545-2553 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2545.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2545

INTRODUCTION

The symptoms of gastroesophageal reflux disease (GERD) are common health problems in industrialized societies. It is a highly prevalent gastrointestinal disorder encountered in clinical practice[1,2]. Erosive esophagitis (EE) is one of the most common forms of GERD. It occurs when excessive reflux of acid and pepsin results in necrosis of surface layers of the esophageal mucosa, thus causing erosions and

ulcers[3]. The etiology of EE may be multifactorial. Esophageal mucosal resistance, gastroesophageal reflux, volume and composition of the gastric contents, contact time for the refluxed material, the degree of incompetence of the intrinsic lower esophageal sphincter, and the presence of a sliding hiatus hernia are likely important determinants[4]. It is a chronic disease that exhausts socioeconomic and medical resources and its symptoms may lower the quality of life of the patients. Additionally, patients with EE are at increasing risk of developing Barrett's esophagus and esophageal adenocarcinoma[5].

During the past several decades, obesity has emerged as a major health concern in the Western world[6]. Several studies have found an increased risk of esophagitis in overweight patients[7-9]. Excess adiposity is a known risk factor for morbidity, including several cancers[10]. Recently, a relationship between obesity and GERD has been reported[11]. One recent population-based case control study reported a strong association between body mass index (BMI) and esophagitis in females, but not in males[12]. Given these associations, it would seem logical that increasing BMI is associated with EE. However, studies on the association between BMI and reflux esophagitis have yielded inconsistent results[13-16], though a few have found a strong relationship between obesity and EE[17,18].

The aim of this study was to investigate the effect of BMI on risk for EE by performing a meta-analysis of all available literature published in PubMed up to April 2011. By performing a meta-analysis of the studies that met our selection criteria, we hoped to better characterize the association between increased BMI and EE.

MATERIALS AND METHODS

Search strategy

Two investigators independently performed a systematic search of all existing English-language literatures published up to April 2011 using PubMed, an electronic search engine for published manuscripts. Search terms included "obesity", "BMI", "overweight" or "BMI", combined with "reflux or EE". A total of 268 articles were identified after the preliminary search was reviewed in further details.

Study selection

Studies were included if they met all the following inclusion criteria: (1) Cross-sectional, case control, or cohort studies that permitted assessment of a causal relationship between BMI and EE; (2) Studies with documented and clearly-defined BMI in kg/m^2 for all participants; (3) Studies that reported a relative risk or odds ratio (OR) with confidence intervals or provided sufficient data to permit their calculation; and (4) Studies with EE diagnosed by upper endoscopy. The inclusion criteria were not otherwise restricted by study size or publication type. The followings were chosen as the exclusion criteria: (1) Studies not limited to humans or not written in English; (2) Studies that did not report risk estimates or raw data to allow independent calculation of these estimates; and (3) Case reports, case series or studies that lacked a control group.

Data abstraction

The abstracted data included information on the source of the study population, study design (case control, cohort, or cross-sectional), length of the study period, primary aim of the study, exposure definitions (BMI definitions of normal, overweight or obese), exposure measurement method (self-reported *vs* measured BMI), outcome definitions (diagnosis of EE with endoscopy), total number of subjects with EE, case and control criteria, ORs or risk ratios with and without adjustment for potential confounders and potential confounders used for adjustment.

Exposure definition

We defined body mass categories using the following BMI [weight (in kilograms)/height (in meters)^2]: "normal" (BMI between 18.5 and 25 kg/m^2), "overweight" (BMI between 25 and 28 kg/m^2), and "obese" (BMI $\geqslant$ 28 kg/m^2). These groupings represented the divisions or quartiles most frequently reported in the literature even though they differed somewhat from BMI categories in common use (overweight, BMI 25-29.9 kg/m^2; obese, BMI $\geqslant$ 30 kg/m^2)[11]. We also created a category that included both overweight and obese (BMI $\geqslant$ 25 kg/m^2). For each study, we selected the BMI classification that most closely approximated each of these categories. We included more than one estimate from the studies (e.g., if a study reported an OR for persons with a BMI 25-28 kg/m^2 and an OR for persons with a BMI $\geqslant$ 28 kg/m^2, both ORs were included in the summary estimate as BMI $\geqslant$ 25 kg/m^2)[11]. We then compared the risk of EE among the BMI categories.

We used estimates adjusted for potential confounders whenever they were available; if no adjusted estimates were provided, unadjusted estimates were used or calculated from the data[11].

Outcome definition

An outcome was defined as EE diagnosed with endoscopy. The severity of EE was graded from A to D according to the LA classification[19] or modified Savary-Miller classification (grade Ⅰ, single or multiple non-confluent erosions; grade Ⅱ, confluent non-circumferential multiple erosion; grade Ⅲ, circumferential erosions; and grade Ⅳ, ulcer and/or stricture)[20].

Statistical analysis

The BMI data were extracted from each study and analyzed with STATA 11.0 (StataCorp, College Station, TX, United States, www.stata.com). Summary OR estimates were calculated using either relative risks (for cohort stud-

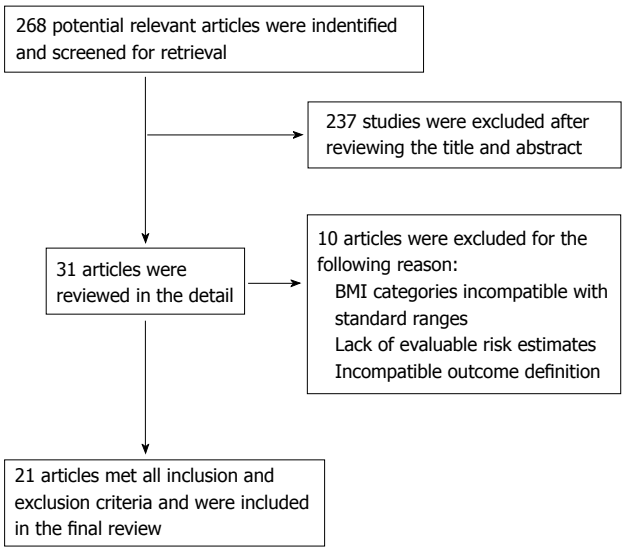


Figure 1 Flow diagram. BMI: Body mass index.

ies) or OR (for case control studies). Summary OR estimates were calculated based on the assumption of fixed effects and heterogeneity was tested using the Mantel-Haenszel method[21]. We evaluated heterogeneity by comparing the results between the fixed effects model and a random effects model[21]. Heterogeneity among the studies was analyzed using χ^2 test and considered present if $P \leqslant 0.05$ or if there was more than a 20% difference in the summary estimates between the two models. To enhance the confidence of the results of the statistics when the number of combined studies was deficient, we used the I^2 metric, which describes the proportion of variability across studies that is due to score heterogeneity. If $I^2 = 0$, there is no heterogeneity. $I^2 > 50\%$ is considered to be indicative of heterogeneity. Larger values indicate greater heterogeneity. If these tests indicated heterogeneity, we explored possible causes[21-24]. Then, to exclude the excessive influence of any single study, we assessed whether exclusion of any single study substantially altered the magnitude or heterogeneity of the summary estimate. We also stratified analyses by several factors[25-31]. Funnel plots were produced and Egger's test[32] was conducted to examine publication bias.

RESULTS

We identified 268 published articles or abstracts (Figure 1). After review of titles and abstracts, 31 articles appeared to meet the initial inclusion criteria. The excluded studies were review articles, animal experiments, case series that lacked appropriate control groups and studies that did not report the subject of interest. These 31 studies underwent a complete data abstraction. Ten additional studies were excluded after data abstraction for the following reasons: BMI categories that were inconsistent with the proposed reference ranges[7,33-36], inconsistent outcome definition[37], lack of proper control group[38], and lack of evaluable risk estimates within the proposed categories[39-41].

The remaining 21 studies[4,8,12,42-59] (i.e., four cross-sectional, three cohort, 14 case control studies) were included in the primary analysis (Tables 1 and 2). Twelve studies were conducted for the primary purpose of evaluating the relationship between BMI and EE[4,44,45,49-52,54,55,57-59], eight studies were conducted to identify the variety of risk factors for EE, including BMI[8,12,42,46-48,53,56], and one study described the clinical characteristics of EE and non-erosive reflux disease, including BMI[43]. In Table 1, controls and normal groups were composed of general population and healthy volunteers. Eighteen studies were included in Table 3 because of their stratification by gender.

The pooled OR of EE related to BMI of 25 kg/m^2 or higher was 1.64-fold greater than that of EE related to BMI less than 25 kg/m^2 (OR, 1.64, 95% CI: 1.45-1.85, test for homogeneity, $P = 0.000$, $I^2 = 65.7\%$) (Figure 2, Table 3).

Stratification by gender and BMI category showed a homogeneous positive association between increased BMI and EE, and the strength of the association with increased BMI (Table 3). The risk for overweight males (OR, 1.40, 95% CI: 1.11-1.75, $P = 0.285$) increased further for obese males (OR, 1.75, 95% CI: 1.02-2.96, $P = 0.099$) (Figure 3). The pooled OR in females and males for BMI greater than 25 kg/m^2 were 1.45 (95% CI: 1.26-1.66) and 1.52 (95% CI: 1.24-1.87), respectively. Therefore, we considered there was a strong positive association between increasing BMI and EE in males, but not in females.

Evaluation of heterogeneity

The initial summary estimates for EE were heterogeneous, as described above. Stratification by BMI category did not substantially resolve the heterogeneity; however, additional stratification by gender provided more homogeneity. Stratification of the entire population by exposure measurement (e.g., self-report *vs* measured), or study design (case control *vs* cohort) did not substantially influence the initial heterogeneity (Table 3).

Publication bias

The rank correlation test did not suggest the presence of publication bias for the main summary estimates for either the overweight ($P = 0.656$) or the obese and overweight ($P = 0.804$). A review of funnel plots did not demonstrate patterns strongly suggestive of publication bias (Figure 4).

DISCUSSION

Our pooled results of observational studies demonstrated a positive association between increased BMI and the risk of EE. The strength of the association increased with increasing BMI and there was a trend towards a stronger association in males than in females. Unlike other non-modifiable risk factors such as age, race and gender, BMI is potentially modifiable. Thus, identifying a relationship

Table 1 Study characteristics

Authors	Yr	Design	Region	Population size	Case population	Reference population	Confounders adjusted for
Ha *et al*[43]	2010	Case-control	South Korea	*n* = 292 (EE), *n* = 500 (NERD)	Single hospital	Hospital controls	G, E, T, J, OD, WHR, TG
Nam *et al*[44]	2010	Cohort	South Korea	*n* = 495 (EE), *n* = 3779 (normal)	General population	General population	WC , WHR , VAT , SAT
Wang *et al*[46]	2010	Case-control	China	*n* = 70 (EE), *n* = 502 (non-EE)	General population	General population	A, G, S, B, T, E, C, tea drinking, spicy food consumption, betel nut use
Koo *et al*[45]	2009	Case-control	South Korea	*n* = 42 (EE), *n* = 987 (control)	General population	General population	G, T, E, TG ,
Koo *et al*[45]	2009	Case-control	South Korea	*n* = 42 (EE), *n* = 1007 (control)	General population	General population	G, T, E, TG ,
Chua *et al*[47]	2009	Case-control	Taiwan, China	*n* = 427 (EE), *n* = 427 (control)	Single hospital	Hospital controls	TG, Glucose intolerance, HDL-C, SBP
Song *et al*[48]	2009	Case-control	South Korea	*n* = 639 (EE), *n* = 5443 (non-EE)	Single hospital	Hospital controls	A, G, T, E, H, TC, HDL-C, LDL-C, TG, BP, fasting glucose
Lien *et al*[49]	2009	Case-control	Taiwan, China	*n* = 102 (EE), *n* = 1942 (non-EE)	Single hospital	Hospital controls	A, G, J
Lien *et al*[49]	2009	Case-control	Taiwan, China	*n* = 240 (EE), *n* = 1662 (non-EE)	Single hospital	Hospital controls	A, G, J
Nam *et al*[50]	2009	Cohort	South Korea	*n* = 552 (EE), *n* = 8019 (non-EE)	General population	General population	A, WC, E, T
Lee *et al*[51]	2009	Case-control	South Korea	*n* = 100 (EE), *n* = 100 (control)	Single hospital	Hospital controls	WHR, T, J, VAT, SAT, VAT/SAT
Chung *et al*[52]	2008	Case-control	South Korea	*n* = 3539 (EE), *n* = 3539 (control)	Single hospital	Hospital controls	E, T, metabolic syndrome
Zagari *et al*[53]	2008	Cross-sectional	Italy	*n* = 122 (EE), *n* = 911 (non-EE)	General population	General population	A, G, E, T, H, J, C, medication use, peptic ulcer
Lee *et al*[54]	2008	Case-control	South Korea	*n* = 292 (EE), *n* = 2896 (control)	Medical center	Medical center	G, TC, TG, WHR, J, T, OD, PBF
Kim *et al*[42]	2008	Case-control	South Korea	*n* = 1810 (EE), *n* = 20154 (normal)	Multiple hospital	Multiple hospital	G, E, J, H, TC, TG, T, medications for liver/heart disease
Moki *et al*[56]	2007	Case-control	Japan	*n* = 191 (EE), *n* = 4968 (non-EE)	General population	General population	A, G, BP, TG, FBG
Kim *et al*[58]	2007	Case-control	South Korea	*n* = 1090 (EE), *n* = 26229 (non-EE)	Single hospital	Hospital controls	A, G, E, T
Nocon *et al*[55]	2007	Cohort	Germany	*n* = 5289 (EE), *n* = 926 (non-EE)	General population	General population	A, T, E,
Kang *et al*[57]	2007	Cross-sectional	South Korea	*n* = 161 (EE), *n* = 2281 (non-EE)	Single hospital	Hospital controls	A, G, J, T, B, hypertensive drugs, lifestyle choices, abdominal obesity
Labenz *et al*[8]	2004	Cross-sectional	Germany	*n* = 2455 (EE), *n* = 2834 (control)	Medical center	Medical center	A, G, R, S, T, E, B, H, concomitant disease, concomitant medicatons
Nilsson *et al*[12]	2002	Case-control	Sweden	*n* = 179 (EE), *n* = 179 (control)	Multiple hospital	Multiple hospital	T, cholecystectomy, I, drugs use
Wilson *et al*[59]	1999	Case-control	United States	*n* = 189 (EE), *n* = 1024 (control)	Single hospital	Single hospital	A, G, J, R
Stene-Larsen *et al*[4]	1988	Cross-sectional	Sweden	*n* = 195 (EE), *n* = 1029 (control)	Single hospital	Single hospital	None

A: Age; B: Aspirin or NSAID intake; C: Coffee; D: Meal size; E: Alcohol/ethanol; F: Family history; G: Gender; H: *Helicobacter pylori* infection; I: Asthma or asthma medication; J: Hiatal hernia; K: Hospital visit or hospitalization; M: Marital status; O: Symptom checklist-90 score; P: Physical activity; Q: Psychosomatic symptoms; R: Race; S: Socioeconomic status, education; T: Tobacco; W: Right handedness; V: Comorbidity; X: Case control status; Y: Birthplace; Z: Hormone replacement therapy; VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue; BP: Blood pressure; SBP: Systolic; DBP: Diastolic blood pressure; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglyceride; HbAlc: Hemoglobin Alc; OD: Obesity degree; WHR: Waist-to-hip ratio; WC: Waist circumference; PBF: Percentage of body fat; FBG: Fasting blood glucose; EE: Erosive esophagitis; NERD: Non-erosive reflux disease; NSAID: Nonsteroidal antiinflammatory drugs.

between obesity and EE might have significant implications for counseling.

A recent meta-analysis of BMI and GERD complications found heterogeneous results and it was not able to identify strata with homogeneous results[60]. It was possibly due to their methods of stratification, the utilization of estimates with markedly different measures of BMI association, the absence of studies included in the current analysis, and the inclusion of studies that did not set up a non-GERD control group. In contrast, in the cur-

Table 2 Exposure and outcome definitions

Authors	Yr	Exposure (source)	BMI reference (kg/m^2)	Exposure (definitions)			Outcome (source)	Outcome (definitions)
				BMI overweight (kg/m^2)	BMI obese (kg/m^2)	BMI overweight + obese (kg/m^2)		
Ha *et al*[43]	2010	Measured BMI	≤ 25			≥ 25	Endoscopy	Los Angeles classification
Nam *et al*[44]	2010	Measured BMI	< 20	25-29.9	≥ 30	≥ 25	Endoscopy	Los Angeles classification
Wang *et al*[46]	2010	Measured BMI	< 25	25-30	> 30	≥ 25	Endoscopy	Los Angeles classification
Koo *et al*[45]	2009	Measured BMI	< 23	23-24.9	≥ 25	≥ 23	Endoscopy	Los Angeles classification
Koo *et al*[45]	2009	Measured BMI	< 23	23-24.9	≥ 25	≥ 23	Endoscopy	Los Angeles classification
Chua *et al*[47]	2009	Self-report	< 25		≥ 25	≥ 25	Endoscopy	Los Angeles classification
Song *et al*[48]	2009	Measured BMI			≥ 30		Endoscopy	Los Angeles classification
Lien *et al*[49]	2009	Self-report	< 24	24-26.9	≥ 27	≥ 24	Endoscopy	Modified Savary-Miller endoscopic classification
Lien *et al*[49]	2009	Self-report	< 24	24-26.9	≥ 27	≥ 24	Endoscopy	Modified Savary-Miller endoscopic classification
Nam *et al*[50]	2009	Self-report	< 20	25-29.9	≥ 30	≥ 25	Endoscopy	Los Angeles classification
Lee *et al*[51]	2009	Measured BMI	20-25	25-30	≥ 30	≥ 25	Endoscopy	Los Angeles classification
Chung *et al*[52]	2008	Measured BMI	< 23	23-24.9	≥ 25	≥ 23	Endoscopy	Los Angeles classification
Zagari *et al*[53]	2008	Self-report	20-24.9	25-29.9	≥ 30	≥ 25	Endoscopy	Modified Savary-Miller endoscopic classification
Lee *et al*[54]	2008	Measured BMI	< 20	25-30	> 30	≥ 25	Endoscopy	Los Angeles classification
Kim *et al*[42]	2008	Measured BMI	< 23			≥ 25	Endoscopy	Los Angeles classification
Moki *et al*[56]	2007	Measured BMI	< 25		≥ 25		Endoscopy	Los Angeles classification
Kim *et al*[58]	2007	Measured BMI	18.9-24.5	25-29.9	≥ 30	≥ 25	Endoscopy	Los Angeles classification
Nocon *et al*[55]	2007	Measured BMI		25-30	> 30		Endoscopy	Los Angeles classification
Kang *et al*[57]	2006	Measured BMI	< 25	25-30	> 30	≥ 25	Endoscopy	Los Angeles classification
Labenz *et al*[8]	2004	Measured BMI	< 25	25-30	> 30		Endoscopy	Los Angeles classification
Nilsson *et al*[12]	2002	Self-report	< 25	25-30	> 30	≥ 25	Endoscopy	Modified Savary-Miller endoscopic classification
Wilson *et al*[59]	1999	Measured BMI	< 20	25-30	> 30	≥ 25	Endoscopy	NA
Stene-Larsen *et al*[4]	1988	Measured BMI	< 25	25-28	> 28		Endoscopy	NA

BMI: Body mass index; NA: Not available.

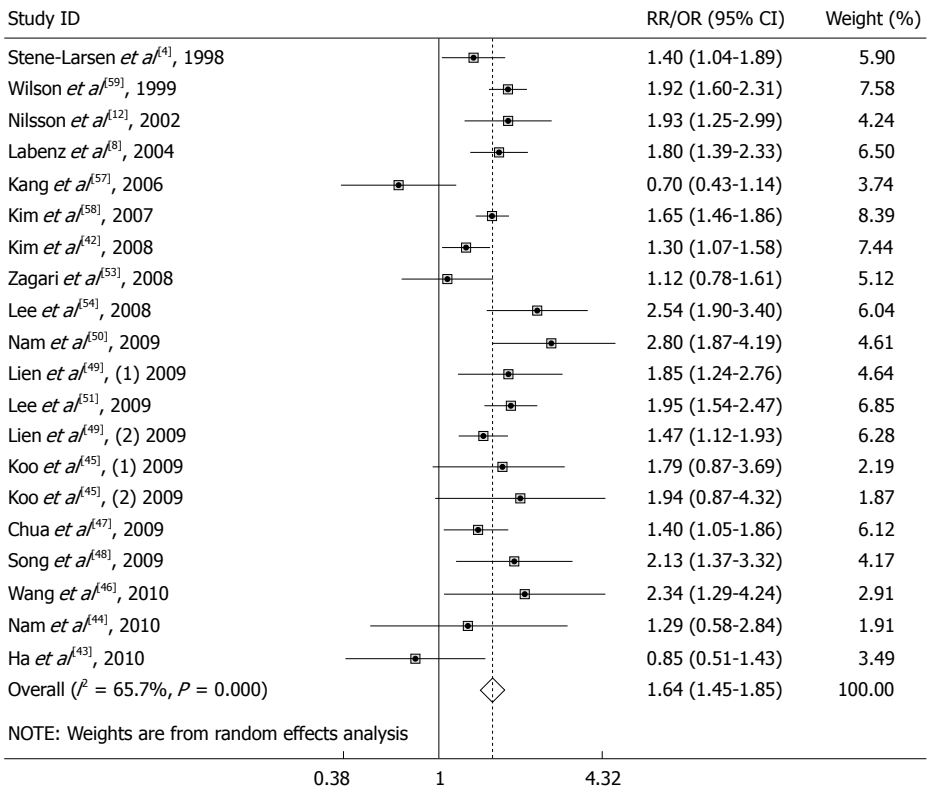


Figure 2 Erosive esophagitis and body mass index (overweight and obese) in males and females. The size of the square represents the weight that the corresponding study exerts in the meta-analysis. RR: Relative risk; OR: Odds ratio.

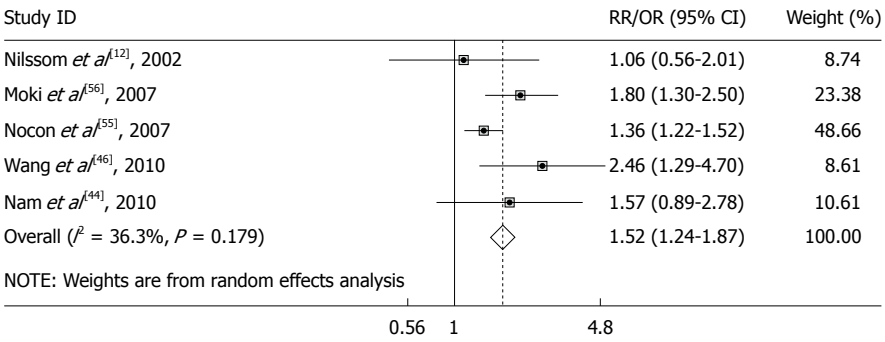


Figure 3 Erosive esophagitis and body mass index (overweight and obese) in males. The size of the square represents the weight that the corresponding study exerts in the meta-analysis. RR: Relative risk; OR: Odds ratio.

Table 3 Meta-analysis results in association between body mass index and erosive esophagitis

BMI category	OR (95% CI)	$P_{homogeneity}$	I^2 (%)	No. of studies
Overall				
Overweight	1.60 (1.35-1.88)	0.003	59.8	12[4,8,12,44,45,50,51,53,54,57-59]
Obese	2.05 (1.65-2.55)	0.000	74.2	15[4,8,12,44-46,50-54,56-59]
Overweight + obese	1.64 (1.45-1.85)	0.000	65.7	18[4,8,12,43-47,49,50-54,56-59]
Females				
Overweight	1.47 (1.15-1.88)	0.011	7.4	3[12,44,55]
Obese	3.76 (0.92-15.28)	0.340	78.0	3[12,44,55]
Overweight + obese	1.45 (1.26-1.66)	0.579	0.0	4[12,44,55,56]
Males				
Overweight	1.40 (1.11-1.75)	0.285	20.8	4[12,44,46,55]
Obese	1.74 (1.02-2.96)	0.099	52.1	4[12,44,46,55]
Overweight + obese	1.52 (1.24-1.87)	0.179	36.3	5[12,44,46,55,56]

BMI: Body mass index; OR: Odds ratio.

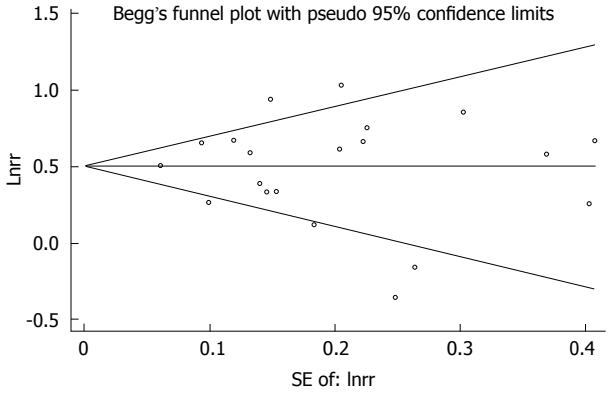


Figure 4 Evaluation of publication bias using a funnel plot. No significant funnel asymmetry was observed which could indicate publication bias. The horizontal line in the funnel plot indicates the random effects summary estimate, while the sloping lines indicate the expected 95% CI for a given standard error, assuming no heterogeneity between studies. Each trial is represented by a circle, the area of which represents the trial's precision. Larger circles represent trials that offer more information.

rent study, after the creation of more categories of BMI among the studies, stratification by gender demonstrated a homogeneous increase in EE with increasing BMI. A study showed a positive correlation between BMI and EE in females, but not in males[12] and a study of reflux patients showed that obese females, but not obese males, had an increased risk of severe esophagitis[55]. The study by Nilsson[12] also found that the association between obesity and EE was further strengthened by the use of oestrogen replacement medication. The prevalence of GERD symptoms as determined in a study investigating a cohort from North America did not differ between males and females[61]. In contrast, in another study, EE was more common in males than in females from Asia[42]. However, in our study, we found a strong positive association between increasing BMI and EE in males, but not in females. This may be because the populations of the included studies were from Asia.

Several hypotheses have been proposed to explain how obesity can cause EE. Abdominal fat may cause reflux through an increase in intrabdominal pressure and subsequent esophageal acid exposure[62,63]. Also, there was a suggestion that hormonal factors related to adiposity are more important than mechanical factors[63]. Obesity is also associated with increased transient lower oesophageal sphincter relaxation[64]. Strengths of this analysis include the use of strict criteria for defining our outcome of interest and the consistency of the BMI-EE association within the males despite different patient populations and different study designs. All the included studies used endoscopy to confirm the diagnosis of EE, which eliminated the possibility of false positive EE cases. Also, we included stratification by study design, location, and source population.

There are potential limitations of this analysis. First, only observational studies were included; study results may be influenced by the presence of measured or unmeasured confounding factors, such as physical activity. Second, bias may also exist in the present study because unpublished data were not included, nor were conference abstracts or articles published in a language other than

English. Third, the exposure definitions (i.e., normal, obese or overweight) differed slightly among the studies. We addressed this, however, by creating more comparable and consistent categories, although few differences still remained. Also, the accuracy of the BMI measurement and its reliability as a measure of adiposity are known to be imperfect.

In summary, based on our extensive review and synthesis of the literature, there appears to be a statistically significant association between elevated BMI and EE. Considering the prevalence of obesity and increasing incidence rates of EE, it is important to pay more attention to further studies that evaluate the influence of gender, ethnicity or age on EE to examine this association. Several studies have found abdominal visceral obesity to be an independent risk factor for EE[44,57]. Nam *et al*[44] demonstrated that association between EE and abdominal visceral adipose tissue volume was consistent among males and females, unlike the association between EE and BMI. However, CT or MRI is needed to test abdominal visceral adipose, which are time consuming and costly. So, measuring BMI may be more feasible. It is also important to determine whether weight loss can decrease the incidence of EE. Further studies are needed to evaluate the relationship between obesity and EE.

COMMENTS

Background

Both obesity and erosive esophagitis (EE) have a high prevalence worldwide. The relationship between them remains controversial.

Research frontiers

Many studies have been performed to evaluate the body mass index (BMI) for gastroesophageal reflux disease risk. It has been found that there was a positive correlation between BMI and EE in females, but not in males.

Innovations and breakthroughs

Findings from this meta-analysis suggested the importance of BMI in EE, especially in males.

Applications

This study provided the potential measurement indicators to identify high-risk groups for EE in obesity population, especially in males.

Terminology

BMI: BMI is a heuristic proxy for human body fat based on an individual's weight and height. It is defined as the individual's body mass divided by the square of his or her height; EE: EE is a term used to indicate any inflammation, swelling, or irritation of the esophagus. The esophagus becomes inflamed (swollen, irritated and red).

Peer review

The meta-analysis presents the data on association between obesity and EE. The topic is interesting and the methodology of the meta-analysis is appropriate.

REFERENCES

1 **Dent J**, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2005; **54**: 710-717
2 **Holtmann G**. Reflux disease: the disorder of the third millennium. *Eur J Gastroenterol Hepatol* 2001; **13** Suppl 1: S5-S11
3 **Kahrilas PJ**. Clinical practice. Gastroesophageal reflux disease. *N Engl J Med* 2008; **359**: 1700-1707
4 **Stene-Larsen G**, Weberg R, Frøyshov Larsen I, Bjørtuft O, Hoel B, Berstad A. Relationship of overweight to hiatus hernia and reflux oesophagitis. *Scand J Gastroenterol* 1988; **23**: 427-432
5 **Pisegna J**, Holtmann G, Howden CW, Katelaris PH, Sharma P, Spechler S, Triadafilopoulos G, Tytgat G. Review article: oesophageal complications and consequences of persistent gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2004; **20** Suppl 9: 47-56
6 **Rennie KL**, Jebb SA. Prevalence of obesity in Great Britain. *Obes Rev* 2005; **6**: 11-12
7 **Fujiwara Y**, Higuchi K, Shiba M, Yamamori K, Watanabe Y, Sasaki E, Tominaga K, Watanabe T, Oshitani N, Arakawa T. Differences in clinical characteristics between patients with endoscopy-negative reflux disease and erosive esophagitis in Japan. *Am J Gastroenterol* 2005; **100**: 754-758
8 **Labenz J**, Jaspersen D, Kulig M, Leodolter A, Lind T, Meyer-Sabellek W, Stolte M, Vieth M, Willich S, Malfertheiner P. Risk factors for erosive esophagitis: a multivariate analysis based on the ProGERD study initiative. *Am J Gastroenterol* 2004; **99**: 1652-1656
9 **Butany VJ**, Singh SH, Lal SK. Study of urinary volume and creatinine excretion in women medical students. *Indian J Physiol Pharmacol* 1975; **19**: 193-198
10 **Vainio H**, Bianchini F. Evaluation of cancer-preventive agents and strategies a new program at the International Agency for Research on Cancer. *Ann N Y Acad Sci* 2001; **952**: 177-180
11 **Corley DA**, Kubo A. Body mass index and gastroesophageal reflux disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2006; **101**: 2619-2628
12 **Nilsson M**, Lundegårdh G, Carling L, Ye W, Lagergren J. Body mass and reflux oesophagitis: an oestrogen-dependent association? *Scand J Gastroenterol* 2002; **37**: 626-630
13 **Nandurkar S**, Locke GR, Fett S, Zinsmeister AR, Cameron AJ, Talley NJ. Relationship between body mass index, diet, exercise and gastro-oesophageal reflux symptoms in a community. *Aliment Pharmacol Ther* 2004; **20**: 497-505
14 **Ruigómez A**, García Rodríguez LA, Wallander MA, Johansson S, Graffner H, Dent J. Natural history of gastro-oesophageal reflux disease diagnosed in general practice. *Aliment Pharmacol Ther* 2004; **20**: 751-760
15 **Locke GR**, Talley NJ, Weaver AL, Zinsmeister AR. A new questionnaire for gastroesophageal reflux disease. *Mayo Clin Proc* 1994; **69**: 539-547
16 **Wang JH**, Luo JY, Dong L, Gong J, Tong M. Epidemiology of gastroesophageal reflux disease: a general population-based study in Xi'an of Northwest China. *World J Gastroenterol* 2004; **10**: 1647-1651
17 **Lagergren J**, Bergström R, Nyrén O. No relation between body mass and gastro-oesophageal reflux symptoms in a Swedish population based study. *Gut* 2000; **47**: 26-29
18 **Incarbone R**, Bonavina L, Szachnowicz S, Saino G, Peracchia A. Rising incidence of esophageal adenocarcinoma in Western countries: is it possible to identify a population at risk? *Dis Esophagus* 2000; **13**: 275-278
19 **Inamori M**, Togawa J, Nagase H, Abe Y, Umezawa T, Nakajima A, Saito T, Ueno N, Tanaka K, Sekihara H, Kaifu H, Tsuboi H, Kayama H, Tominaga S, Nagura H. Clinical characteristics of Japanese reflux esophagitis patients as determined by Los Angeles classification. *J Gastroenterol Hepatol* 2003; **18**: 172-176
20 **Savary M**, Miller G. The oesophagus Handbook and atlas of endoscopy. Switzerland: Verlag Gassman AG, 1978
21 **Petitti DB**. Meta-analysis, decision analysis, and cost-effectiveness analysis. 2nd ed. New York: Oxford University Press, 2000
22 **Olkin I**. Re: "A critical look at some popular meta-analytic methods". *Am J Epidemiol* 1994; **140**: 297-299; discussion 300-301

23 **Poole C**, Greenland S. Random-effects meta-analyses are not always conservative. *Am J Epidemiol* 1999; **150**: 469-475
24 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188
25 **Chalmers TC**, Celano P, Sacks HS, Smith H. Bias in treatment assignment in controlled clinical trials. *N Engl J Med* 1983; **309**: 1358-1361
26 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12
27 **Schulz KF**, Chalmers I, Hayes RJ, Altman DG. Empirical evidence of bias. Dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA* 1995; **273**: 408-412
28 **Schulz KF**, Chalmers I, Grimes DA, Altman DG. Assessing the quality of randomization from reports of controlled trials published in obstetrics and gynecology journals. *JAMA* 1994; **272**: 125-128
29 **Imperiale TF**. Meta-analysis: when and how. *Hepatology* 1999; **29**: 26S-31S
30 **Gerbarg ZB**, Horwitz RI. Resolving conflicting clinical trials: guidelines for meta-analysis. *J Clin Epidemiol* 1988; **41**: 503-509
31 **Kleinbaum DG**, Kupper LL, Morgenstern H. Epidemiologic research: Principles and quantitative methods. New York: John Wiley and Sons, 1982
32 **Banki F**, Demeester SR, Mason RJ, Campos G, Hagen JA, Peters JH, Bremner CG, Demeester TR. Barrett's esophagus in females: a comparative analysis of risk factors in females and males. *Am J Gastroenterol* 2005; **100**: 560-567
33 **Gunji T**, Sato H, Iijima K, Fujibayashi K, Okumura M, Sasabe N, Urabe A, Matsuhashi N. Risk factors for erosive esophagitis: a cross-sectional study of a large number of Japanese males. *J Gastroenterol* 2011; **46**: 448-455
34 **Patel NR**, Ward MJ, Beneck D, Cunningham-Rundles S, Moon A. The Association between Childhood Overweight and Reflux Esophagitis. *J Obes* 2010; **2010**: 136909
35 **Tai CM**, Lee YC, Tu HP, Huang CK, Wu MT, Chang CY, Lee CT, Wu MS, Lin JT, Wang WM. The relationship between visceral adiposity and the risk of erosive esophagitis in severely obese Chinese patients. *Obesity* (Silver Spring) 2010; **18**: 2165-2169
36 **Yasuhara H**, Miyake Y, Toyokawa T, Matsumoto K, Takahara M, Imada T, Yagi S, Miyatake H, Nakatsu M, Ando M, Hirohata M. Large waist circumference is a risk factor for reflux esophagitis in Japanese males. *Digestion* 2010; **81**: 181-187
37 **Elitsur Y**, Dementieva Y, Elitsur R, Rewalt M. Obesity is not a risk factor in children with reflux esophagitis: a retrospective analysis of 738 children. *Metab Syndr Relat Disord* 2009; **7**: 211-214
38 **Nozu T**, Komiyama H. Clinical characteristics of asymptomatic esophagitis. *J Gastroenterol* 2008; **43**: 27-31
39 **Lee SJ**, Song CW, Jeen YT, Chun HJ, Lee HS, Um SH, Lee SW, Choi JH, Kim CD, Ryu HS, Hyun JH. Prevalence of endoscopic reflux esophagitis among Koreans. *J Gastroenterol Hepatol* 2001; **16**: 373-376
40 **Chang CS**, Poon SK, Lien HC, Chen GH. The incidence of reflux esophagitis among the Chinese. *Am J Gastroenterol* 1997; **92**: 668-671
41 **Furukawa N**, Iwakiri R, Koyama T, Okamoto K, Yoshida T, Kashiwagi Y, Ohyama T, Noda T, Sakata H, Fujimoto K. Proportion of reflux esophagitis in 6010 Japanese adults: prospective evaluation by endoscopy. *J Gastroenterol* 1999; **34**: 441-444
42 **Kim N**, Lee SW, Cho SI, Park CG, Yang CH, Kim HS, Rew JS, Moon JS, Kim S, Park SH, Jung HC, Chung IS. The prevalence of and risk factors for erosive oesophagitis and non-erosive reflux disease: a nationwide multicentre prospective study in Korea. *Aliment Pharmacol Ther* 2008; **27**: 173-185
43 **Ha NR**, Lee HL, Lee OY, Yoon BC, Choi HS, Hahm JS, Ahn YH, Koh DH. Differences in clinical characteristics between patients with non-erosive reflux disease and erosive esophagitis in Korea. *J Korean Med Sci* 2010; **25**: 1318-1322
44 **Nam SY**, Choi IJ, Ryu KH, Park BJ, Kim HB, Nam BH. Abdominal visceral adipose tissue volume is associated with increased risk of erosive esophagitis in men and women. *Gastroenterology* 2010; **139**: 1902-1911.e2
45 **Koo JS**, Lee SW, Park SM, Jung SW, Yim HJ, Park JJ, Chun HJ, Lee HS, Choi JH, Kim CD, Ryu HS. Abdominal obesity as a risk factor for the development of erosive esophagitis in subjects with a normal esophago-gastric junction. *Gut Liver* 2009; **3**: 276-284
46 **Wang FW**, Tu MS, Chuang HY, Yu HC, Cheng LC, Hsu PI. Erosive esophagitis in asymptomatic subjects: risk factors. *Dig Dis Sci* 2010; **55**: 1320-1324
47 **Chua CS**, Lin YM, Yu FC, Hsu YH, Chen JH, Yang KC, Shih CH. Metabolic risk factors associated with erosive esophagitis. *J Gastroenterol Hepatol* 2009; **24**: 1375-1379
48 **Song HJ**, Shim KN, Yoon SJ, Kim SE, Oh HJ, Ryu KH, Ha CY, Yeom HJ, Song JH, Jung SA, Yoo K. The prevalence and clinical characteristics of reflux esophagitis in koreans and its possible relation to metabolic syndrome. *J Korean Med Sci* 2009; **24**: 197-202
49 **Lien HC**, Chang CS, Yeh HZ, Ko CW, Chang HY, Cheng KF, Sung FC. Increasing prevalence of erosive esophagitis among Taiwanese aged 40 years and above: a comparison between two time periods. *J Clin Gastroenterol* 2009; **43**: 926-932
50 **Nam SY**, Choi IJ, Nam BH, Park KW, Kim CG. Obesity and weight gain as risk factors for erosive oesophagitis in men. *Aliment Pharmacol Ther* 2009; **29**: 1042-1052
51 **Lee HL**, Eun CS, Lee OY, Jeon YC, Han DS, Yoon BC, Choi HS, Hahm JS, Ahn YH, Song SY. Association between erosive esophagitis and visceral fat accumulation quantified by abdominal CT scan. *J Clin Gastroenterol* 2009; **43**: 240-243
52 **Chung SJ**, Kim D, Park MJ, Kim YS, Kim JS, Jung HC, Song IS. Metabolic syndrome and visceral obesity as risk factors for reflux oesophagitis: a cross-sectional case-control study of 7078 Koreans undergoing health check-ups. *Gut* 2008; **57**: 1360-1365
53 **Zagari RM**, Fuccio L, Wallander MA, Johansson S, Fiocca R, Casanova S, Farahmand BY, Winchester CC, Roda E, Bazzoli F. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano-Monghidoro study. *Gut* 2008; **57**: 1354-1359
54 **Lee HL**, Eun CS, Lee OY, Jeon YC, Sohn JH, Han DS, Yoon BC, Choi HS, Hahm JS, Lee MH, Lee DH. Association between GERD-related erosive esophagitis and obesity. *J Clin Gastroenterol* 2008; **42**: 672-675
55 **Nocon M**, Labenz J, Jaspersen D, Meyer-Sabellek W, Stolte M, Lind T, Malfertheiner P, Willich SN. Association of body mass index with heartburn, regurgitation and esophagitis: results of the Progression of Gastroesophageal Reflux Disease study. *J Gastroenterol Hepatol* 2007; **22**: 1728-1731
56 **Moki F**, Kusano M, Mizuide M, Shimoyama Y, Kawamura O, Takagi H, Imai T, Mori M. Association between reflux oesophagitis and features of the metabolic syndrome in Japan. *Aliment Pharmacol Ther* 2007; **26**: 1069-1075
57 **Kang MS**, Park DI, Oh SY, Yoo TW, Ryu SH, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI. Abdominal obesity is an independent risk factor for erosive esophagitis in a Korean population. *J Gastroenterol Hepatol* 2007; **22**: 1656-1661
58 **Kim HJ**, Yoo TW, Park DI, Park JH, Cho YK, Sohn CI, Jeon WK, Kim BI. Influence of overweight and obesity on upper endoscopic findings. *J Gastroenterol Hepatol* 2007; **22**: 477-481
59 **Wilson LJ**, Ma W, Hirschowitz BI. Association of obesity

with hiatal hernia and esophagitis. *Am J Gastroenterol* 1999; **94**: 2840-2844
60 **Hampel H**, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. *Ann Intern Med* 2005; **143**: 199-211
61 **Locke GR**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456
62 **El-Serag HB**, Ergun GA, Pandolfino J, Fitzgerald S, Tran T, Kramer JR. Obesity increases oesophageal acid exposure. *Gut* 2007; **56**: 749-755
63 **El-Serag HB**, Tran T, Richardson P, Ergun G. Anthropometric correlates of intragastric pressure. *Scand J Gastroenterol* 2006; **41**: 887-891
64 **Wu JC**, Mui LM, Cheung CM, Chan Y, Sung JJ. Obesity is associated with increased transient lower esophageal sphincter relaxation. *Gastroenterology* 2007; **132**: 883-889

S- Editor Shi ZF **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2554

World J Gastroenterol 2012 May 28; 18(20): 2554-2560
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Serum inter-cellular adhesion molecule 1 is an early marker of diagnosis and prediction of severe acute pancreatitis

Hai-Hang Zhu, Lin-Lin Jiang

Hai-Hang Zhu, Lin-Lin Jiang, Department of Gastroenterology, Northern Jiangsu Hospital, College of Clinical Medical Medicine, Yangzhou University, Yangzhou 225001, Jiangsu Province, China
Author contributions: Zhu HH designed the research, analyzed the data and wrote the paper; Jiang LL performed the research.
Correspondence to: Hai-Hang Zhu, Chief, Professor of Medicine, Department of Gastroenterology, Northern Jiangsu Hospital, College of Clinical Medicine, Yangzhou University, 98 West Nantong Road, Yangzhou 225001, Jiangsu Province, China. zhuhaihang@medmail.com.cn
Telephone: +86-514-87937475 Fax: +86-514-87937406
Received: August 2, 2011 Revised: September 28, 2011
Accepted: February 16, 2012
Published online: May 28, 2012

Abstract

AIM: To determine if serum inter-cellular adhesion molecule 1 (ICAM-1) is an early marker of the diagnosis and prediction of severe acute pancreatitis (SAP) within 24 h of onset of pain, and to compare the sensitivity, specificity and prognostic value of this test with those of acute physiology and chronic health evaluation (APACHE) Ⅱ score and interleukin-6 (IL-6).

METHODS: Patients with acute pancreatitis (AP) were divided into two groups according to the Ranson's criteria: mild acute pancreatitis (MAP) group and SAP group. Serum ICAM-1, APACHE Ⅱ and IL-6 levels were detected in all the patients. The sensitivity, specificity and prognostic value of the ICAM-1, APACHE Ⅱ score and IL-6 were evaluated.

RESULTS: The ICAM-1 level in 36 patients with SAP within 24 h of onset of pain was increased and was significantly higher than that in the 50 patients with MAP and the 15 healthy volunteers ($P < 0.01$). The ICAM-1 level (25 ng/mL) was chosen as the optimum cutoff to distinguish SAP from MAP, and the sensitivity, specificity, positive predictive value, negative predictive value (NPV), positive likelihood ratio and negative likelihood ratio were 61.11%, 71.42%, 0.6111, 0.7142, 2.1382 and 0.5445, respectively. The area under the curve demonstrated that the prognostic accuracy of ICAM-1 (0.712) was similar to the APACHE-Ⅱ scoring system (0.770) and superior to IL-6 (0.508) in distinguishing SAP from MAP.

CONCLUSION: ICAM-1 test is a simple, rapid and reliable method in clinical practice. It is an early marker of diagnosis and prediction of SAP within the first 24 h after onset of pain or on admission. As it has a relatively low NPV and does not allow it to be a stand-alone test for the diagnosis of AP, other conventional diagnostic tests are required.

Key words: Intercellular adhesion molecule-1; Severe acute pancreatitis; Early prediction

Peer reviewer: Toshiyuki Ishiwata, Associate Professor, Department of Pathology, Integrative Oncological Pathology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan

Zhu HH, Jiang LL. Serum inter-cellular adhesion molecule 1 is an early marker of diagnosis and prediction of severe acute pancreatitis. *World J Gastroenterol* 2012; 18(20): 2554-2560 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2554.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2554

INTRODUCTION

Most cases of acute pancreatitis (AP) are mild and self limiting, and recover spontaneously, but approximately 20% of attacks turn to severe acute pancreatitis (SAP) with a life-threatening morbidity and a mortality rate

of 20%-30%. Hence, early diagnosis and prediction of the severity of AP are of particular significance[1]. Early prediction of the severity of AP is still difficult in clinical practice. Ranson's score can only be evaluated 48 h after admission. The acute physiology and chronic health evaluation Ⅱ (APACHE Ⅱ) score can be used within a few hours after admission, but its complex and cumbersome performance limits its clinical use[2]. Several laboratory markers have been developed over the past decade for the early diagnosis and prediction of SAP. Since there is no correlation found between the degree of structural damage to pancreas and clinical manifestation of the disease, no ideal predictive system or biochemical marker has been available. Several reports showed that serum intercellular adhesion molecule-1 (ICAM-1) levels were elevated during the course of AP and correlated with the severity of the disease and patient outcome. ICAM-1 can be detected as an early marker in the diagnosis of lung injury[3,4]. However, the details of the clinical use of this test for early diagnosis and prediction of SAP remain obscure, especially within the first 24 h in the patients after admission. The aim of this prospective study was to evaluate the use of the ICAM-1 in early diagnosis and prediction of SAP.

MATERIALS AND METHODS

Study population

All patients with AP were included in the primary analysis according to the guidelines of diagnosis and treatment of AP established by Branch of Gastroenterology, Chinese Medical Association in 2003[5]. The diagnosis of AP was based on the following features: (1) Prolonged abdominal pain characteristic of AP; (2) Elevated serum amylase and/or lipase levels by at least 3-folds that of normal range; and (3) Characteristic findings of AP on abdominal ultrasonography and/or computed tomography (CT) scan. Patients who were admitted within the first 24 h of the onset of abdominal pain were not included in the study. Patients with an accompanying disease that might influence the outcome data were excluded, such as postoperative, post-traumatic, post-endoscopic retrograde cholangiopancreatographic pancreatitis. Other causes of acute abdominal pain were ruled out. Eighty-six patients with AP included in this pilot study were analyzed in a prospective 1-year investigation performed at a single institution.

Clinical assessment

The study was approved by the Committee of Research Ethics of our hospital, and informed consent was obtained from all the patients and healthy volunteers before enrollment. Demographics (gender, age, occupation, course and characteristics of symptom) and the cause of the pancreatitis (cholelithiasis, alcohol abuse, hyperlipidemia and others) were recorded. Routine clinical observation, laboratory test and treatment were performed. The APACHE-Ⅱ score was determined within the first 24 h and 48 h of the onset of pain after admission[6]. Ultrasonography was performed every other day in all the patients and/or spiral CT with intravenous contrast was performed in some patients within 48-72 h after admission to assess the extent of inflammation and the degree of pancreas necrosis according to Balthazar's classification. After the examinations, patients' data were reviewed to determine the eligibility for inclusion into the study.

Clinical classification

Ranson's score was recorded in the first 24 h and 48 h after admission. Since Ranson *et al*[7] in 1974 identified 11 prognostic factors, considerable researches have been undertaken to find the ideal predictor(s) that allow rapid and correct assessment of the severity of AP to suit different clinical and regional settings. SAP was categorized based on the clinical and laboratory data using Ranson's score. Cases meeting less than three positive criteria were classified as mild acute pancreatitis (MAP) and those meeting three or more positive criteria were classified as SAP.

Sample collection and enzyme-linked immunosorbent assay for ICAM-1 and interleukin-6

To check the time kinetics of rise in plasma ICAM-1 and interleukin-6 (IL-6) during AP, its levels were quantified in two time-points. The potential of ICAM-1 and IL-6 to predict SAP within the first 24 h and 48 h after the onset of symptoms or on admission was examined. A 5-mL sample of peripheral venous blood was collected twice from the 85 patients with AP. Blood samples obtained from the 15 healthy volunteers served as controls. The plasma was separated by centrifugation at 3000 r/min for 10 min at 4 ℃ and stored at -20 ℃ until assayed. Plasma ICAM-1 and IL-6 were quantified with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Sen-Xiong Technology Limited Company, Shanghai, China). ELISA plates were read at 450 nm and data was collected. Measurement was performed according to the instructions of the manufacturer.

Presentation of data and statistical analysis

After the examinations, data from each patient were reviewed to ascertain the eligibility for inclusion into the study. The data of gender, etiology of SAP and MAP, and the categorical variables were compared using χ^2 test. Analysis of variance was performed in the continuous variables of age, ICAM-1, IL-6 levels and other relative laboratory tests for SAP and MAP using Student's t test. The differences were considered statistically significant if $P \leqslant 0.05$. In order to differentiate SAP from MAP within the first 24 h of the onset of symptoms, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive likelihood ratio (PLR) and negative likelihood ratio (NLR) at different serum levels of ICAM-1 compared with APACHE Ⅱ and IL-6 levels were calculated, respectively. The level of higher

Table 1 Characteristics of 86 patients with acute pancreatitis

	SAP, n = 36	MAP, n = 50	P value
Age (mean ± SE, yr)	53.19 ± 14.9	49.4 ± 10.9	> 0.05
Gender (male/female)	18/18	30/20	> 0.05
Length of hospital stay (d)	18.3 ± 1.6	7.3 ± 1.4	< 0.001
Etiology, n (%)			
Gall stones	19 (52.7)	20 (40.8)	< 0.05
Alcohol	4 (11.1)	8 (16.3)	
Idiopathic	13 (36.1)	21 (42.8)	
Test at first 24 h			
Blood amylase (U/L)	456.8 ± 175.7	389 ± 125.7	< 0.05
Total bilirubin (mmol/mL)	39.10 ± 22.42	29.15 ± 25.88	> 0.05
Blood glucose (mmol/mL)	9.61 ± 4.77	6.87 ± 2.63	< 0.05
Blood calculus	2.41 ± 0.33	2.63 ± 2.16	> 0.05
Test at 48-72 h			
Pancreatic necrosis	16	2	< 0.01
Ranson's score	5.3 ± 0.5	1.5 ± 0.24	< 0.001

SAP: Severe acute pancreatitis; MAP: Mild acute pancreatitis.

PLR and lower NLR was chosen as the optimum cutoff point. A receiver operating characteristic (ROC) curves were constructed to determine the reference cutoff point for ICAM-1, APACHE Ⅱ and IL-6 levels that could distinguish between SAP and MAP. An index of the goodness of the test of area under the curve (AUC) was used to measure the ability of distinguishing SAP from MAP. A perfect test had an area of 1.0, while a non-discriminating test had an area of 0.5. All statistical analyses were performed using SPSS v8.00 statistical analysis software (SPSS Inc., Cary, NC).

RESULTS

Clinical characteristics of AP

The prospective study population consisted of 86 consecutive patients with AP (48 men, 38 women with a mean age of 50.7 years; range, 17-79 years) and 15 healthy volunteers (10 men, 5 women with a mean age of 48.7 years; range, 28-58 years). Forty-nine (56.9%) patients were diagnosed as having MAP and 36 (41.8%) as having SAP according to the Ranson's criteria. The clinical characteristics of the patients with AP are summarized in Table 1. Gallstones were the most common cause of both SAP and MAP (n = 19, 52.7% and n = 20, 40.8%, respectively). There was no difference in the sex, race and etiology of the disease between the SAP and MAP patients. The mean serum amylase and glucose levels were significantly higher in SAP than in MAP (P < 0.05). There was no significant difference in the serum level of total bilirubin between the two groups at the first 24 h after the onset of pain.

Mean scores of APACHE-Ⅱ, serum ICAM-1 and IL-1 levels in patients with SAP and MAP

The mean scores of APACHE-Ⅱ in the SAP patients within the first 24 h after the onset of pain were also significantly higher than in the MAP patients (P < 0.001). The mean serum level of ICAM-1 in the SAP patients within the first 24 h after the onset of pain was significantly higher than in the MAP patients (P < 0.001) and the healthy controls (P < 0.001). Significantly higher levels of IL-6 were found in the SAP patients as compared with the MAP patients at the 24 h and the healthy controls (P < 0.001). The APACHE-Ⅱ scores within the first 24 h after admission to hospital were significantly higher in SAP than in MAP patients (P < 0.001) (Table 2). The mean serum level of ICAM-1 in SAP patients at 48-72 h after admission was obviously higher than in SAP patients within the first 24 h after admission (P < 0.001). The level of IL-6 declined in the AP patients at 48-72 h after admission, but it was still obviously higher in the SAP patients than in the MAP patients (P < 0.001). The mean scores of APACHE-Ⅱ in SAP and MAP at the 48-72 h after the onset of pain were slightly increased, but the scores in SAP were significantly higher than that in MAP in the first 24 h (Table 3).

Table 2 Acute physiology and chronic health evaluation Ⅱ score, serum intercellular adhesion molecule-1 and interleukin-6 levels in severe acute pancreatitis and mild acute pancreatitis patients within the first 24 h after admission

	SAP, n = 36	MAP, n = 50	Control	t	P value
APACHE-Ⅱ	14.47 ± 5.81	7.57 ± 1.44		7.9132	< 0.001
ICAM-1 (ng/mL)	29.68 ± 8.04	16.77 ± 4.37	8.12 ± 2.33	9.5028	< 0.001
IL-6 (ng/mL)	68.76 ± 28.62	35.95 ± 11.56	14.46 ± 3.53	7.2700	< 0.001

SAP: Severe acute pancreatitis; MAP: Mild acute pancreatitis; APACHE-Ⅱ: Acute physiology and chronic health evaluation Ⅱ; ICAM-1: Intercellular adhesion molecule-1; IL-6: Interleukin-6.

Table 3 Changes of serum intercellular adhesion molecule-1 and interleukin-6 levels in severe acute pancreatitis patients after admission

	Type	< 24 h	48-72 h	t	P value
APACHE-Ⅱ	SAP	14.47 ± 5.81	16.6 ± 2.03	2.0776	< 0.05
	MAP	7.57 ± 1.44	9.51 ± 1.74	6.0216	< 0.001
ICAM-1 (ng/mL)	SAP	29.68 ± 8.04	44.76 ± 12.08	6.2353	< 0.001
	MAP	16.77 ± 4.37	24.10 ± 5.88	7.0038	< 0.001
IL-6 (ng/mL)	SAP	68.76 ± 28.62	42.19 ± 12.77	5.0868	< 0.001
	MAP	35.95 ± 11.56	21.76 ± 8.65	6.8798	< 0.001

SAP: Severe acute pancreatitis; MAP: Mild acute pancreatitis; APACHE-Ⅱ: Acute physiology and chronic health evaluation Ⅱ; ICAM-1: Intercellular adhesion molecule-1; IL-6: Interleukin-6.

Sensitivity, specificity, PPV, NPV, PLR and NLR of ICAM-1 in distinguishing SAP from MAP

The sensitivity, specificity, PPV, NPV, PLR and NLR at different serum ICAM-1 levels (10, 15, 25, 30, 35, 40 and 45 ng/mL) were determined respectively. The ICAM-1 level (25 ng/mL) with higher PLR and lower NLR was chosen as the optimum cutoff to distinguish SAP from MAP within first 24 h of the onset of symptoms, and the sensitivity, specificity, PPV, NPV, PLR and NLR were 61.11%, 71.42%, 0.6111, 0.7142, 2.1382

Table 4 Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of acute physiology and chronic health evaluation Ⅱ, intercellular adhesion molecule-1 and interleukin-6 in distinguishing severe acute pancreatitis from mild acute pancreatitis

	Cutoffs	Sensitivity (%)	Specificity (%)	PPV	NPV	PLR	NLR
APACHE-Ⅱ	> 8	77.77	55.10	0.5600	0.7714	1.7320	0.2223
ICAM-1	25 ng/mL	61.11	71.42	0.6111	0.7142	2.1382	0.5445
IL-6	50 ng/mL	36.11	63.26	0.6190	0.5740	0.8826	1.1680

APACHE-Ⅱ: Acute physiology and chronic health evaluation Ⅱ; ICAM-1: Intercellular adhesion molecule-1; IL-6: Interleukin-6; PPV: Positive predictive value; NPV: Negative predictive value; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio.

and 0.5445, respectively. The APACHE-Ⅱ scores (< 4, 4-8, 9-12, 13-16, 17-20 and > 20) were also calculated. The sensitivity, specificity, PPV, NPV, PLR and NLR at the optimum cutoffs of APACHE-Ⅱ score > 8 were 77.77%, 55.10%, 0.5600, 0.7714, 1.7320 and 0.2223, respectively. The different serum IL-6 levels (20, 30, 40, 50, 60, 70 and 80 ng/mL) were respectively calculated using the same method. The sensitivity, specificity, PPV, NPV, PLR and NLR at the optimum cutoffs of IL-6 50 ng/mL were 36.11%, 63.26%, 0.6169, 0.5740, 1.5869 and 0.9180, respectively (Table 4).

ROC curves and AUC of ICAM-1, APACHE-Ⅱ and IL-6 in distinguishing SAP from MAP

The optimum cutoffs of APACHE-Ⅱ score > 8, serum ICAM-1 (25 ng/mL) and IL-6 (50 ng/mL) which were used to distinguish SAP from MAP within the first 24 h of the onset of symptoms were analyzed by constructing a ROC curve. The AUC of ICAM-1, APACHE-II and IL-6 in predicting SAP was 0.712, 0.770 and 0.508, respectively (Figure 1). The AUC demonstrated that the prognostic accuracy of ICAM-1 was similar to the APACHE-Ⅱ scoring system. The AUC of serum ICAM-1 level was obviously superior to that of IL-6. The serum IL-6 level was not capable of distinguishing SAP from MAP within the first 24 h of the onset of symptoms.

DISCUSSION

In China, the exact incidence of AP is still unknown, but it is increasing gradually in recent years. AP is a life-threatening illness with an annual incidence of 30-50 attacks per 100 000 inhabitants[8]. It is an inflammatory process that presents different severity degrees, ranging from a mild self-limiting disease with interstitial edema in the pancreas, to a severe disease with extensive necrosis[9]. It progresses to a severe illness with a prolonged course in about 15%-20% of the patients. These severely ill patients may develop organ failure and/or local complications such as pancreatic necrosis. Approximately, 75% of the cases are mild with a mortality below 1%. Eighty percent of the patients

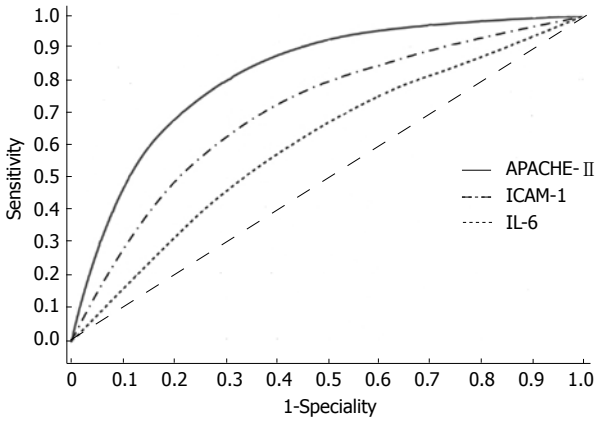


Figure 1 Cutoffs of acute physiology and chronic health evaluation Ⅱ score > 8, serum intercellular adhesion molecule-1 (25 ng/mL) and interleukin-6 (50 pg/mL) levels that can distinguish severe acute pancreatitis from mild acute pancreatitis within 24 h of the onset of symptoms were analyzed by constructing a receiver operating characteristic curve. The area under the curve for intercellular adhesion molecule-1 (ICAM-1), acute physiology and chronic health evaluation Ⅱ (APACHE-Ⅱ) score > 8 and interleukin-6 (IL-6) in predicting severe acute pancreatitis was 0.712, 0.770 and 0.508, respectively.

could recover completely, while 20% had their disease worsened according to the Atlanta criteria. The mortality increases up to 20% if the disease progresses into a severe necrotizing form[10] and the mortality can be as high as 30%-40%[11]. Current management strategies for patients with SAP include early admission to intensive care units, vigorous intravenous resuscitation, and urgent endoscopic retrograde cholangiopancreatography when cholangitis or biliary obstruction is present, antibiotic prophylaxis in patients with pancreatic necrosis, and close patient monitoring.

Thus, the management of AP is still challenging mainly due to the delay in admission to hospital after the onset of symptoms and the difficulty in discriminating MAP from SAP, especially within the first 24 h. There is an urgent need for an early and accurate prediction of SAP to ensure timely interventions in a specialized care setting[12]. The early recognition and diagnosis are an important goal in the optimal management of SAP. Severity assessment is indispensable to the selection of proper initial treatment in the management of AP.

Several prognostic scoring systems are being used in predicting SAP: Ranson's, Glasgow's, APACHE-Ⅱ, the bedside index for severity of AP (BISAP), and computed tomography severity index (CTSI). Papachristou *et al*[13] reported that the AUC for BISAP, Ranson's, APACHE-Ⅱ and CTSI in predicting SAP are 0.81, 0.94, 0.78 and 0.84, respectively in 185 patients with AP and found that all these scoring systems had a high accuracy in predicting SAP in the first 24 h after admission. The components of the BISAP score are clinically relevant and easy to obtain. Lujano-Nicolás *et al*[14] evaluated the severity of AP according to the Ranson's and APACHE-Ⅱ scores on admission in 28 patients with AP and correlated these scales with the local pancreatic complications according to the Balthazar classification. They found that

these scales have discrepancies when compared with tomographic Balthazar and these scales were not correlated well with the tomographic Balthazar degrees. The Ranson's prognostic signs and the Glasgow's score can only be applied 48 h after admission. The APACHE-Ⅱ score has the invaluable advantage of being useful within a few hours after admission, and it can be assessed serially. However, it is cumbersome, which limits its use in clinical practice. The current gold standard for staging AP combines the clinical criteria with CT, but because of the high cost, exposure to ionizing radiation, and lack of sensitivity and specificity in the early stage of the disease, it has limited availability.

Numerous efforts have been made in recent years to identify objective markers that can predict the severity of AP on admission. Various biochemical tests, such as C-reactive protein (CRP), tumor necrosis factor, IL-2 and IL 6, have been developed over the past decade for early diagnosis and prediction of severity of AP[15-17]. However, except for CRP, none of them can accurately predict the disease severity within 24 h of onset, and the outcome in triage to the intensive care unit has not been reported[18]. As there is no correlation between the degree of structural damage to pancreas and clinical manifestation of the disease, there has been no ideal predictive system or biochemical marker for this disease. Little is known about clinical predictors of early readmission for AP. The ideal predictors of the severity of AP are described as being simple, quick, highly sensitive, highly specific, safe, reproducible, and cheap. An immediate test with a high specificity, AUC and low NLR is required. Unfortunately, this ideal predictor is still not available[19].

Adhesion molecules are involved in the inflammatory response during AP. ICAM-1, a single-chain transmembrane glycoprotein with a molecular weight of 80-110 kDa, consists of five Ig-like domains, a hydrophobic transmembrane domain and a short cytoplasmic C-terminal domain. Its ligand includes lymphocyte function-associated antigen-1 and macrophage antigen-1. Under physiological conditions, ICAM-1 is expressed at a low level in endothelial cells and epithelial cells or constitutively on the surface of alveolar cells, providing the underlying molecular basis for cell recognition, activation, proliferation, differentiation and motility, thereby helping stabilize the internal environment of the body. ICAM-1 also plays a key role in pathological events, such as inflammatory reactions, including acute renal failure and acute pancreatitis[20].

One of the most common complications of AP is acute lung injury, during which ICAM-1 plays an important role by participating in leukocyte adhesion and activation as well as by inducing the "cascade effect" of inflammatory mediators, pulmonary microcirculation dysfunction and even acute respiratory distress syndrome, multiple organ failure or death. The upregulation of ICAM-1 expression in the lung during acute lung injury is one of main pathogeneses; the early detection of the ICAM-1 expression level may contribute to the prevention and treatment of acute lung injury[3,4]. Sun *et al*[20] investigated the ICAM-1 in mediating the development of AP from a local disease to a systemic illness in rats and found that upregulation of ICAM-1 and subsequent leukocyte infiltration appear to be significant events of pancreatic and pulmonary injuries. Intracapillary leucocyte accumulation represents a novel protective and potentially lifesaving mechanism of hemostasis in acute pancreatitis. This process depends on the expression of lymphocyte function-associated antigen 1 and ICAM-1 and precedes the classical steps of the leucocyte recruitment cascade[21]. Chooklin *et al*[22] measured the serum levels of pro-inflammatory and anti-inflammatory cytokines in 51 AP patients who were diagnosed with pancreatitis-associated lung injury with and without the development of organ dysfunction and found that in the pathogenesis of respiratory complications in AP cytokines, chemokines and adhesion molecules, in particular ICAM-1, play major roles. High ICAM-1 concentration was found in plasma during AP, which was not reduced by Dx treatment. Dexamethasone down-regulates ICAM-1 expression, but it does not completely prevent leukocyte recruitment during sodium taurocholate-induced AP[23]. Pancreatic ICAM-1 expression was increased in single-nucleotide polymorphism as compared with the controls. After calcitonin gene-related peptide application, pancreatic ICAM-1 expression was attenuated[24]. Graft pancreatitis is induced by ischemia/reperfusion injury in which neutrophil infiltration is believed to be a crucial early event. The data suggested that ICAM-1 was already up-regulated during cold ischemia, possibly representing the mechanism of early neutrophil infiltration observed in human pancreatic ischemia/reperfusion injury[25].

Irrespective of the underlying etiology, the immune response is almost identical in severe cases of AP. While the triggering factors of AP are still poorly understood, cytokines are considered as important mediators in the pathophysiology of SAP. Perejaslov *et al*[26] reported that peak levels of sICAM-1 on admission in AP 87 patients had a subsequent decrease and these mediators are correlated with the disease severity, development of multiple organ dysfunction syndrome and necrosis and may be used as prognostic markers. Several reports showed that serum ICAM-1 levels are elevated during the course of AP and correlate with the severity of the disease and patient outcome. ICAM-1 can be detected as an early marker for the diagnosis of lung injury[20]. Although serum ICAM-1 level was increased in AP patients and closely related to the development of SAP in many clinical studies, the details of the use of ICAM-1 for early diagnosis and prediction of SAP remain obscure, especially in the first 24 h after the admission. The aim of our prospective study was to evaluate the use of the ICAM-1 in early diagnosis and prediction of SAP, and to compare the sensitivity, specificity and prognostic value of this test with those of APACHE-Ⅱ score and IL-6.

Among the 86 patients with AP in this study, 49 patients were diagnosed as having MAP and 36 as having SAP according to the Ranson's criteria. The mean serum

level of ICAM-1 in the SAP patients within the first 24 h of the onset of pain was significantly higher than in MAP patients and the healthy controls. The mean serum level of ICAM-1 in the SAP patients at 48-72 h after admission was obviously higher than in the SAP group. The result showed that ICAM-1 is a simple, rapid and reliable method for use within the first 24 h after admission. It has a higher specificity and a sensitivity for early diagnosis and prediction of SAP. The AUC value demonstrated that the prognostic accuracy of ICAM-1 is similar to the APACHE-Ⅱ scoring system and obviously superior to IL-6 in distinguishing SAP from MAP. Previous reports showed the usefulness of serum IL-6 level in determining the severity of acute pancreatitis[18], but our data indicated that the serum IL-6 level could not distinguish SAP from MAP within the first 24 h. The exact reason for the difference is still unknown. Although the APACHE-Ⅱ scoring system has a high prognostic accuracy, it is too complex and cumbersome, which limits its clinical use. However, ICAM-1 has a relatively low NPV and does not allow it to be a stand-alone tool for diagnosis of acute pancreatitis; and the use of other conventional diagnostic tools remains a requirement.

In conclusion, we found that serum ICAM-1 levels rose within the first 24 h after the onset of AP and that early measurement of serum ICAM-1 levels could distinguish SAP from MAP. It has a higher sensitivity, specificity and NPV. It could be used as a test in screening patients with AP and predicting the outcome in patients with SAP. The predictive accuracy of ICAM-1 is similar to APACHE-Ⅱ scoring system and obviously superior to IL-6. But its low NPV does not allow it to be a stand-alone tool for the diagnosis and prediction of AP. To identify a novel predictor of severity and outcome of AP is needed so as to improve the predictive accuracy.

COMMENTS

Background

Most cases of acute pancreatitis (AP) are mild and self limiting, and recover spontaneously, but approximately 20% of attacks turn to severe acute pancreatitis (SAP) with a life-threatening morbidity and a mortality rate of 20%-30%. There is no established biomarker for early diagnosis and prediction of severe pancreatitis. Early diagnosis and prediction of the severity of AP is of particular significance.

Research frontiers

Serum intercellular adhesion molecule-1 (ICAM-1) levels are elevated during the course of AP and correlate with the severity of the disease. ICAM-1 can be detected as an early marker for the diagnosis of lung injury. The details of the clinical use of ICAM-1 for early diagnosis and prediction of severity in AP remain obscure, especially in the first 24 h after the admission. This study evaluated the use of the ICAM-1 in early diagnosis and prediction of SAP, and compared the sensitivity, specificity and prognostic value of this test with those of acute physiology and chronic health evaluation (APACHE) Ⅱ score and interleukin-6 (IL-6).

Innovations and breakthroughs

Serum ICAM-1 is elevated within the first 24 h after the onset of symptoms. Elevated ICAM-1 levels are associated with the severity of AP with a higher sensitivity, specificity and negative predictive value for early diagnosis and prediction of severity in AP. The predictive accuracy of ICAM-1 is similar to the APACHE-Ⅱ scoring system and obviously superior to IL-6.

Applications

Serum ICAM-1 is an early marker for the diagnosis and prediction of SAP, and the test is simple, rapid and reliable that can be used in clinic practice. The results obtained are important for both early diagnosis and treatment of pancreatitis.

Terminology

ICAM-1 is a single-chain transmembrane glycoprotein. Under physiological conditions, it is expressed at a low level in endothelial cells providing the underlying molecular basis for cell recognition, activation, proliferation, differentiation and motility. ICAM-1 also plays a key role in pathological events, such as inflammatory reactions, including acute renal failure and acute pancreatitis

Peer review

The manuscript reports that ICAM-1 is a useful marker for early diagnosis and a potential predictor of severe acute pancreatitis. These data are important for early treatment for pancreatitis patients.

REFERENCES

1 **Kamer E**, Unalp HR, Derici H, Tansug T, Onal MA. Early diagnosis and prediction of severity in acute pancreatitis using the urine trypsinogen-2 dipstick test: a prospective study. *World J Gastroenterol* 2007; **13**: 6208-6212

2 **Lempinen M**, Stenman UH, Finne P, Puolakkainen P, Haapiainen R, Kemppainen E. Trypsinogen-2 and trypsinogen activation peptide (TAP) in urine of patients with acute pancreatitis. *J Surg Res* 2003; **111**: 267-273

3 **Zhang X**, Wu D, Jiang X. Icam-1 and acute pancreatitis complicated by acute lung injury. *JOP* 2009; **10**: 8-14

4 **Zhao X**, Andersson R, Wang X, Dib M, Wang X. Acute pancreatitis-associated lung injury: pathophysiological mechanisms and potential future therapies. *Scand J Gastroenterol* 2002; **37**: 1351-1358

5 **Branch of digestive diseases of Chinese Medical Association**. Guidelines for diagnosis and treatment of acute pancreatitis(draft). *Zhonghua Neike Zazhi* 2004; **43**: 236-238

6 **Knaus WA**, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-829

7 **Ranson JH**, Rifkind KM, Roses DF, Fink SD, Eng K, Spencer FC. Prognostic signs and the role of operative management in acute pancreatitis. *Surg Gynecol Obstet* 1974; **139**: 69-81

8 **McKay CJ**, Imrie CW. The continuing challenge of early mortality in acute pancreatitis. *Br J Surg* 2004; **91**: 1243-1244

9 **Targarona Modena J**, Barreda Cevasco L, Arroyo Basto C, Orellana Vicuña A, Portanova Ramírez M. Total enteral nutrition as prophylactic therapy for pancreatic necrosis infection in severe acute pancreatitis. *Pancreatology* 2006; **6**: 58-64

10 **Slavin J**, Ghaneh P, Sutton R, Hartley M, Rowlands P, Garvey C, Hughes M, Neoptolemos J. Management of necrotizing pancreatitis. *World J Gastroenterol* 2001; **7**: 476-481

11 **Spanier BWM,** Bruno MJ, Mathus-Vliegen EMH. Enteral nutrition and acute pancreatitis: a review. *Gastroenterol Res Practice* 2010; **2011**: 1155-1153

12 **Bollen TL**, Singh VK, Maurer R, Repas K, van Es HW, Banks PA, Mortele KJ. A comparative evaluation of radiologic and clinical scoring systems in the early prediction of severity in acute pancreatitis. *Am J Gastroenterol* 2012; **107**: 612-619

13 **Papachristou GI**, Muddana V, Yadav D, O'Connell M, Sanders MK, Slivka A, Whitcomb DC. Comparison of BISAP, Ranson's, APACHE-II, and CTSI scores in predicting organ failure, complications, and mortality in acute pancreatitis. *Am J Gastroenterol* 2010; **105**: 435-441; quiz 442

14 **Lujano-Nicolás LA**, Pérez-Hernández JL, Durán-Pérez EG, Serralde-Zúñiga AE. Corelation among clinical, biochemical and tomographic criteria in order to evaluate the severity in acute pancreatitis. *Rev Esp Enferm Dig* 2010; **102**: 376-380

15 **Schütte K**, Malfertheiner P. Markers for predicting severity and progression of acute pancreatitis. *Best Pract Res Clin Gastroenterol* 2008; **22**: 75-90

16 **Digalakis MK**, Katsoulis IE, Biliri K, Themeli-Digalaki K. Serum profiles of C-reactive protein, interleukin-8, and tu-

mor necrosis factor-alpha in patients with acute pancreatitis. *HPB Surg* 2009; **2009**: 878490
17 **Rau B**, Steinbach G, Baumgart K, Gansauge F, Grünert A, Beger HG. The clinical value of procalcitonin in the prediction of infected necrosis in acute pancreatitis. *Intensive Care Med* 2000; **26** Suppl 2: S159-S164
18 **Nathens AB**, Curtis JR, Beale RJ, Cook DJ, Moreno RP, Romand JA, Skerrett SJ, Stapleton RD, Ware LB, Waldmann CS. Management of the critically ill patient with severe acute pancreatitis. *Crit Care Med* 2004; **32**: 2524-2536
19 **Brisinda G**, Vanella S, Crocco A, Mazzari A, Tomaiuolo P, Santullo F, Grossi U, Crucitti A. Severe acute pancreatitis: advances and insights in assessment of severity and management. *Eur J Gastroenterol Hepatol* 2011; **23**: 541-551
20 **Sun W**, Watanabe Y, Wang ZQ. Expression and significance of ICAM-1 and its counter receptors LFA-1 and Mac-1 in experimental acute pancreatitis of rats. *World J Gastroenterol* 2006; **12**: 5005-5009
21 **Ryschich E**, Kerkadze V, Deduchovas O, Salnikova O, Parseliunas A, Märten A, Hartwig W, Sperandio M, Schmidt J. Intracapillary leucocyte accumulation as a novel antihaemorrhagic mechanism in acute pancreatitis in mice. *Gut* 2009; **58**: 1508-1516
22 **Chooklin S**. Pathogenic aspects of pulmonary complications in acute pancreatitis patients. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 186-192
23 **Ramudo L**, Yubero S, Manso MA, Sanchez-Recio J, Weruaga E, De Dios I. Effects of dexamethasone on intercellular adhesion molecule 1 expression and inflammatory response in necrotizing acute pancreatitis in rats. *Pancreas* 2010; **39**: 1057-1063
24 **Schneider L**, Hartwig W, Flemming T, Hackert T, Fortunato F, Heck M, Gebhard MM, Nawroth PP, Bierhaus A, Buchler MW, Werner J. Protective effects and anti-inflammatory pathways of exogenous calcitonin gene-related peptide in severe necrotizing pancreatitis. *Pancreatology* 2009; **9**: 662-669
25 **Wiessner R**, Eisold S, Linnebacher M, Bünger C, Nizze H, Wacke R, Benz S, Schareck W, Klar E. Up-regulation of ICAM-1 during cold ischemia triggers early neutrophil infiltration in human pancreas allograft reperfusion. *Transplant Proc* 2009; **41**: 3622-3627
26 **Perejaslov A**, Chooklin S, Bihalskyy I. Implication of interleukin 18 and intercellular adhesion molecule (ICAM)-1 in acute pancreatitis. *Hepatogastroenterology* 2008; **55**: 1806-1813

S- Editor Gou SX **L- Editor** Ma JY **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2561

World J Gastroenterol 2012 May 28; 18(20): 2561-2568
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Prevalence of depressive and anxiety disorders in Chinese gastroenterological outpatients

Xiao-Jing Li, Yan-Ling He, Hong Ma, Zhe-Ning Liu, Fu-Jun Jia, Ling Zhang, Lan Zhang

Xiao-Jing Li, Ling Zhang, Lan Zhang, Mental Health Center of West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Yan-Ling He, Shanghai Mental Health Center, Shanghai Jiao Tong University, Shanghai 200030, China
Hong Ma, Peking University Institute of Mental Health, Beijing 100007, China
Zhe-Ning Liu, Mental Health Institute of the Second Xiangya Hospital, Central South University, Changsha 410011, Hunan Province, China
Fu-Jun Jia, Guangdong Mental Health Center, Guangdong General Hospital, Guangzhou 510120, Guangdong Province, China
Author contributions: Li XJ performed statistical analysis and wrote the manuscript; He YL, Ma H, Liu ZN, Jia FJ and Zhang L carried out the study; Zhang L provided the analytical assistance; He YL was responsible for study design; both Zhang L and He YL were involved in editing the manuscript.
Supported by The former Wyeth Pharmaceutical Co., Ltd., Madison, NJ, United States
Correspondence to: Dr. Lan Zhang, Mental Health Center of West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China. lanzhang3@gmail.com
Telephone: +86-28-85422633 Fax: +86-28-85422632
Received: November 10, 2011 Revised: February 20, 2012
Accepted: March 9, 2012
Published online: May 28, 2012

Abstract

AIM: To investigate the prevalence and physicians' detection rate of depressive and anxiety disorders in gastrointestinal (GI) outpatients across China.

METHODS: A hospital-based cross-sectional survey was conducted in the GI outpatient departments of 13 general hospitals. A total of 1995 GI outpatients were recruited and screened with the Hospital Anxiety and Depression Scale (HADS). The physicians of the GI departments performed routine clinical diagnosis and management without knowing the HADS score results. Subjects with HADS scores $\geqslant$ 8 were subsequently interviewed by psychiatrists using the Mini International Neuropsychiatric Interview (MINI) to make further diagnoses.

RESULTS: There were 1059 patients with HADS score $\geqslant$ 8 and 674 (63.64%) of them undertook the MINI interview by psychiatrists. Based on the criteria of Diagnostic and Statistical Manual of Mental Disorders (4th edition), the adjusted current prevalence for depressive disorders, anxiety disorders, and comorbidity of both disorders in the GI outpatients was 14.39%, 9.42% and 4.66%, respectively. Prevalence of depressive disorders with suicidal problems [suicide attempt or suicide-related ideation prior or current; module C (suicide) of MINI score $\geqslant$ 1] was 5.84% in women and 1.64% in men. The GI physicians' detection rate of depressive and anxiety disorders accounted for 4.14%.

CONCLUSION: While the prevalence of depressive and anxiety disorders is high in Chinese GI outpatients, the detection rate of depressive and anxiety disorders by physicians is low.

Key words: Depression; Anxiety; Prevalence; Gastrointestinal outpatients; Mini International Neuropsychiatric Interview

Peer reviewer: Maha Maher Shehata, Professor, Department of Internal Medicine, Gastroenterology and Hepatology Unit, Medical Specialized Hospital, Mansoura 35516, Egypt

Li XJ, He YL, Ma H, Liu ZN, Jia FJ, Zhang L, Zhang L. Prevalence of depressive and anxiety disorders in Chinese gastroenterological outpatients. *World J Gastroenterol* 2012; 18(20): 2561-2568 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2561.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2561

INTRODUCTION

Gastrointestinal (GI) disease is a serious illness, which

frequently affects a patient's physical and emotional wellbeing as well as being heavily affected by stress[1-3]. Meanwhile depression and anxiety have been identified as risk factors for some GI diseases[4-6].

Various studies using a variety of assessment methods have demonstrated that high levels of depression and anxiety exist in patients with GI symptoms[7-9]. It has also been shown that patients with comorbid anxiety and depressive disorders tend towards more severe symptoms, longer recovery times, poorer outcomes, and greater use of healthcare resources[10-12]. Despite the likelihood of GI patients to suffer from emotional distress, it has been reported that physicians in the GI department often fail to identify most cases of depression and/or anxiety, leading to under-treatment in 40%-90% of patients[13-16].

Patients with depressive and anxiety disorders often have one or more somatic symptoms (e.g., cardiopulmonary or gastrointestinal), which may be partly induced by emotional disorders[17-19]. On the other hand, many patients with depression or anxiety visit non-psychiatric departments, especially the GI department, for their physical complaints[20-22]. All these facts contribute to the low detection rate of emotional disorders among GI patients.

It is necessary to determine prevalence estimates of emotional disorders in GI patients to facilitate reasonable medical resources allocation. These have been assessed in a number of studies throughout America, Europe, and China, including the Hong Kong and Taiwan regions[15,23-25]. However, the economic status and cultural traditions of mainland China are unique, and likely to make the situation of mainland Chinese GI patients distinctive.

This is the first large-sample, multicenter study based on a mainland Chinese population to estimate the prevalence of depression and anxiety in adult GI outpatients. This cross-sectional study was carried out with GI outpatients from 13 tertiary general hospitals in Beijing, Shanghai, Guangzhou, Changsha and Chengdu. The purpose of this study was to characterize the prevalence of depressive and/or anxiety disorders among GI outpatients and to determine the non-psychiatric physician identification rate of these disorders in GI outpatients.

MATERIALS AND METHODS

Ethics

This survey was approved by the Shanghai Mental Health Center Ethics Committee. All patients provided informed written consent.

Subjects

A multi-center, cross-sectional study was carried out in the outpatient departments of 15 tertiary general hospitals in Beijing, Shanghai, Guangzhou, Changsha, and Chengdu to estimate the prevalence of depressive and anxiety disorders in adult outpatients from gastroenterology, gynecology, cardiovascular and neurology departments. However, only 13 hospitals provided complete data for the GI department (one lacked a GI department, and the other incomplete patient data). Consecutive patients visiting outpatient departments were recruited for the study. Patients were included if they were over 18 years, consented to study participation, and were able to complete the questionnaires. Exclusion criteria included being previously screened, serious physical or mental condition, language or hearing problem, incomplete records, or ongoing psychological treatment. About 140 consecutive GI outpatients were investigated in each hospital during 4-5 consecutive working days randomly selected from the 22 normal workdays in a month by using SAS (v9.0) software.

Research instruments

The somatic symptoms, as well as depression/anxiety, were assessed with the Patient Health Questionnaire 15-Item (PHQ-15)[26], Hospital Anxiety and Depression Scale (HADS)[27,28], and Mini International Neuropsychiatric Interview (MINI)[29]. PHQ-15 is a self-report questionnaire used to screen and assess somatic symptoms. It consists of 15 physical symptoms, scaled 0-2 points for each. The higher the score, the more severe the symptom. The 14-item HADS[27,28] questionnaire evaluates severity of anxiety and depression using 7 items for each affliction, and is widely used in general hospitals. Each item's severity is rated from 0 (none) to 3 (severe). Scores $\geqslant$ 8 indicate probable anxiety or depression with great reliability and validity[30], and were regarded as positive in the preliminary screening of this study. MINI[29], a structured diagnostic instrument, is used to make diagnoses according to the Diagnostic and Statistical Manual of Mental Health Disorders (4th edition, DSM-IV) and the International Statistical Classification of Disease-10. The MINI Chinese version has good reliability and validity[31-33].

Study design

This multi-center, cross-sectional study was carried out in five cities: Beijing, Shanghai, Guangzhou, Chengdu, and Changsha (representing north, east, south, west and central China, respectively). In the first stage, outpatients were screened with PHQ-15 and HADS scales, and then visited GI physicians for their original complaints. The coordinators calculated scores of both scales, kept physicians blind to results, and recorded physicians' diagnosis and treatment. In the second stage, subjects with HADS scores $\geqslant$ 8 were assessed and diagnosed by psychiatrists with MINI. The study design is shown in Figure 1.

Statistical analysis

Data analysis and management were performed using the Statistical Package for Social Sciences v17 (SPSS Inc., Chicago, IL, United States). Demographic data were described by frequency and percentage, and the lack of data in one item, such as sex or diagnosis, was treated as a missing value. Subjects who were positive in the preliminary screening stage but did not complete the psy-

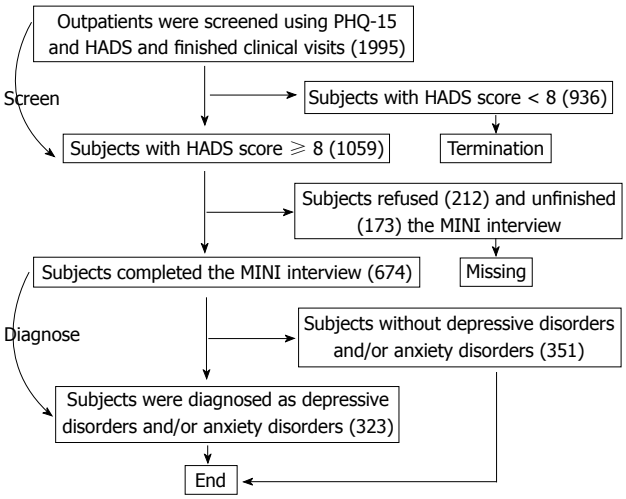


Figure 1 Flowchart of the study on prevalence of depressive disorders and/or anxiety disorders among gastrointestinal outpatients from 13 general hospitals in China. PHQ-15: Patient Health Questionnaire 15-Item; HADS: Hospital Anxiety and Depression Scale; MINI: Mini International Neuropsychiatric Interview.

chiatrists' interview were excluded.

According to a previous publication[34], prevalence was described as the percentage of positive subjects among those who completed the trial, and adjusted prevalence was calculated according to the HADS score distribution among all eligible subjects. The 95% CI of adjusted prevalence were computed using the Gaussian approximation to the log-likelihood. Categorical data, such as differences of prevalence in sex and age, or differences between individuals with and without depressive and/or anxiety disorders, were compared using the χ^2 test at the < 0.05 significance level. Consecutive data with normal distribution, such as age, were expressed as mean ± SD and analyzed using the *t* test.

"Recognized" or "detected" indicated diagnosis of depressive or anxiety disorders according to a physician's clinical judgment, prescription of antidepressants or antianxiety drugs, or referral to psychiatry or psychology departments.

RESULTS

Demographic characteristics

The study comprised 1995 outpatients, aged 18-89 (45.2 ± 15.6) years and 54.19% were female. The patients' demographic characteristics are presented in the first two columns of Table 1. One thousand and fifty-nine subjects screened positive (HADS score ⩾ 8), 580 (54.77%) of whom were female. Among these 1059, 674 completed the second stage screening of psychiatrists' interview with MINI. Among the missing subjects (n = 385), 173 (44.9%) did not complete the further interview and 212 (55.1%) refused to attend the interview (Figure 1). There were no significant differences between missed and followed-up cases in sex (χ^2 = 0.066, P = 0.797) or age (43.72 ± 15.45 *vs* 42.89 ± 14.92, t = -0.860, P = 0.390).

Of the completed subjects, 371 (55.0%) were women and 323 (47.9%) were diagnosed with one or more types of depressive disorders and/or anxiety disorders. Among the 323 confirmed subjects, 194 (60.1%) were women (other characteristics are described in the third column of Table 1). Subjects with depressive and/or anxiety disorders were more likely to be female and younger than those without such disorders (Table 1).

Prevalence of depressive and anxiety disorders in gastrointestinal outpatients

The adjusted prevalence of depressive and anxiety disorders are shown in Table 2. One hundred and eighty-one subjects had current depression disorders, 117 had current anxiety disorders, and 59 had current comorbidity. This indicates that 32.6% (59/181) of individuals with current depressive disorders had at least one type of anxiety disorder, and 50.4% (59/117) of subjects with current anxiety disorders were affected by depressive disorders as well.

The prevalence of all types of depressive disorders and anxiety disorders, according to DSM-Ⅳ criteria, are detailed in Table 2. Among the depressive disorders, depressive episode was the most common with an adjusted current prevalence of 11.23%, while substance-induced mood disorder had the lowest adjusted current prevalence (0.35%). Among 181 outpatients with depressive disorders, 51 (28.2%) had suicidal problems [suicide attempt or suicide-related ideation prior or current; module C (suicide) of MINI, score ⩾ 1], indicating that over a quarter of individuals with depressive disorders were at suicide risk.

Sex differences among current prevalence of depressive disorders and/or anxiety disorders

The current adjusted prevalence of depressive disorders and/or anxiety disorders was significantly different between male and female outpatients (Table 3). The prevalence of depressive disorders, anxiety disorders, and either depressive or anxiety disorders was significantly (P < 0.05) higher in female GI outpatients. The adjusted current prevalence of depressive disorders with suicidal problems was statistically significantly higher in women (mean 5.84%; 95% CI: 4.44-7.24) than in men (mean 1.64%; 95% CI: 0.82-2.46) (χ^2 = 23.096, P = 0.00), and the mean relative risk was 3.71 (95% CI: 2.10-6.56, P < 0.01).

Physicians' detection and treatment of depressive and anxiety disorders in gastrointestinal outpatients

Among 323 digestive outpatients who were diagnosed with depressive disorders and/or anxiety disorders by MINI, complete information of physicians' diagnoses and treatments was available for 290 cases (n = 13 missing diagnosis information, and n = 21 missing treatment information).

The detection rate by physicians was 4.14% (12/290). Among the 12 detected subjects, five were treated with psychotropic drugs, including amitriptyline or doxepin (n = 2). Another seven were referred to the psychiatry de-

Table 1 Baseline characteristics and comparison of subjects with and without depressive disorders and/or anxiety disorders *n* (%)

Characteristic	Screened subjects (*n* = 1995)	Frequency and percentage of subjects with and without depressive disorders and/or anxiety disorders according to the MINI (*n* = 674)			
		With depressive disorders and/or anxiety disorders (*n* = 323)	Without depressive disorders and/or anxiety disorders (*n* = 351)		
Sex				χ^2	*P* value
Male	914 (45.81)	29 (39.94)	173 (49.29)	6.116	0.013[a]
Female	1081 (54.19)	194 (60.06)	178 (50.71)		
Occupation				χ^2	*P* value
Laborer/attendant	282 (14.14)	52 (16.10)	46 (13.11)	5.595	0.588
Office worker	227 (11.38)	38 (11.76)	45 (12.82)		
Businessman	139 (6.97)	25 (7.74)	25 (7.12)		
Teacher	110 (5.51)	17 (5.26)	19 (5.41)		
Manager	244 (12.23)	36 (11.15)	48 (13.68)		
Farmer	264 (13.23)	43 (13.31)	40 (11.40)		
Soldier	9 (0.45)	0 (0)	3 (0.85)		
Other	720 (36.09)	112 (34.67)	124 (35.33)		
Age groups, yr		mean ± SD	mean ± SD	*t*	*P* value
		43.01 ± 14.80	46.12 ± 15.74	3.330	0.001[b]
18-29	377 (18.90)	69 (21.36)	85 (24.22)		
30-44	659 (33.03)	115 (35.60)	114 (32.48)		
45-59	562 (28.17)	93 (28.80)	95 (27.07)		
60-	397 (19.90)	46 (14.24)	57 (16.24)		

The last four columns present the characteristics and the comparison of the two groups (outpatients with and without depressive disorders and/or anxiety disorders). There are statistically significant differences between the two groups in sex ([a]$P < 0.05$ *vs* with depressive disorders group) and age ([b]$P < 0.01$ *vs* with depressive disorders group) by χ^2 test and *t* test, respectively. MINI: Mini International Neuropsychiatric Interview.

Table 2 Adjusted prevalence of depressive and anxiety disorders among gastrointestinal outpatients in 13 general hospitals in mainland China and Diagnostic and Statistical Manual of Mental Health Disorders (4th edition) by using the Mini International Neuropsychiatric Interview

Diagnosis	Frequency, adjusted prevalence (%) and 95% CI (%) based on results of the MINI			
	Current		Lifetime	
GI outpatients in 13 general hospitals				
Depressive disorders	181	14.39 (12.85-15.93)	228	18.35 (16.65-20.05)
Anxiety disorders	117	9.42 (8.14-10.70)	122	9.82 (8.51-11.13)
Comorbid depressive and anxiety disorders	59	4.66 (3.74-5.58)	69	5.46 (4.46-6.46)
Depressive disorders or anxiety disorders	239	19.20 (17.47-20.93)	281	22.71 (20.87-24.55)
DSM-IV by using the MINI interview				
Depressive episode	141	11.23 (9.84-12.62)	183	14.79 (13.23-16.35)
Depressive disorder with suicidal problems	51	3.91 (3.06-4.76)	58	4.46 (3.55-5.37)
Mood disorders due to physical disease	26	2.01 (1.39-2.63)	32	2.51 (1.87-3.15)
Dysthymia	16	1.25 (0.76-1.74)	34	2.66 (1.95-3.37)
Substance-induced mood disorders	4	0.35 (0.10-0.61)	4	0.35 (0.10-0.61)
General anxiety disorder	57	4.66 (3.74-5.58)	57	4.66 (3.74-5.58)
Specific phobia	20	1.65 (1.09-2.21)	20	1.65 (1.09-2.21)
Social phobia (social anxiety disorder)	20	1.60 (1.05-2.15)	20	1.60 (1.05-2.15)
Panic disorder	17	1.35 (0.84-1.86)	24	1.95 (1.34-2.56)
Agoraphobia	17	1.35 (0.84-1.86)	21	1.75 (1.17-2.33)
Obsessive-compulsive disorder	16	1.30 (0.80-1.80)	16	1.30 (0.80-1.80)

Depressive episode is the most common depressive disorder, while substance-induced mood disorder has the lowest adjusted current prevalence. The most common anxiety disorder is general anxiety disorder, followed by social anxiety disorder, panic disorder, agoraphobia, and obsessive-compulsive disorders. MINI: Mini International Neuropsychiatric Interview; GI: Gastrointestinal; DSM-IV: Diagnostic and Statistical Manual of Mental Health Disorders (4th edition).

partment. Meanwhile, three out of 67 (4.48%) subjects with suicide risk were identified and received psychiatric management, including psychiatry department referral (*n* = 1) and doxepin treatment (*n* = 2).

DISCUSSION

The current study evaluated the prevalence of depressive and anxiety disorders among mainland Chinese outpatients visiting GI clinics, regardless of confirmed GI diagnosis. The adjusted current prevalence of depressive disorders, anxiety disorders, and comorbid disorders was 14.39%, 9.42% and 4.66%, respectively.

It is well recognized that depressive and anxiety disorders impair life quality and cause a heavy disease burden[35-38]. Nevertheless, more than half of patients with depression or anxiety visit non-psychiatric departments, especially the GI department, for somatic symp-

Table 3 Current adjusted prevalence of depressive disorders and/or anxiety disorders and comparison between men and women

Diagnosis based on MINI	Frequency, adjusted current prevalence (%) and 95% CI (%) based on results of MINI exam				χ^2	*P* value
	Men		Women			
Depressive disorders	70	12.06 (9.95-14.17)	111	16.40 (14.19-18.61)	7.555	0.006[b]
Anxiety disorders	46	8.00 (6.24-9.76)	116	10.75 (8.90-12.60)	4.339	0.037[a]
Depressive disorders and anxiety disorders	24	4.06 (2.78-5.34)	35	5.10 (3.79-6.41)	1.224	0.269
Depressive disorders or anxiety disorders	92	16.01 (13.63-18.39)	147	21.96 (19.49-24.43)	11.285	0.001[b]

There are significant differences between male and female patients in the current prevalence of depressive disorders ([b]$P < 0.01$), anxiety disorders ([a]$P < 0.05$) and either depressive or anxiety disorders ([b]$P < 0.01$), respectively. MINI: Mini International Neuropsychiatric Interview.

toms[20,21,39,40]. However, most general physicians are not appropriately trained in psychiatry and cannot diagnose or treat depressive and anxiety disorders. Thus, GI physicians tend towards a low detection rate[41-43]. It is meaningful to investigate overall prevalence of depressive and/or anxiety disorders in GI outpatients to understand the actual patient population involved and the importance of diagnosing such disorders.

According to our knowledge, this is the largest study investigating the prevalence of depressive and anxiety disorders in GI outpatients from tertiary general hospitals in mainland China. The reliability of the current prevalence figures was assured by the use of experienced psychiatrists administering a structured diagnostic instrument. The tertiary general hospitals enrolled in this study were distributed in north (Beijing), east (Shanghai), south (Guangzhou), west (Chengdu) and central (Changsha) China, and represent the majority of national tertiary general hospitals. In addition, the DSM-Ⅳ-based MINI was used by experienced psychiatrists to produce accurate and consistent diagnoses. Finally, the study was carried out in two stages, preliminary screening and diagnostic interview.

Prevalence of depressive disorders and/or anxiety disorders in general hospitals or primary care

The overall prevalence figures of depressive disorders and/or anxiety disorders in general medical care have been reported previously[15,44]. The current adjusted prevalence of depressive disorders in our study was 14.39%. However, this value was 19.5% in a meta-analysis of primary care patients in ten countries[41]. The current adjusted prevalence of anxiety disorders reported in our study was 9.42%, which was lower than the 19.0% prevalence reported among Belgian outpatients in 86 general practices[45] and the 19.5% prevalence reported in 15 United States general medical care centers[15]. These apparent discrepancies may be a result of subjects in the previous studies being from primary care and the Primary Care Evaluation of Mental Disorders being used for diagnosis.

Furthermore, other previous domestic investigations have reported varying prevalence of depressive disorders and anxiety disorders. Qin *et al*[46] reported prevalence of 11.01% for depressive disorders in internal medical outpatients from 23 general hospitals in Shenyang. The prevalence of depression was 12.5% in family practices in Taiwan[25], while the prevalence of anxiety disorders was 11.61% in six tertiary general hospitals in Shenyang[42]. Generally speaking, these different results were due to variances in subjects and investigation instruments. The prevalence of depressive disorders and/or anxiety disorders in our study and other domestic studies are lower than results from abroad, which may relate to differences in ethnicity or culture[47,48].

The 1.25% current prevalence of dysthymia, the third top depressive disorder in our study, was higher than the 0.6% prevalence in Shanghai subjects reported by the WHO[43] in 1990, but was similar to the 2.1% mean prevalence for all international sites that participated in the research and the 2.8% prevalence of dysthymia in the study of Qin *et al*[46]. It was lower than the 12.6% prevalence of dysthymia among outpatients from 86 general practices in Belgium[45].

It is well-known that comorbidity of depressive disorders and anxiety disorders can exacerbate symptoms, and co-occurrence of anxiety is an independent risk factor of suicide among depressive patients[35,49]. In the current study, anxiety disorders were comorbid in 32.6% of depressive individuals. This comorbid proportion in depressive patients was found to be 68.9% in a study conducted in 15 centers of China[50], and 50.6% in the United States[51]. It is a common phenomenon that depressive disorders and anxiety disorders are in comorbidity among outpatients in general medical care.

Detection rate by physicians in general hospitals or primary care

Detection rate in this study was 4.14%, similar to the 4% reported for Shenyang[42,46]. A United States-based study of outpatients with GI symptoms revealed that 52% of anxious patients and 26% of depressive patients were recognized by gastroenterologists[16]. Family practices surveyed in Taiwan[25] indicated that the recognition rate of depression disorders was 12.5%, and that of general anxiety disorder was 8.0%. Prevalence of depression disorders in internal medicine inpatients was 26.9% and only 40% of these patients received antidepressant treatment[52]. Another MINI-based study of internal medicine inpatients revealed that prevalence of depressive disorders was 26%, and 43.8% of them were treated with antidepressants[53]. A meta-analysis conducted by

Mitchell *et al*[41] indicated that correct diagnosis rate of clinicians was 47.3%-50.1%. The remarkable difference in detection rate between other investigations and ours suggests the urgent need to improve the diagnosing rates in mainland China.

Meanwhile, comorbid disorders deserve great attention due to their significant correlation to suicide risk. Current prevalence of depressive disorders with suicidal problems was 3.91% in our study, suggesting that over a quarter of patients with depressive disorders were at suicide risk, while only 4.48% of those patients were recognized. Carson *et al*[54] indicated that morbidity of major depression with suicide ideation was 29.9%, while its recognition rate by physicians was 58%. Moreover, prevalence of depression and/or anxiety disorders in our study was higher in females than in males, which is consistent with results in Qin's study[42,46], and reminds physicians to pay more attention to female outpatients with mood problems.

Discrepancies of prevalence and detection rate between our study and previous studies likely reflect the limitations of methodology, which require significant effort to be overcome in subsequent research.

These findings confirm the high prevalence of depressive and anxiety disorders and disappointing detection and treatment rate in the GI departments, and highlight the particular challenge posed by the contrasts between these two rates. Although all 13 tertiary hospitals represent the top general hospitals in China, low recognition and treatment rates raise significant concerns and indicate the need to improve the physician's abilities to diagnose and identify emotional disorders in GI patients.

Several potential explanations exist for the high prevalence of depressive and anxiety disorders and low detection rate in GI outpatients. Physicians are less specialized than psychiatrists in recognizing mental disorders correctly. Furthermore, culture may limit physicians' abilities in this regard. In the Chinese traditional culture, social and cognitive processes or mental status are closely related, which contributes to interpreting emotional distress and anxiety as social or ethical problems rather than mental disorders. Somatic symptoms can also serve as cultural idioms of depressive emotion[55-57]. Depressed or anxious people are inclined to experience physical symptoms, masking the underlying mental disorder[39]. In addition, there is a distorted cognition of mental disorders. It is common to consider depressive individuals as having no self-control and weak. Jorm *et al*[58] reported that around a quarter of Australian adults consider antidepressants as harmful to suicidal depressive patients, who are more likely to reject relevant treatments, including psychotherapy. Finally, the established stigma of mental disorders causes hiding of emotional problems and rationalization to resist therapy. Dramatic reports in the mainstream media of aggressive behavior by mental disorder sufferers prejudice both patients and physicians against the disorder[59-61].

Previous studies have proven that depressive and anxiety disorders influence prognosis of physical diseases, raise medical risk, and increase economic burden[62,63]. However, appropriate treatment does benefit recovery from physical disease and maintenance of social function[64-66]. Therefore, clinicians should improve their ability to diagnose depression and anxiety, especially in patients with complaints of unexplained GI symptoms.

Limitations

Several limitations exist in the current study. Firstly, excluding outpatients who could not complete the investigation due to severe physical or mental dysfunction may have biased the results since severity of physical symptoms is positively related to depression, anxiety or other mental problems[67,68]. Secondly, the 385 missing cases (due to busy schedules and denial of mental issues) from the diagnostic interview accounted for 19.3% of the total. There were no statistically significant differences between missing and follow-up cases in sex ($\chi^2 = 0.066$, $P = 0.797$) or age ($t = -0.860$, $P = 0.390$). Although statistical adjustment was performed, representation of the sample in the study may have been impacted.

COMMENTS

Background

Depressive disorders and anxiety disorders are common in general hospitals and represent significant risks to patients' quality-of-life. Patients visiting non-psychiatric departments may have at least one somatic symptom which is partly of emotional origin, challenging non-psychiatric physicians to detect emotional disorders.

Research frontiers

Emotional disorders in gastrointestinal (GI) patients have been assessed in a number of studies in America, Europe and China, including non-mainland regions of Hong Kong and Taiwan. However, the economic status and cultural traditions of mainland China are quite distinctive from foreign countries and even the non-mainland regions of China. It is important to understand the mental health situation of mainland Chinese GI patients.

Innovations and breakthroughs

The current study determined the prevalence of depressive and/or anxiety disorders and physicians' detection rates in tertiary care hospitals across mainland China. In particular, this is the first multi-center study from the mainland of China with a large number of patients to report the prevalence of depression and anxiety in adult GI outpatients. Furthermore, the diagnosis of depressive and anxiety disorders was made with the Mini International Neuropsychiatric Interview diagnostic instrument.

Applications

The results of this study suggest that clinicians should improve their abilities to detect emotional disorders. Furthermore, they serve to remind the government or medical institutions of the importance of promoting productive interactions between psychiatry and other departments.

Peer review

This study is well designed including group analysis and statistics. In particular, this is the first multi-center study from the mainland of China with a large number of patients to report the prevalence of depression and anxiety in adult GI outpatients.

REFERENCES

1 **Levenstein S**, Ackerman S, Kiecolt-Glaser JK, Dubois A. Stress and peptic ulcer disease. *JAMA* 1999; **281**: 10-11
2 **Mayer EA**. The neurobiology of stress and gastrointestinal disease. *Gut* 2000; **47**: 861-869

3 **Bhatia V**, Tandon RK. Stress and the gastrointestinal tract. *J Gastroenterol Hepatol* 2005; **20**: 332-339
4 **Goodwin RD**, Stein MB. Generalized anxiety disorder and peptic ulcer disease among adults in the United States. *Psychosom Med* 2002; **64**: 862-866
5 **De la Roca-Chiapas JM**, Solís-Ortiz S, Fajardo-Araujo M, Sosa M, Córdova-Fraga T, Rosa-Zarate A. Stress profile, coping style, anxiety, depression, and gastric emptying as predictors of functional dyspepsia: a case-control study. *J Psychosom Res* 2010; **68**: 73-81
6 **Levy RL**, Olden KW, Naliboff BD, Bradley LA, Francisconi C, Drossman DA, Creed F. Psychosocial aspects of the functional gastrointestinal disorders. *Gastroenterology* 2006; **130**: 1447-1458
7 **Mussell M**, Kroenke K, Spitzer RL, Williams JB, Herzog W, Löwe B. Gastrointestinal symptoms in primary care: prevalence and association with depression and anxiety. *J Psychosom Res* 2008; **64**: 605-612
8 **Addolorato G**, Mirijello A, D'Angelo C, Leggio L, Ferrulli A, Abenavoli L, Vonghia L, Cardone S, Leso V, Cossari A, Capristo E, Gasbarrini G. State and trait anxiety and depression in patients affected by gastrointestinal diseases: psychometric evaluation of 1641 patients referred to an internal medicine outpatient setting. *Int J Clin Pract* 2008; **62**: 1063-1069
9 **Walker EA**, Katon WJ, Jemelka RP, Roy-Bryne PP. Comorbidity of gastrointestinal complaints, depression, and anxiety in the Epidemiologic Catchment Area (ECA) Study. *Am J Med* 1992; **92**: 26S-30S
10 **Löwe B**, Spitzer RL, Williams JB, Mussell M, Schellberg D, Kroenke K. Depression, anxiety and somatization in primary care: syndrome overlap and functional impairment. *Gen Hosp Psychiatry* 2008; **30**: 191-199
11 **Haag S**, Senf W, Häuser W, Tagay S, Grandt D, Heuft G, Gerken G, Talley NJ, Holtmann G. Impairment of health-related quality of life in functional dyspepsia and chronic liver disease: the influence of depression and anxiety. *Aliment Pharmacol Ther* 2008; **27**: 561-571
12 **Katon W**, Lin EH, Kroenke K. The association of depression and anxiety with medical symptom burden in patients with chronic medical illness. *Gen Hosp Psychiatry* 2007; **29**: 147-155
13 **Cepoiu M**, McCusker J, Cole MG, Sewitch M, Belzile E, Ciampi A. Recognition of depression by non-psychiatric physicians--a systematic literature review and meta-analysis. *J Gen Intern Med* 2008; **23**: 25-36
14 **Wittchen HU**, Höfler M, Meister W. Prevalence and recognition of depressive syndromes in German primary care settings: poorly recognized and treated? *Int Clin Psychopharmacol* 2001; **16**: 121-135
15 **Kroenke K**, Spitzer RL, Williams JB, Monahan PO, Löwe B. Anxiety disorders in primary care: prevalence, impairment, comorbidity, and detection. *Ann Intern Med* 2007; **146**: 317-325
16 **Keefer L**, Sayuk G, Bratten J, Rahimi R, Jones MP. Multicenter study of gastroenterologists' ability to identify anxiety and depression in a new patient encounter and its impact on diagnosis. *J Clin Gastroenterol* 2008; **42**: 667-671
17 **Caballero L**, Aragonès E, García-Campayo J, Rodríguez-Artalejo F, Ayuso-Mateos JL, Polavieja P, Gómez-Utrero E, Romera I, Gilaberte I. Prevalence, characteristics, and attribution of somatic symptoms in Spanish patients with major depressive disorder seeking primary health care. *Psychosomatics* 2008; **49**: 520-529
18 **Kroenke K**, Spitzer RL, Williams JB, Linzer M, Hahn SR, deGruy FV, Brody D. Physical symptoms in primary care. Predictors of psychiatric disorders and functional impairment. *Arch Fam Med* 1994; **3**: 774-779
19 **Vaccarino AL**, Sills TL, Evans KR, Kalali AH. Prevalence and association of somatic symptoms in patients with Major Depressive Disorder. *J Affect Disord* 2008; **110**: 270-276
20 **Wang T**, Chen YL, Lu YR. A survey of depression patients with physical symptoms as the first symptom attending gastroenterology outpatient clinic of a general hospital: an analysis of 5754 cases. *Shijie Huaren Xiaohua Zazhi* 2010; **18**: 851-853
21 **Simon GE**, VonKorff M, Piccinelli M, Fullerton C, Ormel J. An international study of the relation between somatic symptoms and depression. *N Engl J Med* 1999; **341**: 1329-1335
22 **Menchetti M**, Belvederi Murri M, Bertakis K, Bortolotti B, Berardi D. Recognition and treatment of depression in primary care: effect of patients' presentation and frequency of consultation. *J Psychosom Res* 2009; **66**: 335-341
23 **Mergl R**, Seidscheck I, Allgaier AK, Möller HJ, Hegerl U, Henkel V. Depressive, anxiety, and somatoform disorders in primary care: prevalence and recognition. *Depress Anxiety* 2007; **24**: 185-195
24 **King M**, Nazareth I, Levy G, Walker C, Morris R, Weich S, Bellón-Saameño JA, Moreno B, Svab I, Rotar D, Rifel J, Maaroos HI, Aluoja A, Kalda R, Neeleman J, Geerlings MI, Xavier M, de Almeida MC, Correa B, Torres-Gonzalez F. Prevalence of common mental disorders in general practice attendees across Europe. *Br J Psychiatry* 2008; **192**: 362-367
25 **Liu CY**, Chen CY, Cheng AT. Mental illness in a general hospital's family medicine clinic in Taiwan. *Psychiatry Clin Neurosci* 2004; **58**: 544-550
26 **Kroenke K**, Spitzer RL, Williams JB. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. *Psychosom Med* 2002; **64**: 258-266
27 **Bjelland I**, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. *J Psychosom Res* 2002; **52**: 69-77
28 **Ye WF**, Xu JM. Application and evaluation of General Hospital Anxiety and Depression Scale in general hospital patients. *Zhongguo Xingwei Yixve Zazhi* 1993; **2**: 17-19
29 **Sheehan DV**, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 1998; **59** Suppl 20: 22-33; quiz 34-57
30 **Herrmann C**. International experiences with the Hospital Anxiety and Depression Scale--a review of validation data and clinical results. *J Psychosom Res* 1997; **42**: 17-41
31 **Lecrubier Y**, Sheehan DV, Weiller E, Amorim P, Bonora I, Sheehan KH, Janavs J, Dunbar GC. The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: Reliability and validity according to the CIDI. *Eur Psychiatry* 1997; **12**: 224-231
32 **Sheehan DV**, Lecrubier Y, Sheehan KH, Janavs J, Weiller E, Keskiner A, Schinka J, Knapp E, Sheehan MF, Dunbar GC. The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. *Eur Psychiatry* 1997; **12**: 232-241
33 **Si TM**, Shu L, Dang WM, Su YA, Chen JX, Dong WT, Kong QM, Zhang WH. Evaluation of the reliability and validity of chinese version of the Mini-International Neuropsychiatric Interview in patients with mental disorders. *Zhongguo Xinli Weisheng Zazhi* 2009; **23**: 493-497
34 **He YL**, Ma H, Zhang L, Liu ZN, Jia FJ, Zhang MY. [A cross-sectional survey of the prevalence of depressive-anxiety disorders among general hospital outpatients in five cities in China]. *Zhonghua Neike Zazhi* 2009; **48**: 748-751
35 **Sareen J**, Cox BJ, Afifi TO, de Graaf R, Asmundson GJ, ten Have M, Stein MB. Anxiety disorders and risk for suicidal ideation and suicide attempts: a population-based longitudinal study of adults. *Arch Gen Psychiatry* 2005; **62**: 1249-1257
36 **Balázs J**, Lecrubier Y, Csiszér N, Koszták J, Bitter I. Prevalence and comorbidity of affective disorders in persons making suicide attempts in Hungary: importance of the first

depressive episodes and of bipolar II diagnoses. *J Affect Disord* 2003; **76**: 113-119
37 **Harris EC**, Barraclough B. Suicide as an outcome for mental disorders. A meta-analysis. *Br J Psychiatry* 1997; **170**: 205-228
38 **Murray CJ**, Lopez AD, Jamison DT. The global burden of disease in 1990: summary results, sensitivity analysis and future directions. *Bull World Health Organ* 1994; **72**: 495-509
39 **Kirmayer LJ**, Robbins JM, Dworkind M, Yaffe MJ. Somatization and the recognition of depression and anxiety in primary care. *Am J Psychiatry* 1993; **150**: 734-741
40 **Han YC**, Zong YH, Zhang YH, Wang WH, Hui XiQ, Wang XQ. Clinical analysis of somatic symptoms in 117 cases with major depression. *Zhongguo Xinli Weisheng Zazhi* 2008; **22**: 874-877
41 **Mitchell AJ**, Vaze A, Rao S. Clinical diagnosis of depression in primary care: a meta-analysis. *Lancet* 2009; **374**: 609-619
42 **Qin X**, Phillips MR, Wang W, Li Y, Jin Q, Ai L, Wei S, Dong G, Liu L. Prevalence and rates of recognition of anxiety disorders in internal medicine outpatient departments of 23 general hospitals in Shenyang, China. *Gen Hosp Psychiatry* 2010; **32**: 192-200
43 **Xiao Z**, Yan H, Xiao S. Depressive disorders among outpatients in general hospitals. *Zhonghua Yixve Zazh*i 1999; **79**: 329-331
44 **Li XY**, Zhang YP, Wang ZQ, Yang SJ, Phillips MR. Prevalence of depressive disorders among patients treated in general hospitals in Beijing. *Zhongguo Shenjing Jingshen Jibing Zazhi* 2010; **36**: 65-69
45 **Ansseau M**, Dierick M, Buntinkx F, Cnockaert P, De Smedt J, Van Den Haute M, Vander Mijnsbrugge D. High prevalence of mental disorders in primary care. *J Affect Disord* 2004; **78**: 49-55
46 **Qin X**, Wang W, Jin Q, Ai L, Li Y, Dong G, Liu L, Phillips MR. Prevalence and rates of recognition of depressive disorders in internal medicine outpatient departments of 23 general hospitals in Shenyang, China. *J Affect Disord* 2008; **110**: 46-54
47 **Jackson-Triche ME**, Greer Sullivan J, Wells KB, Rogers W, Camp P, Mazel R. Depression and health-related quality of life in ethnic minorities seeking care in general medical settings. *J Affect Disord* 2000; **58**: 89-97
48 **Riolo SA**, Nguyen TA, Greden JF, King CA. Prevalence of depression by race/ethnicity: findings from the National Health and Nutrition Examination Survey III. *Am J Public Health* 2005; **95**: 998-1000
49 **Moffitt TE**, Harrington H, Caspi A, Kim-Cohen J, Goldberg D, Gregory AM, Poulton R. Depression and generalized anxiety disorder: cumulative and sequential comorbidity in a birth cohort followed prospectively to age 32 years. *Arch Gen Psychiatry* 2007; **64**: 651-660
50 **Shi SX**, Zhang MY, Wu WY, Lu Z, Xin ZT, n ZHi, Liu YL, Zhao JP, Sun XL, Li M, Zhang N, Liu SW, Tao M, Li HC, PingYang Y, Wei J, Ji JL, Zhao BL, Chen SQ, Qu ZW. Multicenter study of the clinical features in depression comorbidity with anxiety disorders. *Shanghai Jingshen Yixve* 2009; **21**: 198-202
51 **Fava M**, Rankin MA, Wright EC, Alpert JE, Nierenberg AA, Pava J, Rosenbaum JF. Anxiety disorders in major depression. *Compr Psychiatry* 2000; **41**: 97-102
52 **Rentsch D**, Dumont P, Borgacci S, Carballeira Y, deTonnac N, Archinard M, Andreoli A. Prevalence and treatment of depression in a hospital department of internal medicine. *Gen Hosp Psychiatry* 2007; **29**: 25-31
53 **Cigognini MA**, Furlanetto LM. Diagnosis and pharmacological treatment of depressive disorders in a general hospital. *Rev Bras Psiquiatr* 2006; **28**: 97-103
54 **Carson AJ**, Best S, Warlow C, Sharpe M. Suicidal ideation among outpatients at general neurology clinics: prospective study. *BMJ* 2000; **320**: 1311-1312
55 **Kirmayer LJ**. Cultural variations in the clinical presentation of depression and anxiety: implications for diagnosis and treatment. *J Clin Psychiatry* 2001; **62** Suppl 13: 22-28; discussion 29-30
56 **Kirmayer LJ**. Cultural variations in the response to psychiatric disorders and emotional distress. *Soc Sci Med* 1989; **29**: 327-339
57 **Lin TY**. Psychiatry and Chinese culture. *West J Med* 1983; **139**: 862-867
58 **Jorm AF**, Christensen H, Griffiths KM. Belief in the harmfulness of antidepressants: results from a national survey of the Australian public. *J Affect Disord* 2005; **88**: 47-53
59 **Prior L**, Wood F, Lewis G, Pill R. Stigma revisited, disclosure of emotional problems in primary care consultations in Wales. *Soc Sci Med* 2003; **56**: 2191-2200
60 **Sirey JA**, Bruce ML, Alexopoulos GS, Perlick DA, Friedman SJ, Meyers BS. Stigma as a barrier to recovery: Perceived stigma and patient-rated severity of illness as predictors of antidepressant drug adherence. *Psychiatr Serv* 2001; **52**: 1615-1620
61 **Crisp AH**, Gelder MG, Rix S, Meltzer HI, Rowlands OJ. Stigmatisation of people with mental illnesses. *Br J Psychiatry* 2000; **177**: 4-7
62 **Weissman MM**, Neria Y, Gameroff MJ, Pilowsky DJ, Wickramaratne P, Lantigua R, Shea S, Olfson M. Positive screens for psychiatric disorders in primary care: a long-term follow-up of patients who were not in treatment. *Psychiatr Serv* 2010; **61**: 151-159
63 **Tian J**, Chen ZC, Hang LF. The effects of psychological status of the patients with digestive system cancers on prognosis of the disease. *Cancer Nurs* 2009; **32**: 230-235
64 **Küchler T**, Bestmann B, Rappat S, Henne-Bruns D, Wood-Dauphinee S. Impact of psychotherapeutic support for patients with gastrointestinal cancer undergoing surgery: 10-year survival results of a randomized trial. *J Clin Oncol* 2007; **25**: 2702-2708
65 **Whooley MA**, Simon GE. Managing depression in medical outpatients. *N Engl J Med* 2000; **343**: 1942-1950
66 **Wells KB**, Sherbourne C, Schoenbaum M, Duan N, Meredith L, Unützer J, Miranda J, Carney MF, Rubenstein LV. Impact of disseminating quality improvement programs for depression in managed primary care: a randomized controlled trial. *JAMA* 2000; **283**: 212-220
67 **Richardson LP**, Lozano P, Russo J, McCauley E, Bush T, Katon W. Asthma symptom burden: relationship to asthma severity and anxiety and depression symptoms. *Pediatrics* 2006; **118**: 1042-1051
68 **Di Marco F**, Verga M, Reggente M, Maria Casanova F, Santus P, Blasi F, Allegra L, Centanni S. Anxiety and depression in COPD patients: The roles of gender and disease severity. *Respir Med* 2006; **100**: 1767-1774

S- Editor Gou SX **L- Editor** Logan S **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2569

World J Gastroenterol 2012 May 28; 18(20): 2569-2575
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Evaluation of malignancy using Ki-67, p53, EGFR and COX-2 expressions in gastrointestinal stromal tumors

Jing Jiang, Mei-Shan Jin, Jian Suo, Yin-Ping Wang, Liang He, Xue-Yuan Cao

Jing Jiang, Division of Clinical Epidemiology, Jilin University First Hospital, Changchun 130021, Jilin Province, China
Mei-Shan Jin, Yin-Ping Wang, Division of Pathology, Jilin University First Hospital, Changchun 130021, Jilin Province, China
Jian Suo, Liang He, Xue-Yuan Cao, Department of Gastrointestinal Surgery, Jilin University First Hospital, Changchun 130021, Jilin Province, China
Author contributions: Jiang J and Cao XY designed the study and carried out most of the experiments; Jin MS and Cao XY collected patients' clinical information; Jin MS and Wang YP analyzed pathological data; Jiang J performed statistical analysis and wrote the manuscript; Suo J and He L provided valuable suggestions in the preparation of the manuscript.
Correspondence to: Xue-Yuan Cao, MD, PhD, Department of Gastrointestinal Surgery, Jilin University First Hospital, 71 Xinmin Street, Changchun 130021, Jilin Province, China. ccmzc32jdyycao@yahoo.com.cn
Telephone: +86-431-887832737 Fax: +86-431-887832889
Received: July 8, 2011 Revised: September 27, 2011
Accepted: October 27, 2011
Published online: May 28, 2012

Abstract

AIM: To investigate the role of expressions of Ki-67, p53, epidermal growth factor receptor (EGFR) and cyclooxygenase-2 (COX-2) in gastrointestinal stromal tumor (GIST) grading and prognosis.

METHODS: Tumor tissue was collected retrospectively from 96 patients with GIST. Antibodies against Ki-67, p53, EGFR and COX-2 were used for immunohistochemical staining. Tumor grading was designated according to a consensus system and the staining was quantified in 3 categories for each antibody in the statistical analysis.

RESULTS: The Ki-67 expression in GISTs was significantly associated with the size of the tumors, mitotic rate and the risk of malignancy (χ^2 = 15.51, P = 0.02; χ^2 = 22.27, P < 0.001; χ^2 = 20.05; P < 0.001). The p53 expression was also significantly correlated with mitotic rate and the risk of malignancy (χ^2 = 9.92, P = 0.04; χ^2 = 9.97; P = 0.04). Over-expression of Ki-67 was strongly correlated with poor survival (χ^2 = 10.44, P = 0.006), but no correlation was found between the expression of p53, EGFR or COX-2 and survival. Multivariate analysis further demonstrated that Ki-67 expression (relative risk = 15.78, 95% CI: 4.25-59.37) could be used as an independent prognostic value for GIST patients. Adjuvant imatinib therapy could improve clinical outcomes in the patients with high risk and intermediate risk of recurrence after complete tumor resections (median survival time: 52 mo *vs* 37 mo, χ^2 = 7.618, P = 0.006).

CONCLUSION: Our results indicated that the expression of Ki-67 could be used as an independent prognostic factor for GIST patients.

Key words: Gastrointestinal stromal tumor; Prognosis; Ki-67 alteration; p53; Epidermal growth factor receptor

Peer reviewer: Yasuhiro Matsumura, MD, PhD, Investigative Treatment Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

Jiang J, Jin MS, Suo J, Wang YP, He L, Cao XY. Evaluation of malignancy using Ki-67, p53, EGFR and COX-2 expressions in gastrointestinal stromal tumors. *World J Gastroenterol* 2012; 18(20): 2569-2575 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2569.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2569

INTRODUCTION

Gastrointestinal stromal tumor (GIST) is one of the most frequent mesenchymal neoplasms of the gastroin-

testinal tract. In the elderly, micro-GIST (the tumor size smaller than 1 cm) is detected in 20%-30% of individuals over 60 years old[1,2]. GIST occurs along the gastrointestinal tract and commonly invades in the stomach and small intestine. The tumors rarely arise from extragastrointestinal sites, such as omentum or mesentery[3]. Most GISTs express c-kit. Monoclonal antibodies against c-kit, DOG1 and protein kinase C theta have been developed as helpful diagnostic adjuncts in pathology[4-6].

GISTs have a wide clinical spectrum, ranging from virtually benign to highly aggressive tumors. Up to 30% of GISTs recur and progress to metastatic disease even after the complete excision of tumors. Despite a remarkable progress in the understanding of GISTs, it is still difficult to make a prognosis due to the variability of disease[7]. According to the National Institutes of Health (NIH) classification system, GISTs are classified into four categories: very low, low, intermediate and high risk[8]. The prognosis of patients is commonly stratified based on tumor size and mitotic counts in the NIH system. Previous studies have demonstrated that nuclear atypia and tumor necrosis all contribute to prognostic outcomes of GIST patients. Further, some studies showed that gastric GISTs had lower risks of recurrence than nongastric tumors with the same size and same mitotic count[9]. The four-point classification only distinguishes GISTs with high-risk from those with low-risk[10]. The system using multiple histopathological parameters for GIST prognosis is subjective and lacks reproducibility[11].

The proliferation marker Ki-67, tumor suppressor gene *p53*, cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR) have been identified as prognostic biomarkers in tumors of epithelial origin. However, there has been no study analyzing these markers systematically in a large cohort of mesenchymal tumors, especially in GISTs[12,13]. In this study, Ki-67, p53, EGFR and COX-2 expressions were fully investigated in the GIST tumor specimens from 96 patients and the grade of the tumor was established based on the immunohistochemical staining of each protein. The grades were then compared with patients' clinical features and roles of prognostic values for GISTs were evaluated. The study indicated that determination of these tumorigenetic and cell proliferative proteins provides alternative measurements for follow-up and prognosis.

MATERIALS AND METHODS

Patients

From January 2005 through December 2009, 134 patients were initially diagnosed as having mesenchymal gastrointestinal tumors at Jilin University First Hospital. Thirty-three patients were excluded from the study due to recurred tumors or the tumors being partially resected. Thus, 101 patients underwent successful surgical operations for complete resection of tumors. Following the surgery, patients with high-risk and intermediate risk were treated with imatinib (Glivec®, Novartis Pharmaceuticals, Basel, Switzerland) at a dose of 400 mg/d for 3 years. No imatinib treatment was given before the surgery. Five cases were lost to follow-up. Ninety-six patients were retrospectively evaluated in the study. Informed consents were obtained from all patients and the study was approved by the local human ethical committee of Jilin University First Hospital. Original hematoxylin and eosin-stained sections were reviewed in each case by two pathologists (Jin MS and Wang YP) according to GIST characteristics described by Miettinen[14]. All tumors from 96 patients were confirmed to be GISTs based on a combination of histological evaluations (highly cellular spindles/epithelioids/mixed cell tumors), and c-kit, DOG1, CD34 positive staining. The clinical information regarding the patients is summarized in Table 1.

Table 1 Clinicopathological features of 96 patients with gastrointestinal stromal tumor

	n	% of 96 GISTs
Age (yr, median age = 55 yr)		
< 40	12	12.5
40-60	52	54.2
> 60	32	33.3
Sex		
Male	57	59.4
Female	39	40.6
Site		
Esophagus	3	3.1
Gastric	45	46.9
Intestine	37	38.5
EGIST	11	11.5
Tumor size (cm, median size = 7.0)		
≤ 2	7	7.3
> 2 to ≤ 5	29	30.2
> 5 to ≤ 10	34	35.4
> 10	26	27.1
Mitotic rate (per 50 HPFs)		
≤ 5	54	56.3
6 to 10	28	29.2
> 10	14	14.6
Risk of malignancy		
High risk	45	46.9
Intermediate risk	24	25.0
Low risk	24	25.0
Very low risk	3	3.1

GISTs: Gastrointestinal stromal tumors; EGIST: Extra GIST; HPFs: High-power fields.

Immunohistochemical analysis

Histological sections (4 μm) of 10% formalin-fixed, paraffin-embedded material were used for immunohistochemical staining. Prior to a primary antibody staining, the slide was pretreated with citric acid or ethylenediaminetetraacetic acid buffer in a pressure cooker for antigen retrieval. Endogenous peroxidase activity was quenched by 3% H_2O_2 blocking reagent for 10 min. The slide was incubated with a primary antibody at 4 ℃ overnight, and then immunostained with the avidin-biotin peroxidase complex (DAKO, CA). Finally, the slide was stained with diaminobenzidine according to the manufacturer's protocol (DAKO, CA). The slide was

rinsed three times with phosphate buffered saline after each step of staining. The sections were stained with primary antibodies against c-kit (Clone: YR145, dilution: 1/50, Cell Marque Corporation, CA), CD34 (QBEnd/10, dilution: 1/100, Neomarkers, CA), Ki-67 (MIB-1, dilution: 1/100, DAKO, Carpinteria, CA), DOG1 (SP31, dilution: 1/100, Spring Bioscience, Pleasanton), SMA (IA4, dilution: 1/200, Cell Marque Corporation, CA), p27 (1B4, dilution: 1/20, Novocastra), p53 (SP5, dilution: 1/100, Zymed Laboratories, San Francisco), S-100 (6E6, dilution: 1/100, Neomarkers, CA), Desmin (D33, dilution:1/50, Cell Marque Corporation, CA), EGFR (EGFR.113, dilution: 1/200, Novocastra Laboratories Ltd, Newcastle, United Kingdom), and COX-2 (SP21, dilution: 1/50, Neomarkers, CA, United States), respectively. All primary antibodies used in the study were biotinylated monoclonal antibodies. The stained slides were evaluated quantitatively or semi-quantitatively by two independent pathologists who were blinded from clinical data. Percentages of positive cells stained with a special antibody observed by two pathologists were consistent and the mean values were determined.

The nuclear staining for Ki-67 and p53, and cytoplasmic immunostaining for EGFR and COX-2, were considered as positive cells of the reaction. According to previous studies[15,16], the following scoring assessments for Ki-67 and p53 were used. The score 0 was assigned for < 5%, 1 for > 5% and < 10%, 2 for > 10% of Ki-67 staining positive cells. The p53 scoring system was 0 assigned for < 5%, 1 for > 5% and < 25%, 2 for > 25% of p53 staining positive cells. EGFR scoring system was 0 assigned for < 10%, 1 for > 10% and < 60%, 2 for > 60% of EGFR positive cells based on the systems described by Nakagawa *et al*[17] and Gumurdulu *et al*[18]. The COX-2 scoring system was 0 assigned for no positive cells; 1 for < 25% and score 2 for > 25% of COX-2 staining positive cells according to Fux *et al*[19].

Statistical analysis

The Chi-square test and Fisher's exact test were used to analyze relationships between clinicopathological features and expression levels of biomarkers. Kaplan-Meier test was applied with a log-rank test to study associations between categorical variables and the mean values of survival among groups. Cox proportional hazards regression analysis was used to estimate a hazard risk for survival and 95% CI was applied. The SPSS program (version 18.0) was used for statistical analysis. A P value of < 0.05 was considered statistically significant.

RESULTS

Clinicopathological findings and follow-up

Clinicopathological features of the patients are summarized in Table 1. The median age of 96 patients was 55 years (range, 26-82 years). Histomorphology showed that the neoplastic cells were predominantly spindle-shaped (83/96, 86.5%). Based on the modified NIH risk consensus system, 45 (46.9%), 24 (25.0%), 24 (25.0%) and 3 (3.1%) cases were classified as high-risk, intermediate-risk, low risk and very low risk categories, respectively. Fifty-three cases (55.2%) had mild nuclear atypia; 32 cases (33.4%) showed severe nuclear atypia, but 11 patients (11.4%) had no nuclear atypia. Tumor necrosis was found in 39 cases of the patients (40.6%).

At the time of study, the mean or the median duration of the follow-up period was 31 mo or 29 mo, respectively. Medical charts were available for 96 of 101 patients (95%). Sixty-nine patients (54.2%) received the imatinib treatment at a dose of 400 mg/d for 13 mo to 36 mo (median, 26 mo). Thirty-seven patients (82%) from the high risk group and 15 patients (62.5%) from the intermediate group required the imatinib treatment. Disease specific 1, 2, 3 and 4 year survival probabilities were 0.97, 0.89, 0.79, and 0.77 (0.65-0.87), respectively. Of the 96 cases, 19 patients (19.8%) died from GISTs and 6 patients (6.3%) died from unrelated causes.

Immunohistochemical findings

Eighty-eight (91.3%) tumor specimens were stained positive for c-kit. The tumors isolated from 8 patients (8.7%) were negative for c-kit but positive for DOG1 and/or CD34 staining. Reactivity with Desmin was found in 3 (3.1%) cases. Positive SMA and S-100 staining were also noted in 46 (47.9%) and 12 (12.5%) cases, respectively. Based on the Ki-67 index, 53.1% of tumors (n = 51) scored 0; 34.4% (n = 33) scored 1; and 12.5% (n = 12) scored 2. 34.4% of tumor specimens (n = 33) were p53 staining positive in the nuclei of over 25% of the cells. EGFR staining was found in most cases. Forty-two (43.8%) cases scored 2, and 27 (28.1%) cases scored 1 for EGFR staining. COX-2 overexpressed in 36 (37.5%) cases (Table 2 and Figure 1).

Clinicopathological features and GIST grades categorized by the staining of Ki-67, p53, EGFR or COX-2 are established in Table 3. The expression of Ki-67 was significantly associated with tumor size (P = 0.02), mitotic rate (P < 0.001) and the risk of malignancy (P < 0.001). The p53 expression was also correlated with mitotic rate (P = 0.04), tumor site (P = 0.02) and the risk of malignancy (P = 0.04). The levels of COX-2 protein were significantly higher in gastric tumors and spindle cell-like tumors (P < 0.001 and P = 0.05, respectively). In contrast, no correlation was found between the EGFR expression and clinicopathological factors or the risk of malignancy.

Survival analysis

The 3-year survival rates for disease specific survival (DSS) were 100%, 89%, 79% and 67% for groups at very low-risk, low-risk, intermediate-risk and high risk by the modified NIH risk categories, respectively. Associations between DSS and different protein biomarkers were analyzed using a multivariate analysis (Table 3 and Figure 2). The survival rates were strongly associated with tumor size (P = 0.004), mitotic count (P = 0.001),

Table 2 Ki-67, P53, epidermal growth factor receptor, cyclooxygenase-2 expression related to clinicopathological features

Variable	Ki-67				P53				EGFR				COX-2			
	0+	1+	2+	*P* value	0+	1+	2+	*P* value	0+	1+	2+	*P* value	0+	1+	2+	*P* value
Tumor size (cm)																
≤ 2	6	1	0	0.02	3	3	1	0.47	1	3	3	0.41	2	2	3	0.07
> 2 to ≤ 5	21	7	1		8	14	7		6	12	11		17	7	5	
> 5 to ≤ 10	15	15	4		9	10	15		11	8	15		20	9	5	
> 10	9	10	7		5	11	10		9	4	13		21	1	4	
Total	51	33	12		25	38	33		27	27	42		60	19	17	
Mitotic rate (per 50 HPFs)																
≤ 5	39	12	3	< 0.001	17	20	17	0.04	16	16	22	0.77	38	8	8	0.41
> 5 to ≤ 10	10	14	4		8	13	7		7	6	15		14	8	6	
> 10	2	7	5		0	5	9		4	5	5		8	3	3	
Risk of malignancy																
Very low + low risk	23	4	0	< 0.001	9	11	7	0.04	6	11	10	0.56	17	6	4	0.50
Intermediate risk	13	9	2		9	11	4		7	5	12		12	5	7	
High risk	15	20	10		7	16	22		14	11	20		31	8	6	

EGFR: Epidermal growth factor receptor; COX-2: Cyclooxygenase-2; HPFs: High-power fields.

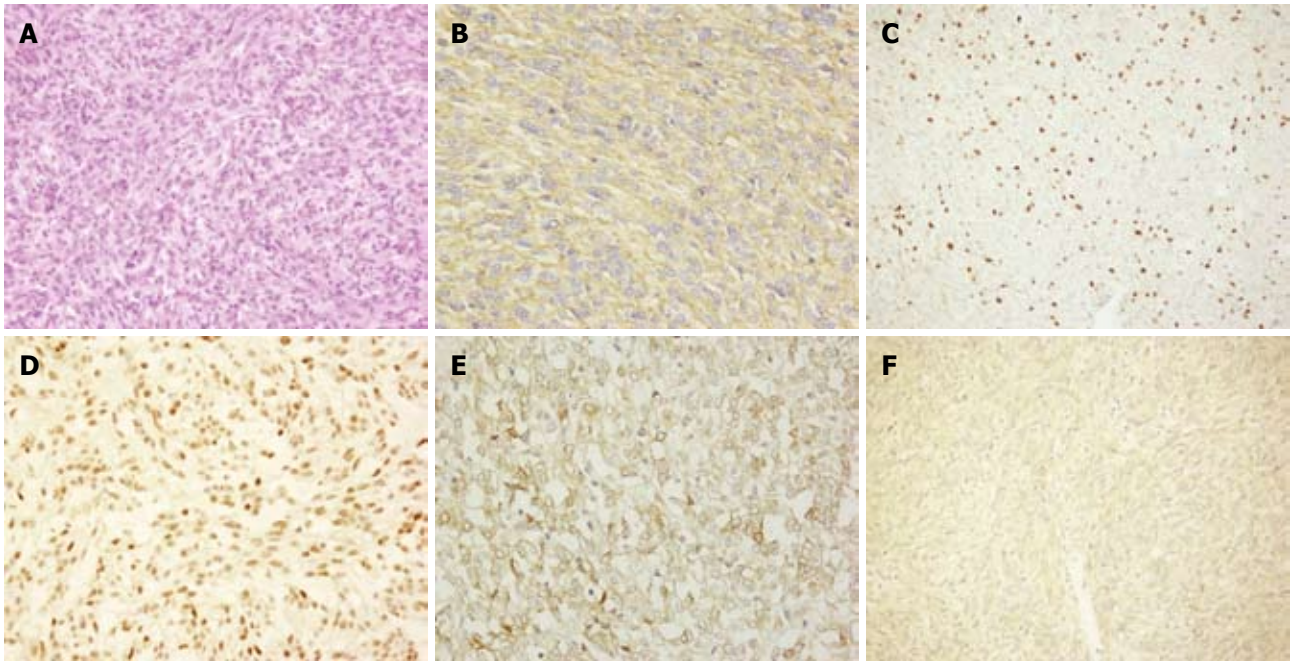


Figure 1 Images of gastrointestinal stromal tumor using hematoxylin-eosin staining and immunohistochemical staining. A: Hematoxylin-eosin stain; B: DOG1 stain; C: Ki-67 stain; D: P53 stain; E: Epidermal growth factor receptor stain; F: Cyclooxygenase-2 stain.

tumor location (P = 0.018), the NIH modified risk criteria (P < 0.001, Figure 2A), Ki-67 amplification (P < 0.001, Figure 2B), and adjuvant imatinib therapy (median survival period 52 mo *vs* 37 mo, χ^2 = 7.618, P = 0.006, Figure 2C). No significance was found when comparing survival rates with the EGFR expression, or the COX-2 expression. In the high-risk group, Ki-67 overexpression was significantly associated with poor survival (χ^2 = 10.44, P = 0.006), but no statistical significance was found between the p53 expression and survival (χ^2 = 4.744, P = 0.089). Using a multivariate analysis, a poor survival was observed in the high risk category [relative risk (RR) = 12.23; 95% CI: 1.61-92.81] graded using the modified NIH risk consensus system or in category 2 scored by the Ki-67 expression (RR = 15.78; 95% CI: 4.25-59.37) (Table 3).

DISCUSSION

In the absence of reliable genetic and immunohistochemical biomarkers in GISTs, the tumor size and mitotic rate are often used to assess risk probabilities in GIST patients. Large retrospective cohort studies have shown that the NIH classification carries substantial prognostic value[20]. Using classical morphological parameters, our results were consistent with previous studies on the prognosis of GIST patients.

Ki-67, a nuclear protein associated with cell prolif-

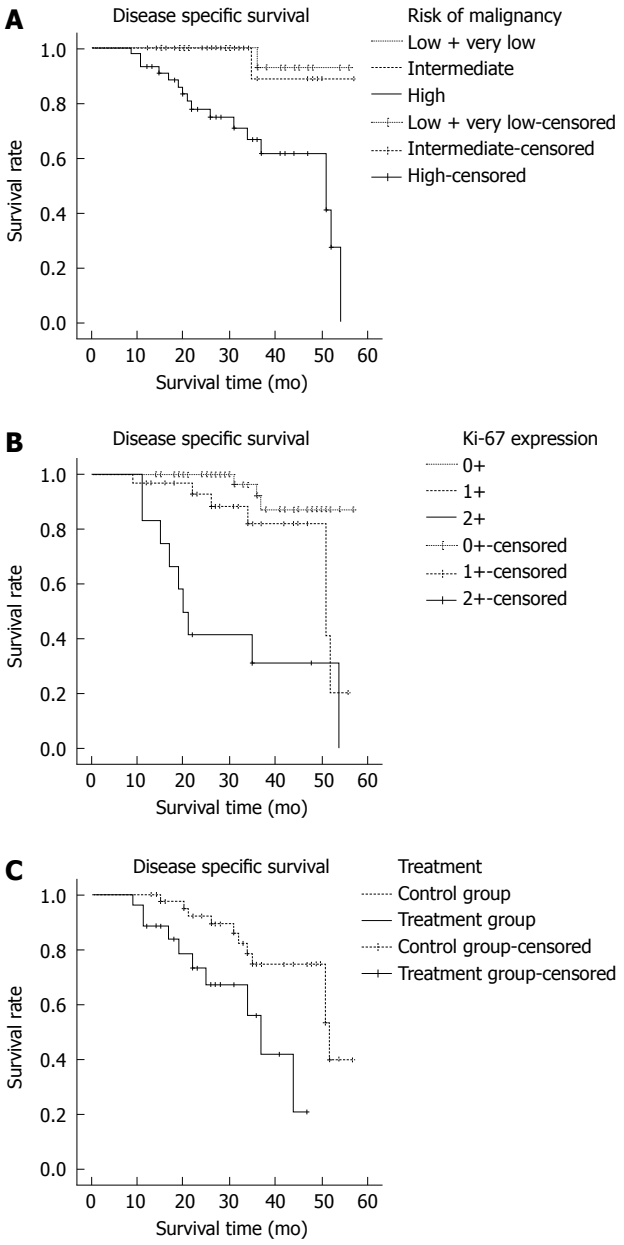


Figure 2 Kaplan-Meier plots for predicting disease specific survival based on the modified National Institutes of Health consensus system (A), Ki-67 expression (B) and adjuvant imatinib therapy (C).

eration, expresses in all cell cycle phases except for G0. A recent study demonstrated that the automated assessments of Ki-67 staining with computing image analysis can be used for prognostic assessments of patients with breast cancer[21]. However, the prognostic value of Ki-67 as a potential biomarker has not been fully investigated in GISTs[22,23]. The present study shows that the expression of Ki-67 or p53 is significantly associated with many clinicopathological features in GISTs; higher score for Ki-67 staining was directly correlated with poor survival; Ki-67 was superior to other protein markers tested in survival assessments, particularly in the high risk group, suggesting that Ki-67 immunostaining is a reliable and independent marker for the prediction of clinical outcomes in patients with GISTs.

Table 3 Multivariate analyses for disease-specific survival

Variable	RR (95% CI)	*P* value
Risk of malignancy		
Very low/low risk	1.00 (reference)	< 0.001
Intermediate risk	1.32 (0.81-21.2)	
High risk	12.23 (1.61-92.81)	
Ki-67 expression		
0+	1.00 (reference)	< 0.001
1+	3.75 (0.97-14.54)	
2+	15.78 (4.25-59.37)	
P53 expression		
0+	1.00 (reference)	0.20
1+	2.11 (0.24-18.37)	
2+	4.49 (0.54-37.11)	
EGFR expression		
0+	1.00 (reference)	0.50
1+	1.97 (0.60-6.50)	
2+	1.17 (0.36-3.77)	
COX-2 expression		
0+	1.00 (reference)	0.19
1+	0.84 (0.42-4.36)	
2+	1.99 (0.55-7.21)	

EGFR: Epidermal growth factor receptor; COX-2: Cyclooxygenase-2; RR: Relative risk.

The tumor suppressor p53 plays an important role in the regulation of cell cycle, DNA repair and programmed cell death. The functional loss of p53 disrupts these pathways and results in the selection of tumor cells with growth advantage[24]. p53 has been reported as a prognostic marker in a wide variety of carcinomas, as well as in GISTs[25]. A study showed that impaired p53 expression was often found in advanced GISTs and a strong effect of p53 on the progression-free survival was also observed[18]. The accumulation of p53 protein was significantly associated with mitotic rate and the risk of malignancy in the present study.

The activation of EGFR is associated with cell growth and transformation. There are few reports analyzing the EGFR expression in GISTs. A study has suggested that a transforming growth factor alpha (TGF-α)/EGFR autocrine loop is present in GISTs, in which TGF-α promotes the proliferation of GIST tumor cells through an interaction of EGFR with HER-1[26]. Co-expressions of EGFR and several EGFR ligands were observed with the upregulation of ADAM17 in GISTs. The authors suggested that the EGFR activation was through shedding of EGFR ligands by ADAM17 and consequently resulted in GIST progression and growth[17]. To our knowledge, there has been no study assessing prognostic values of EGFR in a large cohort of GISTs. However, no significant association was found between the EGFR expression and prognostic analysis of GISTs in our study.

Increased COX-2 expression has been observed in colorectal adenoma and carcinoma[27]. The induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility and adhesion[28]. Over-expression of COX-2 has tumorigenic effects in animal models[29]. Expression levels of COX-2 and vascular endothelial growth factor were found to

be significantly higher in malignant GISTs than those in benign and intermediate GISTs[30]. A study reported a correlation between the COX-2 expression and tumor cell proliferation in GISTs, but no association was found between COX-2 expression and mortality, metastasis, tumor size, the risk of stages, the dose of tyrosine kinase inhibitors, or the rate of complete resection[31]. Our results demonstrated that levels of COX-2 expression were significantly different between gastric tumors and nongastric tumors ($P < 0.001$), but no significant relationship was found between the COX-2 expression and risk factors, or survival.

Imatinib therapy reduces rates of recurrence in GISTs. Nevertheless, it remains unclear how to screen patients who would be more likely to benefit from the adjuvant therapy. In our study, we found that imatinib treatment could significantly improve 3-year DSS rates in the intermediate and high risk categories of patients after a complete tumor resection.

In conclusion, Ki-67 expression is significantly associated with GIST malignancy and can be used as a putative prognostic marker in GISTs. p53 and COX-2 also provide additional valuable information in the evaluation of malignancy and types of GISTs.

COMMENTS

Background

Gastrointestinal stromal tumors (GISTs) are known to have a wide variability in malignancy and prognosis. Risk grading based on tumor size, location and mitotic counts has been proposed to predict adverse outcomes of GIST in the literature.

Research frontiers

Recent molecular studies have found that the deregulations of Ki-67, cyclin A, B1, D1, E, cdc2 and other cell-cycle regulators were significantly associated with a shorter disease-free survival in GISTs.

Innovations and breakthroughs

In this study, expressions of Ki-67, p53, epidermal growth factor receptor (EGFR) and COX-2 were investigated in a large cohort of GIST patients and their roles as prognostic values for GISTs were also evaluated. To our knowledge, this is the first assessment of the prognostic value of EGFR in patients with GISTs.

Applications

The immunohistochemical staining of these tumorigenetic and cell proliferative proteins provides an alternative approach for follow-up and clinical decisions regarding the treatment for GISTs.

Peer review

This paper describes a retrospective clinicopathological study of GIST.

REFERENCES

1 **Rubin BP**, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. *Lancet* 2007; **369**: 1731-1741

2 **Rossi S**, Gasparotto D, Toffolatti L, Pastrello C, Gallina G, Marzotto A, Sartor C, Barbareschi M, Cantaloni C, Messerini L, Bearzi I, Arrigoni G, Mazzoleni G, Fletcher JA, Casali PG, Talamini R, Maestro R, Dei Tos AP. Molecular and clinicopathologic characterization of gastrointestinal stromal tumors (GISTs) of small size. *Am J Surg Pathol* 2010; **34**: 1480-1491

3 **Nilsson B**, Bümming P, Meis-Kindblom JM, Odén A, Dortok A, Gustavsson B, Sablinska K, Kindblom LG. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era-a population-based study in western Sweden. *Cancer* 2005; **103**: 821-829

4 **Andersson J**, Bümming P, Meis-Kindblom JM, Sihto H, Nupponen N, Joensuu H, Odén A, Gustavsson B, Kindblom LG, Nilsson B. Gastrointestinal stromal tumors with KIT exon 11 deletions are associated with poor prognosis. *Gastroenterology* 2006; **130**: 1573-1581

5 **Liegl B**, Hornick JL, Corless CL, Fletcher CD. Monoclonal antibody DOG1.1 shows higher sensitivity than KIT in the diagnosis of gastrointestinal stromal tumors, including unusual subtypes. *Am J Surg Pathol* 2009; **33**: 437-446

6 **Miettinen M**, Wang ZF, Lasota J. DOG1 antibody in the differential diagnosis of gastrointestinal stromal tumors: a study of 1840 cases. *Am J Surg Pathol* 2009; **33**: 1401-1408

7 **Kang YN**, Jung HR, Hwang I. Clinicopathological and immunohistochemical features of gastointestinal stromal tumors. *Cancer Res Treat* 2010; **42**: 135-143

8 **Nakamura N**, Yamamoto H, Yao T, Oda Y, Nishiyama K, Imamura M, Yamada T, Nawata H, Tsuneyoshi M. Prognostic significance of expressions of cell-cycle regulatory proteins in gastrointestinal stromal tumor and the relevance of the risk grade. *Hum Pathol* 2005; **36**: 828-837

9 **Joensuu H**. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. *Hum Pathol* 2008; **39**: 1411-1419

10 **Dorn J**, Spatz H, Schmieder M, Barth TF, Blatz A, Henne-Bruns D, Knippschild U, Kramer K. Cyclin H expression is increased in GIST with very-high risk of malignancy. *BMC Cancer* 2010; **10**: 350

11 **Blay JY**, Bonvalot S, Casali P, Choi H, Debiec-Richter M, Dei Tos AP, Emile JF, Gronchi A, Hogendoorn PC, Joensuu H, Le Cesne A, McClure J, Maurel J, Nupponen N, Ray-Coquard I, Reichardt P, Sciot R, Stroobants S, van Glabbeke M, van Oosterom A, Demetri GD. Consensus meeting for the management of gastrointestinal stromal tumors. Report of the GIST Consensus Conference of 20-21 March 2004, under the auspices of ESMO. *Ann Oncol* 2005; **16**: 566-578

12 **Song Z**, Wang JL, Pan YL, Tao DY, Gan MF, Huang KE. Survival and prognostic factors analysis in surgically resected gastrointestinal stromal tumor patients. *Hepatogastroenterology* 2009; **56**: 149-153

13 **Ruiz-Tovar J**, Diez-Tabernilla M, Housari G, Martinez-Molina E, Sanjuanbenito A. Gastrointestinal stromal tumors: actin expression, a new prognostic factor? *Am Surg* 2010; **76**: 1244-1250

14 **Miettinen M**, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am J Surg Pathol* 2005; **29**: 52-68

15 **Di Vizio D**, Demichelis F, Simonetti S, Pettinato G, Terracciano L, Tornillo L, Freeman MR, Insabato L. Skp2 expression is associated with high risk and elevated Ki67 expression in gastrointestinal stromal tumours. *BMC Cancer* 2008; **8**: 134

16 **Meara RS**, Cangiarella J, Simsir A, Horton D, Eltoum I, Chhieng DC. Prediction of aggressiveness of gastrointestinal stromal tumours based on immunostaining with bcl-2, Ki-67 and p53. *Cytopathology* 2007; **18**: 283-289

17 **Nakagawa M**, Nabeshima K, Asano S, Hamasaki M, Uesugi N, Tani H, Yamashita Y, Iwasaki H. Up-regulated expression of ADAM17 in gastrointestinal stromal tumors: coexpression with EGFR and EGFR ligands. *Cancer Sci* 2009; **100**: 654-662

18 **Gumurdulu D**, Erdogan S, Kayaselcuk F, Seydaoglu G, Parsak CK, Demircan O, Tuncer I. Expression of COX-2, PCNA, Ki-67 and p53 in gastrointestinal stromal tumors and its relationship with histopathological parameters. *World J Gastroenterol* 2007; **13**: 426-431

19 **Fux R**, Schwab M, Thon KP, Gleiter CH, Fritz P. Cyclooxygenase-2 expression in human colorectal cancer is unrelated to overall patient survival. *Clin Cancer Res* 2005; **11**: 4754-4760

20 **Huang HY**, Huang WW, Lin CN, Eng HL, Li SH, Li CF, Lu

D, Yu SC, Hsiung CY. Immunohistochemical expression of p16INK4A, Ki-67, and Mcm2 proteins in gastrointestinal stromal tumors: prognostic implications and correlations with risk stratification of NIH consensus criteria. *Ann Surg Oncol* 2006; **13**: 1633-1644

21 **Konsti J**, Lundin M, Joensuu H, Lehtimäki T, Sihto H, Holli K, Turpeenniemi-Hujanen T, Kataja V, Sailas L, Isola J, Lundin J. Development and evaluation of a virtual microscopy application for automated assessment of Ki-67 expression in breast cancer. *BMC Clin Pathol* 2011; **11**: 3

22 **Romeo S**, Debiec-Rychter M, Van Glabbeke M, Van Paassen H, Comite P, Van Eijk R, Oosting J, Verweij J, Terrier P, Schneider U, Sciot R, Blay JY, Hogendoorn PC. Cell cycle/apoptosis molecule expression correlates with imatinib response in patients with advanced gastrointestinal stromal tumors. *Clin Cancer Res* 2009; **15**: 4191-4198

23 **Gunji Y**, Nikaidou T, Okazumi S, Matsubara H, Shimada H, Nabeya Y, Aoki T, Makino H, Miyazaki S, Ochiai T. Evaluation of Ki-67 and p53 expression in primary and repeated liver metastases of GISTs. *Hepatogastroenterology* 2005; **52**: 829-832

24 **Vousden KH**, Prives C. Blinded by the Light: The Growing Complexity of p53. *Cell* 2009; **137**: 413-431

25 **Hata Y**, Ishigami S, Natsugoe S, Nakajo A, Okumura H, Miyazono F, Matsumoto M, Hokita S, Aikou T. P53 and MIB-1 expression in gastrointestinal stromal tumor (GIST) of the stomach. *Hepatogastroenterology* 2006; **53**: 613-615

26 **Cai YC**, Jiang Z, Vittimberga F, Xu X, Savas L, Woda B, Callery M, Banner B. Expression of transforming growth factor-alpha and epidermal growth factor receptor in gastrointestinal stromal tumours. *Virchows Arch* 1999; **435**: 112-115

27 **Chan AT**. COX-2 expression in adenoma: an imperfect marker for chemoprevention. *Gut* 2010; **59**: 568-569

28 **Wu WK**, Sung JJ, Lee CW, Yu J, Cho CH. Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: an update on the molecular mechanisms. *Cancer Lett* 2010; **295**: 7-16

29 **Spugnini EP**, Porrello A, Citro G, Baldi A. COX-2 overexpression in canine tumors: potential therapeutic targets in oncology. *Histol Histopathol* 2005; **20**: 1309-1312

30 **Miao R**, Liu N, Wang Y, Li L, Yu X, Jiang Y, Li J. Coexpression of cyclooxygenase-2 and vascular endothelial growth factor in gastrointestinal stromal tumor: possible relations to pathological parameters and clinical behavior. *Hepatogastroenterology* 2008; **55**: 2012-2015

31 **Sevinc A**, Camci C, Sari I, Kalender ME, Er O, Soyuer I, Dikilitas M, Yilmaz U, Sagol O, Alacacioglu A. Cyclooxygenase-2 expression in gastrointestinal stromal tumours. *Asian Pac J Cancer Prev* 2010; **11**: 849-853

S- Editor Tian L **L- Editor** Logan S **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2576

World J Gastroenterol 2012 May 28; 18(20): 2576-2581
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Effects of glycine on phagocytosis and secretion by Kupffer cells *in vitro*

Hui-Wen Wu, Ke-Ming Yun, De-Wu Han, Rui-Ling Xu, Yuan-Chang Zhao

Hui-Wen Wu, Ke-Ming Yun, De-Wu Han, Rui-Ling Xu, Yuan-Chang Zhao, Institute of Hepatology, Shanxi Medical University, Taiyuan 030001, Shanxi Province, China
Author contributions: Yun KM and Han DW designed the research; Wu HW, Yun KM, Xu RL and Zhao YC performed the research; Wu HW and Yun KM wrote the paper.
Supported by The Natural Science Foundation of Taiyuan City, China, No. 09121014
Correspondence to: Ke-Ming Yun, Professor, Institute of Hepatology, Shanxi Medical University, 56 Xin Jian Nan Road, Taiyuan 030001, Shanxi Province, China. yunkeming5142@163.com
Telephone: +86-351-4135537 Fax: +86-351-4135197
Received: September 26, 2011 Revised: February 10, 2012
Accepted: March 10, 2012
Published online: May 28, 2012

Abstract

AIM: To investigate the effects and mechanisms of action of glycine on phagocytosis and tumor necrosis factor (TNF)-α secretion by Kupffer cells *in vitro*.

METHODS: Kupffer cells were isolated from normal rats by collagenase digestion and Percoll density gradient differential centrifugation. After culture for 24 h, Kupffer cells were incubated in fresh Dulbecco's Modification of Eagle's Medium containing glycine (G1: 1 mmol/L, G2: 10 mmol/L, G3: 100 mmol/L and G4: 300 mmol/L) for 3 h, then used to measure phagocytosis by a bead test, TNF-α secretion after lipopolysaccharide stimulation by radioactive immunoassay, and microfilament and microtubule expression by staining with phalloidin-fluorescein isothiocyanate (FITC) or a monoclonal anti-α tubulin-FITC antibody, respectively, and evaluated under a ultraviolet fluorescence microscope.

RESULTS: Glycine decreased the phagocytosis of Kupffer cells at both 30 min and 60 min ($P < 0.01$, $P < 0.05$). The numbers of beads phagocytosed by Kupffer cells in 30 min were 16.9 ± 4.0 (control), 9.6 ± 4.1 (G1), 12.1 ± 5.7 (G2), 8.1 ± 3.2 (G3) and 7.5 ± 2.0 (G4), and were 22.5 ± 7.9 (control), 20.1 ± 5.8 (G1), 19.3 ± 4.8 (G2), 13.5 ± 4.7 (G3) and 9.2 ± 3.1 (G4) after 60 min. TNF-α secretion by Kupffer cells in G1 (0.19 ± 0.03), G2 (0.16 ± 0.04), G3 (0.14 ± 0.03) and G4 (0.13 ± 0.05) was significantly less than that in controls (0.26 ± 0.03, $P < 0.01$), and the decrease in secretion was dose-dependent ($P < 0.05$). Microfilaments of Kupffer cells in G2, G3 and G4 groups were arranged in a disorderly manner. The fluorescence densities of microtubules in G1 (53.4 ± 10.5), G2 (54.1 ± 14.6), G3 (64.9 ± 12.1) and G4 (52.1 ± 14.2) were all lower than those in the controls (102.2 ± 23.7, $P < 0.01$), but the decrease in microtubule fluorescence density was not dose-dependant.

CONCLUSION: Glycine can decrease the phagocytosis and secretion by Kupffer cells *in vitro*, which may be related to the changes in the expression of microfilaments and microtubules induced by Kupffer cells.

Key words: Glycine; Kupffer cell; Phagocytosis; Secretion

Peer reviewer: Yujin Hoshida, MD, PhD, Cancer Program, Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, United States

Wu HW, Yun KM, Han DW, Xu RL, Zhao YC. Effects of glycine on phagocytosis and secretion by Kupffer cells *in vitro*. *World J Gastroenterol* 2012; 18(20): 2576-2581 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2576.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2576

INTRODUCTION

Glycine has been well characterized in the spinal cord as an inhibitory neurotransmitter which activates expression of the glycine-gated chloride channel (GlyR) in postsyn-

aptic membranes. Kupffer cells contain a GlyR similar to that described previously in the central nervous system[1,2]. Many studies have shown that dietary or intravenous glycine has a protective effect in rat models against endotoxic shock, hemorrhagic shock, liver ischemia-reperfusion, liver transplantation, and alcohol-induced liver injury and is most likely to exert this effect by inactivating the Kupffer cells *via* this newly identified GlyR[3-10]. Our previous studies also indicated that glycine protected rats from thioacetamide-induced liver injury and intestinal endotoxemia[11,12]. The mechanism involved may be related to inhibition of the release of pro-inflammatory cytokines by Kupffer cells induced by glycine. *In vivo* and *in vitro* experiments have found that glycine inhibits the secretion of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in Kupffer cells[13-15]. However, the impact of glycine on phagocytosis by Kupffer cells has not been reported, and the mechanisms underlying the effect of glycine on TNF-α secretion by Kupffer cells have not been fully understood. Our *in vitro* study showed that lipopolysaccharide (LPS) probably enhanced or inhibited the phagocytosis of Kupffer cells by acting through mechanisms involving microfilaments or microtubules[16]. This study aimed to investigate the effects of glycine on phagocytosis and the mechanisms underlying TNF-α secretion by Kupffer cells *in vitro*.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 300-330 g were obtained from the Experimental Animal Center of Shanxi Medical University (China). All animals were fed with standard laboratory chow and water was available *ad libitum*. The experimental protocols were approved by the Shanxi Animal Research Ethics Committee.

Reagents

Polystyrene beads (1.1 μm), monoclonal anti-α tubulin-fluorescein isothiocyanate (FITC) conjugate, LPS (*Escherichia coli* Serotype 0128:B12), collagenase Ⅳ, phalloidin-FITC, hydroxyethyl piperazine ethanesulfonic acid (HEPES) Percoll, and Dulbecco's Modification of Eagle's Medium (DMEM) were purchased from Sigma (St. Louis, United States); a radioimmunoassay kit for TNF-α measurement was purchased from the Radio-Immunity Institute of the Chinese Liberation Army Omni-hospital (Beijing, China); glycine, sodium pentobarbital, fetal bovine serum (FBS), penicillin G, streptaquaine, insulin, glutamine, trypan blue, and all other reagents not specifically mentioned elsewhere were prepared by Beijing Chemical Inc. (Beijing, China).

Isolation and culture of Kupffer cells

Kupffer cells from Wistar rats were isolated by collagenase digestion and differential centrifugation, using Percoll density gradients as described previously with slight modifications[17]. Briefly, the liver was perfused *in situ* through the portal vein with Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution (HBSS) containing 0.5 mmol/L ethylene glycol-bis (β-aminoethyl ether)-N,N,N,N- tetraacetic acid (EGTA) at 37 ℃ for 5 min at a flow rate of 26 mL/min. Subsequently, perfusion was performed with HBSS containing 0.05% collagenase Ⅳ at 37 ℃ for 5 min. After the liver was digested, it was excised and cut into small pieces in collagenase buffer. The suspension was filtered through nylon gauze, and the filtrate was centrifuged twice at 50 × *g* at 4 ℃ for 3 min to remove parenchymal cells. The nonparenchymal cell fraction was washed with buffer and centrifuged on a density cushion of Percoll at 1000 × *g* at 4 ℃ for 20 min to obtain the Kupffer cell fraction, and the cells obtained were washed with buffer again. The viability of isolated Kupffer cells was determined by trypan blue exclusion and routinely exceeded 90%. Cells were seeded onto 24-well culture plates (Corning, NY) or 25 mm × 25 mm glass coverslips at a density of 1×10^6 or 5×10^5 and cultured in DMEM supplemented with 10% FBS, antibiotics (100 U/mL penicillin G and 100 μg/mL streptomycin sulfate), 0.1 U/100 mL insulin and 15 mmol/L glutamine at 37 ℃ with 5% CO_2. Non-adherent cells were removed after 1 h by replacing the culture medium. All adherent cells phagocytosed latex beads and stained positive for catalase, confirming that they were Kupffer cells, and cells were cultured for 24 h before experiment.

Effects of glycine on Kupffer cells

Cells were seeded onto 24-well plates and 12 mm × 12 mm glass coverslips, and incubated with fresh medium containing glycine (G1: 1 mmol/L, G2: 10 mmol/L, G3: 100 mmol/L and G4: 300 mmol/L) at 37 ℃ with 5% CO_2 for 3 h. Phagocytosis and expression of microfilaments and microtubules by Kupffer cells were measured by the bead phagocytosis test, fluorescence staining and immunofluorescence staining, as described below.

Measurement of phagocytosis by Kupffer cells

Phagocytosis by Kupffer cells was evaluated by the Kupffer cell's ability to ingest polystyrene beads according to the modified method of Hirose *et al*[18]. Briefly, cells were seeded onto 12 mm × 12 mm glass coverslips or glass plates and incubated with fresh medium containing 0.05% polystyrene beads for 30 min or 60 min at 37 ℃ with 5% CO_2. Following vigorous pipetting to remove non-phagocytosed latex beads, the coverslips or glass plates were washed 3 times with PBS and fixed with 2% formaldehyde or methanol for 5 min. After staining by Giemsa's method for 15 min at room temperature and washing 3 times with PBS, the coverslips were inverted onto glass slides and observed under phase contrast microscope. The mean number of latex beads phagocytosed by each Kupffer cell was counted in at least 20 Kupffer cells per field at magnification of 200 times, 5 fields per coverslip in 6 coverslips.

Measurement of Kupffer cell secretion

Kupffer cells were seeded into 24-well plates at a density of 1×10^6/well and incubated with fresh DMEM

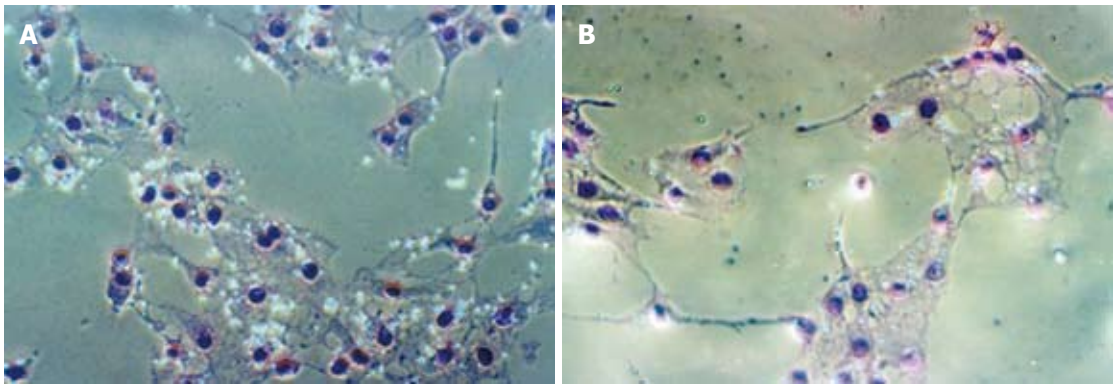


Figure 1 Effects of glycine on phagocytosis by Kupffer cells *in vitro*. A: Phagotosis by Kupffer cells in the control group 30 min after the addition of latex beads, 200×; B: Phagotosis by Kupffer cells in group G3 30 min after the addition of latex beads, 200×.

containing 100 ng/mL LPS for 60 min at 37 ℃ with 5% CO_2. At the end of this period, the medium was collected, centrifuged at 1000 × *g* at 4 ℃ for 10 min, and the supernatant was stored at -80 ℃ until used for TNF-α assay. TNF-α in medium was measured using the radioimmunoassay kit. The levels of TNF-α in the wells represented the secretion of Kupffer cells.

Measurement of microfilament expression by Kupffer cells

Kupffer cells were stained with phalloidin-FITC according to the modified method of Wulf *et al*[19]. Briefly, Kupffer cells were seeded onto 12 mm × 12 mm glass coverslips at a density of 5×10^5 (1×10^4 to 2×10^4 cells/coverslip), fixed with 2% formaldehyde for 20 min and extracted with 0.5% Triton X-100 for 15 min. The fixed cells were then washed 3 times with PBS (10 mmol/L, pH 7.4) and stained with phalloidin-FITC for 45 min at room temperature in the dark. They were then washed for a further 3 times with PBS, the coverslips were inverted onto mounting medium applied to glass slides, and they were observed and photographed under a ultraviolet (UV) fluorescence microscope with a high magnification of 400 times. Mounted preparations could be stored in the dark at 2 ℃-8 ℃.

Measurement of microtubules in Kupffer cells

Microtubules in Kupffer cells were stained with a monoclonal anti-α tubulin-FITC antibody according to the method recommended by the producer. Briefly, Kupffer cells were seeded onto 12 mm × 12 mm glass coverslips at a density of 5×10^5 (1×10^4 to 2×10^4 cells/coverslip). They were then fixed with cold methanol for 10 min at -20 ℃ and rinsed twice with cold acetone (-20 ℃) for 10 s, then the cell layer was rehydrated in PBS (10 mmol/L, pH 7.4) for at least 30 min and stained with monoclonal anti-α tubulin-FITC (1:25 diluted with PBS containing 1% bovine serum albumin) in a dark-room for 60 min at room temperature. The stained cells were washed 3 times with PBS, the coverslips were inverted onto mounting medium applied to glass slides and observed and photographed under a UV fluorescence microscope. Mounted preparations could be stored in the dark at 2-8 ℃. The fluorescence density was measured in 10 cells using the MIAS-300 picture analysis system from at least 5 fields in each picture at a high magnification of 400 times.

Table 1 Effects of glycine on phagocytosis by Kupffer cells *in vitro* (mean ± SD)

Groups	Beads observed in Kupffer cells (n = 6)	
	30 min	60 min
Control	16.9 ± 4.0	22.5 ± 7.9
G1	9.6 ± 4.1[b]	20.1 ± 5.8
G2	12.1 ± 5.7[a]	19.3 ± 4.8
G3	8.1 ± 3.2[b]	13.5 ± 4.7[b,c]
G4	7.5 ± 2.0[b]	9.2 ± 3.1[b,d]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* control; [c]$P < 0.05$, [d]$P < 0.01$ *vs* G1.

Statistical analysis

All results were expressed as mean ± SD. Statistical differences between means were analyzed by one-way analysis of variance or *t* test using the SPSS 12.0 statistical package. Statistical significance level was set at $P < 0.05$.

RESULTS

Effects of glycine on phagocytosis by Kupffer cells

When incubated in 5% CO_2 with fresh medium containing glycine at 37 ℃ for 30 min or 60 min, phagocytosis by Kupffer cells decreased significantly. The number of beads phagocytosed by Kupffer cells in groups G3 and G4 was less than that of group G1 in 60 min. There were no significant differences in the amount of beads phagocytosed by Kupffer cells among the G2, G3 and G4 groups (Table 1 and Figure 1).

Effects of glycine on TNF-α secretion by Kupffer cells

When incubated in 5% CO_2 with fresh medium containing glycine at 37 ℃ for 3 h, TNF-α secretion by Kupffer cells decreased significantly, and the decrease in secretion was dose dependent. TNF-α concentrations detected in the medium of groups G3 and G4 were significantly lower than in the medium of group G1 (Table 2).

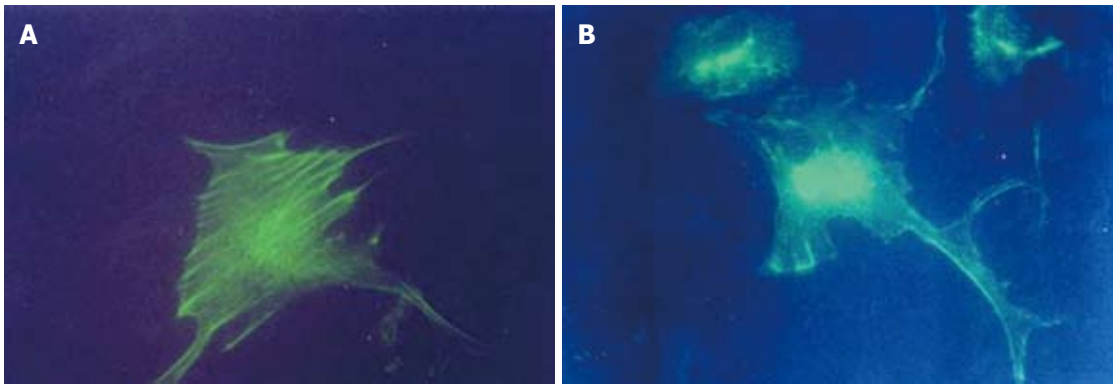


Figure 2 Effects of glycine on expression of microfilaments by Kupffer cells *in vitro*. A: The expression of microfilaments by Kupffer cells in the control group, stained with Phalloidin-fluorescein isothiocyanate (FITC), 400×; B: The expression of microfilaments by Kupffer cells in group G3, stained with Phalloidin-FITC, 400×.

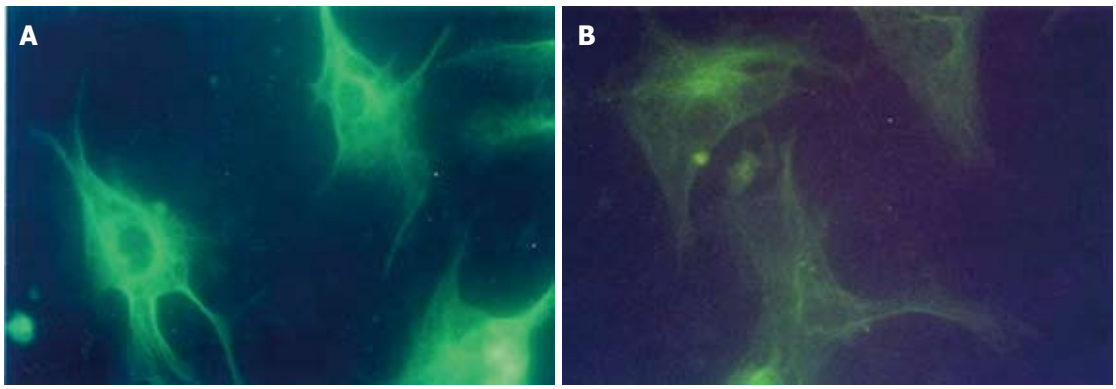


Figure 3 Effects of glycine on expression of microtubules by Kupffer cells *in vitro*. A: The expression of microtubules by Kupffer cells in the control group, stained with monoclonal anti-α tubulin-fluorescein isothiocyanate (FITC) conjugate, 400×; B: The expression of microtubules by Kupffer cells in group G3, stained with monoclonal anti-α tubulin-FITC conjugate, 400×.

Table 2 Effects of glycine on tumor necrosis factor-α secretion and microtubule density of Kupffer cells *in vitro* (n = 6) (mean ± SD)

Groups	TNF-α detected in medium (μg/mL)	Microtubule density of Kupffer cells
Control	0.26 ± 0.03	102.2 ± 23.7
G1	0.19 ± 0.03[d]	53.4 ± 10.5[d]
G2	0.16 ± 0.04[d]	54.1 ± 14.6[d]
G3	0.14 ± 0.03[a,d]	64.9 ± 12.1[d]
G4	0.13 ± 0.05[a,d]	52.1 ± 11.4[d]

[a]P < 0.05 *vs* G1; [d]P < 0.01 *vs* control. TNF: Tumor necrosis factor.

Effects of glycine on microfilaments of Kupffer cells

After 3 h incubation in 5% CO_2 with fresh medium containing glycine at 37 ℃, Kupffer cells stained with FITC-Phalloidin did not demonstrate organized microfilaments in groups G2, G3 or G4. There were no significant differences in the microfilament fluorescence densities among Kupffer cells in control, G1, G2, G3 and G4 groups (Figure 2).

Effects of glycine on microtubules of Kupffer cells

Following 3 h incubation in fresh medium containing glycine at 37 ℃ with 5% CO_2, Kupffer cells were stained with monoclonal anti-α tubulin-FITC. A significant decrease in the fluorescence density of microtubules was observed in Kupffer cells incubated with glycine as compared with the controls. However, the fluorescence density of the microtubules did not show a dose-dependent decrease among G1, G2, G3 and G4 groups (Table 2 and Figure 3).

DISCUSSION

Kupffer cells are the main component of the host monocyte-macrophage system, and their two main functions are phagocytosis and secretion. There is much evidence indicating that activation of Kupffer cells and their production of pro-inflammatory cytokines contribute to the pathogenesis of different liver injuries, including alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and liver failure among others[20-22]. Tsujimoto *et al*[23] showed that phagocytic activity of Kupffer cells was decreased in a rat model of nonalcoholic steatohepatitis. Glycine is a non-essential amino acid and an inhibitory neurotransmitter in the central nervous system. Many studies have shown that dietary or intravenous glycine can protect against a variety of liver injuries[3-10]. In this study, we found that glycine decreases the phagocytosis and secretion of Kupffer cells *in vitro*.

Effects of glycine on phagocytosis by Kupffer cells

The mechanisms of Kupffer cell phagocytosis are still not completely understood. The ruffling of the cell membrane and formation of pseudopodia may play an important role and these effects are believed to be accomplished by the cytoskeleton. In the cytoskeleton, actin-myosin interaction through the calcium-calmodulin system plays a major role in this activity[24]. In this system, intracellular Ca^{2+} combines with calmodulin to form the active calcium-calmodulin complex, which activates an enzyme, myosin light chain kinase, to phosphorylate the light chain of myosin. Phosphorylated myosin, but not unphosphorylated myosin, can interact with actin to induce activity of the cell membrane and pseudopodia, leading to phagocytosis. This process is reversible, in that a phosphatase can catalyze dephosphorylation of myosin, restoring it to a form that can not be activated by actin.

Previous studies have shown that integrity of the cytoskeletal system is important for phagocytosis of Kupffer cells. Depolymerization of the cytoskeleton decreased phagocytosis by Kupffer cells[25-27]. However, the effects of glycine on phagocytosis by Kupffer cells have not been reported.

The present experiments show that glycine decreases phagocytosis by Kupffer cells *in vitro*, causes disordering of the microfilaments in Kupffer cells, and reduces their expression of microtubules. All these results show that glycine can decrease the phagocytosis of Kupffer cells by acting on the microfilaments and microtubules.

Effects of glycine on secretion by Kupffer cells

Some studies have shown that both CD_{14} and non-CD_{14} mechanisms are involved in the TNF-α secretion of monocytes and Kupffer cells, and that both endocytosis and Ca^{2+} are required for endotoxin-stimulated TNF-α release by Kupffer cells in rats[28-30]. Previous studies have shown that glycine can protect against many injuries and illnesses in rat models, most likely by inactivating Kupffer cells and decreasing TNF-α secretion[3-15]. An *in vitro* study has shown that glycine prevents the increases in $[Ca^{2+}]_i$ caused to LPS by activating chloride influx-reduced synthesis and release of toxic mediators by Kupffer cells[2]. Thus, glycine can activate the chloride influx, prevent the increases in $[Ca^{2+}]_i$ and reduce the TNF-α secretion of Kupffer cells.

Other studies have demonstrated the involvement of a microtubule-dependent mechanism in TNF-α secretion by monocytes. Taxol, a microtubule-stabilizing antineoplastic agent, induced expression of tumor TNF-α in macrophages[31]. Microtubule-disrupting agents such as colchicine had opposite effects on TNF-α production[32-34]. The present experiments showed that glycine significantly decreased TNF-α secretion and microtubule expression. Some of our results are consistent with previous reports[13-15], leading us to believe that glycine can prevent TNF-α secretion by Kupffer cells through disruption of microtubules.

In summary, glycine decreases both phagocytosis and secretion by Kupffer cells *in vitro*, which is probably related to glycine-induced changes in expression of microfilaments and microtubules in Kupffer cells.

ACKNOWLEDGMENTS

The authors thank Chun-Ni Zhao for performing the statistical analysis.

COMMENTS

Background

Activated Kupffer cells are most likely involved in the pathogenesis of different liver injuries. Glycine generally is considered as a protective agent for liver injuries. The mechanism may be related to the fact that glycine inhibits the release of pro-inflammatory cytokines by Kupffer cells. So, it is very important to clarify the impact of glycine on the phagocytosis and secretion by Kupffer cells.

Research frontiers

It is believed that cytoskeleton plays a vital physiological role in phagocytosis by Kupffer cells, and depolymerization of cytoskeleton decreases the phagocytosis by Kupffer cells. Glycine protects against liver injuries by preventing the elevation of intracellular Ca^{2+} and reducing pro-inflammatory cytokines production by Kupffer cells. But the impact of glycine on phagocytosis by Kupffer cells is still unclear, and the mechanisms of glycine on tumor necrosis factor-α secretion by Kupffer cells have not been completely understood.

Innovations and breakthroughs

This is the first study to report that glycine decreases the phagocytosis of Kupffer cells by acting on the microfilaments and microtubules *in vitro*.

Applications

This study suggests that glycine may be an effective agent which could provide a future strategy for therapeutic intervention in the treatment of liver injuries induced by activated Kupffer cells.

Peer review

It is an interesting study with appropriate methodology and the results are clear and of great importance.

REFERENCES

1 **Froh M**, Thurman RG, Wheeler MD. Molecular evidence for a glycine-gated chloride channel in macrophages and leukocytes. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G856-G863
2 **Ikejima K**, Qu W, Stachlewitz RF, Thurman RG. Kupffer cells contain a glycine-gated chloride channel. *Am J Physiol* 1997; **272**: G1581-G1586
3 **Matilla B**, Mauriz JL, Culebras JM, González-Gallego J, González P. [Glycine: a cell-protecting anti-oxidant nutrient]. *Nutr Hosp* 2002; **17**: 2-9
4 **Ikejima K**, Iimuro Y, Forman DT, Thurman RG. A diet containing glycine improves survival in endotoxin shock in the rat. *Am J Physiol* 1996; **271**: G97-G103
5 **Zhong Z**, Enomoto N, Connor HD, Moss N, Mason RP, Thurman RG. Glycine improves survival after hemorrhagic shock in the rat. *Shock* 1999; **12**: 54-62
6 **Currin RT**, Caldwell-Kenkel JC, Lichtman SN, Bachmann S, Takei Y, Kawano S, Thurman RG, Lemasters JJ. Protection by Carolina rinse solution, acidotic pH, and glycine against lethal reperfusion injury to sinusoidal endothelial cells of rat livers stored for transplantation. *Transplantation* 1996; **62**: 1549-1558
7 **Thurman RG**, Schemmer P, Zhong Z, Bunzendahl H, von Frankenberg M, Lemasters JJ. Kupffer cell-dependent reperfusion injury in liver transplantation: new clinically relevant use of glycine. *Langenbecks Arch Chir Suppl Kongressbd* 1998; **115**: 185-190
8 **Schemmer P**, Bradford BU, Rose ML, Bunzendahl H, Raleigh JA, Lemasters JJ, Thurman RG. Intravenous glycine

improves survival in rat liver transplantation. *Am J Physiol* 1999; **276**: G924-G932
9 **Iimuro Y**, Bradford BU, Forman DT, Thurman RG. Glycine prevents alcohol-induced liver injury by decreasing alcohol in the rat stomach. *Gastroenterology* 1996; **110**: 1536-1542
10 **Yin M**, Ikejima K, Arteel GE, Seabra V, Bradford BU, Kono H, Rusyn I, Thurman RG. Glycine accelerates recovery from alcohol-induced liver injury. *J Pharmacol Exp Ther* 1998; **286**: 1014-1019
11 **Zhang W**,Yun KM, Han DW. [Study on the protective mechanism of glycine on rat intestinal endotoxemia]. *Shanxi Yike Daxue Xuebao* 2003; **34**: 97-98
12 **Zhang W**, Yun KM, Han DW. [Study on protection of glycine from thioacetamide induced acute liver injury]. *Shanxi Yike Daxue Xuebao* 2003; **34**: 210-211
13 **Alarcon-Aguilar FJ**, Almanza-Perez J, Blancas G, Angeles S, Garcia-Macedo R, Roman R, Cruz M. Glycine regulates the production of pro-inflammatory cytokines in lean and monosodium glutamate-obese mice. *Eur J Pharmacol* 2008; **599**: 152-158
14 **Wang G**, Wang Y, Guan FL, Ren GC, Wang YZ. [The effect of combination of glycine and methylprednisolone on Kupffer cells of liver after hemorrhagic shock in rats]. *Zhonghua Waike Zazhi* 2006; **44**: 349-352
15 **Garcia-Macedo R**, Sanchez-Muñoz F, Almanza-Perez JC, Duran-Reyes G, Alarcon-Aguilar F, Cruz M. Glycine increases mRNA adiponectin and diminishes pro-inflammatory adipokines expression in 3T3-L1 cells. *Eur J Pharmacol* 2008; **587**: 317-321
16 **Yuan KM**, Han DW, Xu RL, Zhao YC. Effect of LPS on phagocytosis of rat Kupffer cells in vitro. *Chin J Pathophysiol* 2003; **19**: 795-798
17 **Smedsrød B**, Pertoft H. Preparation of pure hepatocytes and reticuloendothelial cells in high yield from a single rat liver by means of Percoll centrifugation and selective adherence. *J Leukoc Biol* 1985; **38**: 213-230
18 **Hirose M**, Watanabe S, Ueno T, Kitami N, Sato N. Pertussis toxin-induced redistribution of cortical actomyosin and inhibition of phagocytosis in rat Kupffer cells. *J Gastroenterol Hepatol* 1993; **8**: 348-352
19 **Wulf E**, Deboben A, Bautz FA, Faulstich H, Wieland T. Fluorescent phallotoxin, a tool for the visualization of cellular actin. *Proc Natl Acad Sci USA* 1979; **76**: 4498-4502
20 **Tsukamoto H**, Lu SC. Current concepts in the pathogenesis of alcoholic liver injury. *FASEB J* 2001; **15**: 1335-1349
21 **Solga SF**, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. *J Hepatol* 2003; **38**: 681-687
22 **Jaeschke H**, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. *Toxicol Sci* 2002; **65**: 166-176
23 **Tsujimoto T**, Kawaratani H, Kitazawa T, Hirai T, Ohishi H, Kitade M, Yoshiji H, Uemura M, Fukui H. Decreased phagocytic activity of Kupffer cells in a rat nonalcoholic steatohepatitis model. *World J Gastroenterol* 2008; **14**: 6036-6043
24 **Bretscher A**. Microfilament structure and function in the cortical cytoskeleton. *Annu Rev Cell Biol* 1991; **7**: 337-374
25 **Sun WB**, Han BL, Peng ZM, Li K, Ji Q, Chen J, Wang HZ, Ma RL. Effect of aging on cytoskeleton system of Kupffer cell and its phagocytic capacity. *World J Gastroenterol* 1998; **4**: 77-79
26 **Watanabe S**, Hirose M, Ueno T, Kominami E, Namihisa T. Integrity of the cytoskeletal system is important for phagocytosis by Kupffer cells. *Liver* 1990; **10**: 249-254
27 **Dijkstra J**, van Galen M, Scherphof G. Effects of (dihydro)-cytochalasin B, colchicine, monensin and trifluoperazine on uptake and processing of liposomes by Kupffer cells in culture. *Biochim Biophys Acta* 1985; **845**: 34-42
28 **Su GL**. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G256-G265
29 **Byrd-Leifer CA**, Block EF, Takeda K, Akira S, Ding A. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur J Immunol* 2001; **31**: 2448-2457
30 **Lichtman SN**, Wang J, Zhang C, Lemasters JJ. Endocytosis and Ca2+ are required for endotoxin-stimulated TNF-alpha release by rat Kupffer cells. *Am J Physiol* 1996; **271**: G920-G928
31 **Wang J**, Kobayashi M, Han M, Choi S, Takano M, Hashino S, Tanaka J, Kondoh T, Kawamura K, Hosokawa M. MyD88 is involved in the signalling pathway for Taxol-induced apoptosis and TNF-alpha expression in human myelomonocytic cells. *Br J Haematol* 2002; **118**: 638-645
32 **Li Z**, Davis GS, Mohr C, Nain M, Gemsa D. Suppression of LPS-induced tumor necrosis factor-alpha gene expression by microtubule disrupting agents. *Immunobiology* 1996; **195**: 640-654
33 **Li Z**, Davis GS, Mohr C, Nain M, Gemsa D. Inhibition of LPS-induced tumor necrosis factor-alpha production by colchicine and other microtubule disrupting drugs. *Immunobiology* 1996; **195**: 624-639
34 **Allen JN**, Herzyk DJ, Wewers MD. Colchicine has opposite effects on interleukin-1 beta and tumor necrosis factor-alpha production. *Am J Physiol* 1991; **261**: L315-L321

S- Editor Lv S **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2582

World J Gastroenterol 2012 May 28; 18(20): 2582-2585
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Exceptionally rare cause of a total stomach resection

Jadwiga Snarska, Krzysztof Jacyna, Jacek Janiszewski, Danuta Shafie, Katarzyna Iwanowicz, Anna Żurada

Jadwiga Snarska, Krzysztof Jacyna, Katarzyna Iwanowicz, Department of General Surgery, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, 10561 Olsztyn, Poland
Jacek Janiszewski, Department of Internal Diseases, Medical Care Centre of the Ministry of the Interior and Administration with the Warmia and Mazury Centre of Oncology in Olsztyn, 10561 Olsztyn, Poland
Danuta Shafie, Department of Pathology, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, 10561 Olsztyn, Poland
Anna Żurada, Department of Anatomy, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, 10561 Olsztyn, Poland
Author contributions: Snarska J was responsible for the research design and wrote the paper; Snarska J, Janiszewski J and Iwanowicz K performed the surgical research; Jacyna K performed internal research; Shafie D performed histopathological research; Snarska J, Jacyna K, Janiszewski J, Shafie D, Iwanowicz K and Żurada A analyzed and interpreted the data; Snarska J and Żurada A designed the paper, drafted the article, revised it, and obtained final approval.
Correspondence to: Jadwiga Snarska, MD, PhD, Department of General Surgery, Faculty of Medical Sciences, University of Warmia and Mazury in Olsztyn, ul. Żołnierska 14C, 10561 Olsztyn, Poland. jadwiga.snarska@uwm.edu.pl
Telephone: +48-89-5277004 Fax: +48-89-5245301
Received: November 16, 2011 Revised: January 18, 2012
Accepted: March 9, 2012
Published online: May 28, 2012

Abstract

The first-ever case of a 54-year-old woman who overdosed on non-steroidal anti-inflammatory drugs in an attempt at suicide. Before that incident, she had not been treated for coexisting diseases such as rheumatoid arthritis or depression. At the time of admission to the General Surgery Department, the patient reported pains in the epigastric region with accompanying nausea and vomiting with mucous content as well as the inability to ingest food orally. Despite parenteral and enteral feeding, the patient exhibited a drop in body mass. The histopathologic examination of a sample taken from the stomach during gastroscopy showed some non-specific necrotic and inflammatory masses with granulation. Intraoperatively, a very small, infiltrated stomach with an initial section of duodenum was identified. A total stomach resection together with the reconstruction of digestive tract continuity was performed using the Roux-Y method. Histopathologic examination of the stomach revealed a deep, chronic and exacerbated inflammatory condition with an extensive ulceration over the entire length of the stomach, reaching up to the pylorus. Additionally, numerous lymphatic glands with inflammatory reaction changes were observed.

Key words: Non-steroidal anti-inflammatory drugs poisoning; Total stomach resection; Roux-Y anastomosis

Peer reviewer: Yasuhiro Kodera, Professor, Department of Surgery Ⅱ, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 4668550, Japan

Snarska J, Jacyna K, Janiszewski J, Shafie D, Iwanowicz K, Żurada A. Exceptionally rare cause of a total stomach resection. *World J Gastroenterol* 2012; 18(20): 2582-2585 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2582.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2582

INTRODUCTION

Currently, total stomach resection is typically performed in adults with stomach cancer[1-9]. Other causes of total stomach resection include chemical burns of the digestive tract caused by the consumption of toxic, most often caustic, substances, which occurs accidentally in children and in adults who have attempted suicide[10]. Most recent studies describe burns in the upper part of the digestive tract occurring after the consumption of concentrated acids, pesticides and bases used as detergents, bleachers or rust-removers[11-13]. Drug poisonings usually cause symptoms in the central nervous system and the circulatory and

respiratory systems as well as bleeding because they most often involve the ingestion of soporific, psychotropic and cardiac medicines. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in treatments globally. In some countries, e.g., in the United States, NSAIDs are used protractedly and in large doses, particularly in elderly patients. The administration of NSAIDs involves the risk of complications occurring in the digestive tract. It has been shown that these medicines may damage mucous membranes in the stomach and induce complications such as ulcerations, hemorrhages, or perforations[14-17]. The aim of the article is to present a female patient who consumed a very large amount of NSAIDs and Tramal at the same time in a suicide attempt.

CASE REPORT

A 54-year-old female was admitted to the General Surgery Department of the University Hospital in Olsztyn from the Internal Medicine Department to undergo surgical treatment of a pylorus narrowing that was induced by the consumption of drugs in a suicide attempt. Approximately five months before that, the woman took 60 NSAID pills (including Ibuprom, Ketonal, Diklofenak, Aspirin) and also ingested 300 mg of Tramal. After that episode, she was treated at the intensive care unit and then at the department of internal diseases. During that time, the patient was fed parenterally and, later, also enterally. Prior to that incident, the woman had not been treated for coexisting diseases such as rheumatoid arthritis or depression. At the time of admission to the surgery department, the patient reported epigastric pain with nausea and vomiting with mucous content as well as the inability to consume food orally. Despite parenteral and enteral feeding, the patient exhibited a 16-kg drop in body mass. Upon admission to the surgery department, her weight was 59 kg. The patient also suffered from pain in the epigastric and umbilical regions. However, the belly was soft with no pathological resistance, and the liver was not enlarged. Biochemical examinations showed minimal anemia (hemoglobin 10.4 g/dL) and increased fibrinogen values (up to 479 mg%) at international normalized ratio 1.10. During gastroscopy, a very small stomach was noticeably covered by scars and numerous fibrin-covered ulcerations. The stomach lacked a mucous membrane and hemorrhaged when touched with the device. The length of the stomach in the upper region was 2-3 cm. The distal part of the stomach was excessively narrowed and inaccessible. The pylorus channel allowed only for the insertion of the Flokar Ch10/130 cm catheter, which was placed properly behind the ligament of Treitz, with resistance. The histopathologic examination of a sample taken from the stomach revealed the existence of necrotic and inflammatory masses with granulation. The patient was qualified to undergo surgery operational treatment. A very small, infiltrated stomach with an initial section of duodenum was identified intraoperatively. Those organs exhibited excessive inflammatory infiltration. There were also numerous enlarged lymphatic glands, which were removed. After preparation and total resection of the stomach (Figure 1) with the initial part of the duodenum, a Roux-Y anastomosis was performed using a 25-mm circular stapler. The esophageal resection line was 10 mm and free from pathological changes; the esophagus was observed to have intact epithelium and stroma with dispersed inflammatory infiltration. The initial duodenum resection line was 12-mm long with a preserved margin of mucous membrane and chronic exacerbated inflammation in the mucous and submucosal membranes. Histopathologic examination of the stomach revealed a deep, chronic and exacerbated inflammatory condition with an extensive ulceration over the entire length, reaching up to the pylorus. Microscopic examination revealed numerous lymphatic glands with inflammatory changes (Figures 2 and 3). The post-operative period was complicated on the fifth day due to bile leakage from the part of the duodenum closed with a linear stapler. A relaparotomy revealed numerous adhesions, as well as an orifice, 2 mm in diameter, on the top of the closed, bloated duodenum

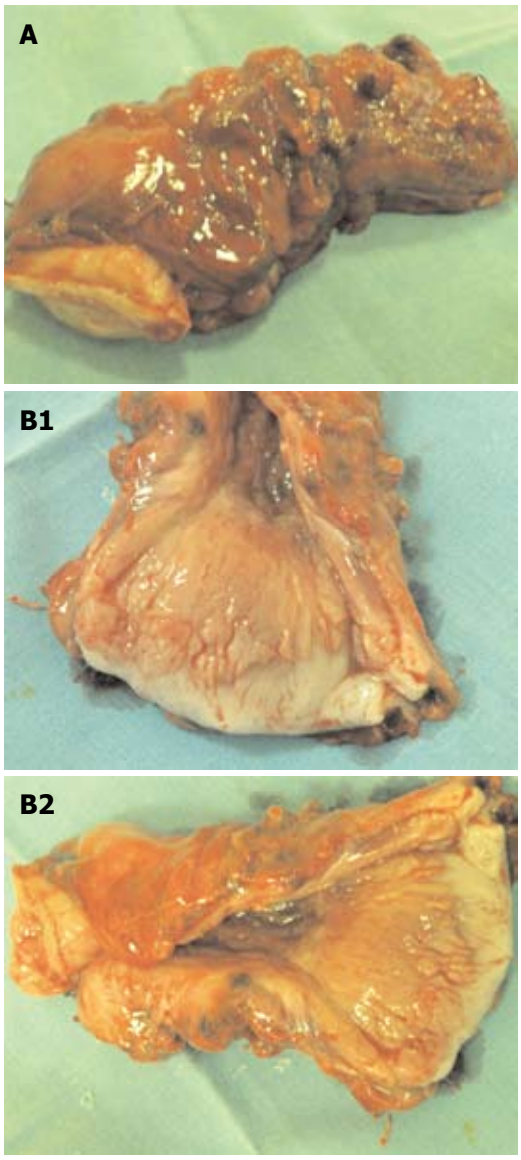


Figure 1 Pathological changes in atrophic gastritis after treatment. A: Tubular resected stomach as a whole, 9 cm long; B1 and B2: Cross-section of stomach with its 2 cm thick wall and its atrophic mucose membrane.

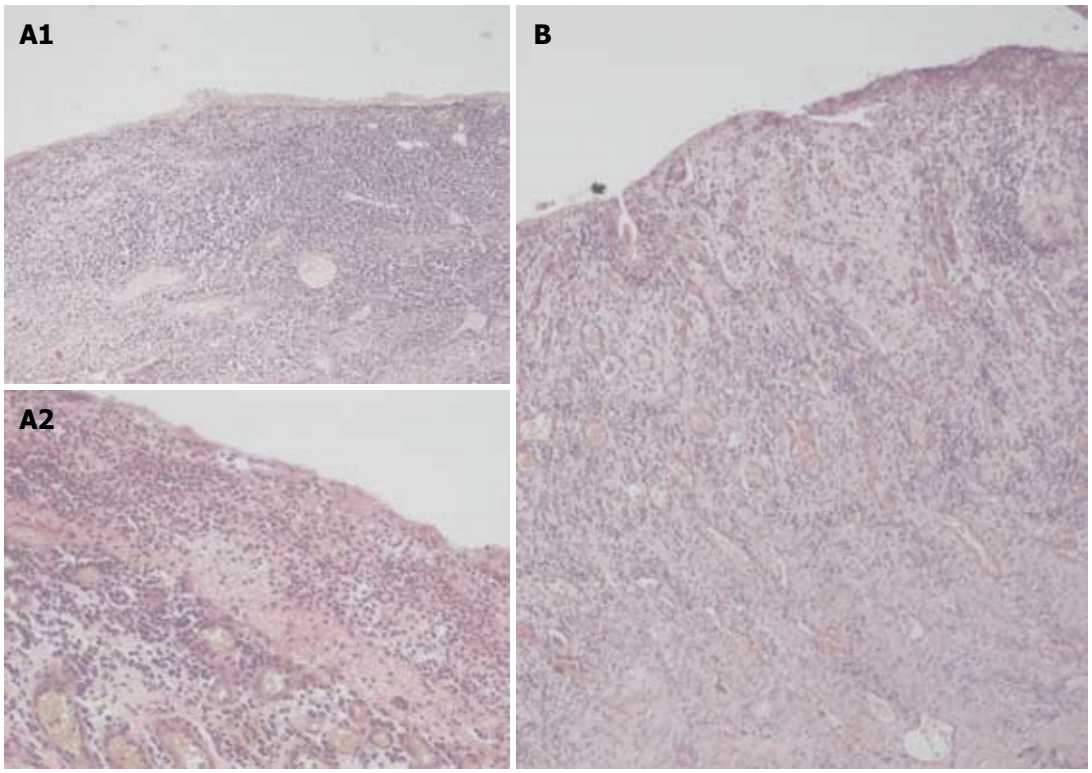


Figure 2 Representative photomicrographs of hematoxylin and eosin stained sections from the stomach's necrosis of submucous membrane. A: The inflammatory infiltration of the submucosal membrane of stomach (A1 magnitude 40× and A2 magnitude 100×); B: The ulceration-granulation in the stomach's submucosal membrane, magnitude 40×.

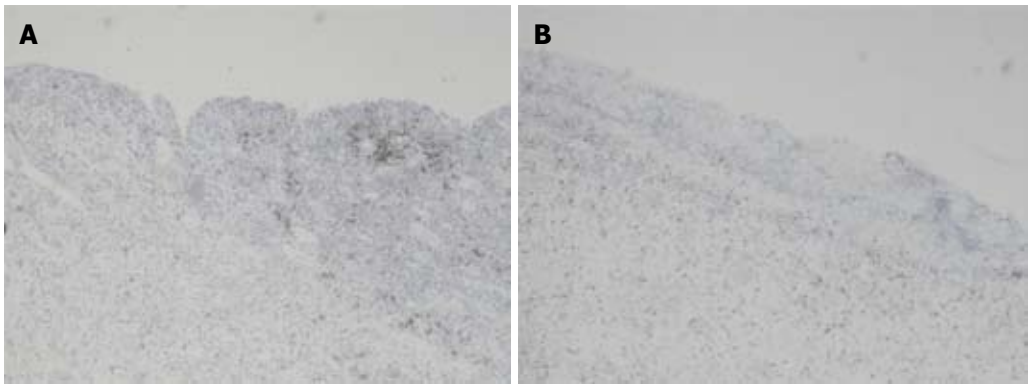


Figure 3 Representative photomicrographs sections from the stomach (magnitude 40×). A: The large, transmural inflammatory cell infiltration of submucosal membrane CD3 (+); B: Mixed inflammatory cell infiltrations CD3 (+).

stump, approximately 10 mm from the stitch line, out of which bile flowed heavily. Through the orifice, a drain of the same diameter was introduced into the duodenum as a duodenostomy. Four days after the surgery, the patient was treated with a respirator. On the following days, apart from some non-edemic and inflammatory changes in the lower segments of the lungs, the course of treatment was satisfactory. The patient was fed parenterally and enterally through a Flocare-type tube. When performing control examinations, the drain was removed from the duodenum stump (14th day). On the 16th day, the patient was administered an oral diet that was extended gradually. On the 21st day, she was discharged from the hospital, in good general condition, with body mass of 61.5 kg.

DISCUSSION

Benzodiazepines and other psychotropic drugs are the most common causes of suicide-related drug poisoning. Few studies have examined suicide attempts that involve NSAID overdoses. The long-term administration of NSAIDs, even in therapeutic doses, results in digestive tract complications in the form of inflammation, ulceration, mucous membrane bleeding and hemorrhage as well as perforations to the stomach and duodenum[14-17]. The consumption of NSAIDs in larger amounts causes damage to the stomach's mucous membrane, even resulting in total necrosis, as observed in the current case. During the six-month-long feeding treatment of the

patient and her endoscopic observation at the Department of Internal Medicine, preparations were made for surgery. The patient was in poor psychological condition and did not agree to undergo surgery. Within the borders of the stomach and duodenum, inflammatory infiltrate almost completely closed the pylorus with numerous adhesions to the surrounding tissues. The mucous membrane had slipped down completely, and its place was taken by necrotic and inflammatory tissues. It has already been proven that high doses of acetylsalicylic acid increase the risk of hemorrhage during the course of peptic ulcer disease[18]. In the current case, there was no information related to the adverse effects of NSAIDs administered to patients with osteoarthritis. When patients overdose for suicidal reasons, the duration of contact between the mucous membrane and the caustic substance is long, as in the case of our patient. The exposure time, type of drug (e.g., hydrophilic properties), concentration in the stomach, contents of the stomach before drug consumption, degree of pylorus contraction and operations performed on the digestive tract that impair its motor activity are of crucial importance with respect to the level of damage and the extent of changes to the stomach. The morphological changes to the stomach upon consumption of large amounts of NSAIDs may be accompanied by damage to the liver, kidneys and heart[17,19]. Nelson *et al*[20] described a case of liver failure requiring transplantation and simultaneous intestinal necrosis upon consumption of large amounts of acetaminophen and ibuprofen by their schizophrenic patient. The toxic activity of those drugs damages the mucous membrane and stops the production of prostaglandins in the intestines. A massive hemorrhage from a duodenum or stomach ulcer may often be observed among patients who have consumed NSAIDs even in therapeutic doses[21,22]. Morphological changes in the digestive tract are usually described in the literature as liquefactive or coagulative necrosis but refer to poisonings with concentrated bases or acids. Regardless of the type of poison consumed orally, chronic ulcerations of the stomach's mucous membrane, excessive inflammation, clots in the micro-circulation, bacterial colonization and increased fibroblastic activity may develop. The toxic activity of any drug depends not only on the consumed dose but also on other circumstances. Having a meal, drinking alcohol, and the ingestion of other medicines influences the patient's clinical condition. Most often, oral poisonings cause changes to the upper part of the digestive tract and lead to damage to the liver and kidneys. The current literature lacks reports on total resection of the stomach due to poisoning with NSAIDs and Tramal.

REFERENCES

1 **Shiraishi N**, Yasuda K, Kitano S. Laparoscopic gastrectomy with lymph node dissection for gastric cancer. *Gastric Cancer* 2006; **9**: 167-176

2 **Kunisaki C**, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono H, Nagahori Y, Hosoi H, Takahashi M, Kito F, Shimada H. Comparison of surgical results of D2 versus D3 gastrectomy (para-aortic lymph node dissection) for advanced gastric carcinoma: a multi-institutional study. *Ann Surg Oncol* 2006; **13**: 659-667

3 **Samarasam I**, Chandran BS, Sitaram V, Perakath B, Nair A, Mathew G. Palliative gastrectomy in advanced gastric cancer: is it worthwhile? *ANZ J Surg* 2006; **76**: 60-63

4 **Kunisaki C**, Ishino J, Nakajima S, Motohashi H, Akiyama H, Nomura M, Matsuda G, Otsuka Y, Ono HA, Shimada H. Outcomes of mass screening for gastric carcinoma. *Ann Surg Oncol* 2006; **13**: 221-228

5 **Dittrich S**, Theuring F. [Carcinoma in the gastric stump--a study of autopsy material]. *Zentralbl Allg Pathol* 1985; **130**: 211-216

6 **Speicher JE**, Thirlby RC, Burggraaf J, Kelly C, Levasseur S. Results of completion gastrectomies in 44 patients with post-surgical gastric atony. *J Gastrointest Surg* 2009; **13**: 874-880

7 **Munson JL**, O'Mahony R. Radical gastrectomy for cancer of the stomach. *Surg Clin North Am* 2005; **85**: 1021-1032, vii

8 **Sierzega M**, Kolodziejczyk P, Kulig J. Impact of anastomotic leakage on long-term survival after total gastrectomy for carcinoma of the stomach. *Br J Surg* 2010; **97**: 1035-1042

9 **Kulig J**, Sierzega M, Kolodziejczyk P, Dadan J, Drews M, Fraczek M, Jeziorski A, Krawczyk M, Starzynska T, Wallner G. Implications of overweight in gastric cancer: A multicenter study in a Western patient population. *Eur J Surg Oncol* 2010; **36**: 969-976

10 **Stiff G**, Alwafi A, Rees BI, Lari J. Corrosive injuries of the oesophagus and stomach: experience in management at a regional paediatric centre. *Ann R Coll Surg Engl* 1996; **78**: 119-123

11 **Herrington JL**. Stenosis of the gastric antrum and proximal duodenum resulting from the ingestion of a corrosive agent. *Am J Surg* 1964; **107**: 580-585

12 **Gago O**, Ritter FN, Martel W, Orvald TO, Delavan JW, Dieterle RV, Kirsh MM, Kahn DR, Sloan H. Aggressive surgical treatment for caustic injury of the esophagus and stomach. *Ann Thorac Surg* 1972; **13**: 243-250

13 **Díaz-Sánchez A**, Carrión G, Barreiro A, Ortiz C, De Fuenmayor ML, Gimeno M, Ponferrada A, Martín S, Aldeguer M. Massive gastric necrosis from hydrochloric acid ingestion. *Rev Esp Enferm Dig* 2009; **101**: 568-570

14 **Fennerty MB**. NSAID-related gastrointestinal injury. Evidence-based approach to a preventable complication. *Postgrad Med* 2001; **110**: 87-88, 91-94

15 **Wolfe MM**, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999; **340**: 1888-1899

16 **Singh G**, Rosen Ramey D. NSAID induced gastrointestinal complications: the ARAMIS perspective--1997. Arthritis, Rheumatism, and Aging Medical Information System. *J Rheumatol Suppl* 1998; **51**: 8-16

17 **Marrone GC**, Silen W. Pathogenesis, diagnosis and treatment of acute gastric mucosal lesions. *Clin Gastroenterol* 1984; **13**: 635-650

18 **Kelly JP**, Kaufman DW, Jurgelon JM, Sheehan J, Koff RS, Shapiro S. Risk of aspirin-associated major upper-gastrointestinal bleeding with enteric-coated or buffered product. *Lancet* 1996; **348**: 1413-1416

19 **Mann NS**, Mann SK. Gastric mucosal barrier, drug-induced acute erosive gastritis and stress ulcer. *South Med J* 1977; **70**: 1179-1182

20 **Nelson H**, Katz D, Dunn T, Singh G, Voigt M, Whitaker E, Thomsen D. Rhabdomyolysis and necrotic bowel after acetaminophen and ibuprofen overdose. *Pharmacotherapy* 2007; **27**: 608-612

21 **Evans C**, Chalmers-Watson TA, Gearry RB. Medical image. Combination NSAID-codeine preparations and gastrointestinal toxicity. *N Z Med J* 2010; **123**: 92-93

22 **Merhav H**, Rothstein H, Simon D, Pfeffermann R. Duodenostomy revisited. *Int Surg* 1988; **73**: 254-256

S- Editor Gou SX **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i20.2586

World J Gastroenterol 2012 May 28; 18(20): 2586-2590
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Lamivudine treatment enabling right hepatectomy for hepatocellular carcinoma in decompensated cirrhosis

Koichi Honda, Masataka Seike, Shin-ichiro Maehara, Koichiro Tahara, Hideaki Anai, Akira Moriuchi, Toyokichi Muro

Koichi Honda, Masataka Seike, First Department of Internal Medicine, Oita University, Oita 879-5593, Japan
Shin-ichiro Maehara, Koichiro Tahara, Hideaki Anai, Department of Surgery, National Hospital Organization Oita Medical Center, Oita 870-0263, Japan
Akira Moriuchi, Department of Pathology, National Hospital Organization Oita Medical Center, Oita 870-0263, Japan
Toyokichi Muro, Department of Gastroenterology and Hepatology, National Hospital Organization Oita Medical Center, Oita 870-0263, Japan
Author contributions: Honda K wrote the manuscript; Honda K, Maehara S, Tahara K, Anai H and Muro T performed the clinical work; Moriuchi A discussed the pathology results; and Seike M reviewed the paper.
Correspondence to: Koichi Honda, MD, PhD, First Department of Internal Medicine, Oita University, 1-1 Hasama-machi, Yufu City, Oita 879-5593, Japan. hondak@oita-u.ac.jp
Telephone: +81-97-5865793 Fax: +81-97-5494480
Received: November 21, 2011 Revised: February 15, 2012
Accepted: March 9, 2012
Published online: May 28, 2012

Abstract

A 69-year-old man was admitted to our hospital in October 2003, for further examination of two liver tumors. He was diagnosed with hepatocellular carcinoma (HCC) arising from decompensated hepatitis B virus (HBV)-related cirrhosis. Long-term lamivudine administration improved liver function dramatically despite repeated treatment for HCC. His Child-Pugh score was 9 points at start of lamivudine treatment, improving to 5 points after 1 year. His indocyanine green at 15 min after injection test score was 48% before lamivudine treatment, improving to 22% after 2 years and to 5% after 4 years. Radiofrequency ablation controlled the HCC foci and maintained his liver function. In April 2009, abdominal computed tomography revealed a tumor thrombus in the right portal vein. Since his indocyanine green test results had improved to less than 10%, we performed a right hepatectomy, which was successful. To our knowledge, there have been no documented reports of patients undergoing successful right hepatectomy for HCC arising from decompensated cirrhosis. The findings observed in our patient indicate the importance of nucleoside analogs for treating HBV-related HCC.

Key words: Hepatitis B virus; Lamivudine; Hepatocellular carcinoma; Decompensated cirrhosis; Hepatectomy

Peer reviewer: Dimitrios E Giakoustidis, Professor, Department of Transplantation, Aristotle University of Thessaloniki, 49 Konstantinoupoleos Street, 54642 Thessaloniki, Greece

Honda K, Seike M, Maehara S, Tahara K, Anai H, Moriuchi A, Muro T. Lamivudine treatment enabling right hepatectomy for hepatocellular carcinoma in decompensated cirrhosis. *World J Gastroenterol* 2012; 18(20): 2586-2590 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i20/2586.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i20.2586

INTRODUCTION

The hepatitis B virus (HBV) infects more than 400 million people worldwide[1] and is an important risk factor for the development of hepatocellular carcinoma (HCC). In Japan, about 1% of individuals in the general population are HBV carriers, accounting for about 14% of patients with liver cirrhosis[2] and 15%-20% of those with HCC[3,4]. The prognosis of patients with HCC arising from chronic liver disease is dependent not only on tumor factors but on hepatic functional reserve. Depending on patient age, liver transplantation may be a good therapeutic option in patients with poor functional reserve. Lamivudine treatment is beneficial in patients with HBV-related HCC because it contributes to improvement of remnant liver function. We describe here a patient with decompensated HBV-related cirrhosis who developed HCC. Lamivudine therapy improved liver function and enabled a right hepatectomy 5 years later.

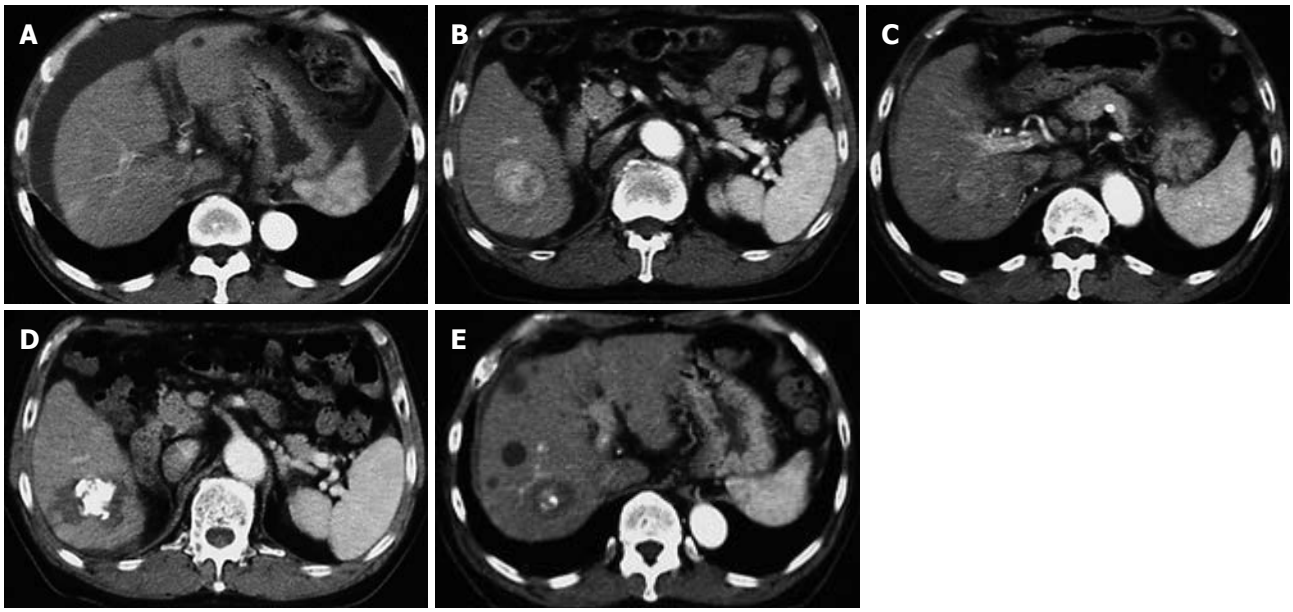


Figure 1 Computed tomography in our patient. It shows a cirrhotic pattern of the liver and massive ascites at first admission (A); Dynamic computed tomography revealed two hepatocellular carcinomas, 4.5 cm (B) and 2.5 cm (C) in diameter, in the right lobe; These two lesions were treated by transcatheter arterial chemoembolization and radiofrequency ablation (D, E).

CASE REPORT

A 69-year old man was admitted to our hospital in October 2003 for examination of two liver tumors. He had been diagnosed with hepatitis B in 1994 and treated with glycyrrhizin. His liver function deteriorated gradually, with ascites appearing in May 2001. He was first admitted to our hospital for treatment of intractable ascites (Figure 1A). Laboratory tests showed that his serum albumin (alb) concentration was 2.7 g/dL, his total bilirubin (T-Bil) was 2.8 mg/dL, his aspartate aminotransferase (AST) was 54 IU/L, his alanine aminotransferase (ALT) was 43 IU/L, and his prothrombin time (PT) was 42%. Administration of diuretic drugs was not effective, but treatment with a preparation of albumin resulted in the disappearance of ascites 1 mo later. Afterward, the ascites was kept under control by administration of diuretics. In October 2003, a computed tomographic (CT) scan of the abdomen revealed two HCCs (4.5 and 2.5 cm in diameter) in the right hepatic lobe (Figure 1B, C). Laboratory tests showed alb 2.5 g/dL, T-Bil 2.4 mg/dL, AST 152 IU/L, ALT 98 IU/L, PT 47%, indocyanine green at 15 min after injection (ICGR15) 48%, alpha-fetoprotein 444 ng/mL, and protein induced by vitamine K absence or antagonist Ⅱ <10 mAU/mL. He was positive for HBe antigen, negative for HBe antibody, and had an HBV-DNA viral load of 6.7 log copies/mL. Beginning in November 2003, he was treated with 100 mg/d lamivudine. The two HCCs were treated by transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA) (Figure 1D, E). Both tumors were treated successfully and the patient's liver function recovered gradually after initiation of lamivudine treatment. In September 2005, an abdominal CT scan revealed a recurrent HCC, located near one of the previously treated tumors; this lesion was treated successfully with TACE and RFA. At this time, laboratory tests showed alb 3.7 g/dL, T-Bil 0.7 mg/dL, AST 23 IU/L, ALT 22 IU/L, PT 84% and ICGR15 22%. All HCC treatments were based on clinical practice guidelines in Japan[5], with the patient providing informed consent.

In May 2006, two HCC recurrences were detected in the right liver lobe and treated with TACE and RFA. Laboratory tests showed good liver function, alb 3.9 g/dL, T-Bil 1.1 mg/dL, AST 22 IU/L, ALT 11 IU/L, PT 95% and ICGR15 25%. In June 2007, a recurrent HCC was treated with TACE and RFA. Liver function was also excellent at this time (alb 4.0 g/dL, T-Bil 0.7 mg/dL and PT 100%). In September 2007, his viral load had again increased, with breakthrough hepatitis, and the YMDD mutation was detected. Treatment with adefovir dipivoxil plus lamivudine resulted in a gradual reduction in viral load. In December 2007, abdominal CT revealed five HCCs in the right lobe; these were treated by TACE and RFA. The patient was then treated with low-dose cisplatin and 5-fluorouracil infused through the hepatic artery. Laboratory tests showed alb 4.1 g/dL, T-Bil 1.0 mg/dL, AST 38 IU/L, ALT 30 IU/L, PT 88% and ICGR15 5%. Due to the development of a pseudoaneurysm in his hepatic artery, infusion of chemotherapy was discontinued. In March 2009, two HCCs were detected in the right lobe and were treated by RFA. Laboratory tests showed alb 3.7 g/dL, T-Bil 0.7 mg/dL, AST 35 IU/L, ALT 31 IU/L, PT 77% and ICGR15 10%. In April 2009, abdominal CT and CT angiography revealed a tumor thrombus in the right portal vein, but no lesion could be detected in the left lobe (Figure 2). Although he was diagnosed with decompensated cirrhosis, of Child-Pugh C, when first hospitalized, lamivudine treatment improved his liver function sufficiently, with an improve-

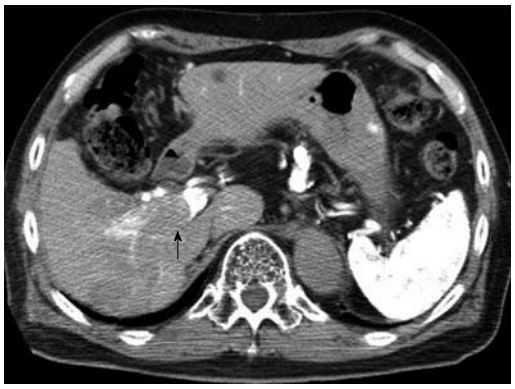


Figure 2 Computed tomography during hepatic arteriography, showing a portal tumor thrombus (arrow) in the right portal vein. Hepatocellular carcinoma was not detected in the left lobe.

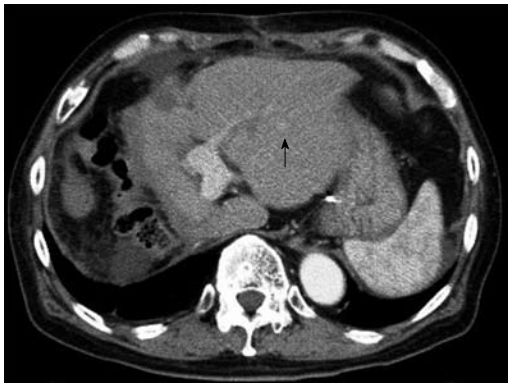


Figure 4 Abdominal computed tomography showing the recurrence of hepatocellular carcinoma (arrow) in the left lobe two months after right hepatectomy.

ment of ICGR15 results to < 10%, to allow the successful performance of a right hepatectomy in April 2009 (Figure 3A). After liver resection, his AST rose to 1220 IU/L, his T-Bil to 1.9 mg/dL, and his PT decreased to 54%, followed by gradual recovery of liver function. He recovered well and left the hospital 1 mo after surgery.

Histologic examination of the extracted specimen showed a moderately differentiated HCC with portal tumor thrombus (Figure 3B) and multiple intrahepatic metastases. Fibrosis of varying extent was observed in the cancer-free area, with some areas showing severe fibrosis with pseudolobules and others showing mild fibrosis (Figure 3C). Abdominal CT in June 2009 suggested the recurrence of HCC in the left lobe (Figure 4), and abdominal angiography revealed multiple HCCs. These tumors were treated by TACE, but this was not effective. The left lobe tumors subsequently enlarged and the patient's liver function deteriorated gradually. The patient died in December 2009 (Clinical course Figure 5).

DISCUSSION

Chronic hepatitis B is a progressive liver disease, leading to cirrhosis and HCC[6]. Before antiviral agents became

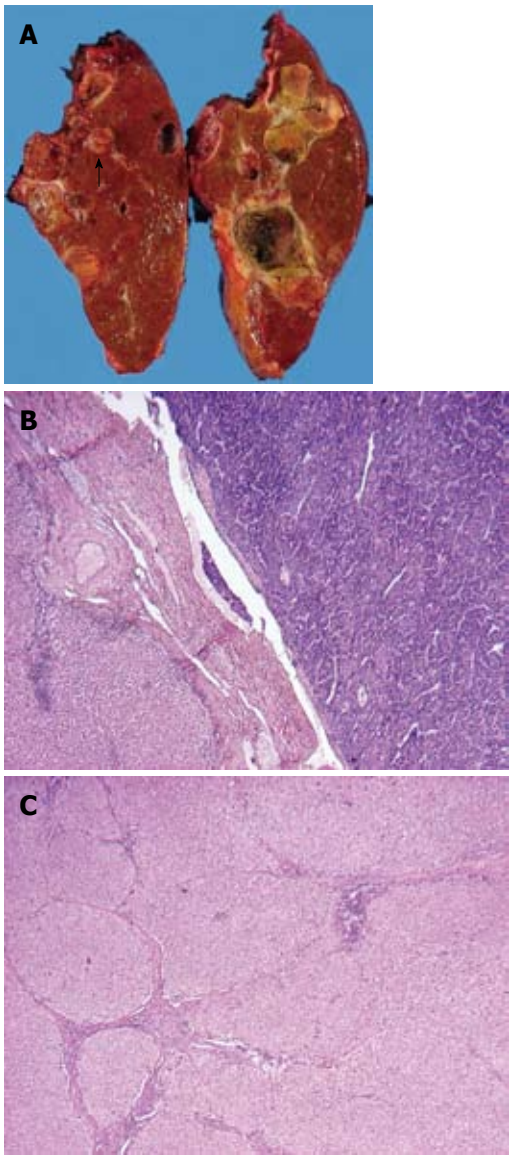


Figure 3 Macroscopic and microscopic appearance of the resected liver. A: A tumor thrombus was detected in the right portal vein (arrow); B: A moderately differentiated hepatocellular carcinoma with a trabecular pattern was seen in the right portal vein [hematoxylin and eosin (HE) stain, ×40]; C: Fibrosis of varying extent was observed in the cancer-free area. Some pseudolobules with severe fibrosis were present in the left part of the photograph (HE stain, ×40).

established as effective treatments for hepatitis B, the prognosis of patients with end-stage HBV infection was generally poor. The 5-year survival rates of patients with compensated and decompensated cirrhosis have been reported to be 55%-84%[7,8] and 14%[9], respectively. Lamivudine, an antiviral drug, is an oral nucleoside analog that inhibits DNA synthesis by terminating the nascent proviral DNA chain. It rapidly reduces both serum HBV-DNA and transaminase concentrations[10]. Prolonged viral suppression can result in histological improvement, including the regression of fibrosis[10-13]. Although a subgroup of individuals with extremely advanced disease require urgent transplantation, lamivudine treatment can achieve significant improvement in liver function and reduce the morbidity of many of patients

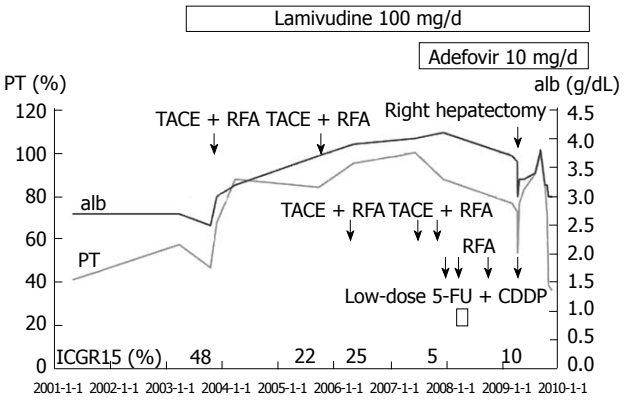


Figure 5 Clinical course of the patient. After lamivudine administration, his liver function gradually improved despite repeated treatments for hepatocellular carcinoma (HCC), and his indocyanine green at 15 min after injection (ICGR15) test score was 5% 4 years after initiation of lamivudine treatment. He underwent a successful right hepatectomy for HCC 5 years after beginning lamivudine treatment. alb: Albumin; PT: Prothrombin time; TACE: Transcatheter arterial chemoembolization; RFA: Radiofrequency ablation; 5-FU: 5-Fluorouracil; CDDP: Cisplatin.

with decompensated cirrhosis[14-19]. At present entecavir is recommended as the primary oral agent for hepatitis B because of its strong antiviral effects and low resistance rate, as well as being effective in treating decompensated cirrhosis[20]. Long-term lamivudine monotherapy can induce the emergence of resistant viruses with an amino acid substitution in the YMDD motif of the viral DNA polymerase[21]. Particularly in patients with decompensated cirrhosis, breakthrough hepatitis resulting from such a mutation may lead to hepatic failure[22] if other antiviral drugs such as adefovir dipivoxil[23,24] are not administered. Long-term treatment with lamivudine has been reported to reduce the incidence of HCC[25,26]. In addition, lamivudine has been found to improve liver function[27,28] and survival[29] in patients with HBV-related HCC after initial treatment of HCC. We have described a patient with HCC arising from decompensated HBV-related cirrhosis. Long-term lamivudine treatment improved his remnant liver function dramatically, despite repeated TACE and RFA sessions for HCC. Although his Child-Pugh score at the start of lamivudine treatment was 9 points, it improved up to 5 points 1 year later. Moreover, he scored 48% on an ICGR15 test performed before his first treatment for HCC, but this score improved to 22% after 2 years and to 5% after 4 years, with the latter considered safe for the performance of a right hepatectomy[30].

Despite repeated RFA, the liver function of this patient was well maintained. Generally, RFA has been regarded as safe and effective for HCC, and has been found to maintain liver function[31-33]. In our patient, lamivudine and RFA were effective in maintaining liver function. A previous case report described a patient with decompensated HBV-related cirrhosis, who, following lamivudine treatment, underwent a hepatectomy for HCC after liver function had improved[34]. That patient, however, underwent a partial hepatectomy for a small HCC. To our knowledge, no prior report has described a successful right hepatectomy for HCC arising from decompensated HBV-associated liver cirrhosis. The findings reported in this patient indicate the importance of nucleoside analogs for treating HBV-related HCC.

In conclusion, we found that lamivudine treatment was beneficial for our patient with decompensated HBV-related cirrhosis and HCC, increasing the likelihood of treatment for HCC.

REFERENCES

1 **Lai CL**, Ratziu V, Yuen MF, Poynard T. Viral hepatitis B. *Lancet* 2003; **362**: 2089-2094
2 **Michitaka K**, Nishiguchi S, Aoyagi Y, Hiasa Y, Tokumoto Y, Onji M. Etiology of liver cirrhosis in Japan: a nationwide survey. *J Gastroenterol* 2010; **45**: 86-94
3 **Edamoto Y**, Tani M, Kurata T, Abe K. Hepatitis C and B virus infections in hepatocellular carcinoma. Analysis of direct detection of viral genome in paraffin embedded tissues. *Cancer* 1996; **77**: 1787-1791
4 **Taura N**, Fukushima N, Yastuhashi H, Takami Y, Seike M, Watanabe H, Mizuta T, Sasaki Y, Nagata K, Tabara A, Komorizono Y, Taketomi A, Matsumoto S, Tamai T, Muro T, Nakao K, Fukuizumi K, Maeshiro T, Inoue O, Sata M. The incidence of hepatocellular carcinoma associated with hepatitis C infection decreased in Kyushu area. *Med Sci Monit* 2011; **17**: PH7-P11
5 **Makuuchi M**, Kokudo N. Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. *World J Gastroenterol* 2006; **12**: 828-829
6 **Park BK**, Park YN, Ahn SH, Lee KS, Chon CY, Moon YM, Park C, Han KH. Long-term outcome of chronic hepatitis B based on histological grade and stage. *J Gastroenterol Hepatol* 2007; **22**: 383-388
7 **Weissberg JI**, Andres LL, Smith CI, Weick S, Nichols JE, Garcia G, Robinson WS, Merigan TC, Gregory PB. Survival in chronic hepatitis B. An analysis of 379 patients. *Ann Intern Med* 1984; **101**: 613-616
8 **Realdi G**, Fattovich G, Hadziyannis S, Schalm SW, Almasio P, Sanchez-Tapias J, Christensen E, Giustina G, Noventa F. Survival and prognostic factors in 366 patients with compensated cirrhosis type B: a multicenter study. The Investigators of the European Concerted Action on Viral Hepatitis (EUROHEP). *J Hepatol* 1994; **21**: 656-666
9 **de Jongh FE**, Janssen HL, de Man RA, Hop WC, Schalm SW, van Blankenstein M. Survival and prognostic indicators in hepatitis B surface antigen-positive cirrhosis of the liver. *Gastroenterology* 1992; **103**: 1630-1635
10 **Lai CL**, Chien RN, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Wu PC, Dent JC, Barber J, Stephenson SL, Gray DF. A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; **339**: 61-68
11 **Honkoop P**, de Man RA, Zondervan PE, Schalm SW. Histological improvement in patients with chronic hepatitis B virus infection treated with lamivudine. *Liver* 1997; **17**: 103-106
12 **Suzuki Y**, Kumada H, Ikeda K, Chayama K, Arase Y, Saitoh S, Tsubota A, Kobayashi M, Koike M, Ogawa N, Tanikawa K. Histological changes in liver biopsies after one year of lamivudine treatment in patients with chronic hepatitis B infection. *J Hepatol* 1999; **30**: 743-748
13 **Dienstag JL**, Goldin RD, Heathcote EJ, Hann HW, Woessner M, Stephenson SL, Gardner S, Gray DF, Schiff ER. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; **124**: 105-117
14 **Kapoor D**, Guptan RC, Wakil SM, Kazim SN, Kaul R, Agarwal SR, Raisuddin S, Hasnain SE, Sarin SK. Beneficial effects

of lamivudine in hepatitis B virus-related decompensated cirrhosis. *J Hepatol* 2000; **33**: 308-312
15 **Villeneuve JP**, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, Leduc R, Peltekian K, Wong F, Margulies M, Heathcote EJ. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. *Hepatology* 2000; **31**: 207-210
16 **Yao FY**, Bass NM. Lamivudine treatment in patients with severely decompensated cirrhosis due to replicating hepatitis B infection. *J Hepatol* 2000; **33**: 301-307
17 **Fontana RJ**, Hann HW, Perrillo RP, Vierling JM, Wright T, Rakela J, Anschuetz G, Davis R, Gardner SD, Brown NA. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. *Gastroenterology* 2002; **123**: 719-727
18 **Hann HW**, Fontana RJ, Wright T, Everson G, Baker A, Schiff ER, Riely C, Anschuetz G, Gardner SD, Brown N, Griffiths D. A United States compassionate use study of lamivudine treatment in nontransplantation candidates with decompensated hepatitis B virus-related cirrhosis. *Liver Transpl* 2003; **9**: 49-56
19 **Tseng PL**, Lu SN, Tung HD, Wang JH, Changchien CS, Lee CM. Determinants of early mortality and benefits of lamivudine therapy in patients with hepatitis B virus-related decompensated liver cirrhosis. *J Viral Hepat* 2005; **12**: 386-392
20 **Shim JH**, Lee HC, Kim KM, Lim YS, Chung YH, Lee YS, Suh DJ. Efficacy of entecavir in treatment-naïve patients with hepatitis B virus-related decompensated cirrhosis. *J Hepatol* 2010; **52**: 176-182
21 **Liaw YF**, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. *Hepatology* 1999; **30**: 567-572
22 **Nishida T**, Kobashi H, Fujioka S, Fujio K, Takaguchi K, Ikeda H, Kawaguchi M, Ando M, Araki Y, Higashi T, Shoji B, Takaki A, Iwasaki Y, Sakaguchi K, Shiratori Y, Yamamoto K. A prospective and comparative cohort study on efficacy and drug resistance during long-term lamivudine treatment for various stages of chronic hepatitis B and cirrhosis. *J Gastroenterol Hepatol* 2008; **23**: 794-803
23 **Perrillo R**, Hann HW, Mutimer D, Willems B, Leung N, Lee WM, Moorat A, Gardner S, Woessner M, Bourne E, Brosgart CL, Schiff E. Adefovir dipivoxil added to ongoing lamivudine in chronic hepatitis B with YMDD mutant hepatitis B virus. *Gastroenterology* 2004; **126**: 81-90
24 **Peters MG**, Hann Hw H, Martin P, Heathcote EJ, Buggisch P, Rubin R, Bourliere M, Kowdley K, Trepo C, Gray Df D, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosgart CL. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. *Gastroenterology* 2004; **126**: 91-101
25 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531
26 **Matsumoto A**, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, Okita K, Hayashi N, Okanoue T, Iino S, Tanikawa K. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; **32**: 173-184
27 **Kuzuya T**, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, Itoh A, Ishigami M, Hayashi K, Honda T, Goto H. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 1929-1935
28 **Kim JH**, Park JW, Koh DW, Lee WJ, Kim CM. Efficacy of lamivudine on hepatitis B viral status and liver function in patients with hepatitis B virus-related hepatocellular carcinoma. *Liver Int* 2009; **29**: 203-207
29 **Kubo S**, Tanaka H, Takemura S, Yamamoto S, Hai S, Ichikawa T, Kodai S, Shinkawa H, Sakaguchi H, Tamori A, Habu D, Nishiguchi S. Effects of lamivudine on outcome after liver resection for hepatocellular carcinoma in patients with active replication of hepatitis B virus. *Hepatol Res* 2007; **37**: 94-100
30 **Imamura H**, Sano K, Sugawara Y, Kokudo N, Makuuchi M. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. *J Hepatobiliary Pancreat Surg* 2005; **12**: 16-22
31 **Livraghi T**, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. *Radiology* 2003; **226**: 441-451
32 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137
33 **N'Kontchou G**, Mahamoudi A, Aout M, Ganne-Carrié N, Grando V, Coderc E, Vicaut E, Trinchet JC, Sellier N, Beaugrand M, Seror O. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475-1483
34 **Nakanishi S**, Michitaka K, Miyake T, Hidaka S, Yoshino I, Konishi I, Iuchi H, Horiike N, Onji M. Decompensated hepatitis B virus-related cirrhosis successfully treated with lamivudine allowing surgery for hepatocellular carcinoma. *Intern Med* 2003; **42**: 416-420

S- Editor Gou SX **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2012 May 28; 18(20): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Acknowledgments to reviewers of *World Journal of Gastroenterology*

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

Masahiro Arai, MD, PhD, Department of Gastroenterology, Toshiba General Hospital, 6-3-22 Higashi-ooi, Shinagawa-ku, Tokyo 140-8522, Japan

Mauro Bernardi, Professor, Internal Medicine, Cardioangiology, Hepatology, University of Bologna, Semeiotica Medical, Policlinico S. Orsola, Malpighi, Via Massarenti, 9, 40138 Bologna, Italy

A Mithat Bozdayi, MD, PhD, Hepatology Institute, Department of Gastroenterology, Ankara Medical Faculty, Ankara University, 06100 Cebeci Ankara, Turkey

Filip Braet, Associate Professor, Australian Key Centre for Microscopy and Microanalysis, Madsen Building (F09), The University of Sydney, Sydney NSW 2006, Australia

Dr. Justin MM Cates, MD, PhD, Department of Pathology, Vanderbilt University Medical Center, Medical Center North, C-3322, 1161 21st Avenue South, Nashville, TN 37232, United States

Wan-Long Chuang, MD, PhD, MS, Professor, Director, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University, No. 100 Shih-Chuan 1st Road, Kaohsiung 807, Taiwan, China

Peter L Lakatos, MD, PhD, Assistant Professor, 1st Department of Medicine, Semmelweis University, Koranyi S 2A, H1083 Budapest, Hungary

Teng-Yu Lee, MD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Taichung Veterans General Hospital, 160, Sec. 3, Taichung Harbor Road, Taichung 407, Taiwan, China

Shin Maeda, Professor and Chairman, Department of Gastroenterology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan

Nobuyuki Matsuhashi, MD, Department of Gastroenterology, Kanto Medical Center, NTT East, 5-9-22 Higashi-gotanda, Shinagawa-ku, Tokyo 141-8625, Japan

Giovanni D De Palma, Professor, Department of Surgery and Advanced Technologies, University of Naples Federico Ⅱ, School of Medicine, 80131 Naples, Italy

Dr. Nagarajan Perumal, Compliance Veterinarian, Center for Life Science, Institutional Animal Care and Use Committee Office, National University of Singapore, Singapore 117456, Singapore

Francisco Rodriguez-Frias, PhD, Proteins Hepatitis Molecular Genetics Unitat, Biochemistry Department, Vall d'Hebron Unicersitary Hospital, 08035 Barcelona, Spain

Dr. Rene Schmidt, MD, Department of Anesthesiology, Freiburg University Medical Center, Hugstetter Strasse 55, 79106 Freiburg, Germany

Yukihiro Shimizu, MD, PhD, Kyoto Katsura Hospital, 17 Yamada-Hirao, Nishikyo, Kyoto 615-8256, Japan

Yoshihisa Takahashi, MD, Department of Pathology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

Pietro Valdastri, MScEE, PhD, Assistant Professor of Industrial BioEngineering, The BioRobotics Institute, Scuola Superiore Sant'Anna, Polo Sant'Anna Valdera, Viale Rinaldo Piaggio, 34, 56025 Pontedera, Italy

Kilian Weigand, MD, Medical Specialist for Internal Medicine, Medizin Ⅳ, Department of Gastroenterology, Infectious Diseases and Intoxications, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany

Frank Ulrich Weiss, PhD, Department of Medicine A, Ernst-Moritz-Arndt University, Friedrich-Loeffler-Str. 23a, 17475 Greifswald, Germany

Dr. Thomas Wex, PhD, Clinic of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany

Satoshi Yamagiwa, MD, PhD, Division of Gastroenterology and Hepatology, Niigata University Graduate School of Medical and Dental Sciences, 757 Asahimachi-dori, Chuo-ku, Niigata 951-8510, Japan

Li Yuyuan, Professor, Department of Gastroenterology, First Municipal People's Hospital of Guangzhou, 1 Panfu Road, Guangzhou 510180, Guangdong Province, China

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2012 May 28; 18(20): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Events Calendar 2012

January 13-15, 2012
Asian Pacific *Helicobacter pylori* Meeting 2012
Kuala Lumpur, Malaysia

January 19-21, 2012
American Society of Clinical Oncology 2012 Gastrointestinal Cancers Symposium
San Francisco, CA 3000, United States

January 19-21, 2012
2012 Gastrointestinal Cancers Symposium
San Francisco, CA 94103, United States

January 20-21, 2012
American Gastroenterological Association Clinical Congress of Gastroenterology and Hepatology
Miami Beach, FL 33141, United States

February 3, 2012
The Future of Obesity Treatment
London, United Kingdom

February 16-17, 2012
4th United Kingdom Swallowing Research Group Conference
London, United Kingdom

February 23, 2012
Management of Barretts Oesophagus: Everything you need to know
Cambridge, United Kingdom

February 24-27, 2012
Canadian Digestive Diseases Week 2012
Montreal, Canada

March 1-3, 2012
International Conference on Nutrition and Growth 2012
Paris, France

March 7-10, 2012
Society of American Gastrointestinal and Endoscopic Surgeons Annual Meeting
San Diego, CA 92121, United States

March 12-14, 2012
World Congress on Gastroenterology and Urology
Omaha, NE 68197, United States

March 17-20, 2012
Mayo Clinic Gastroenterology and Hepatology
Orlando, FL 32808, United States

March 26-27, 2012
26th Annual New Treatments in Chronic Liver Disease
San Diego, CA 92121, United States

March 30-April 2, 2012
Mayo Clinic Gastroenterology and Hepatology
San Antonio, TX 78249, United States

March 31-April 1, 2012
27th Annual New Treatments in Chronic Liver Disease
San Diego, CA 92121, United States

April 8-10, 2012
9th International Symposium on Functional GI Disorders
Milwaukee, WI 53202, United States

April 13-15, 2012
Asian Oncology Summit 2012
Singapore, Singapore

April 15-17, 2012
European Multidisciplinary Colorectal Cancer Congress 2012
Prague, Czech

April 18-20, 2012
The International Liver Congress 2012
Barcelona, Spain

April 19-21, 2012
Internal Medicine 2012
New Orleans, LA 70166, United States

April 20-22, 2012
Diffuse Small Bowel and Liver Diseases
Melbourne, Australia

April 22-24, 2012
EUROSON 2012 EFSUMB Annual Meeting
Madrid, Spain

April 28, 2012
Issues in Pediatric Oncology
Kiev, Ukraine

May 3-5, 2012
9th Congress of The Jordanian Society of Gastroenterology
Amman, Jordan

May 7-10, 2012
Digestive Diseases Week
Chicago, IL 60601, United States

May 17-21, 2012
2012 ASCRS Annual Meeting-American Society of Colon and Rectal Surgeons
Hollywood, FL 1300, United States

May 18-19, 2012
Pancreas Club Meeting
San Diego, CA 92101, United States

May 18-23, 2012
SGNA: Society of Gastroenterology Nurses and Associates Annual Course
Phoenix, AZ 85001, United States

May 19-22, 2012
2012-Digestive Disease Week
San Diego, CA 92121, United States

June 2-6, 2012
American Society of Colon and Rectal Surgeons Annual Meeting
San Antonio, TX 78249, United States

June 18-21, 2012
Pancreatic Cancer: Progress and Challenges
Lake Tahoe, NV 89101, United States

July 25-26, 2012
PancreasFest 2012
Pittsburgh, PA 15260, United States

September 1-4, 2012
OESO 11th World Conference
Como, Italy

September 6-8, 2012
2012 Joint International Neurogastroenterology and Motility Meeting
Bologna, Italy

September 7-9, 2012
The Viral Hepatitis Congress
Frankfurt, Germany

September 8-9, 2012
New Advances in Inflammatory Bowel Disease
La Jolla, CA 92093, United States

September 8-9, 2012
Florida Gastroenterologic Society 2012 Annual Meeting
Boca Raton, FL 33498, United States

September 15-16, 2012
Current Problems of Gastroenterology and Abdominal Surgery
Kiev, Ukraine

September 20-22, 2012
1st World Congress on Controversies in the Management of Viral Hepatitis
Prague, Czech

October 19-24, 2012
American College of Gastroenterology 77th Annual Scientific Meeting and Postgraduate Course
Las Vegas, NV 89085, United States

November 3-4, 2012
Modern Technologies in Diagnosis and Treatment of Gastroenterological Patients
Dnepropetrovsk, Ukraine

November 4-8, 2012
The Liver Meeting
San Francisco, CA 94101, United States

November 9-13, 2012
American Association for the Study of Liver Diseases
Boston, MA 02298, United States

December 1-4, 2012
Advances in Inflammatory Bowel Diseases
Hollywood, FL 33028, United States

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2012 May 28; 18(20): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a weekly, open-access (OA), peer-reviewed journal supported by an editorial board of 1352 experts in gastroenterology and hepatology from 64 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

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

Aims and scope

The major task of *WJG* is to report rapidly the most recent results in basic and clinical research on esophageal, gastrointestinal, liver, pancreas and biliary tract diseases, *Helicobacter pylori*, endoscopy and gastrointestinal surgery, including: gastroesophageal reflux disease, gastrointestinal bleeding, infection and tumors; gastric and duodenal disorders; intestinal inflammation, microflora and immunity; celiac disease, dyspepsia and nutrition; viral hepatitis, portal hypertension, liver fibrosis, liver cirrhosis, liver transplantation, and metabolic liver disease; molecular and cell biology; geriatric and pediatric gastroenterology; diagnosis and screening, imaging and advanced technology.

Columns

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

Name of journal

World Journal of Gastroenterology

ISSN and EISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Editorial office
World Journal of Gastroenterology
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-59080039
Fax: +86-10-85381893

Indexed and abstracted in
Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Thomson Reuters, 2010 Impact Factor: 2.240 (35/71 Gastroenterology and Hepatology).

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

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

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

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJG* 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.

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 Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the 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.wjgnet.com/1007-9327office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjg@wjgnet.com, or by telephone: +86-10-5908-0039. 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

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

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

Abstract

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

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections.

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

Key words

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

Text

For articles of these sections, original articles 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. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm.

Illustrations

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

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

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

PMID and DOI

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

Style for journal references

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

Style for book references

Authors: the name of the first author should be typed in boldfaced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

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

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

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

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunolog-

ic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

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

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.00000 35706.28494.09]

Both personal authors and an organization as author

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

No author given

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

Volume with supplement

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

Issue with no volume

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

No volume or issue

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

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

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

Conference proceedings

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

Conference paper

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

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*

Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*

Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/1007-9327/g_info_20100315220036.htm

Frontier: http://www.wjgnet.com/1007-9327/g_info_20100315220305.htm

Topic highlight: http://www.wjgnet.com/1007-9327/g_info_20100315220601.htm

Observation: http://www.wjgnet.com/1007-9327/g_info_20100312232427.htm

Guidelines for basic research: http://www.wjgnet.com/1007-9327/g_info_20100315220730.htm

Guidelines for clinical practice: http://www.wjgnet.com/1007-9327/g_info_20100315221301.htm

Review: http://www.wjgnet.com/1007-9327/g_info_20100315221554.htm

Original articles: http://www.wjgnet.com/1007-9327/g_info_20100315221814.htm

Brief articles: http://www.wjgnet.com/1007-9327/g_info_20100312231400.htm

Case report: http://www.wjgnet.com/1007-9327/g_info_20100315221946.htm

Letters to the editor: http://www.wjgnet.com/1007-9327/g_info_20100315222254.htm

Book reviews: http://www.wjgnet.com/1007-9327/g_info_20100312231947.htm

Guidelines: http://www.wjgnet.com/1007-9327/g_info_20100312232134.htm

RESUBMISSION OF THE REVISED MANUSCRIPTS

Please revise your article according to the revision policies of *WJG*. The revised version including manuscript and high-resolution image figures (if any) should be re-submitted online (http://www.wjgnet.com/1007-9327office/). The author should send the copyright transfer letter, responses to the reviewers, English language Grade B certificate (for non-native speakers of English) and final manuscript checklist to wjg@wjgnet.com.

Language evaluation

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

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1007-9327/g_info_20100315222818.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/1007-9327/g_info_20100315222607.htm.

Proof of financial support

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

Links to documents related to the manuscript

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

Science news releases

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

Publication fee

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

World Journal of Gastroenterology®

Volume 18 Number 20
May 28, 2012

百世登
Baishideng®

Published by Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No. 90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpg@baishideng.com
http://www.wjgnet.com

ISSN 1007-9327

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