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

World Journal of *Gastroenterology*

World J Gastroenterol 2017 January 7; 23(1): 1-190

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

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

Editorial Board

2014-2017

The *World Journal of Gastroenterology* Editorial Board consists of 1375 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 68 countries, including Algeria (2), Argentina (7), Australia (31), Austria (9), Belgium (11), Brazil (20), Brunei Darussalam (1), Bulgaria (2), Cambodia (1), Canada (25), Chile (4), China (165), Croatia (2), Cuba (1), Czech (6), Denmark (2), Egypt (9), Estonia (2), Finland (6), France (20), Germany (58), Greece (31), Guatemala (1), Hungary (14), Iceland (1), India (33), Indonesia (2), Iran (10), Ireland (9), Israel (18), Italy (194), Japan (149), Jordan (1), Kuwait (1), Lebanon (7), Lithuania (1), Malaysia (1), Mexico (11), Morocco (1), Netherlands (5), New Zealand (4), Nigeria (3), Norway (6), Pakistan (6), Poland (12), Portugal (8), Puerto Rico (1), Qatar (1), Romania (10), Russia (3), Saudi Arabia (2), Singapore (7), Slovenia (2), South Africa (1), South Korea (69), Spain (51), Sri Lanka (1), Sudan (1), Sweden (12), Switzerland (5), Thailand (7), Trinidad and Tobago (1), Tunisia (2), Turkey (55), United Kingdom (49), United States (180), Venezuela (1), and Vietnam (1).

Gordon Stanley Howarth, *Roseworthy*
Seungha Kang, *Brisbane*
Alfred King Lam, *Gold Coast*
Ian C Lawrance, *PerthFremantle*
Barbara Anne Leggett, *Brisbane*
Daniel A Lemberg, *Sydney*
Rupert W Leong, *Sydney*
Finlay A Macrae, *Victoria*
Vance Matthews, *Melbourne*
David L Morris, *Sydney*
Reme Mountifield, *Bedford Park*
Hans J Netter, *Melbourne*
Nam Q Nguyen, *Adelaide*
Liang Qiao, *Westmead*
Rajvinder Singh, *Adelaide*
Ross Cyril Smith, *StLeonards*
Kevin J Spring, *Sydney*
Debbie Trinder, *Fremantle*
Daniel R van Langenberg, *Box Hill*
David Ian Watson, *Adelaide*
Desmond Yip, *Garran*
Li Zhang, *Sydney*

Austria

Felix Aigner, *Innsbruck*
Gabriela A Berlakovich, *Vienna*
Herwig R Cerwenka, *Graz*
Peter Ferenci, *Wien*
Alfred Gangl, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Markus Raderer, *Vienna*
Stefan Riss, *Vienna*

Belgium

Michael George Adler, *Brussels*
Benedicte Y De Winter, *Antwerp*
Mark De Ridder, *Jette*
Olivier Detry, *Liege*
Denis Dufrane Dufrane, *Brussels*
Sven M Francque, *Edegem*
Nikos Kotzampassakis, *Liège*
Geert KMM Robaeys, *Genk*
Xavier Sagaert, *Leuven*
Peter Starkel, *Brussels*
Eddie Wisse, *Keerbergen*

Brazil

SMP Balzan, *Santa Cruz do Sul*
JLF Caboclo, *Sao jose do rio preto*
Fábio Guilherme Campos, *Sao Paulo*
Claudia RL Cardoso, *Rio de Janeiro*
Roberto J Carvalho-Filho, *Sao Paulo*
Carla Daltro, *Salvador*
José Sebastiao dos Santos, *Ribeirao Preto*
Eduardo LR Mello, *Rio de Janeiro*
Sthela Maria Murad-Regadas, *Fortaleza*
Claudia PMS Oliveira, *Sao Paulo*
Júlio C Pereira-Lima, *Porto Alegre*
Marcos V Perini, *Sao Paulo*
Vietla Satyanarayana Rao, *Fortaleza*
Raquel Rocha, *Salvador*
AC Simoes e Silva, *Belo Horizonte*
Mauricio F Silva, *Porto Alefre*
Aytan Miranda Sipahi, *Sao Paulo*
Rosa Leonôra Salerno Soares, *Niterói*
Cristiane Valle Tovo, *Porto Alegre*
Eduardo Garcia Vilela, *Belo Horizonte*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Tanya Kirilova Kadiyska, *Sofia*
Mihaela Petrova, *Sofia*

Cambodia

Francois Rouet, *Phnom Penh*

Canada

Brian Bressler, *Vancouver*
Frank J Burczynski, *Winnipeg*
Wangxue Chen, *Ottawa*
Francesco Crea, *Vancouver*
Jane A Foster, *Hamilton*
Hugh J Freeman, *Vancouver*
Shahrokh M Ghobadloo, *Ottawa*
Yuewen Gong, *Winnipeg*
Philip H Gordon, *Quebec*
Rakesh Kumar, *Edmonton*
Wolfgang A Kunze, *Hamilton*
Patrick Labonte, *Laval*
Zhikang Peng, *Winnipeg*
Jayadev Raju, *Ottawa*
Maitreyi Raman, *Calgary*
Giada Sebastiani, *Montreal*
Maida J Sewitch, *Montreal*
Eldon A Shaffer, *Alberta*
Christopher W Teshima, *Edmonton*
Jean Sévigny, *Québec*
Pingchang Yang, *Hamilton*
Pingchang Yang, *Hamilton*
Eric M Yoshida, *Vancouver*
Bin Zheng, *Edmonton*

Chile

Marcelo A Beltran, *La Serena*
Flavio Nervi, *Santiago*
Adolfo Parra-Blanco, *Santiago*
Alejandro Soza, *Santiago*

China

Zhao-Xiang Bian, *Hong Kong*
San-Jun Cai, *Shanghai*
Guang-Wen Cao, *Shanghai*
Long Chen, *Nanjing*
Ru-Fu Chen, *Guangzhou*
George G Chen, *Hong Kong*
Li-Bo Chen, *Wuhan*
Jia-Xu Chen, *Beijing*
Hong-Song Chen, *Beijing*
Lin Chen, *Beijing*
Yang-Chao Chen, *Hong Kong*
Zhen Chen, *Shanghai*
Ying-Sheng Cheng, *Shanghai*
Kent-Man Chu, *Hong Kong*
Zhi-Jun Dai, *Xi'an*
Jing-Yu Deng, *Tianjin*
Yi-Qi Du, *Shanghai*
Zhi Du, *Tianjin*
Hani El-Nezami, *Hong Kong*
Bao-Ying Fei, *Hangzhou*
Chang-Ming Gao, *Nanjing*
Jian-Ping Gong, *Chongqing*
Zuo-Jiong Gong, *Wuhan*
Jing-Shan Gong, *Shenzhen*
Guo-Li Gu, *Beijing*
Yong-Song Guan, *Chengdu*
Mao-Lin Guo, *Luoyang*
Jun-Ming Guo, *Ningbo*
Yan-Mei Guo, *Shanghai*
Xiao-Zhong Guo, *Shenyang*
Guo-Hong Han, *Xi'an*
Ming-Liang He, *Hong Kong*
Peng Hou, *Xi'an*
Zhao-Hui Huang, *Wuxi*
Feng Ji, *Hangzhou*
Simon Law, *Hong Kong*
Yan-Chang Lei, *Hangzhou*
Yu-Yuan Li, *Guangzhou*
Meng-Sen Li, *Haikou*
Shu-De Li, *Shanghai*
Zong-Fang Li, *Xi'an*
Qing-Quan Li, *Shanghai*
Kang Li, *Lasa*
Han Liang, *Tianjin*
Xing'e Liu, *Hangzhou*
Zheng-Wen Liu, *Xi'an*
Xiao-Fang Liu, *Yantai*
Bin Liu, *Tianjin*
Quan-Da Liu, *Beijing*
Hai-Feng Liu, *Beijing*
Fei Liu, *Shanghai*
Ai-Guo Lu, *Shanghai*
He-Sheng Luo, *Wuhan*
Xiao-Peng Ma, *Shanghai*
Yong Meng, *Shantou*
Ke-Jun Nan, *Xi'an*
Siew Chien Ng, *Hong Kong*
Simon SM Ng, *Hong Kong*
Zhao-Shan Niu, *Qingdao*
Di Qu, *Shanghai*
Ju-Wei Mu, *Beijing*
Rui-Hua Shi, *Nanjing*
Bao-Min Shi, *Shanghai*
Xiao-Dong Sun, *Hangzhou*
Si-Yu Sun, *Shenyang*
Guang-Hong Tan, *Haikou*
Wen-Fu Tang, *Chengdu*
Anthony YB Teoh, *Hong Kong*
Wei-Dong Tong, *Chongqing*
Eric Tse, *Hong Kong*
Hong Tu, *Shanghai*

Stavros Gourgiotis, *Athens*
Leontios J Hadjileontiadis, *Thessaloniki*
Thomas Hyphantis, *Ioannina*
Ioannis Kanellos, *Thessaloniki*
Stylianos Karatapanis, *Rhodes*
Michael Koutsilieris, *Athens*
Spiros D Ladas, *Athens*
Theodoros K Liakakos, *Athens*
Emanuel K Manesis, *Athens*
Spilios Manolakopoulos, *Athens*
Gerassimos John Mantzaris, *Athens*
Athanasios D Marinis, *Piraeus*
Nikolaos Ioannis Nikiteas, *Athens*
Konstantinos X Papamichael, *Athens*
George Sgourakis, *Athens*
Konstantinos C Thomopoulos, *Patras*
Konstantinos Triantafyllou, *Athens*
Christos Triantos, *Patras*
Georgios Zacharakis, *Athens*
Petros Zezos, *Alexandroupolis*
Demosthenes E Ziogas, *Ioannina*

Guatemala

Carlos Maria Parellada, *Guatemala*

Hungary

Mihaly Boros, *Szeged*
Tamás Decsi, *Pécs*
Gyula Farkas, *Szeged*
Andrea Furka, *Debrecen*
Y vette Mandi, *Szeged*
Peter L Lakatos, *Budapest*
Pal Miheller, *Budapest*
Tamás Molnar, *Szeged*
Attila Olah, *Gyor*
Maria Papp, *Debrecen*
Ferenc Sipos, *Budapest*
Miklós Tanyi, *Debrecen*
Tibor Wittmann, *Szeged*

Iceland

Tryggvi Bjorn Stefánsson, *Reykjavík*

Indiad

Brij B Agarwal, *New Delhi*
Deepak N Amarapurkar, *Mumbai*
Shams ul Bari, *Srinagar*
Sriparna Basu, *Varanasi*
Runu Chakravarty, *Kolkata*
Devendra C Desai, *Mumbai*
Nutan D Desai, *Mumbai*
Suneela Sunil Dhaneshwar, *Pune*
Radha K Dhiman, *Chandigarh*
Pankaj Garg, *Mohali*
Uday C Ghoshal, *Lucknow*
Kalpesh Jani, *Vadodara*
Premashis Kar, *New Delhi*
Jyotdeep Kaur, *Chandigarh*
Rakesh Kochhar, *Chandigarh*
Pradyumna K Mishra, *Mumbai*
Asish K Mukhopadhyay, *Kolkata*
Imtiyaz Murtaza, *Srinagar*
P Nagarajan, *New Delhi*
Samiran Nundy, *Delhi*
Gopal Pande, *Hyderabad*
Benjamin Perakath, *Vellore*
Arun Prasad, *New Delhi*
D Nageshwar Reddy, *Hyderabad*
Lekha Saha, *Chandigarh*
Sundeep Singh Saluja, *New Delhi*
Mahesh Prakash Sharma, *New Delhi*
Sadiq Saleem Sikora, *Bangalore*
Sarman Singh, *New Delhi*
Rajeev Sinha, *Jhansi*
Rupjyoti Talukdar, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*
Narayanan Thirumoorthy, *Coimbatore*

Indonesia

David Handojo Muljono, *Jakarta*
Andi Utama, *Jakarta*

Iran

Arezoo Aghakhani, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Ahad Eshraghian, *Shiraz*
Hossein Khedmat, *Tehran*
Sadegh Massarrat, *Tehran*
Marjan Mohammadi, *Tehran*
Roja Rahimi, *Tehran*
Farzaneh Sabahi, *Tehran*
Majid Sadeghizadeh, *Tehran*
Farideh Siavoshi, *Tehran*

Ireland

Gary Alan Bass, *Dublin*
David J Brayden, *Dublin*
Ronan A Cahill, *Dublin*
Glen A Doherty, *Dublin*
Liam J Fanning, *Cork*
Barry Philip McMahon, *Dublin*
RossMcManus, *Dublin*
Dervla O'Malley, *Cork*
Sinead M Smith, *Dublin*

Israel

Dan Carter, *Ramat Gan*
Jorge-Shmuel Delgado, *Metar*
Eli Magen, *Ashdod*
Nitsan Maharshak, *Tel Aviv*
Shaul Mordechai, *Beer Sheva*
Menachem Moshkowitz, *Tel Aviv*
William Bahij Nseir, *Nazareth*
Shimon Reif, *Jerusalem*
Ram Reifen, *Rehovot*
Ariella Bar-Gil Shitrit, *Jerusalem*
Noam Shussman, *Jerusalem*
Igor Sukhotnik, *Haifa*
Nir Wasserberg, *Petach Tiqwa*
Jacob Yahav, *Rehovot*
Doron Levi Zamir, *Gedera*
Shira Zelber-Sagi, *Haifa*
Romy Zemel, *Petach-Tikva*

Italy

Ludovico Abenavoli, *Catanzaro*
Luigi Elio Adinolfi, *Naples*
Carlo Virginio Agostoni, *Milan*
Anna Alisi, *Rome*
Piero Luigi Almasio, *Palermo*
Donato Francesco Altomare, *Bari*
Amedeo Amedei, *Florence*
Pietro Andreone, *Bologna*
Imerio Angriman, *Padova*
Vito Annese, *Florence*
Paolo Aurello, *Rome*
Salavtore Auricchio, *Naples*
Gian Luca Baiocchi, *Brescia*
Gianpaolo Balzano, *Milan*
Antonio Basoli, *Rome*
Gabrio Bassotti, *San Sisto*
Mauro Bernardi, *Bologna*
Alberto Biondi, *Rome*
Ennio Biscaldi, *Genova*
Massimo Bolognesi, *Padua*
Luigi Bonavina, *Milano*
Aldo Bove, *Chieti*
Raffaele Bruno, *Pavia*
Luigi Brusciano, *Napoli*
Giuseppe Cabibbo, *Palermo*
Carlo Calabrese, *Bologna*
Daniele Calistri, *Meldola*
Vincenza Calvaruso, *Palermo*
Lorenzo Camellini, *Reggio Emilia*
Marco Candela, *Bologna*
Raffaele Capasso, *Naples*
Lucia Carulli, *Modena*
Renato David Caviglia, *Rome*
Luigina Cellini, *Chieti*
Giuseppe Chiarioni, *Verona*
Claudio Chiesa, *Rome*
Michele Cicala, *Roma*
Rachele Ciccocioppo, *Pavia*
Sandro Contini, *Parma*
Gaetano Corso, *Foggia*
Renato Costi, *Parma*
Alessandro Cucchetti, *Bologna*
Rosario Cuomo, *Napoli*
Giuseppe Currò, *Messina*
Paola De Nardi, *Milano*
Giovanni D De Palma, *Naples*
Raffaele De Palma, *Napoli*
Giuseppina De Petro, *Brescia*
Valli De Re, *Aviano*
Paolo De Simone, *Pisa*
Giuliana Decorti, *Trieste*
Emanuele Miraglia del Giudice, *Napoli*
Isidoro Di Carlo, *Catania*
Matteo Nicola Dario Di Minno, *Naples*
Massimo Donadelli, *Verona*
Mirko D'Onofrio, *Verona*
Maria Pina Dore, *Sassari*
Luca Elli, *Milano*
Massimiliano Fabozzi, *Aosta*
Massimo Falconi, *Ancona*

Ezio Falletto, *Turin*
Silvia Fargion, *Milan*
Matteo Fassan, *Verona*
Gianfranco Delle Fave, *Roma*
Alessandro Federico, *Naples*
Francesco Feo, *Sassari*
Davide Festi, *Bologna*
Natale Figura, *Siena*
Vincenzo Formica, *Rome*
Mirella Fraquelli, *Milan*
Marzio Frazzoni, *Modena*
Walter Fries, *Messina*
Gennaro Galizia, *Naples*
Andrea Galli, *Florence*
Matteo Garcovich, *Rome*
Eugenio Gaudio, *Rome*
Paola Ghiorzo, *Genoa*
Edoardo G Giannini, *Genova*
Luca Gianotti, *Monza*
Maria Cecilia Giron, *Padova*
Alberto Grassi, *Rimini*
Gabriele Grassi, *Trieste*
Francesco Greco, *Bergamo*
Luigi Greco, *Naples*
Antonio Grieco, *Rome*
Fabio Grizzi, *Rozzano*
Laurino Grossi, *Pescara*
Simone Guglielmetti, *Milan*
Tiberiu Hershcovici, *Jerusalem*
Calogero Iacono, *Verona*
Enzo Ierardi, *Bari*
Amedeo Indriolo, *Bergamo*
Raffaele Iorio, *Naples*
Paola Iovino, *Salerno*
Angelo A Izzo, *Naples*
Loreta Kondili, *Rome*
Filippo La Torre, *Rome*
Giuseppe La Torre, *Rome*
Giovanni Latella, *L'Aquila*
Salvatore Leonardi, *Catania*
Massimo Libra, *Catania*
Anna Licata, *Palermo*
C armela Loguercio, *Naples*
Amedeo Lonardo, *Modena*
Carmelo Luigiano, *Catania*
Francesco Luzza, *Catanzaro*
Giovanni Maconi, *Milano*
Antonio Macrì, *Messina*
Mariano Malaguarnera, *Catania*
Francesco Manguso, *Napoli*
Tommaso Maria Manzia, *Rome*
Daniele Marrelli, *Siena*
Gabriele Masselli, *Rome*
Sara Massironi, *Milan*
Giuseppe Mazzarella, *Avellino*
Michele Milella, *Rome*
Giovanni Milito, *Rome*
Antonella d'Arminio Monforte, *Milan*
Fabrizio Montecucco, *Genoa*
Giovanni Monteleone, *Rome*
Mario Morino, *Torino*
Vincenzo La Mura, *Milan*
Gerardo Nardone, *Naples*
Riccardo Nascimbeni, *Brescia*
Gabriella Nesi, *Florence*
Giuseppe Nigri, *Rome*
Erica Novo, *Turin*
Veronica Ojetti, *Rome*
Michele Orditura, *Naples*
Fabio Pace, *Seriate*
Lucia Pacifico, *Rome*
Omero Alessandro Paoluzi, *Rome*
Valerio Pazienza, *San Giovanni Rotondo*
Rinaldo Pellicano, *Turin*
Adriano M Pellicelli, *Rome*
Nadia Peparini, *Ciampino*
Mario Pescatori, *Rome*
Antonio Picardi, *Rome*
Alberto Pilotto, *Padova*
Alberto Piperno, *Monza*
Anna Chiara Piscaglia, *Rome*
Maurizio Pompili, *Rome*
Francesca Romana Ponziani, *Rome*
Cosimo Prantera, *Rome*
Girolamo Ranieri, *Bari*
Carlo Ratto, *Tome*
Barbara Renga, *Perugia*
Alessandro Repici, *Rozzano*
Maria Elena Riccioni, *Rome*
Lucia Ricci-Vitiani, *Rome*
Luciana Rigoli, *Messina*
Mario Rizzetto, *Torino*
Ballarin Roberto, *Modena*
Roberto G Romanelli, *Florence*
Claudio Romano, *Messina*
Luca Roncucci, *Modena*
Cesare Ruffolo, *Treviso*
L ucia Sacchetti, *Napoli*
Rodolfo Sacco, *Pisa*
Lapo Sali, *Florence*
Romina Salpini, *Rome*
Giulio Aniello, *Santoro Treviso*
Armando Santoro, *Rozzano*
Edoardo Savarino, *Padua*
Marco Senzolo, *Padua*
Annalucia Serafino, *Rome*
Giuseppe S Sica, *Rome*
Pierpaolo Sileri, *Rome*
Cosimo Sperti, *Padua*
Vincenzo Stanghellini, *Bologna*
Cristina Stasi, *Florence*
Gabriele Stocco, *Trieste*
Roberto Tarquini, *Florence*
Mario Testini, *Bari*
Guido Torzilli, *Milan*
Guido Alberto Massimo, *Tiberio Brescia*
Giuseppe Toffoli, *Aviano*
Alberto Tommasini, *Trieste*
Francesco Tonelli, *Florence*
Cesare Tosetti Porretta, *Terme*
Lucio Trevisani, *Cona*
Guglielmo M Trovato, *Catania*
Mariapia Vairetti, *Pavia*
Luca Vittorio Valenti, *Milano*
Mariateresa T Ventura, *Bari*
Giuseppe Verlato, *Verona*
Marco Vivarelli, *Ancona*
Giovanni Li Volti, *Catania*
Giuseppe Zanotti, *Padua*
Vincenzo Zara, *Lecce*
Gianguglielmo Zehender, *Milan*
Anna Linda Zignego, *Florence*
Rocco Antonio Zoccali, *Messina*
Angelo Zullo, *Rome*

Japan

Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Masahiro Arai, *Tokyo*
Makoto Arai, *Chiba*
Takaaki Arigami, *Kagoshima*
Itaru Endo,*Yokohama*
Munechika Enjoji, *Fukuoka*
Shunji Fujimori, *Tokyo*
Yasuhiro Fujino, *Akashi*
Toshiyoshi Fujiwara, *Okayama*
Yosuke Fukunaga, *Tokyo*
Toshio Fukusato, *Tokyo*
Takahisa Furuta, *Hamamatsu*
Osamu Handa, *Kyoto*
Naoki Hashimoto, *Osaka*
Yoichi Hiasa, *Toon*
Masatsugu Hiraki, *Saga*
Satoshi Hirano, *Sapporo*
Keiji Hirata, *Fukuoka*
Toru Hiyama, *Higashihiroshima*
Akira Hokama, *Nishihara*
Shu Hoteya, *Tokyo*
Masao Ichinose, *Wakayama*
Tatsuya Ide, *Kurume*
Masahiro Iizuka, *Akita*
Toshiro Iizuka, *Tokyo*
Kenichi Ikejima, *Tokyo*
Tetsuya Ikemoto, *Tokushima*
Hiroyuki Imaeda, *Saitama*
Atsushi Imagawa, *Kan-onji*
Hiroo Imazu, *Tokyo*
Shuji Isaji, *Tsu*
Toru Ishikawa, *Niigata*
Toshiyuki Ishiwata, *Tokyo*
Soichi Itaba, *Kitakyushu*
Yoshiaki Iwasaki, *Okayama*
Tatehiro Kagawa, *Isehara*
Satoru Kakizaki, *Maebashi*
Naomi Kakushima, *Shizuoka*
Terumi Kamisawa, *Tokyo*
Akihide Kamiya, *Isehara*
Osamu Kanauchi, *Tokyo*
Tatsuo Kanda, *Chiba*
Shin Kariya, *Okayama*
Shigeyuki Kawa, *Matsumoto*
Takumi Kawaguchi, *Kurume*
Takashi Kawai, *Tokyo*
Soo Ryang Kim, *Kobe*
Shinsuke Kiriyama, *Gunma*
Tsuneo Kitamura, *Urayasu*
Masayuki Kitano, *Osakasayama*
Hirotoshi Kobayashi, *Tokyo*
Hironori Koga, *Kurume*
Takashi Kojima, *Sapporo*
Satoshi Kokura, *Kyoto*
Shuhei Komatsu, *Kyoto*
Tadashi Kondo, *Tokyo*
Yasuteru Kondo, *Sendai*
Yasuhiro Kuramitsu, *Yamaguchi*
Yukinori Kurokawa, *Osaka*
Shin Maeda, *Yokohama*
Koutarou Maeda, *Toyoake*

Hitoshi Maruyama, *Chiba*
Atsushi Masamune, *Sendai*
Hiroyuki Matsubayashi, *Suntogun*
Akihisa Matsuda, *Inzai*
Hirofumi Matsui, *Tsukuba*
Akira Matsumori, *Kyoto*
Yoichi Matsuo, *Nagoya*
Y Matsuzaki, *Ami*
Toshihiro Mitaka, *Sapporo*
Kouichi Miura, *Akita*
Shinichi Miyagawa, *Matumoto*
Eiji Miyoshi, *Suita*
Toru Mizuguchi, *Sapporo*
Nobumasa Mizuno, *Nagoya*
Zenichi Morise, *Nagoya*
Tomohiko Moriyama, *Fukuoka*
Kunihiko Murase, *Tusima*
Michihiro Mutoh, *Tsukiji*
Akihito Nagahara, *Tokyo*
Hikaru Nagahara, *Tokyo*
Hidenari Nagai, *Tokyo*
Koichi Nagata, *Shimotsuke-shi*
Masaki Nagaya, *Kawasaki*
Hisato Nakajima, *Nishi-Shinbashi*
Toshifusa Nakajima, *Tokyo*
Hiroshi Nakano, *Kawasaki*
Hiroshi Nakase, *Kyoto*
Toshiyuki Nakayama, *Nagasaki*
Takahiro Nakazawa, *Nagoya*
Shoji Natsugoe, *Kagoshima City*
Tsutomu Nishida, *Suita*
Shuji Nomoto, *Naogya*
Sachiyo Nomura, *Tokyo*
Takeshi Ogura, *Takatsukishi*
Nobuhiro Ohkohchi, *Tsukuba*
Toshifumi Ohkusa, *Kashiwa*
Hirohide Ohnishi, *Akita*
Teruo Okano, *Tokyo*
Satoshi Osawa, *Hamamatsu*
Motoyuki Otsuka, *Tokyo*
Michitaka Ozaki, *Sapporo*
Satoru Saito, *Yokohama*
Naoaki Sakata, *Sendai*
Ken Sato, *Maebashi*
Toshiro Sato, *Tokyo*
Tomoyuki Shibata, *Toyoake*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Nanto*
Tadashi Shimoyama, *Hirosaki*
Masayuki Sho, *Nara*
Ikuo Shoji, *Kobe*
Atsushi Sofuni, *Tokyo*
Takeshi Suda, *Niigata*
M Sugimoto, *Hamamatsu*
Ken Sugimoto, *Hamamatsu*
Haruhiko Sugimura, *Hamamatsu*
Shoichiro Sumi, *Kyoto*
Hidekazu Suzuki, *Tokyo*
Masahiro Tajika, *Nagoya*
Hitoshi Takagi, *Takasaki*
Toru Takahashi, *Niigata*
Yoshihisa Takahashi, *Tokyo*
Shinsuke Takeno, *Fukuoka*
Akihiro Tamori, *Osaka*
Kyosuke Tanaka, *Tsu*
Shinji Tanaka, *Hiroshima*
Atsushi Tanaka, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Shinji Tanaka, *Tokyo*
Minoru Tomizawa, *Yotsukaido City*
Kyoko Tsukiyama-Kohara, *Kagoshima*
Takuya Watanabe, *Niigata*
Kazuhiro Watanabe, *Sendai*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yamamoto, *Otsu*
Kosho Yamanouchi, *Nagasaki*
Ichiro Yasuda, *Gifu*
Yutaka Yata, *Maebashi-city*
Shin-ichi Yokota, *Sapporo*
Norimasa Yoshida, *Kyoto*
Hiroshi Yoshida, *Tama-City*
Hitoshi Yoshiji, *Kashihara*
Kazuhiko Yoshimatsu, *Tokyo*
Kentaro Yoshioka, *Toyoake*
Nobuhiro Zaima, *Nara*

Jordan
Khaled Ali Jadallah, *Irbid*

Kuwait
Islam Khan, *Kuwait*

Lebanon
Bassam N Abboud, *Beirut*
Kassem A Barada, *Beirut*
Marwan Ghosn, *Beirut*
Iyad A Issa, *Beirut*
Fadi H Mourad, *Beirut*
AIa Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania
Antanas Mickevicius, *Kaunas*

Malaysia
Huck Joo Tan, *Petaling Jaya*

Mexico
Richard A Awad, *Mexico City*
Carlos R Camara-Lemarroy, *Monterrey*
Norberto C Chavez-Tapia, *Mexico City*
Wolfgang Gaertner, *Mexico City*
Diego Garcia-Compean, *Monterrey*
Arturo Panduro, *Guadalajara*
OT Teramoto-Matsubara, *Mexico City*
Felix Tellez-Avila, *Mexico City*
Omar Vergara-Fernandez, *Mexico City*
Saúl Villa-Trevino, *Cuidad de México*

Morocco
Samir Ahboucha, *Khouribga*

Netherlands
Robert J de Knegt, *Rotterdam*
Tom Johannes Gerardus Gevers, *Nijmegen*
Menno Hoekstra, *Leiden*
BW Marcel Spanier, *Arnhem*
Karel van Erpecum, *Utrecht*

New Zealand
Leo K Cheng, *Auckland*
Andrew Stewart Day, *Christchurch*
Jonathan Barnes Koea, *Auckland*
Max Petrov, *Auckland*

Nigeria
Olufunmilayo Adenike Lesi, *Lagos*
Jesse Abiodun Otegbayo, *Ibadan*
Stella Ifeanyi Smith, *Lagos*

Norway
Trond Berg, *Oslo*
Trond Arnulf Buanes, *Krokkleiva*
Thomas de Lange, *Rud*
Magdy El-Salhy, *Stord*
Rasmus Goll, *Tromso*
Dag Arne Lihaug Hoff, *Aalesund*

Pakistan
Zaigham Abbas, *Karachi*
Usman A Ashfaq, *Faisalabad*
Muhammad Adnan Bawany, *Hyderabad*
Muhammad Idrees, *Lahore*
Saeed Sadiq Hamid, *Karachi*
Yasir Waheed, *Islamabad*

Poland
Thomas Brzozowski, *Cracow*
Magdalena Chmiela, *Lodz*
Krzysztof Jonderko, *Sosnowiec*
Anna Kasicka-Jonderko, *Sosnowiec*
Michal Kukla, *Katowice*
Tomasz Hubert Mach, *Krakow*
Agata Mulak, *Wroclaw*
Danuta Owczarek, *Kraków*
Piotr Socha, *Warsaw*
Piotr Stalke, *Gdansk*
Julian Teodor Swierczynski, *Gdansk*
Anna M Zawilak-Pawlik, *Wroclaw*

Portugal
Marie Isabelle Cremers, *Setubal*
Ceu Figueiredo, *Porto*
Ana Isabel Lopes, *LIsbon*
M Paula Macedo, *Lisboa*
Ricardo Marcos, *Porto*
Rui T Marinho, *Lisboa*
Guida Portela-Gomes, *Estoril*

Filipa F Vale, *Lisbon*

Puerto Rico

Caroline B Appleyard, *Ponce*

Qatar

Abdulbari Bener, *Doha*

Romania

Mihai Ciocirlan, *Bucharest*
Dan LucianDumitrascu, *Cluj-Napoca*
Carmen Fierbinteanu-Braticevici, *Bucharest*
Romeo G Mihaila, *Sibiu*
Lucian Negreanu, *Bucharest*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Ioan Sporea, *Timisoara*
Letiţia Adela Maria Streba, *Craiova*
Anca Trifan, *Iasi*

Russia

Victor Pasechnikov, *Stavropol*
Vasiliy Ivanovich Reshetnyak, *Moscow*
Vitaly Skoropad, *Obninsk*

Saudi Arabia

Abdul-Wahed N Meshikhes, *Dammam*
M Ezzedien Rabie, *Khamis Mushait*

Singapore

Brian KP Goh, *Singapore*
Richie Soong, *Singapore*
Ker-Kan Tan, *Singapore*
Kok-Yang Tan, *Singapore*
Yee-Joo Tan, *Singapore*
Mark Wong, *Singapore*
Hong Ping Xia, *Singapore*

Slovenia

Matjaz Homan, *Ljubljana*
Martina Perse, *Ljubljana*

South Korea

Sang Hoon Ahn, *Seoul*
Seung Hyuk Baik, *Seoul*
Soon Koo Baik, *Wonju*
Soo-Cheon Chae, *Iksan*
Byung-Ho Choe, *Daegu*
Suck Chei Choi, *Iksan*
Hoon Jai Chun, *Seoul*
Yeun-Jun Chung, *Seoul*
Young-Hwa Chung, *Seoul*
Ki-Baik Hahm, *Seongnam*
Sang Young Han, *Busan*
Seok Joo Han, *Seoul*
Seung-Heon Hong, *Iksan*
Jin-Hyeok Hwang, *Seoungnam*
Jeong Won Jang, *Seoul*
Jin-Young Jang, *Seoul*
Dae-Won Jun, *Seoul*
Young Do Jung, *Kwangju*
Gyeong Hoon Kang, *Seoul*
Sung-Bum Kang, *Seoul*
Koo Jeong Kang, *Daegu*
Ki Mun Kang, *Jinju*
Chang Moo Kang, *Seodaemun-gu*
Gwang Ha Kim, *Busan*
Sang Soo Kim, *Goyang-si*
Jin Cheon Kim, *Seoul*
Tae Il Kim, *Seoul*
Jin Hong Kim, *Suwon*
Kyung Mo Kim, *Seoul*
Kyongmin Kim, *Suwon*
Hyung-Ho Kim, *Seongnam*
Seoung Hoon Kim, *Goyang*
Sang Il Kim, *Seoul*
Hyun-Soo Kim, *Wonju*
Jung Mogg Kim, *Seoul*
Dong Yi Kim, *Gwangju*
Kyun-Hwan Kim, *Seoul*
Jong-Han Kim, *Ansan*
Sang Wun Kim, *Seoul*
Ja-Lok Ku, *Seoul*
Kyu Taek Lee, *Seoul*
Hae-Wan Lee, *Chuncheon*
Inchul Lee, *Seoul*
Jung Eun Lee, *Seoul*
Sang Chul Lee, *Daejeon*
Song Woo Lee, *Ansan-si*
Hyuk-Joon Lee, *Seoul*
Seong-Wook Lee, *Yongin*
Kil Yeon Lee, *Seoul*
Jong-Inn Lee, *Seoul*
Kyung A Lee, *Seoul*
Jong-Baeck Lim, *Seoul*
Eun-Yi Moon, *Seoul*
SH Noh, *Seoul*
Seung Woon Paik, *Seoul*
Won Sang Park, *Seoul*
Sung-Joo Park, *Iksan*
Kyung Sik Park, *Daegu*
Se Hoon Park, *Seoul*
Yoonkyung Park, *Gwangju*
Seung-Wan Ryu, *Daegu*
Il Han Song, *Cheonan*
Myeong Jun Song, *Daejeon*
Yun Kyoung Yim, *Daejeon*
Dae-Yeul Yu *Daejeon*

Spain

Mariam Aguas, *Valencia*
Raul J Andrade, *Málaga*
Antonio Arroyo, *Elche*
Josep M Bordas, *Barcelona*
Lisardo Boscá, *Madrid*
Ricardo Robles Campos, *Murcia*
Jordi Camps, *Reus*
Carlos Cervera *Barcelona*
Alfonso Clemente, *Granada*
Pilar Codoner-Franch, *Valencia*
Fernando J Corrales, *Pamplona*
Fermin Sánchez de Medina, *Granada*
Alberto Herreros de Tejada, *Majadahonda*
Enrique de-Madaria, *Alicante*
JE Dominguez-Munoz, *Santiago de Compostela*
Vicente Felipo, *Valencia*
CM Fernandez-Rodriguez, *Madrid*
Carmen Frontela-Saseta, *Murcia*
Julio Galvez, *Granada*
Maria Teresa García, *Vigo*
MI Garcia-Fernandez, *Málaga*
Emilio Gonzalez-Reimers, *La Laguna*
Marcel Jimenez, *Bellaterra*
Angel Lanas, *Zaragoza*
Juan Ramón Larrubia, *Guadalajara*
Antonio Lopez-Sanroman, *Madrid*
Vicente Lorenzo-Zuniga, *Badalona*
Alfredo J Lucendo, *Tomelloso*
Vicenta Soledad Martinez-Zorzano, *Vigo*
José Manuel Martin-Villa, *Madrid*
Julio Mayol, *Madrid*
Manuel Morales-Ruiz, *Barcelona*
Alfredo Moreno-Egea, *Murcia*
Albert Pares, *Barcelona*
Maria Pellise, *Barcelona*
José Perea, *Madrid*
Miguel Angel Plaza, *Zaragoza*
María J Pozo, *Cáceres*
Enrique Quintero, *La Laguna*
Jose M Ramia, *Madrid*
Francisco Rodriguez-Frias, *Barcelona*
Silvia Ruiz-Gaspa, *Barcelona*
Xavier Serra-Aracil, *Barcelona*
Vincent Soriano, *Madrid*
Javier Suarez, *Pamplona*
Carlos Taxonera, *Madrid*
M Isabel Torres, *Jaén*
Manuel Vazquez-Carrera, *Barcelona*
Benito Velayos, *Valladolid*
Silvia Vidal, *Barcelona*

Sri Lanka

Arjuna Priyadarsin De Silva, *Colombo*

Sudan

Ishag Adam, *Khartoum*

Sweden

Roland G Andersson, *Lund*
Bergthor Björnsson, *Linkoping*
Johan Christopher Bohr, *Örebro*
Mauro D'Amato, *Stockholm*
Thomas Franzen, *Norrkoping*
Evangelos Kalaitzakis, *Lund*
Riadh Sadik, *Gothenburg*
Per Anders Sandstrom, *Linkoping*
Ervin Toth, *Malmö*
Konstantinos Tsimogiannis, *Vasteras*
Apostolos V Tsolakis, *Uppsala*

Switzerland

Gieri Cathomas, *Liestal*
Jean Louis Frossard, *Geneve*
Christian Toso, *Geneva*
Stephan Robert Vavricka, *Zurich*
Dominique Velin, *Lausanne*

Thailand

Thawatchai Akaraviputh, *Bangkok*
P Yoysungnoen Chintana, *Pathumthani*
Veerapol Kukongviriyapan, *Muang*
Vijittra Leardkamolkarn, *Bangkok*
Varut Lohsiriwat, *Bangkok*
Somchai Pinlaor, *Khaon Kaen*
D Wattanasirichaigoon, *Bangkok*

Trinidad and Tobago

B Shivananda Nayak, *Mount Hope*

Tunisia

Ibtissem Ghedira, *Sousse*
Lilia Zouiten-Mekki, *Tunis*

Turkey

Inci Alican, *Istanbul*
Mustafa Altindis, *Sakarya*
Mutay Aslan, *Antalya*
Oktar Asoglu, *Istanbul*
Yasemin Hatice Balaban, *Istanbul*
Metin Basaranoglu, *Ankara*
Yusuf Bayraktar, *Ankara*
Süleyman Bayram, *Adiyaman*
Ahmet Bilici, *Istanbul*
Ahmet Sedat Boyacioglu, *Ankara*
Züleyha Akkan Cetinkaya, *Kocaeli*
Cavit Col, *Bolu*
Yasar Colak, *Istanbul*
Cagatay Erden Daphan, *Kirikkale*
Mehmet Demir, *Hatay*
Ahmet Merih Dobrucali, *Istanbul*
Gülsüm Ozlem Elpek, *Antalya*
Ayse Basak Engin, *Ankara*
Eren Ersoy, *Ankara*
Osman Ersoy, *Ankara*
Yusuf Ziya Erzin, *Istanbul*
Mukaddes Esrefoglu, *Istanbul*
Levent Filik, *Ankara*
Ozgur Harmanci, *Ankara*
Koray Hekimoglu, *Ankara*
Abdurrahman Kadayifci, *Gaziantep*
Cem Kalayci, *Istanbul*
Selin Kapan, *Istanbul*
Huseyin Kayadibi, *Adana*
Sabahattin Kaymakoglu, *Istanbul*
Metin Kement, *Istanbul*
Mevlut Kurt, *Bolu*
Resat Ozaras, *Istanbul*
Elvan Ozbek, *Adapazari*
Cengiz Ozcan, *Mersin*
Hasan Ozen, *Ankara*
Halil Ozguc, *Bursa*
Mehmet Ozturk, *Izmir*
Orhan V Ozkan, *Sakarya*
Semra Paydas, *Adana*
Ozlem Durmaz Suoglu, *Istanbul*
Ilker Tasci, *Ankara*
Müge Tecder-ünal, *Ankara*
Mesut Tez, *Ankara*
Serdar Topaloglu, *Trabzon*
Murat Toruner, *Ankara*
Gokhan Tumgor, *Adana*
Oguz Uskudar, *Adana*
Mehmet Yalniz, *Elazig*
Mehmet Yaman, *Elazig*
Veli Yazisiz, *Antalya*
Yusuf Yilmaz, *Istanbul*
Ozlem Yilmaz, *Izmir*
Oya Yucel, *Istanbul*
Ilhami Yuksel, *Ankara*

United Kingdom

Nadeem Ahmad Afzal, *Southampton*
Navneet K Ahluwalia, *Stockport*
Yeng S Ang, *Lancashire*
Ramesh P Arasaradnam, *Coventry*
Ian Leonard Phillip Beales, *Norwich*
John Beynon, *Swansea*
Barbara Braden, *Oxford*
Simon Bramhall, *Birmingham*
Geoffrey Burnstock, *London*
Ian Chau, *Sutton*
Thean Soon Chew, *London*
Helen G Coleman, *Belfast*
Anil Dhawan, *London*
Sunil Dolwani, *Cardiff*
Piers Gatenby, *London*
Anil T George, *London*
Pasquale Giordano, *London*
Paul Henderson, *Edinburgh*
Georgina Louise Hold, *Aberdeen*
Stefan Hubscher, *Birmingham*
Robin D Hughes, *London*
Nusrat Husain, *Manchester*
Matt W Johnson, *Luton*
Konrad Koss, *Macclesfield*
Anastasios Koulaouzidis, *Edinburgh*
Simon Lal, *Salford*
John S Leeds, *Aberdeen*
JK K Limdi, *Manchester*
Hongxiang Liu, *Cambridge*
Michael Joseph McGarvey, *London*
Michael Anthony Mendall, *London*
Alexander H Mirnezami, *Southampton*
J Bernadette Moore, *Guildford*
Claudio Nicoletti, *Norwich*
Savvas Papagrigoriadis, *London*
Sylvia LF Pender, *Southampton*
David Mark Pritchard, *Liverpool*
James A Ross, *Edinburgh*
Kamran Rostami, *Worcester*
Xiong Z Ruan, *London*
Frank I Tovey, *London*
Dhiraj Tripathi, *Birmingham*
Vamsi R Velchuru, *Great Yarmouth*
Nicholas T Ventham, *Edinburgh*
Diego Vergani, *London*
Jack Westwood Winter, *Glasgow*
Terence Wong, *London*
Ling Yang, *Oxford*

United States

Daniel E Abbott, *Cincinnati*
Ghassan K Abou-Alfa, *New York*
Julian Abrams, *New York*
David William Adelson, *Los Angeles*
Jonathan Steven Alexander, *Shreveport*
Tauseef Ali, *Oklahoma City*
Mohamed R Ali, *Sacramento*
Rajagopal N Aravalli, *Minneapolis*
Hassan Ashktorab, *Washington*
Shashi Bala, *Worcester*
Charles F Barish, *Raleigh*
P Patrick Basu, *New York*
Robert L Bell, *Berkeley Heights*
David Bentrem, *Chicago*
Henry J Binder, *New Haven*
Joshua Bleier, *Philadelphia*
Wojciech Blonski, *Johnson City*
Kenneth Boorom, *Corvallis*
Brian Boulay, *Chicago*
Carla W Brady, *Durham*
Kyle E Brown, *Iowa City*
Adeel A Butt, *Pittsburgh*
Weibiao Cao, *Providence*
Andrea Castillo, *Cheney*
Fernando J Castro, *Weston*
Adam S Cheifetz, *Boston*
Xiaoxin Luke Chen, *Durham*
Ramsey Cheung, *Palo Alto*
Parimal Chowdhury, *Little Rock*
Edward John Ciaccio, *New York*
Dahn L Clemens, *Omaha*
Yingzi Cong, *Galveston*
Laura Iris Cosen-Binker, *Boston*
Joseph John Cullen, *Lowa*
Mark J Czaja, *Bronx*
Mariana D Dabeva, *Bronx*
Christopher James Damman, *Seattle*
Isabelle G De Plaen, *Chicago*
Punita Dhawan, *Nashville*
Hui Dong, *La Jolla*
Wael El-Rifai, *Nashville*
Sukru H Emre, *New Haven*
Paul Feuerstadt, *Hamden*
Josef E Fischer, *Boston*
Laurie N Fishman, *Boston*
Joseph Che Forbi, *Atlanta*
Temitope Foster, *Atlanta*
Amy E Foxx-Orenstein, *Scottsdale*
Daniel E Freedberg, *New York*
Shai Friedland, *Palo Alto*
Virgilio George, *Indianapolis*
Ajay Goel, *Dallas*
Oliver Grundmann, *Gainesville*
Stefano Guandalini, *Chicago*
Chakshu Gupta, *St. Joseph*
Grigoriy E Gurvits, *New York*

World Journal of Gastroenterology

Contents

Weekly Volume 23 Number 1 January 7, 2017

Contents

World Journal of Gastroenterology
Volume 23 Number 1 January 7, 2017

NAME OF JOURNAL
World Journal of Gastroenterology

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

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Yuan Qi, Vice Director
Ze-Mao Gong, Vice Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
January 7, 2017

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.1

World J Gastroenterol 2017 January 7; 23(1): 1-10
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Clinical role of non-invasive assessment of portal hypertension

Massimo Bolognesi, Marco Di Pascoli, David Sacerdoti

Massimo Bolognesi, Marco Di Pascoli, David Sacerdoti, Department of Medicine, University of Padova, I-35128 Padova, Italy

Author contributions: Bolognesi M wrote the manuscript; Di Pascoli M and Sacerdoti D contributed to the intellectual content and revision of the manuscript; all authors approved the final version of the manuscript.

Conflict-of-interest statement: The authors have no conflict of interests.

Manuscript source: Invited manuscript

Correspondence to: Massimo Bolognesi, MD, PhD, Associate Professor of Internal Medicine, Department of Medicine, University of Padova, Azienda Ospedaliera Università di Padova, Clinica Medica 5, Via Giustininani 2, I-35128 Padova, Italy. massimo.bolognesi@unipd.it
Telephone: +39-49-8212383
Fax: +39-49-8754179

Received: August 22, 2016
Peer-review started: August 24, 2016
First decision: September 12, 2016
Revised: September 27, 2016
Accepted: October 30, 2016
Article in press: October 31, 2016
Published online: January 7, 2017

Abstract

Measurement of portal pressure is pivotal in the evaluation of patients with liver cirrhosis. The measurement of the hepatic venous pressure gradient represents the reference method by which portal pressure is estimated. However, it is an invasive procedure that requires significant hospital resources, including experienced staff, and is associated with considerable cost. Non-invasive methods that can be reliably used to estimate the presence and the degree of portal hypertension are urgently needed in clinical practice. Biochemical and morphological parameters have been proposed for this purpose, but have shown disappointing results overall. Splanchnic Doppler ultrasonography and the analysis of microbubble contrast agent kinetics with contrast-enhanced ultrasonography have shown better accuracy for the evaluation of patients with portal hypertension. A key advancement in the non-invasive evaluation of portal hypertension has been the introduction in clinical practice of methods able to measure stiffness in the liver, as well as stiffness/congestion in the spleen. According to the data published to date, it appears to be possible to rule out clinically significant portal hypertension in patients with cirrhosis (*i.e.*, hepatic venous pressure gradient $\geqslant$ 10 mmHg) with a level of clinically-acceptable accuracy by combining measurements of liver stiffness and spleen stiffness along with Doppler ultrasound evaluation. It is probable that the combination of these methods may also allow for the identification of patients with the most serious degree of portal hypertension, and ongoing research is helping to ensure progress in this field.

Key words: Portal hypertension; Splenic stiffness; Liver stiffness; Splenic arterial resistance indices; Sonography; Doppler ultrasound; Cirrhosis; Transient elastography; Esophageal varices

Core tip: This Editorial analyzes the newest and promising methods for estimating portal pressure non-

invasively in cirrhotic patients with portal hypertension. Measurements of liver and spleen stiffness, combined with Doppler ultrasound evaluation, allow for the identification of patients without clinically-significant portal hypertension and are also promising for estimation of the degree of portal pressure in patients with portal hypertension.

Bolognesi M, Di Pascoli M, Sacerdoti D. Clinical role of non-invasive assessment of portal hypertension. *World J Gastroenterol* 2017; 23(1): 1-10 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/1.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.1

INTRODUCTION

Measurement of portal pressure is pivotal in the evaluation of patients with liver cirrhosis. Indeed, portal hypertension is a complication of cirrhosis that affects prognosis and the natural history (disease stage). Portal hypertension is the main etiology underlying the opening of collateral circulation and the onset of hyperdynamic circulatory syndrome, which can result in esophageal varices (EV), gastrointestinal bleeding, ascites, hepatorenal syndrome, spontaneous bacterial peritonitis and/or hepatic encephalopathy[1]. Therefore, upper gastrointestinal endoscopy and measurement of portal pressure are recommended for patients with suspected liver cirrhosis and portal hypertension[1]. Moreover, the measurement of portal pressure represents the only valid method currently available for evaluation of effectiveness of portal hypertension therapies (pharmacological, surgical, interventional radiology)[2].

CLINICALLY-SIGNIFICANT PORTAL HYPERTENSION

Increase in portal pressure has been shown to be clinically significant (clinically significant portal hypertension, CSPH) when it corresponds to a porto-hepatic gradient of $\geqslant$ 10 mmHg[2,3]. CSPH is considered the threshold beyond which complications like EV and ascites may develop[2,4]. However, in the evaluation of portal hypertension, it is not sufficient to merely determine the presence or absence of CSPH. Indeed, the degree of portal hypertension defines different levels of risk, with progressively worse prognostic significance.

Risk of EV, ascites and decompensation after surgery for hepatocellular carcinoma is associated with portal pressure > 10 mmHg, while risk of bleeding of EV is associated with a portal pressure of $\geqslant$ 12 mmHg[5]. Portal pressure > 16 mmHg is reported as correlated with survival, first clinical decompensation in patients with varices, and higher risk of esophageal rebleeding and mortality in patients with decompensated cirrhosis; higher than 20 mmHg is correlated with failure to control bleeding in patients with active bleeding from varices and to mortality, > 22 mmHg is correlated with mortality in patients with alcoholic cirrhosis and acute alcoholic hepatitis, and > 30 mmHg is correlated with spontaneous bacterial peritonitis[3,5,6]. In contrast, improvement of portal hypertension is associated with improved prognosis. In particular, a reduction in portal pressure to a level of < 12 mmHg, or to at least of 20% of the baseline values, is necessary to obtain clinical efficacy of portal hypertension therapy[2,7,8]. It is clear, therefore, that when portal hypertension is suspected in patients with liver cirrhosis, it is not only necessary to know whether CSPH is present but also to quantify the level of portal hypertension and, further, to evaluate the change in portal pressure over time.

MEASUREMENT OF PORTAL PRESSURE: THE HEPATIC VENOUS PRESSURE GRADIENT

The actual reference method for the measurement of portal pressure is measurement of the hepatic venous pressure gradient (HVPG), an indirect estimate of portal pressure obtained by use of catheterization of the hepatic veins. It allows measurement of the level of sinusoidal pressure by calculating the difference between the pressure in a hepatic vein that has been inserted with the occluding catheter and free pressure[5,7]. In the cirrhosis condition, portal hypertension is mainly due to sinusoidal and post-sinusoidal hypertension, with the sinusoidal pressure corresponding to the pressure in the portal vein. Introduction of balloon catheters to this measurement approach has resulted in marked improvement in reliability of the measurement[9].

HVPG is an indirect method, which is only able to correctly evaluate portal pressure in patients with increased portal pressure at the sinusoidal level (*i.e.,* cirrhosis), and it has no value in patients with pre-sinusoidal and pre-hepatic portal hypertension. Yet, detection of its normality can sometimes help in differential diagnosis of those forms of pre-hepatic portal hypertension.

With this limitation, HVPG, if executed according to the guidelines, is a safe and reproducible technique, and has emerged as the reference method for measurement of the pressure gradient between the portal vein and the inferior vena cava in cirrhosis (sinusoidal portal hypertension)[2]. Unfortunately, the method is invasive and relies on the commitment of significant hospital resources, equating to a considerable cost and requiring experienced staff. As such, it is routinely performed in only a few centers, particularly those specializing in the treatment of portal

hypertension[10].

NON-INVASIVE ESTIMATION OF PORTAL PRESSURE

Non-invasive methods that can be used reliably to determine the presence and estimate the degree of portal hypertension have been in great demand for at least 30 years. Despite substantial efforts to generate such a method, up until a few years ago only disappointing and unsatisfactory results were obtained.

Since the primary cause of portal hypertension is the mechanical increase in intrahepatic resistance due to fibrosis and distortion of liver architecture, it is reasonable to assume that non-invasive parameters of liver fibrosis may indicate the presence of portal hypertension.

ESTIMATION OF PORTAL HYPERTENSION BY BIOCHEMICAL AND MORPHOLOGICAL PARAMETERS

A number of the biochemical and morphological parameters that have been proposed for evaluation of the degree of liver fibrosis have been analyzed for their potential in use for the evaluation of portal hypertension and/or the presence of EV[11]. Even if there is a broad correlation between these indices and portal pressure or the presence of EV, confirming the role of liver fibrosis in the genesis of this condition, it is a fact that the low coefficients of the correlations do not support clinical use of these parameters for this purpose. Various indices have also been proposed[3,12,13]. The platelet count/spleen diameter ratio (Plt/Spl) was reported to be independently associated with the presence of EV, as shown in a multivariate analysis. A Plt/Spl cut-off value of 909 had 100% negative predictive value for diagnosis of EV[14,15]. Another study determined that this parameter is also related to the presence of portal hypertension[16]. A model combining albumin, aspartate aminotransferase (AST) and the international normalized ratio (INR) had an area under the receiver operating characteristic curve (AUROC) of 0.952 for prediction of CSPH in a group of patients with compensated cirrhosis[17].

In a study by Sebastiani *et al*[18], a combination of the Lok index (an index derived by AST and alanine aminotransferase (ALT) levels, platelet counts and prothrombin time (PT)-INR; using a cut-off of 1.5) and the Forns' index (an index derived by age, platelet counts, gamma-glutamyl transferase (GGT) and cholesterol; using a cut-off of 8.8) had an AUROC of 0.80 (95%CI: 0.76-0.84) and a high negative predictive value (> 90%) for excluding clinically-relevant EV[18].

Overall, the results for the proposed parameters and indices have not been satisfactory[3]. Serum markers may be useful as a first-line tool to identify cirrhotic patients in whom the risk of clinically-relevant EV is trivial[17]. However, the possibility of replacing upper endoscopy with simple serum non-invasive markers is still not practical for the vast majority of patients[3,11,13,17,18]. Accordingly, the biochemical/morphological tests may be of help to diagnose patients with suspected CSPH, but not to estimate the degree of portal pressure. These tests do not allow for clinical decisions on their own, nor can they be used alone in a clinical context; although, they may be sufficient in use as a first-level test[19] (laboratory tests require no clinical skillfulness, distinctive from Doppler ultrasound and measurement of tissue stiffness), but their use would not exempt a clinician from undertaking further analysis with more accurate tests.

ESTIMATION OF PORTAL HYPERTENSION BY DOPPLER ULTRASOUND TECHNIQUES

The introduction of ultrasound and Doppler techniques generated great expectations in the 1990s for non-invasive assessment of portal hypertension. Doppler ultrasound evaluation was a major step forward in the clinical evaluation of patients with portal hypertension. Indeed, many parameters indicating the presence of portal hypertension could be identified non-invasively, including the presence of collateral vessels, spleen enlargement, ascites, change in the portal vein parameters (*e.g.* increase in diameter, disappearance of caliper variation during respiration, decrease in blood flow velocity, increase in the congestion index), increase in hepatic and splenic arterial resistance indices, and decrease in the damping index of hepatic veins[5,12,20-22]. Scores obtained by the combinations of measuring portal vein blood velocity, portal vein diameter, the hepatic artery resistance index and splenic artery resistance index[4,12,23-26] were proposed and demonstrated to be useful in the clinical monitoring of patients with cirrhosis and portal hypertension.

Some of these parameters, such as the presence of collateral circulation in patients with cirrhosis, can be considered as having a specificity of 100% for the diagnosis of CSPH[2]; although, all of these parameters have low diagnostic sensitivity for identifying the condition.

In patients with known cirrhosis, Doppler ultrasound has > 80% specificity for diagnosis of CSPH, but sensitivity does not exceed 40%-70%, particularly in compensated patients[5]. Therefore, while the presence of one or more Doppler ultrasound signs can

establish the presence of CSPH, their absence cannot exclude it. Moreover, Doppler ultrasound is not useful for evaluating the effect of pharmacological therapy on portal hypertension, as vasoactive drugs used in the therapy of portal hypertension modify Doppler parameters (*i.e.*, vascular blood velocity, resistance indices) *per se*, in a manner independent of the final modification of portal pressure.

On the contrary, Doppler parameters may have major utility in the evaluation of the effect of surgical therapy and of liver transplantation on portal hypertension. Indeed, in these conditions, normalization of portal hemodynamics and of splenic Doppler resistance indices has been proposed as confirmatory for having achieved a good resolution of portal hypertension after surgery[27].

Thus, although indispensable in the evaluation and monitoring of patients with cirrhosis and portal hypertension, Doppler ultrasonography cannot be used on its own as a screening method to exclude CSPH, nor as a method for monitoring portal pressure over time. Doppler ultrasound, however, does detect signs, such as portal-collateral circulation, ascites and portal vein thrombosis, that, if present, allow for a certain diagnosis of CSPH.

Color Doppler ultrasonography is a useful non-invasive modality for assessing gastric, duodenal and rectal varices[28-31]. Contrast-enhanced ultrasonography analysis of transit time of microbubble contrast agent through the liver has demonstrated that a decrease in the transit time between the hepatic vein and the hepatic artery or the portal vein (a sign of porto-hepatic shunting) is related to the degree of portal hypertension in cirrhosis[12,21,32]. Moreover, a relation has been reported for the presence of portal hypertension and a number of other parameters derived by the analysis of time-intensity curves of contrast agent in the various liver structures; these parameters include regional hepatic perfusion[33], portal vein/hepatic artery strength ratio, area under the portal vein/hepatic artery time-intensity curve ratio, and portal vein/hepatic artery wash-in perfusion slope ratio[34]. Unfortunately, most of the correlations reported between these parameters and portal pressure are weak, indicating that they cannot predict the presence of CSPH in single patients with sufficient accuracy.

Recently, a new non-invasive approach to quantify portal pressure has been proposed that is based upon subharmonic emission from ultrasound contrast agent[35,36]. The changes of subharmonic signal amplitude are reported as correlating with portal pressure changes[36]; moreover, Eisenbrey *et al*[37] demonstrated that subharmonic-aided pressure estimation (SHAPE) was in good overall agreement with HVPG (r = 0.82). This method seems promising and deserves further study.

ESTIMATION OF PORTAL PRESSURE BY THE MEASUREMENT OF LIVER STIFFNESS

Another important advancement in the non-invasive assessment of portal hypertension has been the introduction of non-invasive measurement of liver stiffness (LS) by transient elastography (TE). Originally proposed and designed as a non-invasive approach for detecting the presence of fibrosis in the liver, after initial doubts, the method has gradually imposed itself as a routine method used in the clinical evaluation of patients with chronic liver disease. TE has proven sensitive for estimating the absence of liver fibrosis or the presence of high-degree liver fibrosis, yet patients with moderate fibrosis remain more difficult to assess[26]. TE has also been shown to be related to the degree of portal pressure[10,38,39]. Such a correlation is somewhat expected because liver fibrosis is the first and main determinant both of tissue stiffness and of intrahepatic resistance to portal blood flow[3]. LS can increase independently of fibrosis due to food ingestion, inflammation, cholestasis and liver congestion[3]. Even with the limitations cited above, a number of studies have demonstrated that the related method allows not only for estimation of liver fibrosis but also determination of CSPH presence[13,40,41]. In patients with chronic liver disease, LS can predict CSPH (HVPG $\geqslant$ 10 mmHg) with a very high accuracy, having an AUROC of 0.945 (95%CI: 0.904-0.987); when the cut-off value was set at 21 kPa, this procedure accurately predicted CSPH in 92% of the patients for whom LS was successful[40]. Lemoine *et al*[41] confirmed that LS can predict CSPH, but highlighted that the cut-off is higher, with a better performance, in alcoholic patients; in particular, the AUROC for diagnosis of CSPH was reported as 0.76 ± 0.07 in patients with hepatitis C virus (HCV) infection (best cut-off at 20.5 kPa) and 0.94 ± 0.03 (best cut-off at 34.9 kPa) in alcoholic patients[41].

These results justify the proposal to use this method in clinical practice for identifying patients with CSPH[5]. Therefore, TE can be used as a screening method for CSPH in patients with compensated liver cirrhosis[2,5].

Vizzutti *et al*[39] showed that the correlation between LS and portal pressure in cirrhosis is very good up to 10-12 mmHg, while it is substantially lacking for higher values. This finding has been explained by the fact that - while in the early stages of the disease the main factor determining portal hypertension is liver fibrosis, therefore it is well related to portal pressure - once CSPH is established, the progression of portal hypertension depends not only on liver fibrosis but also on other factors, especially those related to the hyperdynamic circulation, the splanchnic vasodilatation and the resistance in portosystemic collaterals[42-44].

Unfortunately, these factors are not estimated by LS[10,16,39].

According to the collective data, TE can be very useful for ruling out or ruling in CSPH[5]; however, the technique is not accurate enough to replace HVPG in quantifying the exact severity of portal hypertension[5]. Furthermore, TE is unlikely to be useful in monitoring hemodynamic response to drug therapy, the effect of which is mediated primarily by decreases in splanchnic blood flow and partially by modifications in hepatic and collateral resistance, and not by improvements in hepatic fibrosis and LS[38].

LS has been demonstrated to be as effective as HVPG for predicting clinical decompensation and portal hypertension-related complications in patients with chronic liver disease[45]. The usefulness of LS in predicting portal hypertensive complications was confirmed by Kitson *et al*[46].

In recent years, additional techniques have been proposed for the evaluation of LS, each of which appear to overcome some of the limitations presented by traditional TE[47,48]; these include acoustic radiation force impulse imaging (ARFI) and shear-wave velocity estimation. In particular, the real-time shear-wave elastography (SWE) allows for real-time viewing of the area under investigation, contrary to TE which is done blindly, as well as integration of the assessment of TE with traditional ultrasound and Doppler[1,12,21,47,49-51]. In this context, it may be possible to integrate the measurement of LS with Doppler ultrasound parameters and, therefore, improve the accuracy of portal hypertension evaluation. The reported technical success rate of SWE is significantly better than that of TE[52]. Choi *et al*[53] also proposed that non-invasive measurement of LS by SWE may be useful for monitoring efficacy of the medical therapy of portal hypertension.

ESTIMATION OF PORTAL HYPERTENSION BY THE MEASUREMENT OF SPLEEN STIFFNESS

A very noteworthy advancement in this field is the application of non-invasive evaluation of parenchymal stiffness (*via* TE, ARFI and SWE) in the spleen[16,54,55]. An interesting study[16] showed that in patients with HCV-related cirrhosis, there is a very good correlation between HVPG and spleen stiffness (SS) (r^2 = 0.78), with a correlation that is maintained even when portal pressure is > 10 mmHg, which contrasts with LS. This study suggests that SS increases in close parallel with the progression of portal hypertension from the early to the late stages of cirrhosis[16].

Similarly to LS, SS measurement has also been reported as useful for predicting of clinical complications in compensated cirrhosis[56]. In patients with HCV-related cirrhosis, a SS and model for end-stage liver disease (MELD) predictive model represented an accurate predictor of clinical decompensation, with accuracy at least equivalent to that of HVPG[56]. A value for SS of < 54 kPa ruled out the risk of complications in the subsequent 2 years[56]. SS has been shown to decrease after orthotopic liver transplantation, when portal hypertension is resolved[57]. This is a behavior similar to splenic resistance indices[27].

Although not all of the subsequent studies yielded such reassuring results[51,52], this study highlighted that spleen parameters probably reflect the levels of portal hypertension more accurately, due to the peculiar modifications that occur in the spleen during portal hypertension as a result of congestion and hyperplasia[58]. In cirrhosis, splenomegaly is not only due to passive congestion but also to tissue hyperplasia, and is characterized by a combination of angiogenesis, fibrogenesis, and enlargement and hyperactivation of the splenic lymphoid compartment[16,58]. This condition of hyperplasia, with increased flow, participates in the hyperdynamic circulatory syndrome of portal hypertension[58].

Stiffness and hemodynamics of the spleen are probably sensitive sensors of portal pressure and of portal vein resistance. Therefore, it seems that the next route to follow will be the combination of SS with the Doppler splenic resistance indices, and possibly platelet count and spleen size. Indeed, individually, these parameters have shown better accuracy in the prediction of portal hypertension. SS is probably related to splenic congestion due to portal hypertension in an organ with a rigid capsule. The platelet count/spleen diameter ratio is probably the simplest index for determining the presence of portal hypertension and EV[14-16,59,60]. Doppler splenic resistance indices are related to portal blood flow resistance and to HVPG[22,24].

As evidence for the central role of splenic hemodynamics in portal hypertension, a few studies have shown the usefulness of combining the value of LS with splenic parameters to improve the identification of patients with portal hypertension. Among these parameters are the LS-spleen diameter to platelet ratio score (LSPS)[13,16,59] and the portal hypertension risk score, the latter of which combines LS, sex and spleen diameter/platelet count ratio[61]. This portal hypertension risk score had the highest AUROC value (0.935), as compared with LS alone or LSPS, for identifying patients with CSPH.

LIMITATIONS OF LS AND SS MEASUREMENT

A limitation of this method is the significant number of patients for whom the measurement of LS and/or SS could not be completed or yielded unreliable results. Reportedly, valid measurement of LS is not obtained

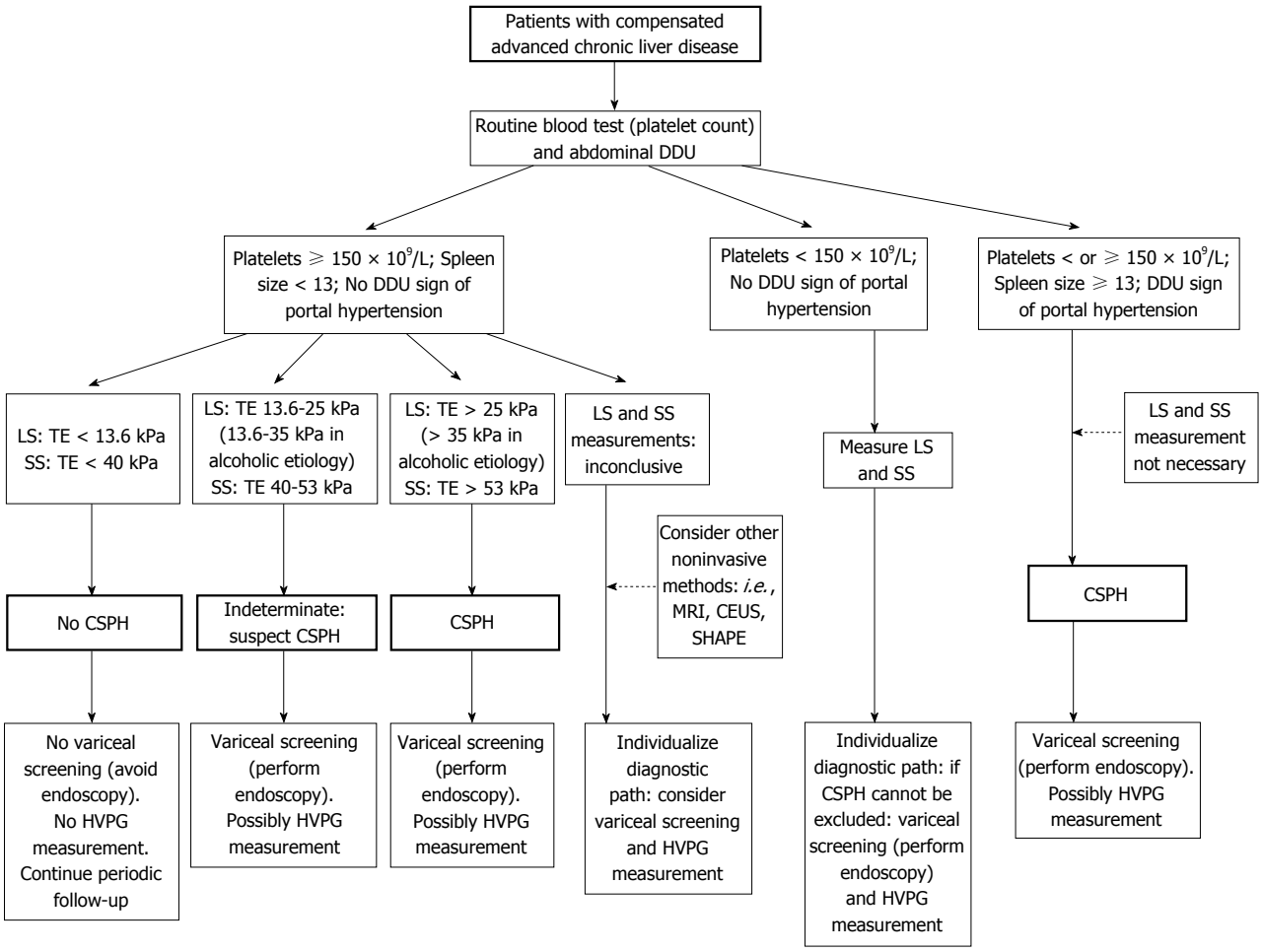


Figure 1 Hypothetical algorithm of non-invasive methods for screening and evaluation of clinically-significant portal hypertension and to discriminate patients with or without a need for endoscopic screening. CEUS: Contrast-enhanced ultrasonography; CSPH: Clinically-significant portal hypertension; DDU: Duplex Doppler ultrasonography; HVPG: Hepatic venous pressure gradient; LS: Liver stiffness; MRI: Magnetic resonance imaging; SHAPE: Subharmonic-aided pressure estimation; SS: Spleen stiffness; TE: Transient elastography.

in approximately 20% of patients[16,19,47]. TE cannot be performed in patients with ascites, and the failure rate of TE is generally higher in obese patients[47]. Aminotransferase flares, food intake, extrahepatic cholestasis, steatosis, increased central venous pressure and the use of beta-blockers can influence the accuracy of LS assessment by TE[36,62].

Moreover, LS and SS measurement are considered reliable for estimating portal hypertension only when the coefficient of variation among the successful measurements in a single patient is low[51,52]. In the study by Elkrief *et al*[52], the designation of excellent accuracy (*i.e.*, patients with variation coefficient of TE measurement < 10%) was achieved in < 50% of the patients. Procopet *et al*[51] proposed that SWE measurement of LS can be considered "highly reliable" only when measurements have a coefficient of variation < 10% and a depth of measurement < 5.6 cm; when these criteria are fulfilled, the rate of patients considered well-classified for the presence or absence of CSPH is close to 100%.

MAGNETIC RESONANCE IMAGING AND COMPUTED TOMOGRAPHY

Other methods have been proposed for non-invasive assessment of LS related to portal hypertension, namely magnetic resonance elastography, quantitative magnetic resonance imaging and computed tomography (CT). Although very interesting, at present these methods cannot be recommended as routine for measuring LS and SS. Magnetic resonance elastography can decompose tissue viscoelastic parameters into different components, including stiffness, elasticity and viscosity, allowing for better differentiation of fibrosis from congestion[63]. CT has the hypothetical capacity to assess portal pressure by using computational fluid dynamic modeling[64], and has already been proposed for use in evaluation of the fractional flow coronary reserve[65]. While magnetic resonance techniques are very promising[66,67], they are too expensive and the use of CT also seems impractical due to the high cost and the time-consuming nature of

the computational fluid dynamic modeling.

NON-INVASIVE ESTIMATION OF THE PRESENCE OF EV

A number of studies have shown that LS and SS would also be able to identify, with acceptable accuracy, patients with EV at risk of bleeding[16,59-61,68]. The findings of these studies, however, have been contradicted by other research groups[13,52,69]. The identification of patients with risk of bleeding from EV may be better with the measurement of SS[16,70,71], and particularly as related to LS[16,60].

Considering that the measurement of LS and SS can be considered a good method to identify patients with CSPH, and that EV develops only in the presence of CSPH, it may be reasonable to propose the measurement of LS and SS as a screening method for identifying chronic liver disease patients with HVPG < 10 mmHg (these patients should not have EV). Also, it is important to note that CSPH is a necessary, but not sufficient, condition for development of EV[19]. Therefore, measurement of LS and SS can exclude the need for a screening esophagogastroduodenoscopy, but cannot identify who among the patients with CSPH is at risk of esophageal bleeding[1,59,72].

According to the Baveno VI criteria, TE and platelet count may be used to discriminate such patients, without the need for screening varices[2]. On the other hand, imaging analyses have shown that collateral circulation is sufficient for ruling-in CSPH in patients with compensated advanced chronic liver diseases of all etiologies[2].

A hypothetical algorithm of non-invasive methods for screening and evaluation of CSPH and to discriminate patients with or without a need for screening varices is presented in Figure 1.

CONCLUSION

According to the data published to date in the publicly available literature, it appears possible to rule-out CSPH with a clinically-acceptable accuracy through the combination of LS and SS measurements[10,16,50,59,73] along with Doppler ultrasound evaluation. It is probable that the combination of these methods may also allow for the identification of patients with the most serious degree of portal hypertension. Indeed, progress is being made in this field.

To conclude, however, advancement in the non-invasive evaluation of portal hypertension has included the introduction of methods to clinical practice that are able to measure stiffness in the liver and stiffness/congestion in the spleen. These methods, combined with Doppler ultrasound evaluation, allow for the identification of patients without CSPH. They are also promising for their ability to estimate the degree of portal pressure in patients with CSPH.

REFERENCES

1 **Castéra L**, García-Tsao G. When the spleen gets tough, the varices get going. *Gastroenterology* 2013; **144**: 19-22 [PMID: 23164570 DOI: 10.1053/j.gastro.2012.11.015]

2 **de Franchis R**. Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol* 2015; **63**: 743-752 [PMID: 26047908 DOI: 10.1016/j.jhep.2015.05.022]

3 **Castera L**, Pinzani M, Bosch J. Non invasive evaluation of portal hypertension using transient elastography. *J Hepatol* 2012; **56**: 696-703 [PMID: 21767510 DOI: 10.1016/j.jhep.2011.07.005]

4 **Stefanescu H**, Procopet B. Noninvasive assessment of portal hypertension in cirrhosis: liver stiffness and beyond. *World J Gastroenterol* 2014; **20**: 16811-16819 [PMID: 25492995 DOI: 10.3748/wjg.v20.i45.16811]

5 **Berzigotti A**, Seijo S, Reverter E, Bosch J. Assessing portal hypertension in liver diseases. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 141-155 [PMID: 23363263 DOI: 10.1586/egh.12.83]

6 **Procopeţ B**, Tantau M, Bureau C. Are there any alternative methods to hepatic venous pressure gradient in portal hypertension assessment? *J Gastrointestin Liver Dis* 2013; **22**: 73-78 [PMID: 23539394]

7 **Bosch J**, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 573-582 [PMID: 19724251 DOI: 10.1038/nrgastro.2009.149]

8 **Merkel C**, Bolognesi M, Berzigotti A, Amodio P, Cavasin L, Casarotto IM, Zoli M, Gatta A. Clinical significance of worsening portal hypertension during long-term medical treatment in patients with cirrhosis who had been classified as early good-responders on haemodynamic criteria. *J Hepatol* 2010; **52**: 45-53 [PMID: 19914730 DOI: 10.1016/j.jhep.2009.10.014]

9 **Maleux G**, Willems E, Fieuws S, Heye S, Vaninbroukx J, Laleman W, Cassiman D, Verslype C, Nevens F. Prospective study comparing different indirect methods to measure portal pressure. *J Vasc Interv Radiol* 2011; **22**: 1553-1558 [PMID: 22024115 DOI: 10.1016/j.jvir.2011.08.003]

10 **Jeong SW**. Liver stiffness measurement: is it a non-invasive substitution for HVPG? *Clin Mol Hepatol* 2013; **19**: 367-369 [PMID: 24459640 DOI: 10.3350/cmh.2013.19.4.367]

11 **Deng H**, Qi X, Guo X. Diagnostic Accuracy of APRI, AAR, FIB-4, FI, King, Lok, Forns, and FibroIndex Scores in Predicting the Presence of Esophageal Varices in Liver Cirrhosis: A Systematic Review and Meta-Analysis. *Medicine* (Baltimore) 2015; **94**: e1795 [PMID: 26496312 DOI: 10.1097/MD.0000000000001795]

12 **Colecchia A**, Marasco G, Taddia M, Montrone L, Eusebi LH, Mandolesi D, Schiumerini R, Di Biase AR, Festi D. Liver and spleen stiffness and other noninvasive methods to assess portal hypertension in cirrhotic patients: a review of the literature. *Eur J Gastroenterol Hepatol* 2015; **27**: 992-1001 [PMID: 26020376 DOI: 10.1097/MEG.0000000000000393]

13 **Cho EJ**, Kim MY, Lee JH, Lee IY, Lim YL, Choi DH, Kim YJ, Yoon JH, Baik SK. Diagnostic and Prognostic Values of Noninvasive Predictors of Portal Hypertension in Patients with Alcoholic Cirrhosis. *PLoS One* 2015; **10**: e0133935 [PMID: 26196942 DOI: 10.1371/journal.pone.0133935]

14 **Giannini E**, Botta F, Borro P, Risso D, Romagnoli P, Fasoli A, Mele MR, Testa E, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. *Gut* 2003; **52**: 1200-1205 [PMID: 12865282]

15 **Giannini EG**, Zaman A, Kreil A, Floreani A, Dulbecco P, Testa E, Sohaey R, Verhey P, Peck-Radosavljevic M, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices: results of a multicenter, prospective, validation study. *Am J Gastroenterol* 2006; **101**: 2511-2519 [PMID: 17029607 DOI: 10.1111/j.1572-0241.2006.00874.x]

16 **Colecchia A**, Montrone L, Scaioli E, Bacchi-Reggiani ML, Colli

A, Casazza G, Schiumerini R, Turco L, Di Biase AR, Mazzella G, Marzi L, Arena U, Pinzani M, Festi D. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 2012; **143**: 646-654 [PMID: 22643348 DOI: 10.1053/j.gastro.2012.05.035]

17 **Berzigotti A**, Gilabert R, Abraldes JG, Nicolau C, Bru C, Bosch J, García-Pagan JC. Noninvasive prediction of clinically significant portal hypertension and esophageal varices in patients with compensated liver cirrhosis. *Am J Gastroenterol* 2008; **103**: 1159-1167 [PMID: 18477345 DOI: 10.1111/j.1572-0241.2008.01826.x]

18 **Sebastiani G**, Tempesta D, Fattovich G, Castera L, Halfon P, Bourliere M, Noventa F, Angeli P, Saggioro A, Alberti A. Prediction of oesophageal varices in hepatic cirrhosis by simple serum non-invasive markers: Results of a multicenter, large-scale study. *J Hepatol* 2010; **53**: 630-638 [PMID: 20615567 DOI: 10.1016/j.jhep.2010.04.019]

19 **Augustin S**, Millán L, González A, Martell M, Gelabert A, Segarra A, Serres X, Esteban R, Genescà J. Detection of early portal hypertension with routine data and liver stiffness in patients with asymptomatic liver disease: a prospective study. *J Hepatol* 2014; **60**: 561-569 [PMID: 24211744 DOI: 10.1016/j.jhep.2013.10.027]

20 **Bolognesi M**, Sacerdoti D, Merkel C, Gerunda G, Maffei-Faccioli A, Angeli P, Jemmolo RM, Bombonato G, Gatta A. Splenic Doppler impedance indices: influence of different portal hemodynamic conditions. *Hepatology* 1996; **23**: 1035-1040 [PMID: 8621130 DOI: 10.1002/hep.510230515]

21 **Kim MY**, Jeong WK, Baik SK. Invasive and non-invasive diagnosis of cirrhosis and portal hypertension. *World J Gastroenterol* 2014; **20**: 4300-4315 [PMID: 24764667 DOI: 10.3748/wjg.v20.i15.4300]

22 **Lee CM**, Jeong WK, Lim S, Kim Y, Kim J, Kim TY, Sohn JH. Diagnosis of Clinically Significant Portal Hypertension in Patients with Cirrhosis: Splenic Arterial Resistive Index versus Liver Stiffness Measurement. *Ultrasound Med Biol* 2016; **42**: 1312-1320 [PMID: 27045219 DOI: 10.1016/j.ultrasmedbio.2016.01.026]

23 **Iwao T**, Toyonaga A, Oho K, Tayama C, Masumoto H, Sakai T, Sato M, Tanikawa K. Value of Doppler ultrasound parameters of portal vein and hepatic artery in the diagnosis of cirrhosis and portal hypertension. *Am J Gastroenterol* 1997; **92**: 1012-1017 [PMID: 9177521]

24 **Bolognesi M**, Sacerdoti D, Merkel C, Bombonato G, Gatta A. Noninvasive grading of the severity of portal hypertension in cirrhotic patients by echo-color-Doppler. *Ultrasound Med Biol* 2001; **27**: 901-907 [PMID: 11476922]

25 **Piscaglia F**, Donati G, Serra C, Muratori R, Solmi L, Gaiani S, Gramantieri L, Bolondi L. Value of splanchnic Doppler ultrasound in the diagnosis of portal hypertension. *Ultrasound Med Biol* 2001; **27**: 893-899 [PMID: 11476921]

26 **Zardi EM**, Di Matteo FM, Pacella CM, Sanyal AJ. Invasive and non-invasive techniques for detecting portal hypertension and predicting variceal bleeding in cirrhosis: a review. *Ann Med* 2014; **46**: 8-17 [PMID: 24328372 DOI: 10.3109/07853890.2013.857831]

27 **Bolognesi M**, Sacerdoti D, Bombonato G, Merkel C, Sartori G, Merenda R, Nava V, Angeli P, Feltracco P, Gatta A. Change in portal flow after liver transplantation: effect on hepatic arterial resistance indices and role of spleen size. *Hepatology* 2002; **35**: 601-608 [PMID: 11870373 DOI: 10.1053/jhep.2002.31352]

28 **Komatsuda T**, Ishida H, Konno K, Hamashima Y, Ohnami Y, Naganuma H, Asanuma Y, Masamune O. Color Doppler findings of gastrointestinal varices. *Abdom Imaging* 1998; **23**: 45-50 [PMID: 9437062]

29 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Suga T. Color Doppler findings of gastric varices compared with findings on computed tomography. *J Gastroenterol* 2002; **37**: 604-610 [PMID: 12203075 DOI: 10.1007/s005350200096]

30 **Sato T**, Yamazaki K, Toyota J, Karino Y, Ohmura T, Akaike J. Diagnosis of rectal varices via color Doppler ultrasonography. *Am J Gastroenterol* 2007; **102**: 2253-2258 [PMID: 17561969 DOI: 10.1111/j.1572-0241.2007.01340.x]

31 **Sato T**, Yamazaki K, Akaike J. Diagnosis of gastric varices and evaluation of the effectiveness of treatment using transabdominal color Doppler ultrasonography. *J Ultrasound Med* 2009; **28**: 1125-1131 [PMID: 19710209]

32 **Maruyama H**, Shiha G, Yokosuka O, Kumar A, Sharma BC, Ibrahim A, Saraswat V, Lesmana CR, Omata M. Non-invasive assessment of portal hypertension and liver fibrosis using contrast-enhanced ultrasonography. *Hepatol Int* 2016; **10**: 267-276 [PMID: 26696585 DOI: 10.1007/s12072-015-9670-9]

33 **Berzigotti A**, Nicolau C, Bellot P, Abraldes JG, Gilabert R, García-Pagan JC, Bosch J. Evaluation of regional hepatic perfusion (RHP) by contrast-enhanced ultrasound in patients with cirrhosis. *J Hepatol* 2011; **55**: 307-314 [PMID: 21167236 DOI: 10.1016/j.jhep.2010.10.038]

34 **Qu EZ**, Zhang YC, Li ZY, Liu Y, Wang JR. Contrast-enhanced sonography for quantitative assessment of portal hypertension in patients with liver cirrhosis. *J Ultrasound Med* 2014; **33**: 1971-1977 [PMID: 25336484 DOI: 10.7863/ultra.33.11.1971]

35 **Dave JK**, Halldorsdottir VG, Eisenbrey JR, Raichlen JS, Liu JB, McDonald ME, Dickie K, Wang S, Leung C, Forsberg F. Noninvasive LV pressure estimation using subharmonic emissions from microbubbles. *JACC Cardiovasc Imaging* 2012; **5**: 87-92 [PMID: 22239898 DOI: 10.1016/j.jcmg.2011.08.017]

36 **Dave JK**, Halldorsdottir VG, Eisenbrey JR, Merton DA, Liu JB, Zhou JH, Wang HK, Park S, Dianis S, Chalek CL, Lin F, Thomenius KE, Brown DB, Forsberg F. Investigating the efficacy of subharmonic aided pressure estimation for portal vein pressures and portal hypertension monitoring. *Ultrasound Med Biol* 2012; **38**: 1784-1798 [PMID: 22920550 DOI: 10.1016/j.ultrasmedbio.2012.06.013]

37 **Eisenbrey JR**, Dave JK, Halldorsdottir VG, Merton DA, Miller C, Gonzalez JM, Machado P, Park S, Dianis S, Chalek CL, Kim CE, Baliff JP, Thomenius KE, Brown DB, Navarro V, Forsberg F. Chronic liver disease: noninvasive subharmonic aided pressure estimation of hepatic venous pressure gradient. *Radiology* 2013; **268**: 581-588 [PMID: 23525208 DOI: 10.1148/radiol.13121769]

38 **Carrión JA**, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006; **12**: 1791-1798 [PMID: 16823833 DOI: 10.1002/lt.20857]

39 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]

40 **Bureau C**, Metivier S, Peron JM, Selves J, Robic MA, Gourraud PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. *Aliment Pharmacol Ther* 2008; **27**: 1261-1268 [PMID: 18397389 DOI: 10.1111/j.1365-2036.2008.03701.x]

41 **Lemoine M**, Katsahian S, Ziol M, Nahon P, Ganne-Carrie N, Kazemi F, Grando-Lemaire V, Trinchet JC, Beaugrand M. Liver stiffness measurement as a predictive tool of clinically significant portal hypertension in patients with compensated hepatitis C virus or alcohol-related cirrhosis. *Aliment Pharmacol Ther* 2008; **28**: 1102-1110 [PMID: 18691352 DOI: 10.1111/j.1365-2036.2008.03825.x]

42 **Bolognesi M**, Di Pascoli M, Verardo A, Gatta A. Splanchnic vasodilation and hyperdynamic circulatory syndrome in cirrhosis. *World J Gastroenterol* 2014; **20**: 2555-2563 [PMID: 24627591 DOI: 10.3748/wjg.v20.i10.2555]

43 **Sacerdoti D**, Pesce P, Di Pascoli M, Brocco S, Cecchetto L, Bolognesi M. Arachidonic acid metabolites and endothelial dysfunction of portal hypertension. *Prostaglandins Other Lipid Mediat* 2015; **120**: 80-90 [PMID: 26072731 DOI: 10.1016/j.prostaglandins.2015.05.008]

44 **Di Pascoli M**, Zampieri F, Verardo A, Pesce P, Turato C, Angeli P, Sacerdoti D, Bolognesi M. Inhibition of epoxyeicosatrienoic acid production in rats with cirrhosis has beneficial effects on portal hypertension by reducing splanchnic vasodilation. *Hepatology* 2016; **64**: 923-930 [PMID: 27312119 DOI: 10.1002/hep.28686]

45 **Robic MA**, Procopet B, Métivier S, Péron JM, Selves J, Vinel JP, Bureau C. Liver stiffness accurately predicts portal hypertension related complications in patients with chronic liver disease: a prospective study. *J Hepatol* 2011; **55**: 1017-1024 [PMID: 21354450 DOI: 10.1016/j.jhep.2011.01.051]

46 **Kitson MT**, Roberts SK, Colman JC, Paul E, Button P, Kemp W. Liver stiffness and the prediction of clinically significant portal hypertension and portal hypertensive complications. *Scand J Gastroenterol* 2015; **50**: 462-469 [PMID: 25623641 DOI: 10.3109/00365521.2014.964758]

47 **Piscaglia F**, Marinelli S, Bota S, Serra C, Venerandi L, Leoni S, Salvatore V. The role of ultrasound elastographic techniques in chronic liver disease: current status and future perspectives. *Eur J Radiol* 2014; **83**: 450-455 [PMID: 23891139 DOI: 10.1016/j.ejrad.2013.06.009]

48 **Piscaglia F**, Salvatore V, Mulazzani L, Cantisani V, Schiavone C. Ultrasound Shear Wave Elastography for Liver Disease. A Critical Appraisal of the Many Actors on the Stage. *Ultraschall Med* 2016; **37**: 1-5 [PMID: 26871407 DOI: 10.1055/s-0035-1567037]

49 **Kim TY**, Jeong WK, Sohn JH, Kim J, Kim MY, Kim Y. Evaluation of portal hypertension by real-time shear wave elastography in cirrhotic patients. *Liver Int* 2015; **35**: 2416-2424 [PMID: 25875718 DOI: 10.1111/liv.12846]

50 **Şirli R**, Sporea I, Popescu A, Dănilă M. Ultrasound-based elastography for the diagnosis of portal hypertension in cirrhotics. *World J Gastroenterol* 2015; **21**: 11542-11551 [PMID: 26556985 DOI: 10.3748/wjg.v21.i41.11542]

51 **Procopet B**, Berzigotti A, Abraldes JG, Turon F, Hernandez-Gea V, García-Pagán JC, Bosch J. Real-time shear-wave elastography: applicability, reliability and accuracy for clinically significant portal hypertension. *J Hepatol* 2015; **62**: 1068-1075 [PMID: 25514554 DOI: 10.1016/j.jhep.2014.12.007]

52 **Elkrief L**, Rautou PE, Ronot M, Lambert S, Dioguardi Burgio M, Francoz C, Plessier A, Durand F, Valla D, Lebrec D, Vilgrain V, Castéra L. Prospective comparison of spleen and liver stiffness by using shear-wave and transient elastography for detection of portal hypertension in cirrhosis. *Radiology* 2015; **275**: 589-598 [PMID: 25469784 DOI: 10.1148/radiol.14141210]

53 **Choi SY**, Jeong WK, Kim Y, Kim J, Kim TY, Sohn JH. Shear-wave elastography: a noninvasive tool for monitoring changing hepatic venous pressure gradients in patients with cirrhosis. *Radiology* 2014; **273**: 917-926 [PMID: 25025464 DOI: 10.1148/radiol.14140008]

54 **Attia D**, Schoenemeier B, Rodt T, Negm AA, Lenzen H, Lankisch TO, Manns M, Gebel M, Potthoff A. Evaluation of Liver and Spleen Stiffness with Acoustic Radiation Force Impulse Quantification Elastography for Diagnosing Clinically Significant Portal Hypertension. *Ultraschall Med* 2015; **36**: 603-610 [PMID: 26565516 DOI: 10.1055/s-0041-107971]

55 **Takuma Y**, Nouso K, Morimoto Y, Tomokuni J, Sahara A, Takabatake H, Matsueda K, Yamamoto H. Portal Hypertension in Patients with Liver Cirrhosis: Diagnostic Accuracy of Spleen Stiffness. *Radiology* 2016; **279**: 609-619 [PMID: 26588019 DOI: 10.1148/radiol.2015150690]

56 **Colecchia A**, Colli A, Casazza G, Mandolesi D, Schiumerini R, Reggiani LB, Marasco G, Taddia M, Lisotti A, Mazzella G, Di Biase AR, Golfieri R, Pinzani M, Festi D. Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study. *J Hepatol* 2014; **60**: 1158-1164 [PMID: 24607624 DOI: 10.1016/j.jhep.2014.02.024]

57 **Chin JL**, Chan G, Ryan JD, McCormick PA. Spleen stiffness can non-invasively assess resolution of portal hypertension after liver transplantation. *Liver Int* 2015; **35**: 518-523 [PMID: 25074281 DOI: 10.1111/liv.12647]

58 **Bolognesi M**, Merkel C, Sacerdoti D, Nava V, Gatta A. Role of spleen enlargement in cirrhosis with portal hypertension. *Dig Liver Dis* 2002; **34**: 144-150 [PMID: 11926560]

59 **Kim BK**, Han KH, Park JY, Ahn SH, Kim JK, Paik YH, Lee KS, Chon CY, Kim DY. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol* 2010; **105**: 1382-1390 [PMID: 20087336 DOI: 10.1038/ajg.2009.750]

60 **Calvaruso V**, Bronte F, Conte E, Simone F, Craxì A, Di Marco V. Modified spleen stiffness measurement by transient elastography is associated with presence of large oesophageal varices in patients with compensated hepatitis C virus cirrhosis. *J Viral Hepat* 2013; **20**: 867-874 [PMID: 24304456 DOI: 10.1111/jvh.12114]

61 **Berzigotti A**, Seijo S, Arena U, Abraldes JG, Vizzutti F, García-Pagán JC, Pinzani M, Bosch J. Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis. *Gastroenterology* 2013; **144**: 102-111.e1 [PMID: 23058320 DOI: 10.1053/j.gastro.2012.10.001]

62 **Petta S**, Maida M, Macaluso FS, Di Marco V, Cammà C, Cabibi D, Craxì A. The severity of steatosis influences liver stiffness measurement in patients with nonalcoholic fatty liver disease. *Hepatology* 2015; **62**: 1101-1110 [PMID: 25991038 DOI: 10.1002/hep.27844]

63 **Ronot M**, Lambert S, Elkrief L, Doblas S, Rautou PE, Castera L, Vilgrain V, Sinkus R, Van Beers BE, Garteiser P. Assessment of portal hypertension and high-risk oesophageal varices with liver and spleen three-dimensional multifrequency MR elastography in liver cirrhosis. *Eur Radiol* 2014; **24**: 1394-1402 [PMID: 24626745 DOI: 10.1007/s00330-014-3124-y]

64 **Qi X**, Li Z, Huang J, Zhu Y, Liu H, Zhou F, Liu C, Xiao C, Dong J, Zhao Y, Xu M, Xing S, Xu W, Yang C. Virtual portal pressure gradient from anatomic CT angiography. *Gut* 2015; **64**: 1004-1005 [PMID: 25398771 DOI: 10.1136/gutjnl-2014-308543]

65 **Taylor CA**, Fonte TA, Min JK. Computational fluid dynamics applied to cardiac computed tomography for noninvasive quantification of fractional flow reserve: scientific basis. *J Am Coll Cardiol* 2013; **61**: 2233-2241 [PMID: 23562923 DOI: 10.1016/j.jacc.2012.11.083]

66 **Guo J**, Büning C, Schott E, Kröncke T, Braun J, Sack I, Althoff C. In vivo abdominal magnetic resonance elastography for the assessment of portal hypertension before and after transjugular intrahepatic portosystemic shunt implantation. *Invest Radiol* 2015; **50**: 347-351 [PMID: 25599282 DOI: 10.1097/RLI.0000000000000136]

67 **Palaniyappan N**, Cox E, Bradley C, Scott R, Austin A, O'Neill R, Ramjas G, Travis S, White H, Singh R, Thurley P, Guha IN, Francis S, Aithal GP. Non-invasive assessment of portal hypertension using quantitative magnetic resonance imaging. *J Hepatol* 2016; **65**: 1131-1139 [PMID: 27475617 DOI: 10.1016/j.jhep.2016.07.021]

68 **Kazemi F**, Kettaneh A, N'kontchou G, Pinto E, Ganne-Carrie N, Trinchet JC, Beaugrand M. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. *J Hepatol* 2006; **45**: 230-235 [PMID: 16797100 DOI: 10.1016/j.jhep.2006.04.006]

69 **Castéra L**, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, de Lédinghen V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. *J Hepatol* 2009; **50**: 59-68 [PMID: 19013661 DOI: 10.1016/j.jhep.2008.08.018]

70 **Takuma Y**, Nouso K, Morimoto Y, Tomokuni J, Sahara A, Toshikuni N, Takabatake H, Shimomura H, Doi A, Sakakibara I, Matsueda K, Yamamoto H. Measurement of spleen stiffness by acoustic radiation force impulse imaging identifies cirrhotic patients with esophageal varices. *Gastroenterology* 2013; **144**: 92-101.e2 [PMID: 23022955 DOI: 10.1053/j.gastro.2012.09.049]

71 **Takuma Y**, Nouso K, Morimoto Y, Tomokuni J, Sahara A, Takabatake H, Doi A, Matsueda K, Yamamoto H. Prediction of oesophageal variceal bleeding by measuring spleen stiffness in patients with liver cirrhosis. *Gut* 2016; **65**: 354-355 [PMID:

26006115 DOI: 10.1136/gutjnl-2015-309741]
72 **Shi KQ**, Fan YC, Pan ZZ, Lin XF, Liu WY, Chen YP, Zheng MH. Transient elastography: a meta-analysis of diagnostic accuracy in evaluation of portal hypertension in chronic liver disease. *Liver Int* 2013; **33**: 62-71 [PMID: 22973991 DOI: 10.1111/liv.12003]
73 **Bureau C**, Di Martino V, Calès P. A major new step in non-invasive evaluation of portal hypertension: elastography. *Liver Int* 2013; **33**: 4-6 [PMID: 23121438 DOI: 10.1111/liv.12008]

P- Reviewer: Fernandez-Rodriguez CM, Fierbinteanu-Braticevici C, La Mura V, Makisalo H, Maruyama H, Sato T, Tantau M
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.11

World J Gastroenterol 2017 January 7; 23(1): 11-24
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Fecal incontinence - Challenges and solutions

Nallely Saldana Ruiz, Andreas M Kaiser

Nallely Saldana Ruiz, Andreas M Kaiser, Department of Surgery, Division of Colorectal Surgery, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, United States

Author contributions: Kaiser AM developed concept; Saldana Ruiz N did literature search; Saldana Ruiz N and Kaiser AM analyzed published data, wrote/edited the paper.

Conflict-of-interest statement: There are not conflicting interests to report.

Manuscript source: Invited manuscript

Correspondence to: Andreas M Kaiser, MD, FACS, FASCRS, Professor of Clinical Surgery, Department of Surgery, Division of Colorectal Surgery, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Suite 7418, Los Angeles, CA 90033, United States. akaiser@usc.edu
Telephone: +1-323-8653690
Fax: +1-323-8653671

Received: August 28, 2016
Peer-review started: September 1, 2016
First decision: September 20, 2016
Revised: October 14, 2016
Accepted: December 8, 2016
Article in press: December 8, 2016
Published online: January 7, 2017

Abstract

Fecal incontinence is not a diagnosis but a frequent and debilitating common final pathway symptom resulting from numerous different causes. Incontinence not only impacts the patient's self-esteem and quality of life but may result in significant secondary morbidity, disability, and cost. Treatment is difficult without any panacea and an individualized approach should be chosen that frequently combines different modalities. Several new technologies have been developed and their specific roles will have to be defined. The scope of this review is outline the evaluation and treatment of patients with fecal incontinence.

Key words: Fecal incontinence; Sphincteroplasty; Sacral nerve stimulation; Endorectal ultrasound; New technologies; Quality of life

Core tip: Fecal incontinence is frequent, under-reported, and lacks a perfect treatment solution. Fecal control is not equivalent to normal sphincter muscles. Other factors such (*e.g.*, stool consistency, rectal reservoir function and elasticity are equally important. Incontinence is rather a symptom than a diagnosis, representing the common final pathway of various etiologies. Measurement of fecal incontinence remains subjective and based on patient reporting. Successful incontinence management combines a thorough understanding of contributing factors, workup and interpretation of individual results, tailoring of individual treatment plan. New technologies are abundant but not indicated for all patients, and objective results often less strong than advertised.

Saldana Ruiz N, Kaiser AM. Fecal incontinence - Challenges and solutions. *World J Gastroenterol* 2017; 23(1): 11-24 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/11.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.11

INTRODUCTION

Continence is one of our fundamental expectations

Table 1 Causes of fecal incontinence

Category	Details
Acquired structural abnormalities	Obstetric injury (vaginal delivery)
	Anorectal surgery (hemorrhoid, fistula, fissure, *etc.*)
	Rectal intussusception/prolapse
	Sphincter-sparing bowel resection
	Trauma (*e.g.*, pelvic fracture, Anal impalement)
Functional disorders	Chronic diarrhea
	Irritable bowel disease
	Inflammatory bowel disease
	Radiation proctitis
	Malabsorption
	Hypersecretory tumors
	Fecal impaction (paradoxical diarrhea)
	Physical disabilities
	Psychiatric disorder
Neurological disorders	Pudendal neuropathy (radiation, diabetes, chemotherapy)
	Spinal surgery
	Multiple sclerosis
	Dementia
	CNS disorder: stroke, trauma, tumor, infection
	Spina bifida
Congenital disorders	Imperforate anus
	Cloacal defect
	Spina bifida (myelomeningocele, meningocele)

and a basic element of quality of life. It reflects the confidence to have in place an adequate perception and control mechanism for stool and urine to allow for a conscious selection of the appropriate timing, location and privacy for voiding and moving the bowels. Continence is the result of a balanced interaction between the anal sphincter complex ("plug"), stool consistency, the rectal reservoir function, and neurological function. Disease processes or structural defects that alter any of these components can lead to the clinical symptom of fecal incontinence.

Fecal incontinence is defined as the involuntary loss of rectal contents (feces, gas) through the anal canal and the inability to postpone an evacuation until socially convenient. Attached to the definition are a time and age component to include a duration of the problem for at least one month and an age of at least 4 years with previously achieved control[1-3]. Depending on the presenting circumstances, fecal incontinence is commonly classified as (1) passive incontinence (involuntary discharge without any awareness); (2) urge incontinence (discharge despite active attempts to retain contents); and (3) fecal seepage (leakage of stool with grossly normal continence and evacuation)[2]. Fecal control is often thought to be synonymous with normal sphincter muscles; however other factors are equally important[4]. Hence, fecal incontinence has to be considered the common final pathway symptom of multiple independent etiologies (Table 1).

Consequences of incontinence (both fecal and urinary) are significant at different levels[4-7]: (1) The patients may develop secondary medical morbidities, such as skin maceration, urinary tract infections, decubitus ulcers, *etc*; (2) There are substantial direct and indirect financial expenses to the patients (*e.g.*, diapers, clothes, loss of productivity), the employers (days off work), and the insurances (health care cost, unemployment, *etc*.)[5]; and (3) Most importantly, there is a significant impact on the quality of life (self-esteem, embarrassment, shame, depression, need to organize life around easy access to bathroom, avoidance of enjoyable activities, *etc*.). Notably, this aspect is not limited to the patient but could to a similar degree affect the patient's significant others[7].

The purpose of our review is to analyze the complexity and limitations of fecal incontinence management and to correlate basic concepts of etiopathogenesis and work-up on one hand with the treatment options on the other hand. The challenges need to be pointed out to define current options and possible solutions.

Challenge

Treatment for fecal incontinence often is demanding and needs to be tailored to the individual circumstances[8]. Unfortunately and despite of a wealth of data, our knowledge about the physiology and pathophysiology of the anorectal continence remains sketchy in many aspects[3,4,9,10]. In particular, it remains difficult if not impossible to correlate subjective and objective parameters in a way to allow for prediction of outcomes. The matter is further complicated by a striking absence of standardization of definitions and of instruments to measure and quantitate fecal incontinence. Even though there are a number of scoring systems that are commonly used [*e.g.*, Wexner/CCF incontinence score; Fecal Incontinence Quality of Life (FIQL) score; Fecal Incontinence Severity Index (FISI); St. Marks Incontinence Score (SMIS); *etc*.)[11], there is none that would include physiologic components or objective test parameters to accurately reflect the clinical severity. Instead, most instruments are based on a subjective patient-reported assessment of severity and frequency.

In the United States, the Cleveland Clinic Florida (Wexner) fecal incontinence score remains the most commonly employed score because of its ease of use (Table 2)[12]: the summary score is derived from 5 parameters whose frequency is each ranked on a scale from 0 (= absent) to 4 (daily): incontinence to gas, to non-formed stool, or to solid stool, need to wear pad, and lifestyle changes. A score of 0 means perfect control, a score of 20 complete incontinence[12]. Unfortunately, the patient's behavior and coping mechanisms are not taken into consideration and can result in substantial variation of the reported score. For example and solely for the purpose of arguments, if a completely incontinent patient hypothetically spent the whole time on the toilet, there would be no incontinence to gas, liquid or formed stool, no need for a diaper, and therefore the only parameter to count would be a "daily impact on his quality of life", *i.e.,* a score of 4 (instead of the more appropriate score of 20).

Table 2 Cleveland Clinic Fecal Incontinence Score[12]

Parameter	Frequency				
	Never	Rarely (< 1/mo)	Sometimes (< 1/wk but ⩾ 1/mo)	Usually (< 1/d but ⩾ 1/wk)	Always (⩾ 1/d)
Incontinence to solid stool	0	1	2	3	4
Incontinence to liquid/loose stool	0	1	2	3	4
Incontinence to gas	0	1	2	3	4
Wears pad	0	1	2	3	4
Lifestyle alteration	0	1	2	3	4

Sum of the five parameters: perfect control = 0; complete incontinence = 20.

Epidemiology

Fecal incontinence is very common but because of the associated embarrassment and a common taboo nature, it is under-reported and its true prevalence difficult to reliably assess[13]. Reported estimates of prevalence rates always have to be interpreted with caution and should be seen within their respective context[14]. Depending on the method and strategy of assessment and the target population, such data may not be representative of the whole population but only reflect selected subsets that may be very different from other population segments. Analysis of 14759 participants in the United States National Health and Nutrition Examination Survey revealed a fecal incontinence prevalence of 8.4% among non-institutionalized United States adults with an age-dependent increase over time[14]. International population-based studies suggested a fecal incontinence prevalence of 0.4%-18%[14-17]. A telephone survey in the United States reported a prevalence of 2.2% with a female to male ratio of 63% *vs* 37%, whereby 30% of the affected interviewees were older than 65 years[18]. Review of outpatient clinic patients revealed a prevalence of 5.6% in general outpatients as opposed to 15.9% in urogynecology patients[16]. A disproportionate fraction of 45%-50% of affected individuals have severe physical and/or mental disabilities, and incontinence is a frequent reason for transfer to nursing homes[19-21].

Etiologies

A vast number of etiologies have been associated with the development of fecal incontinence (see Table 1), including acquired structural abnormalities or congenital malformations, degenerative and functional conditions, or neurological disorders[13]. Diarrhea and altered bowel habits [*e.g.*, from irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), diet intolerance, constipation with paradoxical diarrhea and overflow incontinence] is one of the most frequent independent risk factors for incontinence[22]. The most common structural causes, however, are the result of obstetrical injury (often decades before onset of symptoms)[23], anorectal surgeries (hemorrhoidectomy, fistulotomy, sphincterotomy)[24], prolapse[25], anoreceptive intercourse[26], or a status post colo-anal or ileo-anal reconstruction[27].

A third or fourth degree obstetrical injury with sphincter disruption is clinically recognized in approximately 3%-8% of all vaginal deliveries. But even uncomplicated first-time vaginal deliveries may reveal an occult sphincter damage in up to 35%, whereby forceps delivery, occipito-posterior presentation of the baby, and prolonged labor are independent risk factors[2]. The controversy whether episiotomies are "good, bad, or ugly" in the first place or because simply done too late in the course of labor goes beyond the focus of this review[28,29]. Occult defects remain silent in two thirds of the individuals, but in one third over time become symptomatic with incontinence or urgency. It is important to note that the extent of a sphincter defect has only limited correlation with the degree of fecal incontinence. Intuitively, a large enough sphincter defect alters the circular muscle contraction with concentric closure of the anal canal into a more curvilinear muscle shortening with decreased force onto the anal canal (Figure 1). Beyond that, however, a sphincter defect rather represents a surrogate parameter for the fact that the entire neuromuscular structures of the pelvic floor have suffered a substantial traumatic impact that goes beyond the simple size measurement of a defect angle. The onset of symptoms may frequently lag behind the time of injury by many years; other factors such as onset of menopause, accelerated aging of the traumatized sphincter structures, or decompensation of coping mechanisms may contribute to that delay.

Similar to obstetrical injuries, anorectal surgeries (hemorrhoidectomy, sphincterotomy, fistula surgeries) are frequently identified in patients with symptoms of incontinence. This is at variance with low percentages of incontinence when outcomes of such surgical series are reported. The explanation for this discrepancy may be found in the fact that such observational cohort studies frequently lack long-term follow-up of more than 10 years and hence fail to capture the delayed onset of symptoms to determine the true incidence of this long-term complication.

From physiology to pathophysiology

Successful management of patients with fecal incontinence depends not only on a fundamental knowledge about etiologies, but requires a good

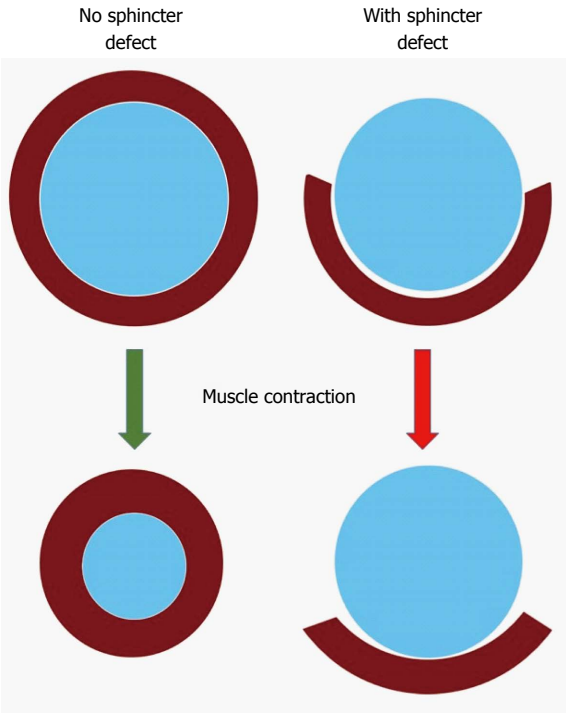


Figure 1 Negative impact of sphincter defect: A normal circumferential muscle configuration results in a concentric contraction and narrowing of the anus (left); if there is a segmental defect in the muscle, contraction may result in shortening of the muscle fibers behind the anus without narrowing it (right).

understanding of the underlying normal mechanisms and the intricate interaction of different components that contribute to achieving fecal control.

Outlet resistance - anal closure function ("plug")

There need to be structures and functions in place to create a dynamic barrier with sufficient outlet resistance against a varying range of intrarectal pressures of the feces at rest, or when there is an increase of the intra-abdominal pressure, be it physiologically during a peristaltic wave, or during physical stress and activity[4,30]: (1) Puborectalis sling and external anal sphincter (EAS): This is an array of striated muscles with slow-twitch, fatigue-resistant muscle fibers that at the center and bottom of the pelvic floor. They are innervated by the inferior branch of the pudendal nerve (S3-S4), contribute to about 30%-40% of the anal resting tone (normal reference value: > 50 mmHg)[31], and provide the voluntary sphincter contraction (squeeze pressure) with roughly a doubling of the resting pressure (normal reference value: > 100 mmHg). Puborectalis dysfunction results in complete incontinence, EAS dysfunction in impaired voluntary control (urge incontinence); (2) Internal anal sphincter (IAS): This smooth muscle represents the thickened end in continuation of the muscularis propria of the rectum. It has an autonomic innervation and contributes to an estimated 50%-55% of the resting tone of the anal canal[31]. IAS dysfunction is associated with impaired fine tuning of fecal control (passive incontinence); (3) Hemorrhoidal cushions: Under normal conditions, these structures provide a fine-tuning seal of the anal canal and can contribute to up to 10%-15% of the overall control[31]. While the basic design is beneficial, deviations from it may quickly flaw their impact, for example if the hemorrhoids either start to protrude or are surgically removed; and (4) Configuration of anal canal: In order to achieve a sufficient closure, the mechanism needs an unhindered ability to generate a strong enough radial force with adequate and concentric pressure values, which are translated to and distributed over a sufficient length of the anal canal (so called high-pressure zone). Altered texture or gross or focal structural deformities of the ano-perineal configuration (*e.g.,* rigid scarring, cloaca, or a keyhole deformity) can be cause to significant symptoms. The latter may result from previous anorectal surgery and - despite a seemingly normal anal pressure profile - may be associated with fecal leakage as capillary forces allow particularly liquid stool components to find their way out (Figure 2). A prolapse of hemorrhoids or the rectum does not only stretch out the sphincter complex and pelvic floor muscles and effectively prevents it from closing the aperture ("shoe in the door"); it also dislocates and everts the crucial sensing zone of the anal canal such that feedback about arriving stool comes too late or not at all.

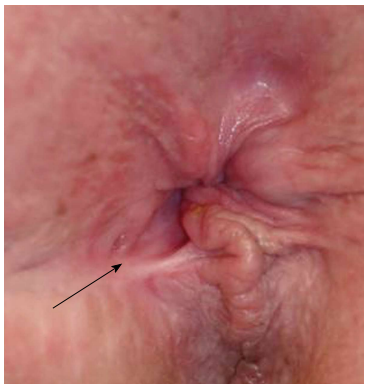


Figure 2 Keyhole deformity: After a previous fistulotomy, the anus is not patulous but appears to have a deformity (arrow).

Stool quality and propulsive force

Formed stool is generally easier to control than liquids or gas (even for a perfectly intact anatomy).

Stool load and extent of gas production: An increase in either one is paralleled by a surge of the pressure in the rectum and the resulting force onto the anal canal. Particularly, when the sphincter resistance is weakened, the increased stool load (for example secondary to supplemental fiber intake) induces a higher probability of accidents. Furthermore, increased gas production often results in higher awareness and reduced self-consciousness.

Increased propulsive axial forces: Diarrhea (for

example as part of IBS or IBD) not only results in an unfavorable change of the stool consistency but often is associated with a more forceful propulsive wave that further challenges the sphincter complex.

Rectal capacity and compliance (reservoir function)

The normal rectum combines an adequate low-pressure space with the ability of an orderly axial propulsion to allow for accumulation and storage of feces until a coordinated and ideally complete evacuation is desired and effectuated[4]. Parameters that are important in this context include[32]: (1) Rectal capacity: parameter to reflect the overall size of the reservoir whereby a more spacious reservoir allows for storage of more stool, but too large of a reservoir (for example, megarectum or excessive size of a J-pouch) may lead to ineffective evacuation (stool clustering); (2) Rectal compliance: parameter to reflect the distensibility of the rectal wall, *i.e.,* the ratio of Δvolume/Δpressure; and (3) Layout and configuration of the original rectum (*e.g.*, absence of pelvic organ descent and prolapse, kinking, enterocele, rectocele) or of a post-surgical neo-reservoir (*e.g.,* J-pouch *vs* straight anastomosis).

Pelvic organ descent and prolapse represent a frequent degenerative pathology disproportionally affecting women. The positional instability of the pelvic structures with ineffective initiation and completion of defecation (and/or urine voiding) over time may result in a functionally reduced reservoir and potentially and more frequent and undesired evacuations. It is of note that IBS is characterized by a typically reduced volume tolerance and hence capacity, however in contrast to structural problems the rectal compliance remains normal (increased visceral sensitivity but absence of structural problem)[33]. Last but not least, an impaired reservoir function with decreased size and compliance is commonly seen after previous rectal surgery (*e.g.,* LAR), pelvic radiation, or in the presence of tumors, strictures, or ongoing rectal wall inflammation (IBD, abscess, *etc*.). Management strategies including surgical efforts to overcome some of these negative impacts by neoadjuvant rather than adjuvant radiation or by creation of a lower pressure reservoir (J-pouch, transverse coloplasty) may result in a short-term benefit with reduced urgency and frequency but in the long run level out and may even be associated with fecal clustering[34].

Neurologic sensory or motor function

Central nervous system: Conscious (awareness) and subconscious networking of information from and to the anorectum are necessary for adequate control. Possible central neurological deficits include focal brain defects from stroke, tumor, trauma, or multiple sclerosis or from more diffuse brain alteration (dementia, multiple sclerosis, infection, drug-induced).

Intact peripheral nerve function: Transmission of the adequate somatic and visceral nerve input to the intestines, as well as the pelvic floor and sphincter muscle complex are needed to allow for correct processing of sensoriceptor information (rectal pressure, sphincter pressure) and pelvic floor function. Peripheral neuropathy may be localized (parity-induced pudendal neuropathy, pelvic radiation, post-surgical), or have a diffuse pattern as a result of diabetes mellitus or neurotoxic drugs such as some chemotherapy agents (*e.g.*, oxaliplatin).

Functional dysfunction: Visceral hypersensitivity is the key concept behind IBS and is characterized by a number of measurable dyssensations (hypersensitivity, spasticity, intensified propulsions) in absence of any morphological correlate.

Symptom analysis

Primary symptoms of fecal incontinence include a worsening lack of control for different rectal components, *i.e.*, solid stool, liquid/semi-formed stool, gas. The degree of content loss is commonly quantitated as staining < soilage < seepage < accidents. Involuntary discharge without any awareness is labeled as passive incontinence, whereas accidents despite awareness and active countermeasures are called urge incontinence. Some patients may report a reduced sensation for arriving stool, a reduced urge-suppressing capacity, and hence a dramatically shortened maximal deferability ("time to bathroom"). It is important to explore and recognize individual variations in relation to other extrinsic factors such as daytime versus nighttime, physical activity, or food intake.

Secondary symptoms of fecal incontinence may develop as a result of leaking stool and include pruritus ani, perianal skin irritation, urinary tract infections, *etc*. In some patients, these secondary symptoms may in fact be their chief complaint without noticing or acknowledging the lack of control as such.

Depending on the etiology, fecal incontinence may have associated symptoms which need to be actively checked with the patient such as urinary incontinence, vaginal bulging (rectocele, cystocele), prolapse (hemorrhoidal, mucosal, full-thickness rectal), rectovaginal fistula, altered bowel habits.

Workup

A structured workup stands at the beginning of any incontinence management (Table 3). There is a need for a careful, thorough, yet sensitive history in every patient[3,4,10]. The details are necessary to define the complaints and their impact, possible triggering or aggravating factors or events, and the time interval to the onset of symptoms. All past evaluations, treatments with response and failures, as well as the current management and day-to-day routine have to be meticulously explored and documented. Related and seemingly unrelated surgeries such as

Table 3 Structured workup of patients with fecal incontinence

Assessment tool	Details
History	Onset
	Quantitation: staining < soilage < seepage < accidents
	Qualitative assessment: passive incontinence *vs* urge incontinence
	Obstetrical history: pregnancies, vaginal deliveries
	Previous surgeries: anorectal surgeries, hysterectomy, bladder surgeries, (colo-)rectal surgeries, spinal surgeries
	Underlying diseases (diabetes, stroke, *etc.*)
	Bowel function and stool quality
	Incomplete evacuation
	Stool/gas passage through vagina
	Medications
Scoring instruments	CCF incontinence score ("Wexner score")
	Fecal Incontinence Quality of Life score
	Fecal Incontinence Severity Index
	St. Marks Incontinence Score
	EORTEC SF-36
	Revised Fecal Incontinence Scale
	Other scoring instruments
Physical exam	Inspection: patulous anus, folds, perineal body, keyhole, skin irritation, perineal descent, prolapse, cloaca, rectovaginal fistula (stool in vagina)?
	Digital exam: sphincter integrity, tone (rest/squeeze), compensatory contraction/discoordination, rectocele, mass?
	Sensation/anal reflex
	Instrumentation/visualization: rule out other pathologies (*e.g.*, rectal tumor, proctitis)
Anophysiology testing	Anal ultrasound
	Anophysiology testing:
	Manometry
	Anorectal sensation and volume tolerance
	Compliance measurement
	Nerve studies: PNTML, occasionally EMG
	Placement of SNS trial electrode (phase I)
Additional evaluations in select cases	Imaging: dynamic pelvic MRI
	Defecating proctogram
	Evaluation by other specialties (Urogynecology, Urology, Gastroenterology, *etc.*)

spinal surgery could be important. Further attention should focus on underlying diseases (diabetes, stroke, chemotherapy), current medications, the dynamics of bowel movements, and associated symptoms. Additional standardized and validated scoring and quality of life instruments are administered to define the severity and impact of the fecal incontinence[4,11,35].

The clinical exam includes a visual inspection, an educated digital rectal exam (sphincter integrity, sphincter tone, compensatory auxiliary muscle contraction, length of anal canal, rectocele, palpable mass), as well as at least a limited visualization of the anorectum. A colonic evaluation may not as such contribute to the incontinence management but should be done according to national guidelines to avoid overlooking other more relevant conditions. More objective data can be obtained from anophysiology studies, but the results have to be interpreted with caution in the context of all other factors.

Anophysiology studies attempt to correlate the subjective complaints and clinical exam findings with objective parameters. It would be desirable to define parameters that would directly dictate appropriate respective treatment options and forecast the outcome. However, the predictability of all tests remains a challenge[36]. Furthermore, the value and timing for issuing such tests remain controversial and need to be defined on an individual basis. In recent years since introduction of sacral nerve stimulation, an increasing number of authors have suggested to skip basic testing and in absence of contraindications to proceed with a trial placement of the sacral nerve stimulation (SNS) electrode as the first diagnostic and therapeutic step[37].

Anorectal ultrasound is generally accepted as the most sensitive tool to assess the sphincter complex for the presence or absence of any defect or structural alteration (see Figure 2).

Anal manometry including anorectal sensation and volume tolerance, as well as determination of the rectal compliance aim at objectively assessing the muscle strength and the reservoir function[38-41]. Conventional multichannel manometry has increasingly been replaced by high-resolution manometry using an integrated probe that allows for 3D-analysis and visualization of pressure profiles[42]. A number of reports have correlated clinical symptoms and/or manometry testing with the degree of subjective impairment[43], however it has remained a major challenge to reliably define the best treatment modality or treatment response, respectively[44].

Nerve studies: Measurement of the pudendal nerve conductivity, also known as pudendal nerve terminal motor latency (PTNML), is used to identify pudendal neuropathy, which may result from direct or indirect impact (*e.g.*, obstetrical stretch injury, abscess formation, surgery, or radiation) or systemic factors (chemotherapy, diabetes, *etc.*). The (controversial) parameter has been associated with poor outcomes after overlapping sphincteroplasties in some but not in other studies[45-48]. Electromyography (EMG) aims at analyzing the neuromuscular motor-units, commonly as summary potential by means of painless but imprecise surface electrodes, rarely through precise but very painful needle electrodes. EMG may play a role in confirming paradoxical puborectalis contraction in patients with obstructed defecation, but otherwise is typically of limited value for workup of fecal incontinence.

Depending on the presentation and previous findings, other work-up steps might be appropriate to evaluate more complex pelvic floor dysfunction, *e.g.*, dynamic pelvic MRI, defecating, proctogram, urodynamics, or referral and evaluation by other specialties.

Nonoperative treatment

Management of patients with fecal incontinence invariably starts with non-operative measures. The

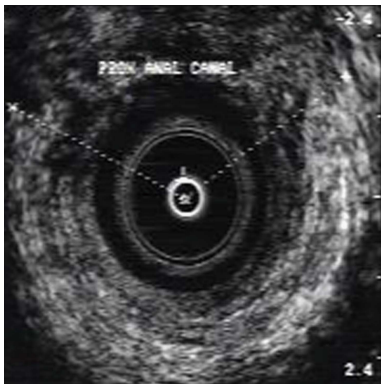


Figure 3 Anorectal ultrasound showing an anterior defect in external anal sphincter.

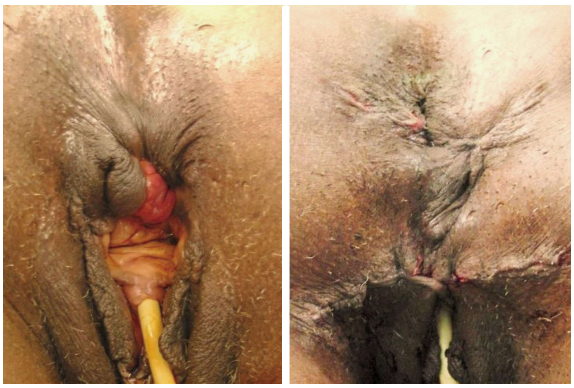


Figure 4 Cloaca-like deformity, corrected with sphincteroplasty and X-flaps.

most pressing goals are (1) to optimize the stool consistency; (2) slow down bowel motility; and (3) to minimize the average stool load in the rectum, particularly prior to leaving the safety of the private home. Specific inflammatory conditions should get the appropriate attention and treatment to correct related diarrhea. Dietary changes are intended to identify and avoid foods that cause diarrhea or urgency. A limited amount of supplementary fibers with limited fluid intake may help to thicken the stool but larger doses tend to unnecessarily increase the stool volumes and may be counterproductive when at the same time the sphincter function is weak. Bowel habit and behavioral training is important to develop regularity while avoiding obsessive patterns. Supportive measures include application of barrier creams to the perianal skin. The stool load may be reduced through rectal washouts (scheduled enemas). Medications are introduced as needed to slow down the bowels (anti-diarrheal medications), bind bile acids (cholestyramine), or to reduce the reflectory sphincter relaxation (antidepressants such as amitriptyline)[49]. There has been speculation about the role of hormone replacement therapy in postmenopausal women[50], but no definitive recommendation has been released.

Physical therapy and biofeedback training aim at strengthening and coordinating the pelvic floor and sphincter function in response to rectal distention, commonly in conjunction with other above mentioned conservative measures[51]. The approach is simple, non-invasive, and without any adverse side effects. Detecting an objective improvement compared to standard care is frequently impossible[52,53], even if the patients report a subjective benefit in 64%-89%[54,55]. In the end, the most significant impact on the patients may be the fact that they are tasked to take an active role in overcoming their incontinence. The use of pelvic floor muscle training (PFMT) and biofeedback for reconditioning of dysfunctional pelvic floor muscles has long been a conservative fecal incontinence modality. A 2012 Cochrane review of 21 studies with a total of 1525 participants found that a limited number of trials did not provide sufficient evidence for the effectiveness of anal sphincter exercise and biofeedback therapy, but suggested that biofeedback and/or PFMT in combination with other modalities (*e.g.*, electrical nerve stimulation techniques) may enhance the overall outcome. But due to the general weakness of the reported data, the authors concluded that the suggested therapeutic effect of some elements of biofeedback therapy and sphincter exercises was not certain[52].

Operative strategies

Surgical options are explored in patients with significant fecal incontinence that is refractory to conservative management[56]. while avoiding obsessive patterns. Obvious and correctable structural deformities that lend themselves to a surgical intervention should always be addressed first. Examples include a cloaca-like deformity (see Figure 3)[57], hemorrhoidal or full-thickness rectal prolapse, keyhole deformity (after fistulotomy or other surgeries, see Figure 4), or a mucosal ectropion. Other conditions that may emulate the symptom of incontinence (perirectal fistula, rectovaginal fistula) unquestionably should be corrected prior to focusing on the workup or management of the "incontinence" as such[4].

If gross morphological pathology is either absent or has been corrected, a number of operative approaches strategies are available to address the incontinence itself[8,58]. Their applicability depends on the individual circumstances, the severity of the patient's symptoms, as well as a clear definition of the treatment goals and priorities[9]. Both the patient and treating physicians need to engage in an optimistic but at the same time honest discussion about the pros and cons, realistic versus unrealistic goals, and the expected outcomes of the various surgeries[3,10]. While this review provides an overview of the concepts (Table 4), a detailed discussion of the techniques and their respective results will be beyond its scope. A task force of the American Society of Colon and Rectal Surgeons, however, has recently reviewed the evidence and

Table 4 Surgical strategies

Goal	Options
Correction of morphological deformities	Prolapse
	Cloaca
	Keyhole deformity
	Perirectal fistula
	Rectovaginal fistula
	Tumor
Sphincter repair	Overlapping sphincteroplasty
Enhancement of impaired sphincter function	Sacral nerve stimulation
	Radiofrequency energy administration (SECCA™)
	Injection of bulking agents (NASHA/DX, beads *etc.*)
Sphincter replacement/ support	Artificial bowel sphincter
	Implantation of magnetic anal sphincter (Fenix™)
	Graciloplasty
	Implantation of Thiersch
	Implantation of pelvic sling system
Diversion	Colostomy
Reduction of fecal load	Malone antegrade continence enema

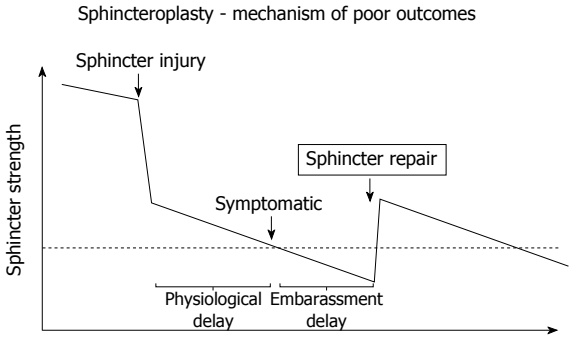


Figure 5 Model for poor outcomes after sphincteroplasty. Hypothetical model to explain poor outcomes after sphincteroplasty: The graph shows a hypothetical time course (x-axis) of the sphincter strength (y-axis) with the dotted line representing the threshold below which incontinence becomes clinically evident. There may be a natural decline of sphincter strength (time before sphincter injury), a dramatic reduction through the injury, followed by an accelerated decline. The physiological delay represents the time until symptoms evolve, while the embarrassment delay reflects the time until a symptomatic patient acknowledges the problem. A sphincter repair may restore some strength, but with continued and possibly accelerated decline of the sphincter function the threshold is again crossed after a period of time.

published a current status on new technologies[9].

Correction of morphological deformities

Reshaping and correction of gross deformities and pathologies (see above).

Sphincter repair

Sphincter repair (sphincteroplasty) seems to be a rational and still probably the most frequently used approach if a segmental sphincter defect is identified (see Figure 2). The goal is to reconstitute the circular configuration of the muscle around the anal canal (see Figure 1) and with that the high pressure zone. The short-term results are generally good with an estimated 75%-86% improvement of incontinence episodes. However, the urgency may persist and over time, the long-term function has been noted to deteriorate with some series reporting only 0%-50% of patients still being fully continent after 5-10 years[59-61]. A systematic review of 16 studies with more than 5 years of follow-up and nearly 900 sphincter repairs noted that most patients remained satisfied with their surgical outcome despite worsening results over time[62].

One might speculate that reasons for this unsatisfactory durability are to be found in the fact that the sphincter defect represents a much larger than measurable injury and leads to a faster degeneration process (Figure 5). It will have to be seen whether systematic combination with physical therapy and/or sacral nerve stimulation could result in more durable outcomes of either technique.

Enhancement of impaired sphincter function

SNS: This is the concept and surgical modality that - in the last two decades - has transformed the management of fecal incontinence in the most dramatic way. In contrast to other interventions, it does not focus at all on the anal canal as such, and yet, it showed remarkable short- and long-term improvements regardless of whether a sphincter defect was present or not[63]. Prior to being introduced for fecal incontinence, it had been widely utilized for patients with urinary incontinence. In 1995, the first trial for bowel control was reported in Europe and set the starting point for a worldwide revolution[64]. The technique involves two short outpatient procedures under superficial anesthesia. During the first, placement of a 4-point electrode at the sacral root S3 is carried out and linked to a temporary external stimulation device. If the patient shows a good response within the subsequent 2-wk trial period, a definitive implantation of the pacemaker-like stimulator device is performed in the second surgery; otherwise the electrode is removed. Although the exact mechanism of this technique is yet to be completely understood, SNS is believed to re-stimulate a dysfunctional pelvic floor and receptor pathway on one hand and in addition to activate the afferent brain pathway related to the continence mechanism[65,66]. Furthermore, there has been some evidence that it might affect the pacing of the colon and potentially even induce retroperistaltic activity[67]. Independent of the true nature of its effect, the results are fascinating insofar as two thirds of the patients have a greater than 50% improvement such that they have the a definitive stimulator implanted[9]. For the most part, the positive experience is sustained, both immediately and over time. After definitive implantation, 86%-87% of patients reported a greater than 50% improvement and about 40% of the patients achieved perfect control, a success than persisted over 3-5 years and beyond[9,68-70]. The complication rate is relatively low, whereby infection and dislocation of the electrode

Table 5 Types of injectable/implantable materials

Category	Details
Conventional	Carbon
	Teflon or silicon biomaterial beads
	Collagen
	Autologous fat
Newer	Non-animal stabilized hyaluronic acid/dextranomer
Pilot	Self-expandable hyexpan (polyacrylonitrile) prosthesis
Future	Stem cells

are the most frequent ones with 3% and 12%, respectively[71]. However, 19%-36% of patients require subsequent interventions for revision or device replacement (battery life)[70,71].

Tibial nerve stimulation: Another related modality of nerve stimulation utilized for the management of fecal incontinence is percutaneous tibial nerve stimulation (PTNS). Similar to the introduction of SNS, PTNS is a technology that was initially studied and used for the treatment of urinary incontinence[72]. Using either transcutaneous or percutaneous electrodes, the posterior tibial nerve is stimulated in sessions of approximately 30 min duration over a minimum of 3 mo[72]. Although the benefit and mechanism of action of tibial nerve stimulation is even less intuitive and far from being understood, it again is believed to impact fecal control through the activation of the central nervous system and supra-sacral neural centers *via* the afferent fibers of the peripheral nervous system. As the posterior tibial nerve originates from the ventral branches of lumbar and sacral nerves, it is furthermore believed that a similar response may be elicited as by means of SNS[73].

Radiofrequency energy administration ("SECCA procedure"): This FDA-approved technique involves the delivery of a thermo-controlled multi-point radio-frequency energy (465 kHz) to the depth of the anal canal without burning the mucosal surface. The purpose is to induce an increase of the outlet resistance by means of a controlled scarring; additionally, a remodeling effect on the sphincter muscle fibers has been postulated[9]. Six prospective series and one retrospective study including a United States multicenter trial with 50 patients summarized the results. With the exception of one series (reported on three separate occasions), the majority of reports noted no or only a moderate clinical benefit with 0%-38% of patients achieving more than 50% improvement, but never perfect control[9,74,75].

Injection/implantation of bulking agents: With the goal to bulk up the anal canal or perianal tissues and increase the passive outlet resistance, a number of different techniques have been used to inject or implant a variety of materials (Table 5). Patient selection has been poorly defined but could include those with mild passive incontinence secondary to internal anal sphincter weakness, or patients with postsurgical deformities and an uneven shape of the anal canal. A systematic review on conventional injectables with 16 studies (13 case series, 1 prospective trial with and 2 without data) and a total pool of 420 patients (5-73 patients per study) found little evidence for the effectiveness in passive fecal incontinence; a greater than > 50% improvement was only achieved in 2 studies, while the others reported a 15%-50% improvement at the longest follow-up[76]. Complications and side effects occurred in up to 10% and 12%, respectively[76]. Subsequently, and seemingly for only a limited period of time, NASHA/Dx gained some momentum and was aggressively marketed to specialist physicians and general practitioners alike. The outpatient/office-based injection received attention after in 2011, a prospective randomized, sham-controlled trial of 206 patients in a 2:1 distribution found a greater than 50% improvement in 53.2% *vs* 30.7% in the intervention versus sham group, respectively[77]. Questions regarding the value of statistical as opposed to clinical significance, a low rate of only 6% complete continence at 6 mo, lack of specific objective data and selection criteria, the durability, and last but not least the cost of the intervention limited the expansion of the technique[9,78,79]. The most recent two strategies that still await broader evaluation include the implantation of self-expandable hyexpan (polyacrylonitrile) prosthesis by means of a applicator gun[80,81], or of stem cells[82,83].

Sphincter replacement

Dynamic sphincter replacement: (1) Implantation of artificial bowel sphincter: This was the only approach that provided a true functional/dynamic solution with excellent results; its limitations were largely related to the risk of infection (5%) and long-term device erosion or dysfunction[9]. Unfortunately, the device is not on the market anymore; (2) Implantation of magnetic anal sphincter: The aim is to augment the sphincter function by increasing the passive outlet resistance whereby a high enough rectal pressure can overcome the anal canal closure for good or for bad[9]. The method has so far been tested in limited feasibility studies and cases series and shown some promising results[84-86], but prospective data are needed at this point[87]; and (3) Dynamic graciloplasty: The autologous gracilis muscle is carefully mobilized, that is disconnected distally while the proximal neurovascular bundle is preserved. A tunnel is created towards and around the anus and the pedicled muscle wrapped around the anal canal. Unfortunately, the ability to consciously use this muscle and learn voluntary contractions is very limited. However, implantation of a pulse generator device (not available in the United States) for continued electrical stimulation of the muscle induces contractions and

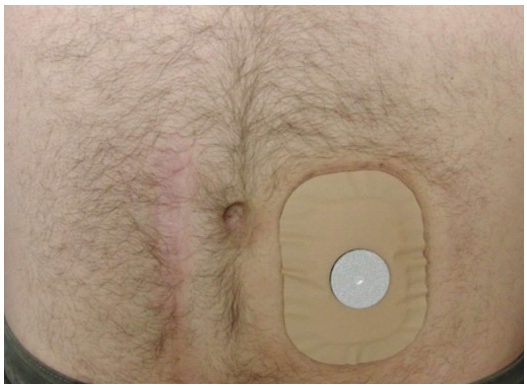


Figure 6 Trained colostomy. Trained colostomy: After observation of cyclic emptying pattern, in conjunction with appropriate supportive measures (*e.g.*, timed enemas), the patient may not need a true bag, but simply covers the stoma with a mini-appliance with a gas filter.

over time converts the fast-twitch, fatigable gracilis muscle to a slow-twitch, fatigue-resistant muscle. The technique has been shown to have a reasonable efficacy, but its associated high morbidity has overall limited its use even in countries where such stimulator is available[88,89].

Nondynamic sphincter and pelvic floor support: (1) Thiersch and related procedures: These utilize the placement of an anal encirclement with the aim of narrowing the anal canal and subsequently increase the passive outlet resistance, even when lacking a dynamic component. Both non-elastic and elastic silicone-based implants have been used. The approach is uncommon, and data are anecdotal at best; (2) Non-dynamic graciloplasty or gluteoplasty: The non-stimulated transposition and wrapping of gracilis or gluteus muscle around the anal canal ("bio-Thiersch") has limited indications because of the high risk of complications and a lack of true functionality. Nonetheless, a retrospective series of 25 patients who underwent unilateral gluteoplasty reported a significant improvement in more than 72%[90]; and (3) Pelvic floor repairs/sling: This fairly old concept of addressing fecal incontinence by correcting the pelvic floor support and restoring the anorectal angle (*e.g.*, posterior Parks repair) was generally unsuccessful. It was hence abandoned, but recently regained some momentum when an investigational trans-obturator posterior anal sling system was introduced and a multi-center trial was launched. A self-fixating poly-propylene mesh is inserted and placed behind the anorectum *via* two small incisions by means of two curved introducer needles[91]. The trial in 14 United States centers with 152 participants and a 1 year follow-up found that 69.1% of participants met the criteria for treatment success and 19% reported complete continence[92].

Fecal diversion

When other therapies have failed or when they are preemptively believed to eventually inevitably fail, or if co-morbidities preclude a more aggressive or time-consuming strategy, fecal diversion with the creation of a diverting well-constructed colostomy at a carefully selected site remains a more satisfying than acknowledged alternative[58,93]. Even if it does not restore continence in a strict sense and has an impact on the body image, it provides the patient with the luxury of a controlled waste management and hence permits resumption of a normal personal and social life style. Patients who hesitate prior to the surgery should be encouraged to list pros/cons for both the status quo as well as the creation of a colostomy; to their own surprise, they often realize that objectively the benefits outweigh the negative impacts. It should be noted that some patients are able to train their colostomy such that they can empty their colon with the help of an enema once daily and cover the stoma for the rest of the 24-h cycle (see Figure 6).

Rarely used tools

Malone antegrade continence enema: A surgery is performed to create a continent one-way appendicostomy or mini colostomy[94]. The location for that access opening can either be placed into the umbilicus or at a very low cosmetically acceptable location in the right lower quadrant. Alternatively, a percutaneous cecostomy with trap-door button can serve the same purpose. A catheter has to be introduced in scheduled intervals to flush the entire colon and eliminate the fecal load at a time and location chosen by the patient. The concept is attractive in some ways, but the daily irrigation is rather time-consuming and the patients may experience some continued leakage immediately following the irrigation[95].

CONCLUSION

Fecal incontinence is the final common pathway symptom of a variety of conditions, but disproportionally affects woman as a result of gravity and parity. The recognition, workup and treatment remain a huge challenge as the functional aspects do not strictly correlate with the morphological findings[9]. Hence, there is not a single technique that would guarantee perfect outcomes without any morbidities (Table 6). One must assume that successful treatment almost always needs to combine a number of different approaches[56]. Development of a treatment algorithms (*e.g.*, as outlined in the ASCRS position paper) have to be based on the severity of the incontinence, anatomical and functional findings[9,58]. Unquestionable, there is space for expanding our knowledge on all aspects of the control organ[9]. It would be highly desirable to plan and carry out good randomized multi-center trials to study work-up parameters and combination treatments in a standardized and scientific fashion.

Table 6 Overview of various surgical options with respective outcomes (as detailed in the text)

Interventions category	Specific technique	Efficacy rate (complete/ > 50% improvement)	Complication rates	Grade
Correction of morphological abnormalities	Depending on underlying condition: Prolapse, cloaca, keyhole deformity, perirectal fistula, rectovaginal fistula, tumor	N/A	N/A	N/A
Sphincter repair	Overlapping sphincteroplasty	75%-85% (short term) 0-50% (after 5-10 yr)	N/A	
Enhancement of sphincter function	Sacral nerve stimulation	0%-56%/51-100%	lead displacement (15%), diarrhea (6%), pain (6-28%), bleeding 11%, infection (3%)	1B
	Tibial nerve simulation	0%-12% (-40%)/0%-67%	59% (infection, mild gastrodynia, temporary leg numbness)	2C
	Radiofrequency energy administration	0%/12%-38% (-84%)	0%-52% (pain, bleeding, infection)	2B
	Injection of: conventional bulking agents NASHA/DX	0%/33%-90% 6%/56%-61%	10%-12% (pain, bleeding, infection)	2A
Sphincter replacement	Artificial bowel sphincter	61%-90%/31%-100%	5%-10% infection rates, 30%-52% long-term failure 9	1B
	Implantation of magnetic ring (Fenix™)	NA/54%	0%-7% obstruction, infection, erosion	1C
	Graciloplasty (dynamic/non-dynamic)	NA/72%	> 40% including urinary tract infection/retention, infections 76	2C
	Implantation of Thiersch	N/A	N/A	N/A
	Pelvic floor repairs/sling	19%/69.1%	17%-30% (pain, infection)	2C
Fecal diversion	Ileostomy, loop colostomy, end colostomy	near 100% FI improvement	5%-10% stoma outlet obstruction, stricture, prolapse, hernia	1C
Fecal load reduction	Malone antegrade continence enema	(0%)/33%-100% FI continence	8%-50% stoma stenosis, leakage	2C

Key points

(1) Continence requires a balanced interaction between the anal sphincter complex, the stool consistency, the rectal reservoir function, and neurological function; (2) Fecal incontinence is frequently under-reported, but the estimated prevalence ranges from 0.4%-18%; (3) Management of patients with fecal incontinence starts with a detailed history and physical exam; (4) Symptom severity should be quantified using one of several validated scoring systems, all of which are based on subjective reporting and lack incorporation or correlation with objective test data; (5) Objective evaluation tools include anorectal ultrasound, anal manometry with anorectal sensation and volume tolerance, compliance and strength/reservoir function testing, and nerve studies. Further imaging (*e.g.*, dynamic pelvic MRI, defecating, proctogram), urodynamics, or referral to associated specialties (urology, gynecology) are indicated on an individualized basis; (6) Non-operative management aims at optimizing stool consistency, dietary and bowel habits, as well as muscle function in conjunction with supportive medications and care measures, as well as scheduled enemas to reduce stool load; physical therapy with pelvic floor muscle and biofeedback training are typically encouraged; and (7) Operative strategies are explored in patients with obvious structural deformities or significant fecal incontinence that is refractory to conservative management. Among various available options, the most common ones are sphincter repair, sacral nerve stimulation, sphincter implants, or creation of a stoma.

REFERENCES

1 **Wald A**. Clinical practice. Fecal incontinence in adults. *N Engl J Med* 2007; **356**: 1648-1655 [PMID: 17442907 DOI: 10.1056/NEJMcp067041]

2 **Rao SS**. Diagnosis and management of fecal incontinence. American College of Gastroenterology Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1585-1604 [PMID: 15307881 DOI: 10.1111/j.1572-0241.2004.40105.x]

3 **Paquette IM**, Varma MG, Kaiser AM, Steele SR, Rafferty JF. The American Society of Colon and Rectal Surgeons' Clinical Practice Guideline for the Treatment of Fecal Incontinence. *Dis Colon Rectum* 2015; **58**: 623-636 [PMID: 26200676 DOI: 10.1097/DCR.0000000000000397]

4 **Kaiser AM**. McGraw-Hill Manual: Colorectal Surgery. 2009 Available from: URL: http://accesssurgery.com/resourceToc.aspx?resourceID=211

5 **Xu X**, Menees SB, Zochowski MK, Fenner DE. Economic cost of fecal incontinence. *Dis Colon Rectum* 2012; **55**: 586-598 [PMID: 22513438 DOI: 10.1097/DCR.0b013e31823dfd6d]

6 **Kamm MA**. Faecal incontinence. *BMJ* 1998; **316**: 528-532 [PMID: 9501717 DOI: 10.1136/bmj.316.7130.528]

7 **Meyer I**, Richter HE. Impact of fecal incontinence and its treatment on quality of life in women. *Womens Health* (Lond) 2015; **11**: 225-238 [PMID: 25776296 DOI: 10.2217/whe.14.66]

8 **Madoff RD**. Surgical treatment options for fecal incontinence. *Gastroenterology* 2004; **126**: S48-S54 [PMID: 14978638 DOI: 10.1053/j.gastro.2003.10.015]

9 **Kaiser AM**, Orangio GR, Zutshi M, Alva S, Hull TL, Marcello PW, Margolin DA, Rafferty JF, Buie WD, Wexner SD. Current status: new technologies for the treatment of patients with fecal incontinence. *Surg Endosc* 2014; **28**: 2277-2301 [PMID: 24609699 DOI: 10.1007/s00464-014-3464-3]

10 **Alavi K**, Chan S, Wise P, Kaiser AM, Sudan R, Bordeianou L. Fecal Incontinence: Etiology, Diagnosis, and Management. *J Gastrointest Surg* 2015; **19**: 1910-1921 [PMID: 26268955 DOI: 10.1007/s11605-015-2905-1]

11 **Vaizey CJ**, Carapeti E, Cahill JA, Kamm MA. Prospective

comparison of faecal incontinence grading systems. *Gut* 1999; **44**: 77-80 [PMID: 9862829 DOI: 10.1136/gut.44.1.77]
12 **Jorge JM**, Wexner SD. Etiology and management of fecal incontinence. *Dis Colon Rectum* 1993; **36**: 77-97 [PMID: 8416784 DOI: 10.1007/BF02050307]
13 **Bharucha AE**, Dunivan G, Goode PS, Lukacz ES, Markland AD, Matthews CA, Mott L, Rogers RG, Zinsmeister AR, Whitehead WE, Rao SS, Hamilton FA. Epidemiology, pathophysiology, and classification of fecal incontinence: state of the science summary for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) workshop. *Am J Gastroenterol* 2015; **110**: 127-136 [PMID: 25533002 DOI: 10.1038/ajg.2014.396]
14 **Ditah I**, Devaki P, Luma HN, Ditah C, Njei B, Jaiyeoba C, Salami A, Ditah C, Ewelukwa O, Szarka L. Prevalence, trends, and risk factors for fecal incontinence in United States adults, 2005-2010. *Clin Gastroenterol Hepatol* 2014; **12**: 636-643.e1-2 [PMID: 23906873 DOI: 10.1016/j.cgh.2013.07.020]
15 **Nelson RL**. Epidemiology of fecal incontinence. *Gastroenterology* 2004; **126**: S3-S7 [PMID: 14978632 DOI: 10.1053/j.gastro.2003.10.010]
16 **Faltin DL**, Sangalli MR, Curtin F, Morabia A, Weil A. Prevalence of anal incontinence and other anorectal symptoms in women. *Int Urogynecol J Pelvic Floor Dysfunct* 2001; **12**: 117-120; discussion 121 [PMID: 11374509 DOI: 10.1007/PL00004031]
17 **Johanson JF**, Lafferty J. Epidemiology of fecal incontinence: the silent affliction. *Am J Gastroenterol* 1996; **91**: 33-36 [PMID: 8561140]
18 **Nelson R**, Norton N, Cautley E, Furner S. Community-based prevalence of anal incontinence. *JAMA* 1995; **274**: 559-561 [PMID: 7629985 DOI: 10.1001/jama.1995.03530070057030]
19 **Nelson R**, Furner S, Jesudason V. Fecal incontinence in Wisconsin nursing homes: prevalence and associations. *Dis Colon Rectum* 1998; **41**: 1226-1229 [PMID: 9788384 DOI: 10.1007/BF02258218]
20 **Wu JM**, Matthews CA, Vaughan CP, Markland AD. Urinary, fecal, and dual incontinence in older U.S. Adults. *J Am Geriatr Soc* 2015; **63**: 947-953 [PMID: 25940401 DOI: 10.1111/jgs.13385]
21 **Jerez-Roig J**, Souza DL, Amaral FL, Lima KC. Prevalence of fecal incontinence (FI) and associated factors in institutionalized older adults. *Arch Gerontol Geriatr* 2015; **60**: 425-430 [PMID: 25721982 DOI: 10.1016/j.archger.2015.02.003]
22 **Andrews CN**, Bharucha AE. The etiology, assessment, and treatment of fecal incontinence. *Nat Clin Pract Gastroenterol Hepatol* 2005; **2**: 516-525 [PMID: 16355157 DOI: 10.1038/ncpgasthep0315]
23 **Shin GH**, Toto EL, Schey R. Pregnancy and postpartum bowel changes: constipation and fecal incontinence. *Am J Gastroenterol* 2015; **110**: 521-529; quiz 530 [PMID: 25803402 DOI: 10.1038/ajg.2015.76]
24 **Ternent CA**, Fleming F, Welton ML, Buie WD, Steele S, Rafferty J. Clinical Practice Guideline for Ambulatory Anorectal Surgery. *Dis Colon Rectum* 2015; **58**: 915-922 [PMID: 26347962 DOI: 10.1097/DCR.0000000000000451]
25 **Wallenhorst T**, Bouguen G, Brochard C, Cunin D, Desfourneaux V, Ropert A, Bretagne JF, Siproudhis L. Long-term impact of full-thickness rectal prolapse treatment on fecal incontinence. *Surgery* 2015; **158**: 104-111 [PMID: 25869649 DOI: 10.1016/j.surg.2015.03.005]
26 **Markland AD**, Dunivan GC, Vaughan CP, Rogers RG. Anal Intercourse and Fecal Incontinence: Evidence from the 2009-2010 National Health and Nutrition Examination Survey. *Am J Gastroenterol* 2016; **111**: 269-274 [PMID: 26753893 DOI: 10.1038/ajg.2015.419]
27 **Walma MS**, Kornmann VN, Boerma D, de Roos MA, van Westreenen HL. Predictors of fecal incontinence and related quality of life after a total mesorectal excision with primary anastomosis for patients with rectal cancer. *Ann Coloproctol* 2015; **31**: 23-28 [PMID: 25745623 DOI: 10.3393/ac.2015.31.1.23]
28 **Handa VL**, Blomquist JL, McDermott KC, Friedman S, Muñoz A. Pelvic floor disorders after vaginal birth: effect of episiotomy, perineal laceration, and operative birth. *Obstet Gynecol* 2012; **119**: 233-239 [PMID: 22227639 DOI: 10.1097/AOG.0b013e318240df4f]
29 **Carroli G**, Mignini L. Episiotomy for vaginal birth. *Cochrane Database Syst Rev* 2009; **(1)**: CD000081 [PMID: 19160176 DOI: 10.1002/14651858.CD000081.pub2]
30 **Kaiser AM**, Ortega AE. Anorectal anatomy. *Surg Clin North Am* 2002; **82**: 1125-1138, v [PMID: 12516843 DOI: 10.1016/S0039-6109(02)00056-7]
31 **Lestar B**, Penninckx F, Kerremans R. The composition of anal basal pressure. An in vivo and in vitro study in man. *Int J Colorectal Dis* 1989; **4**: 118-122 [PMID: 2746132 DOI: 10.1007/BF01646870]
32 **Felt-Bersma RJ**, Sloots CE, Poen AC, Cuesta MA, Meuwissen SG. Rectal compliance as a routine measurement: extreme volumes have direct clinical impact and normal volumes exclude rectum as a problem. *Dis Colon Rectum* 2000; **43**: 1732-1738 [PMID: 11156459 DOI: 10.1007/BF02236859]
33 **Zar S**, Benson MJ, Kumar D. Rectal afferent hypersensitivity and compliance in irritable bowel syndrome: differences between diarrhoea-predominant and constipation-predominant subgroups. *Eur J Gastroenterol Hepatol* 2006; **18**: 151-158 [PMID: 16394796 DOI: 10.1097/00042737-200602000-00007]
34 **Fazio VW**, Zutshi M, Remzi FH, Parc Y, Ruppert R, Fürst A, Celebrezze J, Galanduik S, Orangio G, Hyman N, Bokey L, Tiret E, Kirchdorfer B, Medich D, Tietze M, Hull T, Hammel J. A randomized multicenter trial to compare long-term functional outcome, quality of life, and complications of surgical procedures for low rectal cancers. *Ann Surg* 2007; **246**: 481-488; discussion 488-490 [PMID: 17717452 DOI: 10.1097/SLA.0b013e3181485617]
35 **Vaizey CJ**. Faecal incontinence: standardizing outcome measures. *Colorectal Dis* 2014; **16**: 156-158 [PMID: 24450821 DOI: 10.1111/codi.12566]
36 **Quezada Y**, Whiteside JL, Rice T, Karram M, Rafferty JF, Paquette IM. Does preoperative anal physiology testing or ultrasonography predict clinical outcome with sacral neuromodulation for fecal incontinence? *Int Urogynecol J* 2015; **26**: 1613-1617 [PMID: 26017894 DOI: 10.1007/s00192-015-2746-1]
37 **Maeda Y**, O'Connell PR, Lehur PA, Matzel KE, Laurberg S. Sacral nerve stimulation for faecal incontinence and constipation: a European consensus statement. *Colorectal Dis* 2015; **17**: O74-O87 [PMID: 25603960 DOI: 10.1111/codi.12905]
38 **Gooneratne ML**, Scott SM, Lunniss PJ. Unilateral pudendal neuropathy is common in patients with fecal incontinence. *Dis Colon Rectum* 2007; **50**: 449-458 [PMID: 17279299 DOI: 10.1007/s10350-006-0839-0]
39 **Fitzpatrick M**, O'brien C, O'connell PR, O'herlihy C. Patterns of abnormal pudendal nerve function that are associated with postpartum fecal incontinence. *Am J Obstet Gynecol* 2003; **189**: 730-735 [PMID: 14526303 DOI: 10.1067/S0002-9378(03)00817-2]
40 **Súilleabháin CB**, Horgan AF, McEnroe L, Poon FW, Anderson JH, Finlay IG, McKee RF. The relationship of pudendal nerve terminal motor latency to squeeze pressure in patients with idiopathic fecal incontinence. *Dis Colon Rectum* 2001; **44**: 666-671 [PMID: 11357026 DOI: 10.1007/BF02234563]
41 **Rasmussen OO**, Christiansen J, Tetzschner T, Sørensen M. Pudendal nerve function in idiopathic fecal incontinence. *Dis Colon Rectum* 2000; **43**: 633-636; discussion 636-637 [PMID: 10826423 DOI: 10.1007/BF02235577]
42 **Soh JS**, Lee HJ, Jung KW, Yoon IJ, Koo HS, Seo SY, Lee S, Bae JH, Lee HS, Park SH, Yang DH, Kim KJ, Ye BD, Byeon JS, Yang SK, Kim JH, Myung SJ. The diagnostic value of a digital rectal examination compared with high-resolution anorectal manometry in patients with chronic constipation and fecal incontinence. *Am J Gastroenterol* 2015; **110**: 1197-1204 [PMID: 26032152 DOI: 10.1038/ajg.2015.153]
43 **Zutshi M**, Salcedo L, Hammel J, Hull T. Anal physiology testing in fecal incontinence: is it of any value? *Int J Colorectal Dis* 2010; **25**: 277-282 [PMID: 19902225 DOI: 10.1007/s00384-009-0830-z]

44 **Pehl C**, Seidl H, Scalercio N, Gundling F, Schmidt T, Schepp W, Labermeyer S. Accuracy of anorectal manometry in patients with fecal incontinence. *Digestion* 2012; **86**: 78-85 [PMID: 22832781 DOI: 10.1159/000338954]

45 **Gilliland R**, Altomare DF, Moreira H, Oliveira L, Gilliland JE, Wexner SD. Pudendal neuropathy is predictive of failure following anterior overlapping sphincteroplasty. *Dis Colon Rectum* 1998; **41**: 1516-1522 [PMID: 9860332 DOI: 10.1007/BF02237299]

46 **Chen AS**, Luchtefeld MA, Senagore AJ, Mackeigan JM, Hoyt C. Pudendal nerve latency. Does it predict outcome of anal sphincter repair? *Dis Colon Rectum* 1998; **41**: 1005-1009 [PMID: 9715157 DOI: 10.1007/BF02237391]

47 **Sangwan YP**, Coller JA, Barrett RC, Roberts PL, Murray JJ, Rusin L, Schoetz DJ. Unilateral pudendal neuropathy. Impact on outcome of anal sphincter repair. *Dis Colon Rectum* 1996; **39**: 686-689 [PMID: 8646958 DOI: 10.1007/BF02056951]

48 **Buie WD**, Lowry AC, Rothenberger DA, Madoff RD. Clinical rather than laboratory assessment predicts continence after anterior sphincteroplasty. *Dis Colon Rectum* 2001; **44**: 1255-1260 [PMID: 11584195 DOI: 10.1007/BF02234781]

49 **Santoro GA**, Eitan BZ, Pryde A, Bartolo DC. Open study of low-dose amitriptyline in the treatment of patients with idiopathic fecal incontinence. *Dis Colon Rectum* 2000; **43**: 1676-1681; discussion 1681-1682 [PMID: 11156450]

50 **Melville JL**, Newton K, Fan MY, Katon W. Health care discussions and treatment for urinary incontinence in U.S. women. *Am J Obstet Gynecol* 2006; **194**: 729-737 [PMID: 16522405 DOI: 10.1016/j.ajog.2005.09.011]

51 **Sjödahl J**, Walter SA, Johansson E, Ingemansson A, Ryn AK, Hallböök O. Combination therapy with biofeedback, loperamide, and stool-bulking agents is effective for the treatment of fecal incontinence in women - a randomized controlled trial. *Scand J Gastroenterol* 2015; **50**: 965-974 [PMID: 25892434 DOI: 10.3109/00365521.2014.999252]

52 **Norton C**, Cody JD. Biofeedback and/or sphincter exercises for the treatment of faecal incontinence in adults. *Cochrane Database Syst Rev* 2012; **(7)**: CD002111 [PMID: 22786479 DOI: 10.1002/14651858.CD002111.pub3]

53 **Norton C**, Chelvanayagam S, Wilson-Barnett J, Redfern S, Kamm MA. Randomized controlled trial of biofeedback for fecal incontinence. *Gastroenterology* 2003; **125**: 1320-1329 [PMID: 14598248 DOI: 10.1016/j.gastro.2003.09.039]

54 **Chiarioni G**, Bassotti G, Stanganini S, Vantini I, Whitehead WE. Sensory retraining is key to biofeedback therapy for formed stool fecal incontinence. *Am J Gastroenterol* 2002; **97**: 109-117 [PMID: 11808933 DOI: 10.1111/j.1572-0241.2002.05429.x]

55 **Lacima G**, Pera M, Amador A, Escaramís G, Piqué JM. Long-term results of biofeedback treatment for faecal incontinence: a comparative study with untreated controls. *Colorectal Dis* 2010; **12**: 742-749 [PMID: 19486084 DOI: 10.1111/j.1463-1318.2009.01881.x]

56 **Brown SR**, Wadhawan H, Nelson RL. Surgery for faecal incontinence in adults. *Cochrane Database Syst Rev* 2013; **(7)**: CD001757 [PMID: 23821339 DOI: 10.1002/14651858.CD001757.pub4]

57 **Kaiser AM**. Cloaca-like deformity with faecal incontinence after severe obstetric injury--technique and functional outcome of ano-vaginal and perineal reconstruction with X-flaps and sphincteroplasty. *Colorectal Dis* 2008; **10**: 827-832 [PMID: 18205849 DOI: 10.1111/j.1463-1318.2007.01440.x]

58 **Madoff RD**, Parker SC, Varma MG, Lowry AC. Faecal incontinence in adults. *Lancet* 2004; **364**: 621-632 [PMID: 15313364 DOI: 10.1016/S0140-6736(04)16856-6]

59 **Johnson E**, Carlsen E, Steen TB, Backer Hjorthaug JO, Eriksen MT, Johannessen HO. Short- and long-term results of secondary anterior sphincteroplasty in 33 patients with obstetric injury. *Acta Obstet Gynecol Scand* 2010; **89**: 1466-1472 [PMID: 20955101 DOI: 10.3109/00016349.2010.519019]

60 **Zutshi M**, Tracey TH, Bast J, Halverson A, Na J. Ten-year outcome after anal sphincter repair for fecal incontinence. *Dis Colon Rectum* 2009; **52**: 1089-1094 [PMID: 19581851 DOI: 10.1007/DCR.0b013e3181a0a79c]

61 **Rothbarth J**, Bemelman WA, Meijerink WJ, Buyze-Westerweel ME, van Dijk JG, Delemarre JB. Long-term results of anterior anal sphincter repair for fecal incontinence due to obstetric injury / with invited commentaries. *Dig Surg* 2000; **17**: 390-393; discussion 394 [PMID: 11053947 DOI: 10.1159/000018883]

62 **Glasgow SC**, Lowry AC. Long-term outcomes of anal sphincter repair for fecal incontinence: a systematic review. *Dis Colon Rectum* 2012; **55**: 482-490 [PMID: 22426274 DOI: 10.1097/DCR.0b013e3182468c22]

63 **Ratto C**, Litta F, Parello A, Donisi L, De Simone V, Zaccone G. Sacral nerve stimulation in faecal incontinence associated with an anal sphincter lesion: a systematic review. *Colorectal Dis* 2012; **14**: e297-e304 [PMID: 22356165 DOI: 10.1111/j.1463-1318.2012.03003.x]

64 **Matzel KE**, Stadelmaier U, Hohenfellner M, Gall FP. Electrical stimulation of sacral spinal nerves for treatment of faecal incontinence. *Lancet* 1995; **346**: 1124-1127 [PMID: 7475602 DOI: 10.1016/S0140-6736(95)91799-3]

65 **Lundby L**, Møller A, Buntzen S, Krogh K, Vang K, Gjedde A, Laurberg S. Relief of fecal incontinence by sacral nerve stimulation linked to focal brain activation. *Dis Colon Rectum* 2011; **54**: 318-323 [PMID: 21304303 DOI: 10.1007/DCR.0b013e31820348ac]

66 **Michelsen HB**, Thompson-Fawcett M, Lundby L, Krogh K, Laurberg S, Buntzen S. Six years of experience with sacral nerve stimulation for fecal incontinence. *Dis Colon Rectum* 2010; **53**: 414-421 [PMID: 20305440 DOI: 10.1007/DCR.0b013e3181ca7dc2]

67 **Patton V**, Wiklendt L, Arkwright JW, Lubowski DZ, Dinning PG. The effect of sacral nerve stimulation on distal colonic motility in patients with faecal incontinence. *Br J Surg* 2013; **100**: 959-968 [PMID: 23536312 DOI: 10.1002/bjs.9114]

68 **Wexner SD**, Coller JA, Devroede G, Hull T, McCallum R, Chan M, Ayscue JM, Shobeiri AS, Margolin D, England M, Kaufman H, Snape WJ, Mutlu E, Chua H, Pettit P, Nagle D, Madoff RD, Lerew DR, Mellgren A. Sacral nerve stimulation for fecal incontinence: results of a 120-patient prospective multicenter study. *Ann Surg* 2010; **251**: 441-449 [PMID: 20160636 DOI: 10.1097/SLA.0b013e3181cf8ed0]

69 **Mellgren A**, Wexner SD, Coller JA, Devroede G, Lerew DR, Madoff RD, Hull T. Long-term efficacy and safety of sacral nerve stimulation for fecal incontinence. *Dis Colon Rectum* 2011; **54**: 1065-1075 [PMID: 21825885 DOI: 10.1097/DCR.0b013e31822155e9]

70 **Hull T**, Giese C, Wexner SD, Mellgren A, Devroede G, Madoff RD, Stromberg K, Coller JA. Long-term durability of sacral nerve stimulation therapy for chronic fecal incontinence. *Dis Colon Rectum* 2013; **56**: 234-245 [PMID: 23303153 DOI: 10.1097/DCR.0b013e318276b24c]

71 **Duelund-Jakobsen J**, Lehur PA, Lundby L, Wyart V, Laurberg S, Buntzen S. Sacral nerve stimulation for faecal incontinence - efficacy confirmed from a two-centre prospectively maintained database. *Int J Colorectal Dis* 2016; **31**: 421-428 [PMID: 26490052 DOI: 10.1007/s00384-015-2411-7]

72 **Wexner SD**, Bleier J. Current surgical strategies to treat fecal incontinence. *Expert Rev Gastroenterol Hepatol* 2015; **9**: 1577-1589 [PMID: 26414494 DOI: 10.1586/17474124.2015.1093417]

73 **Peña Ros E**, Parra Baños PA, Benavides Buleje JA, Muñoz Camarena JM, Escamilla Segade C, Candel Arenas MF, Gonzalez Valverde FM, Albarracín Marín-Blázquez A. Short-term outcome of percutaneous posterior tibial nerve stimulation (PTNS) for the treatment of faecal incontinence. *Tech Coloproctol* 2016; **20**: 19-24 [PMID: 26499791 DOI: 10.1007/s10151-015-1380-8]

74 **Efron JE**, Corman ML, Fleshman J, Barnett J, Nagle D, Birnbaum E, Weiss EG, Nogueras JJ, Sligh S, Rabine J, Wexner SD. Safety and effectiveness of temperature-controlled radio-frequency energy delivery to the anal canal (Secca procedure) for the treatment

of fecal incontinence. *Dis Colon Rectum* 2003; **46**: 1606-1616; discussion 1616-1618 [PMID: 14668584 DOI: 10.1097/01.DCR.0000098927.47914.D0]

75 **Abbas MA**, Tam MS, Chun LJ. Radiofrequency treatment for fecal incontinence: is it effective long-term? *Dis Colon Rectum* 2012; **55**: 605-610 [PMID: 22513440 DOI: 10.1097/DCR.0b013e3182415406]

76 **Luo C**, Samaranayake CB, Plank LD, Bissett IP. Systematic review on the efficacy and safety of injectable bulking agents for passive faecal incontinence. *Colorectal Dis* 2010; **12**: 296-303 [PMID: 19320664 DOI: 10.1111/j.1463-1318.2009.01828.x]

77 **Graf W**, Mellgren A, Matzel KE, Hull T, Johansson C, Bernstein M. Efficacy of dextranomer in stabilised hyaluronic acid for treatment of faecal incontinence: a randomised, sham-controlled trial. *Lancet* 2011; **377**: 997-1003 [PMID: 21420555 DOI: 10.1016/S0140-6736(10)62297-0]

78 **Maeda Y**, Laurberg S, Norton C. Perianal injectable bulking agents as treatment for faecal incontinence in adults. *Cochrane Database Syst Rev* 2013; **(2)**: CD007959 [PMID: 23450581 DOI: 10.1002/14651858.cd007959.pub3]

79 **Wald A**. New treatments for fecal incontinence: update for the gastroenterologist. *Clin Gastroenterol Hepatol* 2014; **12**: 1783-1788 [PMID: 24534548 DOI: 10.1016/j.cgh.2014.02.013]

80 **Ratto C**, Donisi L, Litta F, Campennì P, Parello A. Implantation of SphinKeeper(TM): a new artificial anal sphincter. *Tech Coloproctol* 2016; **20**: 59-66 [PMID: 26658726 DOI: 10.1007/s10151-015-1396-0]

81 **Ratto C**, Buntzen S, Aigner F, Altomare DF, Heydari A, Donisi L, Lundby L, Parello A. Multicentre observational study of the Gatekeeper for faecal incontinence. *Br J Surg* 2016; **103**: 290-299 [PMID: 26621029 DOI: 10.1002/bjs.10050]

82 **Salcedo L**, Sopko N, Jiang HH, Damaser M, Penn M, Zutshi M. Chemokine upregulation in response to anal sphincter and pudendal nerve injury: potential signals for stem cell homing. *Int J Colorectal Dis* 2011; **26**: 1577-1581 [PMID: 21706136 DOI: 10.1007/s00384-011-1269-6]

83 **Lane FL**, Jacobs SA, Craig JB, Nistor G, Markle D, Noblett KL, Osann K, Keirstead H. In vivo recovery of the injured anal sphincter after repair and injection of myogenic stem cells: an experimental model. *Dis Colon Rectum* 2013; **56**: 1290-1297 [PMID: 24105005 DOI: 10.1097/DCR.0b013e3182a4adfb]

84 **Lehur PA**, McNevin S, Buntzen S, Mellgren AF, Laurberg S, Madoff RD. Magnetic anal sphincter augmentation for the treatment of fecal incontinence: a preliminary report from a feasibility study. *Dis Colon Rectum* 2010; **53**: 1604-1610 [PMID: 21178853 DOI: 10.1007/DCR.0b013e3181f5d5f7]

85 **Barussaud ML**, Mantoo S, Wyart V, Meurette G, Lehur PA. The magnetic anal sphincter in faecal incontinence: is initial success sustained over time? *Colorectal Dis* 2013; **15**: 1499-1503 [PMID: 24103055 DOI: 10.1111/codi.12423]

86 **Pakravan F**, Helmes C. Magnetic anal sphincter augmentation in patients with severe fecal incontinence. *Dis Colon Rectum* 2015; **58**: 109-114 [PMID: 25489702 DOI: 10.1097/DCR.0000000000000263]

87 **Lehur PA**, Wyart V, Riche VP. SaFaRI: sacral nerve stimulation versus the Fenix® magnetic sphincter augmentation for adult faecal incontinence: a randomised investigation. *Int J Colorectal Dis* 2016; **31**: 1505 [PMID: 26861637 DOI: 10.1007/s00384-016-2531-8]

88 **Matzel KE**, Madoff RD, LaFontaine LJ, Baeten CG, Buie WD, Christiansen J, Wexner S. Complications of dynamic graciloplasty: incidence, management, and impact on outcome. *Dis Colon Rectum* 2001; **44**: 1427-1435 [PMID: 11598470 DOI: 10.1007/BF02234593]

89 **Rongen MJ**, Uludag O, El Naggar K, Geerdes BP, Konsten J, Baeten CG. Long-term follow-up of dynamic graciloplasty for fecal incontinence. *Dis Colon Rectum* 2003; **46**: 716-721 [PMID: 12794571 DOI: 10.1007/s10350-004-6645-7]

90 **Hultman CS**, Zenn MR, Agarwal T, Baker CC. Restoration of fecal continence after functional gluteoplasty: long-term results, technical refinements, and donor-site morbidity. *Ann Plast Surg* 2006; **56**: 65-70; discussion 70-71 [PMID: 16374099]

91 **Rosenblatt P**. New developments in therapies for fecal incontinence. *Curr Opin Obstet Gynecol* 2015; **27**: 353-358 [PMID: 26308196 DOI: 10.1097/GCO.0000000000000201]

92 **Mellgren A**, Zutshi M, Lucente VR, Culligan P, Fenner DE. A posterior anal sling for fecal incontinence: results of a 152-patient prospective multicenter study. *Am J Obstet Gynecol* 2016; **214**: 349.e1-349.e8 [PMID: 26493933 DOI: 10.1016/j.ajog.2015.10.013]

93 **Hendren S**, Hammond K, Glasgow SC, Perry WB, Buie WD, Steele SR, Rafferty J. Clinical practice guidelines for ostomy surgery. *Dis Colon Rectum* 2015; **58**: 375-387 [PMID: 25751793 DOI: 10.1097/DCR.0000000000000347]

94 **Patel AS**, Saratzis A, Arasaradnam R, Harmston C. Use of Antegrade Continence Enema for the Treatment of Fecal Incontinence and Functional Constipation in Adults: A Systematic Review. *Dis Colon Rectum* 2015; **58**: 999-1013 [PMID: 26347973 DOI: 10.1097/DCR.0000000000000428]

95 **Koch SM**, Uludağ O, El Naggar K, van Gemert WG, Baeten CG. Colonic irrigation for defecation disorders after dynamic graciloplasty. *Int J Colorectal Dis* 2008; **23**: 195-200 [PMID: 17896111 DOI: 10.1007/s00384-007-0375-y]

P- Reviewer: Awad RA, Chiarioni G, Lakatos PL, Lee JT, Santoro GA
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.25

World J Gastroenterol 2017 January 7; 23(1): 25-41
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Maximizing the endosonography: The role of contrast harmonics, elastography and confocal endomicroscopy

Andrada Seicean, Ofelia Mosteanu, Radu Seicean

Andrada Seicean, Ofelia Mosteanu, Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Hatieganu", 400162 Cluj-Napoca, Romania

Radu Seicean, First Surgical Clinic Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Hatieganu", 400162 Cluj-Napoca, Romania

Author contributions: Seicean A conceived and designed the study; Seicean A, Mosteanu O and Seicean R analyzed and interpreted the data, drafted the article, and gave their approval for the final version of the article to be published.

Supported by National Grant of the Romanian Education Ministry, No. PN-II-PT-PCCA.2O 1 3-4-1 1 O5/2014.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Ofelia Mosteanu, MD, PhD, Regional Institute of Gastroenterology and Hepatology Cluj-Napoca, University of Medicine and Pharmacy "Iuliu Hatieganu", Croitorilor Street 19-21, 400162 Cluj-Napoca, Romania. ofeliamosteanu@gmail.com
Telephone: +40-742-073020
Fax: +40-264-431758

Received: September 23, 2016
Peer-review started: September 24, 2016
First decision: October 20, 2016
Revised: November 17, 2016
Accepted: December 8, 2016

Article in press: December 8, 2016
Published online: January 7, 2017

Abstract

New technologies in endoscopic ultrasound (EUS) evaluation have been developed because of the need to improve the EUS and EUS-fine needle aspiration (EUS-FNA) diagnostic rate. This paper reviews the principle, indications, main literature results, limitations and future expectations for each of the methods presented. Contrast-enhanced harmonic EUS uses a low mechanical index and highlights slow-flow vascularization. This technique is useful for differentiating solid and cystic pancreatic lesions and assessing biliary neoplasms, submucosal neoplasms and lymph nodes. It is also useful for the discrimination of pancreatic masses based on their qualitative patterns; however, the quantitative assessment needs to be improved. The detection of small solid lesions is better, and the EUS-FNA guidance needs further research. The differentiation of cystic lesions of the pancreas and the identification of the associated malignancy features represent the main indications. Elastography is used to assess tissue hardness based on the measurement of elasticity. Despite its low negative predictive value, elastography might rule out the diagnosis of malignancy for pancreatic masses. Needle confocal laser endomicroscopy offers useful information about cystic lesions of the pancreas and is still under evaluation for use with solid pancreatic lesions of lymph nodes.

Key words: Endosonography; Endosonography-fine needle aspiration; Contrast-enhanced; Harmonics; Elastography; Endomicroscopy; Pancreas; Lymph nodes; Cyst

Core tip: Contrast-enhanced harmonic endoscopic ultrasound, elastography and needle confocal laser endomicroscopy represent new, emerging technologies for improving the diagnosis obtained using endoscopic ultrasonography. This paper reviews the principle, indications, main literature results, limitations and future expectations for each of these methods, such as their use in the guidance or orientation of endoscopic ultrasound fine needle aspiration, molecular imaging and neurophysiology assessment in gastroenterology.

Seicean A, Mosteanu O, Seicean R. Maximizing the endosonography: The role of contrast harmonics, elastography and confocal endomicroscopy. *World J Gastroenterol* 2017; 23(1): 25-41 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/25.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.25

INTRODUCTION

New technologies in endosonography assessment are under development due to the limitations of standard endoscopic ultrasound-fine needle aspiration (EUS-FNA): the diagnostic accuracy for pancreatic masses is as high as 95% for cytology[1,2] and 86% for histology[3], the sensitivity for diagnosing lymph nodes is 90%[4], correct diagnosis is difficult for submucosal neoplasms[5], the differential diagnosis of pancreatic cysts is approximately 20%-30%[6], and the malignancy potential is sometimes challenging[7].

CONTRAST HARMONICS ENDOSONOGRAPHY

The use of Doppler contrast-enhanced EUS using a high mechanical index has been abandoned due to artifacts such as blooming, motion artifacts, poor spatial resolution, and low sensitivity to slow-flow structures[8].

The principle of contrast-enhanced harmonic imaging is to selectively depict signals from the microbubbles of ultrasound contrast agents, which resonate non-linearly when exposed to ultrasonic beams[9]. Background tissue signals are automatically subtracted, and only signals from the contrast agent are enhanced. The mechanical index (MI), which represents the ratio between the peak negative pressure amplitude and the square of the frequency, is related to the oscillation of the microbubbles. For an MI value of lower than 0.1, the bubble oscillation is linear, and no harmonics are produced. For an MI value of higher than 0.6, the microbubbles are destroyed. For this reason, an MI value of 0.14-0.4 is used during contrast-enhanced harmonics EUS (CH-EUS). This method enables the dynamic observation of microvessels with slow flows that are not revealed by Doppler color, which differentiates perfused and non-perfused tissue; however, image resolution is reduced compared to that in B-mode harmonic images.

EQUIPMENT AND CONTRAST SUBSTANCE

CH-EUS can be performed using dynamic contrast harmonic imaging (dCHI) implemented onthe Hitachi platform and using the extended pure harmonic (ExpH) as technique implemented on Aloka platforms. The principle behind the first method is based on the emission of consecutive waves in phase inversion, followed by non-linear oscillation of the microbubbles[10]. The second system produces two transmitted pulses that consecutively reach the microbubble, yielding a phase shift between the two received waves[10]. The acoustic power used is low to avoid rapid destruction of the microbubbles (0.2-0.4). A suitable dynamic range that enables good visualization of small differences between vessels and the parenchyma and the focus under the target lesion should be fixed before the contrast injection starts.

Two major contrast substances have been used. Sonovue (Bracco Imaging, Milan, Italy) contains microbubbles of sulfur hexafluoride gas enclosed in a lipid shell. Its injection is followed by an arterial phase (the first 25-30 s after the injection) and a venous phase (30-45 s after the injection). Sonazoid (Daiichi-Sankyo, Tokyo, Japan), which is unavailable in Europe and comprises perfluorobutane in a lipid shell, is uptaken by Kupffer cells, conferring a longer duration than Sonovue.

INDICATIONS FOR THE USE OF CH-EUS

(1) Assessment of solid and cystic lesions of the pancreas; (2) characterization of submucosal neoplasms; (3) assessment of biliary neoplasms; and (4) assessment of lymph nodes.

SOLID PANCREATIC MASSES

CH-EUS helps in mass differentiation, the differentiation between vascular (solid) and avascular (liquid/necrotic) components of the lesion, and the depiction of the dimensions and margins of the pancreatic mass, including its relationship with adjacent vessels.

Mass differentiation

CH-EUS examination has to report three descriptors[11,12], and the resulting pattern differs between lesions, enabling differentiation before the pathology result is available (Tables 1 and 2).

Qualitative assessment: Adenocarcinoma. Contrast uptake by small vessels reveals the low vascularity of these lesions. The hypoenhanced aspect has been

Table 1 Descriptors used in harmonic contrast-enhanced endoscopic ultrasound examination

Descriptors	Enhancement	Pattern of distribution	Wash-out
	Hyper/iso/hypoenhancement	Homogenous/inhomogenous	Slow/Fast
Corresponding feature	Arteriolar density compared to the adjacent normal parenchyma	Vascularity architecture	Velocity of the venous blood flow
Phase	Arterial	Arterial	Venous

Arterial phase: 20 s to 30-45 s after injection; Venous phase: Later than 30-45 s after contrast injection.

Table 2 Description of solid and cystic pancreatic lesions during harmonic contrast-enhanced endoscopic ultrasound examination

		Enhancement	Pattern of distribution	Wash-out
Solid pancreatic lesion	Adenocarcinoma	Hypoenhanced	Homogenous/non-homogenous	Fast
	NET	Hyperenhanced > hypoenhanced	Homogenous/non-homogenous	Slow > Fast
	Chronic pancreatitis	Isoenhanced/hyperenhanced > hypoenhanced	Homogenous/non-homogenous	Fast
	Autoimmune pancreatitis	Isoenhanced/hyperenhanced	Homogenous/non-homogenous	Fast
Cystic pancreatic lesion	SCA	Hyperenhancement of the vascularized septae, honeycomb aspect highlighted	Homogenous	Slow
	MCN	Hyperenhanced thick walls, thick septa and nodules are predictive for malignancy		Fast
	Pseudocyst	Avascular wall + solid component without any contrast uptake		-
	IPMN	Hyperenhanced septae and vascularized neoplastic nodules		Fast
	NET cystic	Hyperenhanced wall and vascularized nodules		Slow

NET: Neuroendocrine tumor; SCA: Serous cystadenoma; MCN: Mucinous cystic neoplasm; IPMN: Intraductal papillary mucinous neoplasm.

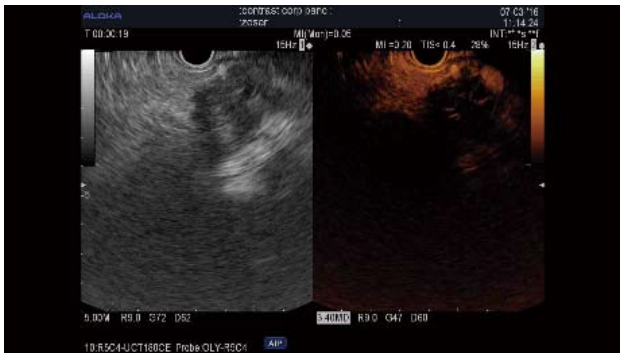


Figure 1 Contrast-enhanced harmonics-endoscopic ultrasound of pancreatic adenocarcinoma. Standard endoscopic ultrasound image (left) of a hypoenhanced lesion of the pancreatic body. The contrast-enhanced image (right) shows a hypoenhanced adenocarcinoma. The dilated pancreatic duct is seen upstream of the lesion.

reported as predictive for malignancy in several series of patients, with sensitivities of 84%-96%, specificities of 64%-94%, and accuracies 82%-92% (Table 3[12-21]); further, two studies reported superior results compared to standard EUS[12,13]. The pattern of contrast uptake can be inhomogenous when necrosis or intensive fibrosis is present, and fast wash-out is generally seen[12]. Some cases (4%-11% of cases) of isoenhanced/hyperenhanced adenocarcinoma aspect have been reported[14-17]. However, CH-EUS cannot yet replace EUS-FNA for the differentiation of solid masses[14,15,17,18] (Figure 1).

A meta-analysis of the diagnosis of adenocarcinoma published in 2012 proved that hypoenhancement has a global sensitivity of 94% and a specificity of 89%. However, the main bias of this study was the combination of Doppler and harmonic contrast EUS[22]. A second meta-analysis on contrast-enhanced ultrasound included a combination of endoscopic and transabdominal methods, and its results cannot be generalized for CH-EUS[23]. In a retrospective study of small pancreatic masses, adenocarcinoma was found in only 40%[21] of cases, and CH-EUS might be useful for the identification of such hypoenhanced lesions, which can be sent for surgery without EUS-FNA.

Tumor types other than adenocarcinoma, such as neuroendocrine tumors (NET), chronic pancreatitis, autoimmune pancreatitis, serous cystadenoma, and metastasis, are iso/hyperenhanced[12,14], with a sensitivity of 39%-86% and a specificity of 98%[12,15]. However, only 69%-100% of NET are hyperenhanced[12,14,15]. An inhomogenous pattern in these tumor types corresponding to hemorrhage or necrosis is suggestive of malignancy[12,24] and can be seen in 15% of cases[24]. Few data are available about the usefulness of CH-EUS compared to standard EUS and were acquired in a very limited number of patients (n = 19) (Sn = 78.9%, Sp = 98%), providing a similar value as CT scanning when the small lesions were taken into account[15] (Figure 2).

Focal inflammatory mass may have a hypoechoic appearance in standard EUS and may exhibit diffuse iso/hyperenhancement using CH-EUS[15,16,19] with homogenous or inhomogenous content and fast wash-out. Sometimes these masses present as hypoenhanced lesions (9%-17%) because they exhibit different

Table 3 Results of contrast-enhanced harmonics-endoscopic ultrasound assessment for solid pancreatic masses in various studies

Ref.	Type of study	Contrast agent	No. of patients	MI	Hypoenhancement as a sign of adenocarcinoma	EUS diagnostic rate	EUS-FNA diagnostic rate
Napoleon *et al*[18] 2010	Endoscopy	Sonovue	35 PC-18 NET-9 CP-7	0.4	Sn = 89% Sp = 88% PPV = 89% NPV = 88% Acc = 88.5%		Sn = 79% Sp = 100% PPV = 100% NPV = 54% Acc = 83%
Fusaroli *et al*[12] 2010	Prospective	Sonovue	90 PC-51, NET-13, CP-13	0.36 radial 0.28 linear	Sn = 96% Sp = 64% Ac = 82%	Sn = 86% Sp = 18% Ac = 57%	
Ang *et al*[19] 2011		Definity	29 (PC-16, CP-4, Other-9)	0.3	Better detection of vascular invasion and tumor margins		-
Matsubara *et al*[20] 2011	Retrospective	Sonazoid	91	0.2	Sn = 87.5% Sp = 77.8%	-	-
Hocke *et al*[13] 2012	Prospective	Sonovue	58	-	Sn = 84% Sp = 76%	Sn = 73% Sp = 61%	-
Kitano *et al*[15] 2012	Prospective	Sonazoid	277 (PC-204, NET-19, CrP-46, Other-8)	0.3	Sn = 95% Sp = 89%	-	Sn = 92%[1] Sp = 100%
Lee *et al*[16] 2013	Prospective	Sonovue	37 (PC-28, NET-5, CP-2)	-	Sn = 93% Sp = 86% PPV = 93% NPV = 75% Acc = 92%	-	-
Gincul *et al*[14] 2014	Prospective	Sonovue	100 (PC-69, NET-10, CP-13, Other-8)	0.4	Sn = 96% Sp = 94% PPV = 94% NPV = 97% Acc = 91%		Sn = 95% Sp = 93% PPV = 100% NPV = 100% Acc = 86%
Park *et al*[17] 2014	Retrospective	Sonovue	90	-	Sn = 91.9% Sp = 67.8%	-	Sn = 90% Sp = 100%
Dietrich *et al*[21] 2016	Retrospective	Sonovue	394 PC-146 NET-156		Sn = 92%	-	-

[1]91 patients with resected lesions. MI: Mechanical index; PC: Pancreatic cancer; Cp: Chronic pancreatitis; NET: Neuroendocrine tumors; PPV: Positive predictive value; NPV: Negative predictive value; Sn: Sensitivity; Sp: Specificity; EUS-FNA: Endoscopic ultrasound-fine needle aspiration.

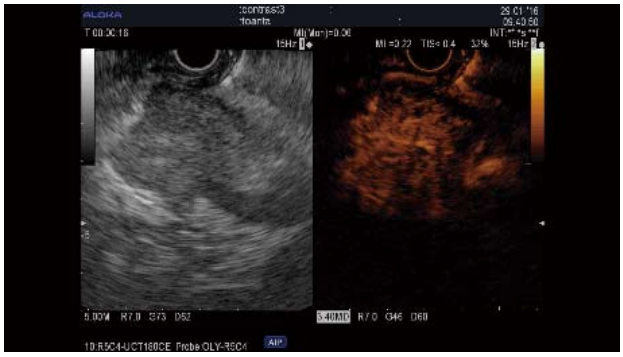


Figure 2 Contrast-enhanced harmonics-endoscopic ultrasound of a neuroendocrine pancreatic tumor. Standard endoscopic ultrasound image (left) of a hypoenhanced, well-delineated lesion of the head of the pancreas. The contrast image (right) shows a hyperenhanced lesion that is suggestive of a neuroendocrine tumor, as later proved by fine needle aspiration.

degrees of fibrosis[14,15,25]. The presence of calcifications is a confounding factor and should be avoided in the region of interest while analyzing contrast uptake. Only one study on solid pancreatic masses compared the low mechanical index of CH-EUS with the high mechanical index of contrast Doppler CEUS, and the second method was found to be superior. The main reason for this was the lack of contrast enhancement in patients with chronic pancreatitis, which was perhaps related to the machine settings; however, this cannot be assessed because the settings were not fully reported[25]. Autoimmune pancreatitis is usually homogenously isoenhanced[26] or hyperenhanced[14].

Cancer metastases are hyperenhanced (renal and thyroid carcinomas, lymphoma, and colon cancer)[14,27,28] or are hypoenhanced (colon cancer, sarcoma, and breast and ovarian cancer)[27,28]; melanoma is isoenhanced[27].

Interobserver agreement for solid pancreatic mass diagnosis showed modest results for the examinations using Sonovue (k = 0.46-0.66) and for the degree of enhancement[14,29]. The k coefficient was 0.94 for Sonazoid, a value that was perhaps related to the signal intensity and its duration. The effect of the endosonographer in CH-EUS was similar for experienced and non-experienced doctors[14,29], except in one study[30].

Combined use of qualitative CH-EUS with EUS-FNA: increased the sensitivity of differential diagnosis using Sonazoid from 92% to 100%, and the specificity was maintained at 92%[15,17]; these findings were similar to results obtained using Sonovue[18]. In

Table 4 Quantitative assessment studies for differentiating pancreatic masses

Ref.	Type of study	Type of mass	Contrast agent	Type of echoendoscope	MI	Quantitative assessment	Features useful for differentiation	Diagnostic rate
Seicean *et al*[31], 2010	Prospective	PC-15 CP-12	Sonovue	Radial	0.36	Hue histogram	Uptake index ratio	Sn = 80% Sp = 91% PPV = 92.8% NPV = 78%
Matsubara *et al*[20], 2011	Retrospective	PC-48 AIP-14 CP-13 NET-16	Sonazoid	Linear	0.20	TIC	Echo intensity reduction rate relative to the peak at 1 min	Sn = 87.5% Sp = 88.9% EUS + TIC Sn = 95.8% Sp = 92.6%
Gheonea *et al*[25], 2012	Prospective	CP-19 PC-32	Sonovue	Linear	0.20	Postprocessing TIC	Peak intensity intensity TTP AUC	Sn = 93.7% Sp = 89.4%
Imazu *et al*[32], 2014	Prospective	AIP-8 PC-22	Sonazoid	Radial	0.25-0.3	TIC	Peak intensity Maximum intensity gain	Sn = 100% Sp = 100%
Săftoiu *et al*[33], 2015	Prospective	PC-112 CP-55	Sonovue	Linear Radial	0.1-0.3	TIC	Peak intensity Wash-in AUC Wash-in rate Wash-in perfusion index	Sn = 87.5% Sp = 92.72%

MI: Mechanical index; PC: Pancreatic cancer; CP: Chronic pancreatitis; NET: Neuroendocrine tumors; AIP: Autoimmune pancreatitis; PPV: Positive predictive value; NPV: Negative predictive value; Sn: Sensitivity; Sp: Specificity.

addition, the hypoenhanced aspect obtained during CH-EUS proved to be useful in false negative cases of adenocarcinoma[17,18].

Quantitative assessment: Several attempts were done to quantify the image obtained during contrast injection. The dedicated software installed with the instruments is difficult to use because respiratory movements cannot be corrected by the software, and the time-intensity curve (TIC) has many artifacts.

The first study using quantitative assessment and harmonics was based on a hue histogram analysis, and the uptake ratio index between the mass and the surrounding parenchyma was 0.17, with a sensitivity of 80%[28] (Table 4[20,25,31-33]). The post-processing analysis of the time-intensity curves using dedicated software showed that the inflammatory mass studied exhibited a dynamic enhancement pattern using CH-EUS that was similar to that obtained for the rest of the parenchyma, while an adenocarcinoma mass presented low contrast enhancement during the early arterial and late venous phases[25]. Two other studies showed that peak intensity and the rate of echo intensity decrease relative to the peak obtained at 1 min were useful for differentiating malignant tumors[13,26].

A large multicentric study showed again that peak intensity and features related to the wash-in phase are good parameters for differentiating pancreatic masses, presenting a similar diagnostic rate to that obtained in studies using Sonazoid. However, the time-to-peak value revealed no significance[33]. Post-processing analysis of the TIC in a neural network showed even better diagnostic value, but further results are expected due to the extensive use of this method[33]. However, the software available for use with ultrasound machines awaits further refinement.

Mass detection

Mass detection is improved only in cases that are poorly seen using standard EUS, such as chronic pancreatitis or biliary stents[12]. CH-EUS is superior to CT for the detection of small tumors (Sn = 91.2%, Sp = 94.4%) but not for the detection of all tumors[15].

Tumor staging

Tumor staging appears to be better assessed using Sonazoid[32], in particular because the portal vein wall is more clearly seen[11]. No superiority in staging was observed when Sonovue was used[28], although vessel invasion and tumor size were more effectively seen in contrast-enhanced images[28].

CH-EUS-FNA

The orientation of the EUS-FNA after contrast injection in the hypoenhanced areas was first described by Kitano and then applied in 26% of cases with mixed adenocarcinoma to aid needle placement in the hypoenhanced area[14].

The guidance of the needle during the venous phase of contrast EUS was reported in some case reports and in three series[34]. The diagnostic value was similar to that of standard EUS-FNA in all three studies[28,35,36]. In one randomized control study, the first pass provided better cytology results than standard puncture and limited the number of passes[35]. Our results showed that a combination of two passes under contrast

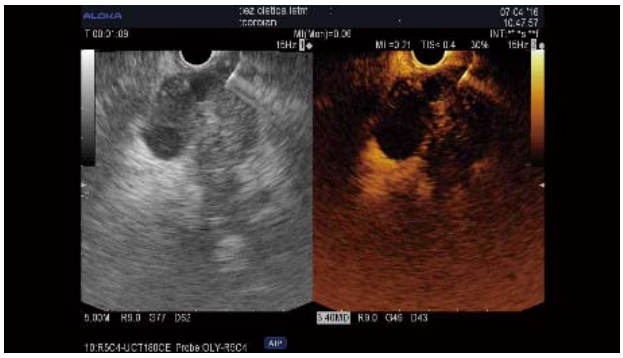


Figure 3 Contrast-enhanced harmonics-endoscopic ultrasound-fine needle aspiration of solid pancreatic adenocarcinoma. Standard endoscopic ultrasound view of a hypoenhanced, inhomogenous adenocarcinoma of the head of the pancreas, showing anechoic parts suggestive of necrosis. The contrast image (right) highlights these avascular parts of the lesion, and the needle inside is clearly seen as avoiding them.

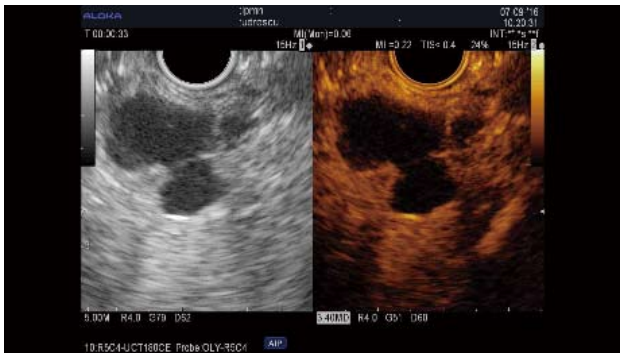


Figure 4 Contrast-enhanced harmonics-endoscopic ultrasound in mucinous cystadenomashowing features suggestive of malignancy. Standard endoscopic ultrasound image (left) of a macrocystic lesion of the pancreas with mural nodules and thin septae inside. The contrast image (right) shows vascularized mural nodules and no contrast uptake in some of the septae.

and two standard passes improved core histology-based diagnosis[28] (Figure 3). However, further larger multicenter studies are needed to establish the value of this method, which is safe, rapid, and entails minimal extra costs.

CYSTIC LESIONS OF THE PANCREAS

In cystic lesions of the pancreas (PCL), the cystic wall, septae and nodules are assessed for vascularization using the contrast-enhancing bubble movement[37], with the goal of obtaining a differential diagnosis of PCL and identifying malignancy risk features (vascularized wall nodules and the intracystic solid component).

Differential diagnosis

The CH-EUS degree and pattern of enhancement aids in the differentiation of PCL when used as an additional examination (Table 2). The method cannot replace EUS-FNA, except for with typical serous cystadenoma (SCA), because in 86% of cases, the aspect is hyperenhanced, with slow wash-out in 78% of cases[38]. Mucinous cystadenoma with neoplastic transformation presents thick septa and hyperenhanced mural nodules but cannot be differentiated from macrocystic SCA using CH-EUS[38]. Pancreatic pseudocysts have an avascular wall. However, in a series of 46 pseudocysts, three exhibited some wall vascularity[37]. These cysts can have a solid component without any contrast uptake during CH-EUS, thus avoiding EUS-FNA with a potential risk of infection[38]. Neuroendocrine tumors (NET) with a cystic aspect, despite the presence of a hyperenhanced rim, should be sampled by EUS-FNA to differentiate them from cystic adenocarcinoma.

The wall nodules of mucinous cystic neoplasms (MCNs) or intraductal papillary mucinous neoplasms (IPMNs) at risk of becoming malignant appear as hyper/isoechoic without a hyperechoic rim or are smooth edged, and the Doppler vascularity is seldom positive (2 of 14 cases were resected)[39]. CH-EUS is superior to EUS because it reveals small vessels by intense uptake of the contrast substance and differentiates the vessels from mucus or debris, which are not enhanced. Moreover, the CH-EUS may orientate/guide the EUS-FNA[38].

Identification of risk features for malignancy

CH-EUS differentiates unenhanced mucus or debris from the malignant nodules of MCNs or IPMNs, which are hyperenhanced, and fast wash-out has been reported in some retrospective studies (Table 5; Figure 4). The CH-EUS detection rate of malignant nodules (84%-98%) was found to be superior to that of standard EUS and CT scan in three studies using Sonazoid[40-42]. The quantitative analysis of contrast uptake may add supplementary data for use in nodule assessment[43]. Several parameters have been described in a retrospective study using Sonazoid, such as the echo intensity change, echo intensity reduction rate and nodule/pancreatic parenchyma contrast ratio. The nodule size on CH-EUS was found to be predictive of malignancy (4 mm and 8 mm)[41,42]; however, another study found that size did not have predictive value[43]. Hyperenhancement of the solid component might orientate/guide EUS-FNA and help the operator to avoid puncturing debris, sludge and mucus plugs[38] (Figure 5).

Interobserver agreement: For cyst assessment was moderate for the uptake (k = 0.557), slight for the pattern (k = 0.083), and fair for the washout (k = 0.350)[29]. Considering mural nodules, interobserver agreement was excellent using Sonazoid as the contrast agent[42].

Submucosal neoplasms: Hyperenhancement of the submucosal neoplasm during contrast injection was considered suggestive of gastrointestinal stromal tumor (GIST) and is useful for differentiation from benign hypoenhanced lesions, such as lipoma and

Table 5 Contrast-enhanced endoscopic ultrasound for use in characterizing mural nodules in cystic pancreatic lesions

Ref.	Type of study	MI	No. of patients	Type of cystic lesions	Contrast substance	Detection of mural nodules accuracy	Diagnosis of malignancy	Cut-off height for malignancy diagnosis(mm)
Yamashita *et al*[40] 2013	Retrospective	0.36	17	IPMN	Sonazoid	EUS-0 CT-71% CH-EUS-94%		
Hocke *et al*[37] 2014	Retrospective	0.02-0.18	125	1 MCN 6 MD-IPMN 16 BD-IPMN 103 others	Sonovue	Not defined	Not defined	-
Harima *et al*[41] 2015	Retrospective	-	50	IPMN BD	Sonazoid	CT-92% EUS-72% CH-EUS-98%		8.8 (AUROC = 0.93)
Kamata *et al*[42] 2016	Retrospective	0.30	70	6 MCN 42 BD-IPMNs 4 SCN 18 other	Sonazoid	EUS-73% CH-EUS-84%	EUS-64 CH-EUS-84	EUS-8 mm (AUROC = 0.84) CH-EUS-4 mm (AUROC = 0.93)
Yamamoto *et al*[43] 2016	Retrospective	0.20	30	6/18/2006 MD/BD/Mixt IPMN	Sonazoid		Echo intensity change-0.8 Echo intensity reduction rate-0.9 Nodule/pancreatic parenchyma contrast ratio-0.89	No effect on malignancy rate

IPMNs: Intraductal papillary mucinous neoplasms; CH-EUS: Contrast-enhanced harmonics-endoscopic ultrasound; MCN: Mucinous cystic neoplasm.

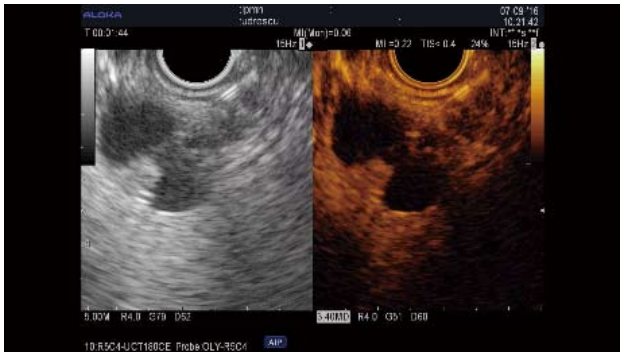


Figure 5 CH-endoscopic ultrasound-fine needle aspiration of a vascularized mural nodules within mucinous cystadenoma. An endoscopic ultrasound-fine needle aspiration needle during the puncture of one of the mural nodules is better seen on the contrast image (right).

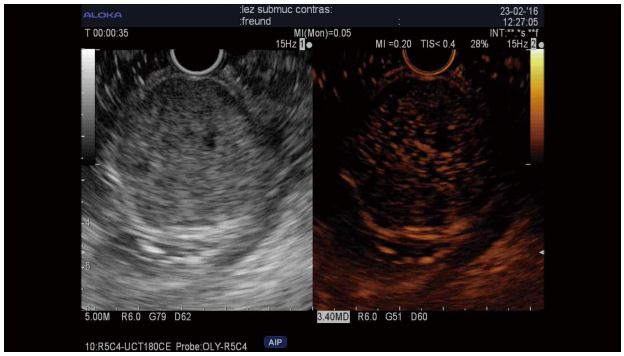


Figure 6 Contrast-enhanced harmonics endoscopic ultrasound of gastric gastrointestinal stromal tumor. Endoscopic ultrasound standard image (right) of a gastric gastrointestinal stromal tumor of the muscularis propria. The contrast image (left) shows hyperenhancement of the lesion.

leiomyoma[44]. The consideration of irregular vessels as predictors of GIST malignancy has shown a sensitivity of 100% and a specificity of 63%[45]. Interobserver agreement was substantial (k = 0.63) for the uptake, slight for the pattern (k = 0.18), and fair for the washout (k = 0.39)[29] (Figure 6).

Biliary tumors: Biliary polyps have been assessed using CH-EUS. Cholesterol polyps revealed heterogenous enhancement, and adenoma exhibited homogenous enhancement with a sensitivity and specificity of 75% and 66%, respectively[46]. However, the heterogenous aspect of a cholesterol polyp could be mistaken for adenoma due to the presence of microvessels with hyaline fibrosis[46].

Ampullary carcinoma and biliary tumors are hyperenhanced with fast wash-out[19,46]. The thick wall of the gallbladder makes it difficult to differentiate between malignant tumors and inflammatory modifications using standard EUS; however, CH-EUS improves the accuracy (94% *vs* 73%)[47]. The interobserver accuracy obtained when Sonazoid was used to examine the gallbladder wall was substantial (k = 0.77)[47].

Lymph node assessment: Round shape, sharp edge, and a short axis exceeding 8.3 mm are significantly associated with malignant cytology in LNs[48]. A retrospective study of CH-EUS use in LNs showed that 83% of malignant nodes presented a heterogenous pattern with distorted vessels and

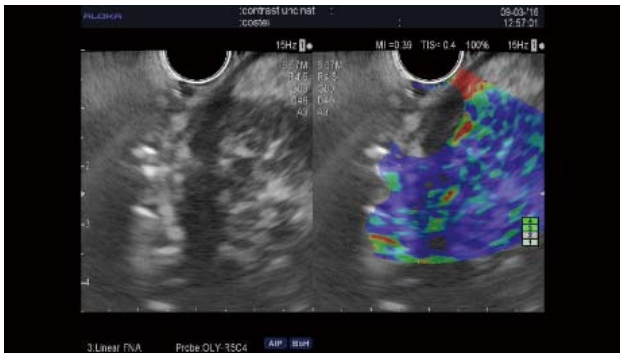


Figure 7 Elastography of pancreatic neuroendocrine tumor - qualitative assessment. Endoscopic ultrasound standard image (right) showing a well-delineated hypoenhanced lesion of the head of the pancreas. The elastography image (left) exhibits a blue pattern in this neuroendocrine tumor.

that a homogenous enhancement was suggestive of reactive lymph nodes[43]. The diagnostic value of CH-EUS for malignancy was characterized by a sensitivity, a specificity and an accuracy of 83%, 91% and 88% respectively[49]. Similar results have been reported for the characterization of intra-abdominal lesions of unknown origin[50]. The interobserver agreement obtained for LN assessment was excellent (k = 0.81)[49].

Limitations: (1) the duration of contrast enhancement is short, especially for Sonovue; (2) quantitative assessment is difficult due to respiratory movements; and (3) technical standardization is lacking.

Future perspectives: The contrast guidance of EUS-FNA could become a routine technique. Because CH-EUS has proven its role to show the change of size and tumor vascularity during chemotherapy for gastric cancers[51], it can be used also assess therapy in other digestive tumors.

Elastography

Principle: This technique assesses the hardness of the tissue by measuring its elasticity, similar to a virtual palpation. The compression of a target tissue by an echo-endoscopic probe produces a displacement of the tissue called "strain", which correlates with the hardness of the structure.

Technique: It is essential to establish a large region of interest (ROI) that half comprises the lesion and half comprises the surrounding tissues such that the hardness of the lesion and that of the surrounding tissue can be compared. The probe of the echoendoscope, when upright, creates some pressure, and very small additional movements are important for obtaining the image.

Qualitative assessment is based on superimposing a colored image over the conventional gray-scale EUS image in a region of interest. The strain level of the hard tissue is colored in blue, and the soft tissue is colored in green. An elastic score has been proposed for the pancreas: homogenously hard, heterogenously hard, mixed, heterogenously soft, and homogenously soft[52].

Two semiquantitative approaches are included in the software and can be accessed during the EUS procedure. One approach calculates the hue histogram (strain histogram) as the ratio of the strain between two areas that are selected by the investigator, which are situated at the same distance from the transducer to obtain a similar compression by the probe[13]. The new generation of EUS elastography enables the operator to calculate the mean strain ratio (SR) within a selected area inside the ROI as the difference in elasticity between the targeted lesion and the surrounding tissue, yielding an objective numeric value. However, it is important to obtain a still image, and for this reason, multiple measurements are performed in each patient[53].

Indications: (1) differentiation of the pancreatic masses; (2) differentiation of the lymph nodes; and (3) assessment of fibrosis.

Differentiation of pancreatic masses: Normal pancreas tissue appears as soft tissue (green color) in E-EUS. A pancreatic mass, which is usually hypoechoic in standard EUS, appears as homogenously or inhomogenously green or blue, depending on tissue hardness[54-57].

Based on this qualitative assessment, the global sensitivity and specificity for pancreatic mass assessment were considered to be 100% and 67%, respectively[58]; however, a later multicentric European study found a sensitivity of 93.4% and a specificity of 66%, with a global accuracy of 85.4%[58]. Other studies obtained similar sensitivities and lower specificities for discriminating malignant pancreatic masses (Table 6). It was hoped that this examination could discriminate inflammatory changes from tumor involvement of the vessel wall[59], which has not yet been demonstrated (Figure 7).

Using the hue histogram, a value of 175 was found to be suggestive of malignancy[60,61]. However, artifacts related to the presence of surrounding structures with excessive or insufficient stiffness[61] are very important; therefore, the selection of appropriate regions of interest is of great importance.

Postprocessing analysis of the hue histogram using an artificial neural network enabled an optimal prediction of all types of pancreatic lesions and provided better results than hue histogram analysis[60,62]. However, the procedure is complex, and further studies on its practical applicability are warranted.

Multiple studies were performed to measure the mean SR and cut-off value for malignancy. No optimal cut-off value has yet been established for use in malignancy diagnosis (Table 6) due to the inter-observer variability of the method and the difficulty of

Table 6 Efficiency of E-endoscopic ultrasound for solid pancreatic mass assessment

Ref.	Type of study	Final diagnosis	No. of patients	E-EUS assessment	Main results
Giovannini *et al*[58] 2006	Prospective Single center	Surgery EUS-FNA	24	Color pattern	Sn = 100% Sp = 67%
Janssen *et al*[75] 2007	Prospective Single center	Surgery EUS-FNA	73	Color pattern	-
Săftoiu *et al*[60] 2008	Prospective Single center	Surgery EUS-FNA	43	Hue histogram cut-off value=175	Sn = 91%, Sp = 87%, PPV = 88%. NPV = 90%, Acc = 89%
Iglesias-Garcia *et al*[72] 2009	Prospective Single center	Surgery EUS-FNA	130	Color pattern	Sn = 100%, Sp = 85%, PPV = 90%, NPV= 100%, Acc = 94%
Giovannini *et al*[79] 2009	Prospective Multicenter	Surgery EUS-FNA	121	Color pattern	Sn = 92% Sp = 80%
Iglesias-Garcia *et al*[57] 2010	Prospective Single center	Surgical FNA	86	SR = 4.62	Sn = 100%, Sp = 92%
Săftoiu *et al*[59] 2011	Prospective Multicenter	Surgery EUS-FNA	258	Hue histogram cut-off value = 175	Sn = 93%, Sp = 66%, PPV = 92%, NPV = 68%, Acc = 85%
Itokawa *et al*[73] 2011	Retrospective		109	SR=39.08	-
Hocke *et al*[13] 2012	Prospective Single center	Surgical EUS-FNA Follow up	58	Color pattern	Sn = 94.7% Sp = 33.4%
Figueiredo *et al*[71] 2012	Prospective Single center	Surgical EUS-FNA Follow up	47	SR = 8	Sn = 90% Sp = 75%
Dawwas *et al*[70] 2012	Prospective	Surgical	111	SR = 4.69 (AUC = 0.69)	Sn = 100%, Sp = 16.7%, PPV = 86%, NPV = 100%, Acc = 86%
	Single center	EUS-FNA		Masks elasticity (AUC= 0.72)	Sn = 95%, Sp = 22%, PPV = 86%, NPV = 50%, Acc = 83%
Lee *et al*[74] 2013	Retrospective	-	15	Color pattern SR = 0.02%	-
Havre *et al*[54] 2014	Prospective	Surgery EUS-FNA Follow-up	48	SR = 4.4	Sn = 67%, Sp = 71%
Rustemovic *et al*[93] 2014	Prospective Single center	Surgery EUS-FNA	149	SR = 7.59	Sn = 100% Sp = 45%
Kongkam *et al*[69] 2015	Prospective Single center	Surgery EUS-FNA	38	SR=3.17	Sn = 86%, Sp = 66%
Opačić *et al*[94] 2015	Prospective	Surgery	105 pancreatic mass	Hue histogram	Sn = 98%, Sp = 50%, PPV = 92%, NPV = 100%, Ac = 69%
	Single center	EUS-FNA	44 controls		
Mayerle *et al*[68] 2016	Prospective Single center	Surgery EUS-FNA Follow-up	85	SR = 24.82 or 10	Sn = 77%, Sp = 65% Sn = 96%, Sp = 43%

E-EUS: Elastography endosonography; SR: Cut-off value of strain ratio; Sn: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; Acc: Accuracy; EUS-FNA: Endoscopic ultrasound-fine needle aspiration.

standardizing the compression.

Two meta-analyses on the differentiation of malignant pancreatic tumors from inflammatory pancreatic masses, each including 13 studies with 1042 and 1044 patients, showed a sensitivity of 95% and a specificity of 67%-69%, with an AUC of 0.86-0.90[63,64]. A third meta-analysis included 7 studies and 752 patients, with a global sensitivity of 97%, a specificity of 76% and an AUC of 0.95[65]. This meta-analysis serves as a reminder that it is difficult to differentiate adenocarcinoma and neuroendocrine tumor, which are both hard lesions, using elastography[65]. A fourth meta-analysis found that the use of a color pattern for elastography EUS interpretation was associated with a sensitivity of 99% and a specificity of 69%-76%[65-67]; moreover, using a hue histogram, the sensitivity was 92% and the specificity was lower, 86%[66]. Both of the semiquantitative assessments evaluated by other meta-analyses showed a sensitivity of 96% and a specificity of 76%[67].

A comparison of B mode EUS, EUS-FNA and EUS elastography favored the standard EUS; the accuracies were 87%, 85% and 73%, respectively[68]. A combination of EUS-FNA with SR was not superior to EUS-FNA alone in a study involving 28 patients[69]. Due to the low negative value of LR (0.09), this method should be indicated to rule out a diagnosis of malignancy and to avoid unnecessary EUS-FNA[63].

Limitations: (1) The control of tissue compression (the degree of compression and the angulation) and motion artifacts determined by respiratory and heart movements is difficult[69-72]; (2) adjacent structures with very low or very high density should be avoided (the heart, major vessels). The interposition of cysts or dilated ducts should be avoided because these

Table 7 Efficiency of E-endoscopic ultrasound for LN assessment

Ref.	Type of study	Final diagnosis	No. of patients	E-EUS assessment	Main results
Giovannini *et al*[58] 2006	Prospective Single center	EUS-FNA	31	Color pattern	Sn = 100% Sp = 50%
Janssen *et al*[75] 2007	Prospective Single center	EUS-FNA	66	Color pattern	Hard - Acc = 81%-86% Soft - Acc = 84%-86%
Săftoiu *et al*[95] 2007	Prospective Single center	Surgery EUS-FNA	78	Hue histogram	Sn = 85% Sp = 91%
Giovannini *et al*[79] 2009	Prospective Multicenter	Surgery EUS-FNA	101	Color pattern	Sn = 91.8% Sp = 82.5%
Larsen *et al*[81] 2012	Prospective Single center	Surgery	56	Color pattern	Sn = 55%-59% Sp = 82%-85%
Paterson *et al*[78] 2012	Prospective Single center	EUS-FNA	53	Strain ratio for malignancy = 7.5	Sn = 83%, Sp = 96%, PPV = 95%, NPV = 86%, Acc = 90%
Knabe *et al*[83] 2013	Prospective	EUS-FNA	40	Color pattern Computed analysis	Sn = 100% Sp = 64% Computed analysis Sn = 88.9% Sp = 86.7%

E-EUS: Elastographic endosonography; SR: Cut-off value of the strain ratio; Sn: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value.

may impair the SR[73,74]; (3) the size and depth of the region of interest should be similar when SR is calculated[73]. This improves with the experience of the endosonographer, and modern equipment automatically selects the best still image that is representative of the mean SR of the lesion; (4) The negative predictive value remains low, at 60%-70%[69]; and (5) The procedure is poorly reproducible, and the coefficient of variance is greater than 0.3[69].

Interobserver variability is good in the case of experienced EUS elastography operators (k = 0.8) and is fair in the case of unexperienced EUS elastography operators (k = 0.24)[73]. Intraobserver variability was also good (k = 0.86-0.94)[59].

Differentiation between benign and malignant lymph nodes: The main problem in assessing lymph nodes is to determine when to apply EUS-FNA. Using EUS elastography, benign LNs appear homogenous and are colored green, whereas malignant LNs are colored blue[75]. The same color differentiation also proved efficient while applying elastography to endobronchial ultrasound[76]. Parts of LNs that appear blue can be targeted by the needle to prove the presence of micrometastasis.

The cut-off SR value for differentiating between malignant and benign LNs in 55 patients was considered to be 3.81[77]; a value of 7.5 was found in another group of 53 LNs[78]. Interobserver agreement was good to excellent, with a K value for ES of 0.58-0.84 and a value of 0.35 for the ES scoring system[79-81](Table 7).

A meta-analysis, including seven studies and 368 patients, revealed a sensitivity of 88% and a specificity of 85% using elastography EUS for differentiating between malignant and benign LNs[82].

Results concerning the superiority of elastography EUS over EUS are conflicting. The color pattern[79,83] and strain ratio were found to be of superior value to conventional EUS criteria[78]. However, these results were not sustained in another study that included a surgical pathology comparison[77].

Assessment of pancreatic fibrosis: The usefulness of elastography for assessing pancreatic fibrosis distal to a tumor and to differentiate chronic pancreatitis from healthy pancreas was previously proven[84,85]. Using a radial scope, an SR cut-off value of 2.25 yielded a diagnosis accuracy of 91% for chronic pancreatitis[1] and increased the EUS evaluation yield in 18% of cases. Autoimmune pancreatitis exhibits a blue pattern involving both mass-forming autoimmune pancreatitis and surrounding tissue[86].

A direct relationship was found between the SR and the probability of pancreatic exocrine insufficiency, as measured using the ^{13}C-MTG breath test, and a probability of 87% was found in patients with an SR of higher than 4.5[87]. The results were similar for patients with calcifying and non-calcifying chronic pancreatitis[87]. No elastographic studies have compared this finding using radial and linear echoendoscopes.

Other indications: Few cases of gastric submucosal tumors have been assessed using elastography[53,88]. A GIST may have a non-homogenous blue-green structure, and a typical lipoma is mostly soft, green and homogenous; however, differentiation between benign and malignant lesions using elastography EUS remains difficult.

Sessile rectal adenoma and adenocarcinoma were better differentiated using elastography compared to

Table 8 Needle confocal laser endosonography features of different cystic lesions of the pancreas

Type of lesion	nCLE features	Diagnostic rate, references
SCA	A vascular network of the cystic wall	Sn = 69%, Sp = 100%, PPV = 100%, NPV = 82%[100]
MCN	A gray band delineated by a thin dark line	Sn = 80%, Sp = 100%[103] Sn = 67%, Sp = 96%[100]
IPMN	Papillary projections: characterized by the alternation of vascular cores (white) and epithelial borders	Sn = 59%, Sp = 100%[102] Sn = 80%, Sp = 92%[100]
Pseudocyst	Inflammatory cells bright, gray and black particles	Sn = 43%, Sp = 100%, Acc = 87%[100]
Cystic NET	Dark irregular clusters of compact cells + gray tissue of fibrovascular stroma	Sn = 67%, Sp = 96%, Acc = 90%[100]

nCLE: Needle confocal laser endosonography; SCA: Serous cystadenoma; MCN: Mucinous cystic neoplasm; IPMN: Intraductal papillary mucinous neoplasm; NET: Neuroendocrine tumor; Sn: Sensitivity; Sp: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; Acc: Accuracy.

standard rectal endosonography, with an SR cut-off mean of 1.25, resulting in a sensitivity of 96% and a specificity of 86%; these results are superior to the use of EUS alone[89,90]. This could be important for the local resection of rectal tumors, but multiple studies confirming this indication are needed.

The discrimination between T2 and T3 tumors based on identifying "softer" inflammatory tissue and "harder" tumor tissue has not yet been published.

Elastography was not found to be more useful compared to rectal EUS in patients with fecal incontinence, previously irradiated or not[91], where the sphincter appears as an interrupted inhomogenous thickened layer[53], or for the discrimination of Crohn strictures from adenocarcinoma.

Sclerosing primary cholangitis might have a hard or mixed type common bile duct wall compared to controls; this might be related to an extension of the fibrotic change of the wall[92].

Future: Site selection for EUS-FNA[59,65,93-95].

Needle-based confocal endomicroscopy

Principle: Standard confocal laser endomicroscopy (CLE) allows the real-time visualization of cellular and subcellular structures with up to 1000 times magnification and a penetration of 100 μm below the mucosal surface[96]. The studied tissue is illuminated with a low-power laser, and the fluorescence of light reflected from the tissue is subsequently detected through a pinhole[96]. The returned light is reflected by the same lens and reaches the detector of the confocal system. The illumination and detection systems are "confocal", meaning that they are aligned in the same focal plane[96]. A contrast agent is administered intravenously (fluorescein) or topically (acriflavine and cresyl violet) to emphasize the cellular, subcellular and vasculature elements.

In clinical practice, two CLE systems are used: an endoscope-integrated CLE and a probe-based CLE (p-CLE), the latter being the widest system used for the assessment of colonic polyps, neoplastic lesions in inflammatory bowel diseases or Barrett's esophagus[97-100].

Needle-CLE (nCLE) represents an improved version of CLE and is performed during EUS; the organs within or adjacent to the GI tract are assessed using a miniprobe, which is passed through an endoscopic needle. nCLE allows *in vivo,* real-time histological diagnosis, thus enhancing EUS performance, mainly in the setting of pancreatic and lymph node lesions[101]. Inconclusive diagnostic procedures can be decreased using this technique, termed "optical needle biopsy"[93].

Technique: The system comprises an AQ-Flex 19 miniprobe, which is inserted through a 19-gauge EUS needle while a fluorescence contrast agent (acriflavine, fluorescein) provides tissue architecture imaging, similar to standard histological examination (depth 40-70 mm, field of view 325 microns, lateral resolution 3.5 mm).

Indications: (1) cystic lesions of the pancreas larger than 1 cm; and (2) solid pancreatic masses and lymph node nCLE remain under evaluation.

Pancreatic cystic lesions: The management of cystic lesions is currently suboptimal mainly due to a lack of accuracy in discriminating among different types of pancreatic cystic lesions.

The nCLE patterns for pancreatic cystic lesions were recently published[96,102,103] and provide a global accuracy for diagnosis ranging from 46% to 90%[102-106], and studies have underlined the importance of nCLE acting as an optical needle biopsy[93] (Table 8). Serous cystadenomas presents a superficial vascular network, which can stop their follow-up[103] or avoid an unnecessary resection (reported as 60% in a multicentric study of 2622 patients[105]). Benign IPMNs appear as finger-like papillary projections with an epithelial border and a vascular core, while malignant IPMNs appear as dark clumps with fluorescent substance leakage due to tumor neo-vascularization[103].

The multicentric INSPECT study[106] showed that the accuracy of differentiating between different types of PCL using nCLE was 41.9%, which is greater than that obtained using a carcinoembryonic antigen (CEA) level > 192 ng/mL (28.6%) or cytology results (29.6%). Epithelial villous structures were found to be predictive of PCL with a specificity of 100%, but the sensitivity

and negative predictive value were only 59% and 50%, respectively[106].

In the DETECT trial[107], nCLE was combined with cystoscopy using a SpyGlass fiberoptic probe in 18 patients with a high probability of having PCNs. Cystoscopy and nCLE were reported to have sensitivities of 90% and 80%, respectively; the combination of the two methods reached a sensitivity of 100% for the clinical diagnosis of mucinous cysts. In addition, both cystoscopy and nCLE exhibited higher sensitivity and accuracy than CEA levels (33% and 61%, respectively) in the entire study population[107,108].

Limitations: Inter-observer agreement is considered as globally low[104] and fair for MCN, moderate for IPMN, and very good for PC and SCA[102]; The operator learning curve of the technique influences the results obtained[103]; Sampling error is limited by the location and size of the cyst, the angulation of the needle, and the use of a transgastric or transduodenal approach; Incomplete evaluation - needle entry site, a solid mass inside the cyst; Better in combination with cystoscopy for cyst evaluation[107]; Complications of nCLE are seen in up to 3.29%-9% of cases such as pancreatitis or bleeding[102,106,107].

Solid pancreatic lesions: Few data are available for nCLE assessment in pancreatic solid lesions, and difficulties have been encountered, especially when using the transduodenal approach.

Normal pancreas has been described as having "an appearance of coffee beans corresponding to acinis"[108]. Adenocarcinoma has an aspect of dark cell aggregates and irregular vessels with the leakage of fluorescein[108]. Chronic pancreatitis presents as residual regular glandular pancreatic structures[108] and white fibrous bands[109,110]. Neuroendocrine tumors appear as black cell aggregates surrounded by vessels and fibrotic areas[108].

The sensitivity, specificity and accuracy of this technique were 77%, 100% and 85%, respectively, supporting the use of nCLE instead of repeating EUS-FNA after a previous inconclusive biopsy[108].

Another study used nCLE as optical guidance for EUS-FNA and found an accuracy rate of 90.9% with good inter-observer agreement (k = 0.82)[109].

Lymph nodes: On nCLE, benign lymph nodes have a reticular background with lymphocytes[111]. Clusters of dark pleomorphic tumor cells are consistent with carcinoma features, while enlarged follicles are less convincing for malignancy[112]. Additionally, significant leakage of fluorescent dye due to tumor angiogenesis is suggestive of malignant nodes[111].

Future: Molecular imaging of the pancreas might be feasible using nCLE: pancreatic histology assessment after the use of a fluorescence-labeled anti-EGF-R antibody[113] has been reported. One study recently used nCLE for the visualization of the Meissner and Auerbach plexus after the submucosal injection of NeuroTrace[114], a new step in the assessment of functional and motility disorders of the gastrointestinal tract.

In vitro imaging of pancreatic carcinogenesis using nCLE combined with molecular markers, such as cathepsin E[96], has also been reported, with important future clinical implications for the monitoring of pancreatic ductal carcinoma.

Despite its high accuracy and the existence of several clinical applications, nCLE is still used only in research trials, probably due to a lack of standardization, availability and reimbursement in some countries, a lengthy physician learning curve and the broad range of histologic diagnoses. A recent study showed the benefit of using nCLE in cases of "diagnostic doubts", impacting diagnosis and management in 40% of cases and the performance of target biopsies in 100% of cases[115].

CONCLUSION

The new derivative modalities described above represent a step forward in maximizing the results of endoscopic ultrasonography procedures. The complementary role of these techniques is becoming clearer, and elastography and harmonic contrast-enhanced EUS are suitable for routine use in the future. However, none of these techniques is yet able to replace EUS-FNA.

REFERENCES

1 **Puli SR**, Bechtold ML, Buxbaum JL, Eloubeidi MA. How good is endoscopic ultrasound-guided fine-needle aspiration in diagnosing the correct etiology for a solid pancreatic mass?: A meta-analysis and systematic review. *Pancreas* 2013; **42**: 20-26 [PMID: 23254913 DOI: 10.1097/MPA.0b013e3182546e79]

2 **Hewitt MJ**, McPhail MJ, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasms: a meta-analysis. *Gastrointest Endosc* 2012; **75**: 319-331 [PMID: 22248600 DOI: 10.1016/j.gie.2011.08.049]

3 **Bang JY**, Hawes R, Varadarajulu S. A meta-analysis comparing ProCore and standard fine-needle aspiration needles for endoscopic ultrasound-guided tissue acquisition. *Endoscopy* 2016; **48**: 339-349 [PMID: 26561917 DOI: 10.1055/s-0034-1393354]

4 **Micames CG**, McCrory DC, Pavey DA, Jowell PS, Gress FG. Endoscopic ultrasound-guided fine-needle aspiration for non-small cell lung cancer staging: A systematic review and metaanalysis. *Chest* 2007; **131**: 539-548 [PMID: 17296659 DOI: 10.1378/chest.06-1437]

5 **Sekine M**, Imaoka H, Mizuno N, Hara K, Hijioka S, Niwa Y, Tajika M, Tanaka T, Ishihara M, Ito S, Misawa K, Ito Y, Shimizu Y, Yatabe Y, Ohnishi H, Yamao K. Clinical course of gastrointestinal stromal tumor diagnosed by endoscopic ultrasound-guided fine-needle aspiration. *Dig Endosc* 2015; **27**: 44-52 [PMID: 25059428 DOI: 10.1111/den.12333]

6 **Brugge WR**, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of

pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology* 2004; **126**: 1330-1336 [PMID: 15131794]
7 **Wang QX**, Xiao J, Orange M, Zhang H, Zhu YQ. EUS-Guided FNA for Diagnosis of Pancreatic Cystic Lesions: a Meta-Analysis. *Cell Physiol Biochem* 2015; **36**: 1197-1209 [PMID: 26138881 DOI: 10.1159/000430290]
8 **Dietrich CF**, Ignee A, Braden B, Barreiros AP, Ott M, Hocke M. Improved differentiation of pancreatic tumors using contrast-enhanced endoscopic ultrasound. *Clin Gastroenterol Hepatol* 2008; **6**: 590-597.e1 [PMID: 18455699 DOI: 10.1016/j.cgh.2008.02.030]
9 **Kitano M**, Kamata K, Imai H, Miyata T, Yasukawa S, Yanagisawa A, Kudo M. Contrast-enhanced harmonic endoscopic ultrasonography for pancreatobiliary diseases. *Dig Endosc* 2015; **27** Suppl 1: 60-67 [PMID: 25639788 DOI: 10.1111/den.12454]
10 **Alvarez-Sánchez MV**, Napoléon B. Contrast-enhanced harmonic endoscopic ultrasound imaging: basic principles, present situation and future perspectives. *World J Gastroenterol* 2014; **20**: 15549-15563 [PMID: 25400439 DOI: 10.3748/wjg.v20.i42.15549]
11 **Kitano M**, Kudo M, Sakamoto H, Komaki T. Endoscopic ultrasonography and contrast-enhanced endoscopic ultrasonography. *Pancreatology* 2011; **11** Suppl 2: 28-33 [PMID: 21464584 DOI: 10.1159/000323493]
12 **Fusaroli P**, Spada A, Mancino MG, Caletti G. Contrast harmonic echo-endoscopic ultrasound improves accuracy in diagnosis of solid pancreatic masses. *Clin Gastroenterol Hepatol* 2010; **8**: 629-634.e1-2 [PMID: 20417721 DOI: 10.1016/j.cgh.2010.04.012]
13 **Hocke M**, Ignee A, Dietrich CF. Advanced endosonographic diagnostic tools for discrimination of focal chronic pancreatitis and pancreatic carcinoma--elastography, contrast enhanced high mechanical index (CEHMI) and low mechanical index (CELMI) endosonography in direct comparison. *Z Gastroenterol* 2012; **50**: 199-203 [PMID: 22298098 DOI: 10.1055/s-0031-1281824]
14 **Gincul R**, Palazzo M, Pujol B, Tubach F, Palazzo L, Lefort C, Fumex F, Lombard A, Ribeiro D, Fabre M, Hervieu V, Labadie M, Ponchon T, Napoléon B. Contrast-harmonic endoscopic ultrasound for the diagnosis of pancreatic adenocarcinoma: a prospective multicenter trial. *Endoscopy* 2014; **46**: 373-379 [PMID: 24532350 DOI: 10.1055/s-0034-1364969]
15 **Kitano M**, Kudo M, Yamao K, Takagi T, Sakamoto H, Komaki T, Kamata K, Imai H, Chiba Y, Okada M, Murakami T, Takeyama Y. Characterization of small solid tumors in the pancreas: the value of contrast-enhanced harmonic endoscopic ultrasonography. *Am J Gastroenterol* 2012; **107**: 303-310 [PMID: 22008892 DOI: 10.1038/ajg.2011.354]
16 **Lee TY**, Cheon YK, Shim CS. Clinical role of contrast-enhanced harmonic endoscopic ultrasound in differentiating solid lesions of the pancreas: a single-center experience in Korea. *Gut Liver* 2013; **7**: 599-604 [PMID: 24073319 DOI: 10.5009/gnl.2013.7.5.599]
17 **Park JS**, Kim HK, Bang BW, Kim SG, Jeong S, Lee DH. Effectiveness of contrast-enhanced harmonic endoscopic ultrasound for the evaluation of solid pancreatic masses. *World J Gastroenterol* 2014; **20**: 518-524 [PMID: 24574720 DOI: 10.3748/wjg.v20.i2.518]
18 **Napoleon B**, Alvarez-Sanchez MV, Gincoul R, Pujol B, Lefort C, Lepilliez V, Labadie M, Souquet JC, Queneau PE, Scoazec JY, Chayvialle JA, Ponchon T. Contrast-enhanced harmonic endoscopic ultrasound in solid lesions of the pancreas: results of a pilot study. *Endoscopy* 2010; **42**: 564-570 [PMID: 20593334 DOI: 10.1055/s-0030-1255537]
19 **Ang TL**, Teo EK, Ang D, Kwek AB, Fock KM. A pilot study of contrast harmonic endosonography using DEFINITY™ in the evaluation of suspected pancreatic and peri-ampullary malignancies. *J Interv Gastroenterol* 2011; **1**: 160-165 [PMID: 22586529 DOI: 10.4161/jig.19958]
20 **Matsubara H**, Itoh A, Kawashima H, Kasugai T, Ohno E, Ishikawa T, Itoh Y, Nakamura Y, Hiramatsu T, Nakamura M, Miyahara R, Ohmiya N, Ishigami M, Katano Y, Goto H, Hirooka Y. Dynamic quantitative evaluation of contrast-enhanced endoscopic ultrasonography in the diagnosis of pancreatic diseases. *Pancreas* 2011; **40**: 1073-1079 [PMID: 21633317 DOI: 10.1097/MPA.0b013e31821f57b7]
21 **Dietrich CF**, Sahai AV, D'Onofrio M, Will U, Arcidiacono PG, Petrone MC, Hocke M, Braden B, Burmester E, Möller K, Săftoiu A, Ignee A, Cui XW, Iordache S, Potthoff A, Iglesias-Garcia J, Fusaroli P, Dong Y, Jenssen C. Differential diagnosis of small solid pancreatic lesions. *Gastrointest Endosc* 2016; **84**: 933-940 [PMID: 27155592 DOI: 10.1016/j.gie.2016.04.034]
22 **Gong TT**, Hu DM, Zhu Q. Contrast-enhanced EUS for differential diagnosis of pancreatic mass lesions: a meta-analysis. *Gastrointest Endosc* 2012; **76**: 301-309 [PMID: 22703697 DOI: 10.1016/j.gie.2012.02.051]
23 **D'Onofrio M**, Biagioli E, Gerardi C, Canestrini S, Rulli E, Crosara S, De Robertis R, Floriani I. Diagnostic performance of contrast-enhanced ultrasound (CEUS) and contrast-enhanced endoscopic ultrasound (ECEUS) for the differentiation of pancreatic lesions: a systematic review and meta-analysis. *Ultraschall Med* 2014; **35**: 515-521 [PMID: 25226455 DOI: 10.1055/s-0034-1385068]
24 **Ishikawa T**, Itoh A, Kawashima H, Ohno E, Matsubara H, Itoh Y, Nakamura Y, Nakamura M, Miyahara R, Hayashi K, Ishigami M, Katano Y, Ohmiya N, Goto H, Hirooka Y. Usefulness of EUS combined with contrast-enhancement in the differential diagnosis of malignant versus benign and preoperative localization of pancreatic endocrine tumors. *Gastrointest Endosc* 2010; **71**: 951-959 [PMID: 20438884 DOI: 10.1016/j.gie.2009.12.023]
25 **Gheonea DI**, Streba CT, Ciurea T, Săftoiu A. Quantitative low mechanical index contrast-enhanced endoscopic ultrasound for the differential diagnosis of chronic pseudotumoral pancreatitis and pancreatic cancer. *BMC Gastroenterol* 2013; **13**: 2 [PMID: 23286918 DOI: 10.1186/1471-230X-13-2]
26 **Imazu H**, Kanazawa K, Mori N, Ikeda K, Kakutani H, Sumiyama K, Hino S, Ang TL, Omar S, Tajiri H. Novel quantitative perfusion analysis with contrast-enhanced harmonic EUS for differentiation of autoimmune pancreatitis from pancreatic carcinoma. *Scand J Gastroenterol* 2012; **47**: 853-860 [PMID: 22507131 DOI: 10.3109/00365521.2012.679686]
27 **Fusaroli P**, D'Ercole MC, De Giorgio R, Serrani M, Caletti G. Contrast harmonic endoscopic ultrasonography in the characterization of pancreatic metastases (with video). *Pancreas* 2014; **43**: 584-587 [PMID: 24713844 DOI: 10.1097/MPA.0000000000000081]
28 **Seicean A**, Badea R, Moldovan-Pop A, Vultur S, Botan EC, Zaharie T, Săftoiu A, Mocan T, Iancu C, Graur F, Sparchez Z, Seicean R. Harmonic Contrast-Enhanced Endoscopic Ultrasonography for the Guidance of Fine-Needle Aspiration in Solid Pancreatic Masses. *Ultraschall Med* 2015; Epub ahead of print [PMID: 26274382 DOI: 10.1055/s-0035-1553496]
29 **Fusaroli P**, Kypraios D, Mancino MG, Spada A, Benini MC, Bianchi M, Bocus P, De Angelis C, De Luca L, Fabbri C, Grillo A, Marzioni M, Reggio D, Togliani T, Zanarini S, Caletti G. Interobserver agreement in contrast harmonic endoscopic ultrasound. *J Gastroenterol Hepatol* 2012; **27**: 1063-1069 [PMID: 22414180 DOI: 10.1111/j.1440-1746.2012.07115.x]
30 **Soares JB**, Iglesias-Garcia J, Gonçalves B, Lindkvist B, Lariño-Noia J, Bastos P, Caetano AC, Ferreira A, Pimentel-Nunes P, Lopes L, Moutinho P, Dominguez-Muñoz JE. Interobserver agreement of contrast-enhanced harmonic endoscopic ultrasonography in the evaluation of solid pancreatic lesions. *Endosc Int Open* 2015; **3**: E205-E209 [PMID: 26171432 DOI: 10.1055/s-0034-1391415]
31 **Seicean A**, Badea R, Stan-Iuga R, Mocan T, Gulei I, Pascu O. Quantitative contrast-enhanced harmonic endoscopic ultrasonography for the discrimination of solid pancreatic masses. *Ultraschall Med* 2010; **31**: 571-576 [PMID: 21080306 DOI: 10.1055/s-0029-1245833]
32 **Imazu H**, Uchiyama Y, Matsunaga K, Ikeda K, Kakutani H, Sasaki Y, Sumiyama K, Ang TL, Omar S, Tajiri H. Contrast-enhanced harmonic EUS with novel ultrasonographic contrast (Sonazoid) in the preoperative T-staging for pancreaticobiliary malignancies. *Scand J Gastroenterol* 2010; **45**: 732-738 [PMID: 20205504 DOI: 10.3109/00365521003690269]

33 **Săftoiu A**, Vilmann P, Dietrich CF, Iglesias-Garcia J, Hocke M, Seicean A, Ignee A, Hassan H, Streba CT, Ioncică AM, Gheonea DI, Ciurea T. Quantitative contrast-enhanced harmonic EUS in differential diagnosis of focal pancreatic masses (with videos). *Gastrointest Endosc* 2015; **82**: 59-69 [PMID: 25792386 DOI: 10.1016/j.gie.2014.11.040]

34 **Ueda K**, Yamashita Y, Itonaga M. Real-time contrast-enhanced endoscopic ultrasonography-guided fine-needle aspiration (with video). *Dig Endosc* 2013; **25**: 631 [PMID: 24112287 DOI: 10.1111/den.12165]

35 **Sugimoto M**, Takagi T, Hikichi T, Suzuki R, Watanabe K, Nakamura J, Kikuchi H, Konno N, Waragai Y, Watanabe H, Obara K, Ohira H. Conventional versus contrast-enhanced harmonic endoscopic ultrasonography-guided fine-needle aspiration for diagnosis of solid pancreatic lesions: A prospective randomized trial. *Pancreatology* 2015; **15**: 538-541 [PMID: 26145837 DOI: 10.1016/j.pan.2015.06.005]

36 **Hou X**, Jin Z, Xu C, Zhang M, Zhu J, Jiang F, Li Z. Contrast-enhanced harmonic endoscopic ultrasound-guided fine-needle aspiration in the diagnosis of solid pancreatic lesions: a retrospective study. *PLoS One* 2015; **10**: e0121236 [PMID: 25793739 DOI: 10.1371/journal.pone.0121236]

37 **Hocke M**, Cui XW, Domagk D, Ignee A, Dietrich CF. Pancreatic cystic lesions: The value of contrast-enhanced endoscopic ultrasound to influence the clinical pathway. *Endosc Ultrasound* 2014; **3**: 123-130 [PMID: 24955342 DOI: 10.4103/2303-9027.131040]

38 **Fusaroli P**, Serrani M, De Giorgio R, D'Ercole MC, Ceroni L, Lisotti A, Caletti G. Contrast Harmonic-Endoscopic Ultrasound Is Useful to Identify Neoplastic Features of Pancreatic Cysts (With Videos). *Pancreas* 2016; **45**: 265-268 [PMID: 26474428 DOI: 10.1097/MPA.0000000000000441]

39 **Zhong N**, Zhang L, Takahashi N, Shalmiyev V, Canto MI, Clain JE, Deutsch JC, DeWitt J, Eloubeidi MA, Gleeson FC, Levy MJ, Mallery S, Raimondo M, Rajan E, Stevens T, Topazian M. Histologic and imaging features of mural nodules in mucinous pancreatic cysts. *Clin Gastroenterol Hepatol* 2012; **10**: 192-198, 198.e1-2 [PMID: 21982970 DOI: 10.1016/j.cgh.2011.09.029]

40 **Yamashita Y**, Ueda K, Itonaga M, Yoshida T, Maeda H, Maekita T, Iguchi M, Tamai H, Ichinose M, Kato J. Usefulness of contrast-enhanced endoscopic sonography for discriminating mural nodules from mucous clots in intraductal papillary mucinous neoplasms: a single-center prospective study. *J Ultrasound Med* 2013; **32**: 61-68 [PMID: 23269711]

41 **Harima H**, Kaino S, Shinoda S, Kawano M, Suenaga S, Sakaida I. Differential diagnosis of benign and malignant branch duct intraductal papillary mucinous neoplasm using contrast-enhanced endoscopic ultrasonography. *World J Gastroenterol* 2015; **21**: 6252-6260 [PMID: 26034360 DOI: 10.3748/wjg.v21.i20.6252]

42 **Kamata K**, Kitano M, Omoto S, Kadosaka K, Miyata T, Yamao K, Imai H, Sakamoto H, Harwani Y, Chikugo T, Chiba Y, Matsumoto I, Takeyama Y, Kudo M. Contrast-enhanced harmonic endoscopic ultrasonography for differential diagnosis of pancreatic cysts. *Endoscopy* 2016; **48**: 35-41 [PMID: 26605974 DOI: 10.1055/s-0034-1393564]

43 **Yamamoto N**, Kato H, Tomoda T, Matsumoto K, Sakakihara I, Noma Y, Horiguchi S, Harada R, Tsutsumi K, Hori K, Tanaka T, Okada H, de Yamamoto K. Contrast-enhanced harmonic endoscopic ultrasonography with time-intensity curve analysis for intraductal papillary mucinous neoplasms of the pancreas. *Endoscopy* 2016; **48**: 26-34 [PMID: 26561919 DOI: 10.1055/s-0034-1393563]

44 **Kannengiesser K**, Mahlke R, Petersen F, Peters A, Ross M, Kucharzik T, Maaser C. Contrast-enhanced harmonic endoscopic ultrasound is able to discriminate benign submucosal lesions from gastrointestinal stromal tumors. *Scand J Gastroenterol* 2012; **47**: 1515-1520 [PMID: 23148660 DOI: 10.3109/00365521.2012.729082]

45 **Sakamoto H**, Kitano M, Matsui S, Kamata K, Komaki T, Imai H, Dote K, Kudo M. Estimation of malignant potential of GI stromal tumors by contrast-enhanced harmonic EUS (with videos). *Gastrointest Endosc* 2011; **73**: 227-237 [PMID: 21295636 DOI: 10.1016/j.gie.2010.10.011]

46 **Park CH**, Chung MJ, Oh TG, Park JY, Bang S, Park SW, Kim H, Hwang HK, Lee WJ, Song SY. Differential diagnosis between gallbladder adenomas and cholesterol polyps on contrast-enhanced harmonic endoscopic ultrasonography. *Surg Endosc* 2013; **27**: 1414-1421 [PMID: 23233003 DOI: 10.1007/s00464-012-2620-x]

47 **Imazu H**, Mori N, Kanazawa K, Chiba M, Toyoizumi H, Torisu Y, Koyama S, Hino S, Ang TL, Tajiri H. Contrast-enhanced harmonic endoscopic ultrasonography in the differential diagnosis of gallbladder wall thickening. *Dig Dis Sci* 2014; **59**: 1909-1916 [PMID: 24664415 DOI: 10.1007/s10620-014-3115-5]

48 **Gill KR**, Ghabril MS, Jamil LH, Hasan MK, McNeil RB, Woodward TA, Raimondo M, Hoffman BJ, Hawes RH, Romagnuolo J, Wallace MB. Endosonographic features predictive of malignancy in mediastinal lymph nodes in patients with lung cancer. *Gastrointest Endosc* 2010; **72**: 265-271 [PMID: 20541192 DOI: 10.1016/j.gie.2010.02.037]

49 **Miyata T**, Kitano M, Omoto S, Kadosaka K, Kamata K, Imai H, Sakamoto H, Nisida N, Harwani Y, Murakami T, Takeyama Y, Chiba Y, Kudo M. Contrast-enhanced harmonic endoscopic ultrasonography for assessment of lymph node metastases in pancreatobiliary carcinoma. *World J Gastroenterol* 2016; **22**: 3381-3391 [PMID: 27022220 DOI: 10.3748/wjg.v22.i12.3381]

50 **Xia Y**, Kitano M, Kudo M, Imai H, Kamata K, Sakamoto H, Komaki T. Characterization of intra-abdominal lesions of undetermined origin by contrast-enhanced harmonic EUS (with videos). *Gastrointest Endosc* 2010; **72**: 637-642 [PMID: 20646696 DOI: 10.1016/j.gie.2010.04.013]

51 **Matsui S**, Kudo M, Kitano M, Asakuma Y. Evaluation of the Response to Chemotherapy in Advanced Gastric Cancer by Contrast-Enhanced Harmonic EUS. *Hepatogastroenterology* 2015; **62**: 595-598 [PMID: 26897935]

52 **Iglesias-Garcia J**, Domínguez-Muñoz JE, Castiñeira-Alvariño M, Luaces-Regueira M, Lariño-Noia J. Quantitative elastography associated with endoscopic ultrasound for the diagnosis of chronic pancreatitis. *Endoscopy* 2013; **45**: 781-788 [PMID: 24019131 DOI: 10.1055/s-0033-1344614]

53 **Dietrich CF**, Săftoiu A, Jenssen C. Real time elastography endoscopic ultrasound (RTE-EUS), a comprehensive review. *Eur J Radiol* 2014; **83**: 405-414 [PMID: 23643030 DOI: 10.1016/j.ejrad.2013.03.023]

54 **Havre RF**, Ødegaard S, Gilja OH, Nesje LB. Characterization of solid focal pancreatic lesions using endoscopic ultrasonography with real-time elastography. *Scand J Gastroenterol* 2014; **49**: 742-751 [PMID: 24713038 DOI: 10.3109/00365521.2014.905627]

55 **Hirche TO**, Ignee A, Barreiros AP, Schreiber-Dietrich D, Jungblut S, Ott M, Hirche H, Dietrich CF. Indications and limitations of endoscopic ultrasound elastography for evaluation of focal pancreatic lesions. *Endoscopy* 2008; **40**: 910-917 [PMID: 19009483 DOI: 10.1055/s-2008-1077726]

56 **Hu DM**, Gong TT, Zhu Q. Endoscopic ultrasound elastography for differential diagnosis of pancreatic masses: a meta-analysis. *Dig Dis Sci* 2013; **58**: 1125-1131 [PMID: 23306838 DOI: 10.1007/s10620-012-2428-5]

57 **Iglesias-Garcia J**, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. *Gastroenterology* 2010; **139**: 1172-1180 [PMID: 20600020 DOI: 10.1053/j.gastro.2010.06.059]

58 **Giovannini M**, Hookey LC, Bories E, Pesenti C, Monges G, Delpero JR. Endoscopic ultrasound elastography: the first step towards virtual biopsy? Preliminary results in 49 patients. *Endoscopy* 2006; **38**: 344-348 [PMID: 16680632 DOI: 10.1055/s-2006-925158]

59 **Săftoiu A**, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, Iglesias-Garcia J, Arcidiacono P, Will U, Giovannini M, Dietrich C, Havre R, Gheorghe C, McKay C, Gheonea DI, Ciurea T. Accuracy of endoscopic ultrasound elastography used for

differential diagnosis of focal pancreatic masses: a multicenter study. *Endoscopy* 2011; **43**: 596-603 [PMID: 21437851 DOI: 10.1055/s-0030-1256314]

60 **Săftoiu A**, Vilmann P, Gorunescu F, Gheonea DI, Gorunescu M, Ciurea T, Popescu GL, Iordache A, Hassan H, Iordache S. Neural network analysis of dynamic sequences of EUS elastography used for the differential diagnosis of chronic pancreatitis and pancreatic cancer. *Gastrointest Endosc* 2008; **68**: 1086-1094 [PMID: 18656186 DOI: 10.1016/j.gie.2008.04.031]

61 **Săftoiu A**, Iordache SA, Gheonea DI, Popescu C, Maloş A, Gorunescu F, Ciurea T, Iordache A, Popescu GL, Manea CT. Combined contrast-enhanced power Doppler and real-time sonoelastography performed during EUS, used in the differential diagnosis of focal pancreatic masses (with videos). *Gastrointest Endosc* 2010; **72**: 739-747 [PMID: 20674916 DOI: 10.1016/j.gie.2010.02.056]

62 **Săftoiu A**, Vilmann P, Gorunescu F, Janssen J, Hocke M, Larsen M, Iglesias-Garcia J, Arcidiacono P, Will U, Giovannini M, Dietrich CF, Havre R, Gheorghe C, McKay C, Gheonea DI, Ciurea T. Efficacy of an artificial neural network-based approach to endoscopic ultrasound elastography in diagnosis of focal pancreatic masses. *Clin Gastroenterol Hepatol* 2012; **10**: 84-90.e1 [PMID: 21963957 DOI: 10.1016/j.cgh.2011.09.014]

63 **Pei Q**, Zou X, Zhang X, Chen M, Guo Y, Luo H. Diagnostic value of EUS elastography in differentiation of benign and malignant solid pancreatic masses: a meta-analysis. *Pancreatology* 2012; **12**: 402-408 [PMID: 23127527 DOI: 10.1016/j.pan.2012.07.013]

64 **Mei M**, Ni J, Liu D, Jin P, Sun L. EUS elastography for diagnosis of solid pancreatic masses: a meta-analysis. *Gastrointest Endosc* 2013; **77**: 578-589 [PMID: 23199646 DOI: 10.1016/j.gie.2012.09.035]

65 **Xu W**, Shi J, Li X, Zeng X, Lin Y. Endoscopic ultrasound elastography for differentiation of benign and malignant pancreatic masses: a systemic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2013; **25**: 218-224 [PMID: 23169307 DOI: 10.1097/MEG.0b013e32835a7f7c]

66 **Li X**, Xu W, Shi J, Lin Y, Zeng X. Endoscopic ultrasound elastography for differentiating between pancreatic adenocarcinoma and inflammatory masses: a meta-analysis. *World J Gastroenterol* 2013; **19**: 6284-6291 [PMID: 24115828 DOI: 10.3748/wjg.v19.i37.6284]

67 **Ying L**, Lin X, Xie ZL, Hu YP, Tang KF, Shi KQ. Clinical utility of endoscopic ultrasound elastography for identification of malignant pancreatic masses: a meta-analysis. *J Gastroenterol Hepatol* 2013; **28**: 1434-1443 [PMID: 23731128 DOI: 10.1111/jgh.12292]

68 **Mayerle J**, Beyer G, Simon P, Dickson EJ, Carter RC, Duthie F, Lerch MM, McKay CJ. Prospective cohort study comparing transient EUS guided elastography to EUS-FNA for the diagnosis of solid pancreatic mass lesions. *Pancreatology* 2016; **16**: 110-114 [PMID: 26602088 DOI: 10.1016/j.pan.2015.10.003]

69 **Kongkam P**, Lakananurak N, Navicharern P, Chantarojanasiri T, Aye K, Ridtitid W, Kritisin K, Angsuwatcharakon P, Aniwan S, Pittayanon R, Sampatanukul P, Treeprasertsuk S, Kullavanijaya P, Rerknimitr R. Combination of EUS-FNA and elastography (strain ratio) to exclude malignant solid pancreatic lesions: A prospective single-blinded study. *J Gastroenterol Hepatol* 2015; **30**: 1683-1689

70 **Dawwas MF**, Taha H, Leeds JS, Nayar MK, Oppong KW. Diagnostic accuracy of quantitative EUS elastography for discriminating malignant from benign solid pancreatic masses: a prospective, single-center study. *Gastrointest Endosc* 2012; **76**: 953-961 [PMID: 22854060 DOI: 10.1016/j.gie.2012.05.034]

71 **Figueiredo FA**, da Silva PM, Monges G, Bories E, Pesenti C, Caillol F, Delpero JR, Giovannini M. Yield of Contrast-Enhanced Power Doppler Endoscopic Ultrasonography and Strain Ratio Obtained by EUS-Elastography in the Diagnosis of Focal Pancreatic Solid Lesions. *Endosc Ultrasound* 2012; **1**: 143-149 [PMID: 24949352 DOI: 10.7178/eus.03.005]

72 **Iglesias-Garcia J**, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. EUS elastography for the characterization of solid pancreatic masses. *Gastrointest Endosc* 2009; **70**: 1101-1108 [PMID: 19647248 DOI: 10.1016/j.gie.2009.05.011]

73 **Itokawa F**, Itoi T, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Umeda J, Tanaka R, Yokoyama N, Moriyasu F, Kasuya K, Nagao T, Kamisawa T, Tsuchida A. EUS elastography combined with the strain ratio of tissue elasticity for diagnosis of solid pancreatic masses. *J Gastroenterol* 2011; **46**: 843-853 [PMID: 21505859 DOI: 10.1007/s00535-011-0399-5]

74 **Lee TH**, Cho YD, Cha SW, Cho JY, Jang JY, Jeong SW, Choi HJ, Moon JH. Endoscopic ultrasound elastography for the pancreas in Korea: a preliminary single center study. *Clin Endosc* 2013; **46**: 172-177 [PMID: 23614128 DOI: 10.5946/ce.2013.46.2.172]

75 **Janssen J**, Schlörer E, Greiner L. EUS elastography of the pancreas: feasibility and pattern description of the normal pancreas, chronic pancreatitis, and focal pancreatic lesions. *Gastrointest Endosc* 2007; **65**: 971-978 [PMID: 17531630 DOI: 10.1016/j.gie.2006.12.057]

76 **Izumo T**, Sasada S, Chavez C, Matsumoto Y, Tsuchida T. Endobronchial ultrasound elastography in the diagnosis of mediastinal and hilar lymph nodes. *Jpn J Clin Oncol* 2014; **44**: 956-962 [PMID: 25121724 DOI: 10.1093/jjco/hyu105]

77 **Larsen MH**, Fristrup CW, Mortensen MB. Intra- and interobserver agreement of endoscopic sonoelastography in the evaluation of lymph nodes. *Ultraschall Med* 2011; **32** Suppl 2: E45-E50 [PMID: 22194049 DOI: 10.1055/s-0031-1273493]

78 **Paterson S**, Duthie F, Stanley AJ. Endoscopic ultrasound-guided elastography in the nodal staging of oesophageal cancer. *World J Gastroenterol* 2012; **18**: 889-895 [PMID: 22408347 DOI: 10.3748/wjg.v18.i9.889]

79 **Giovannini M**, Thomas B, Erwan B, Christian P, Fabrice C, Benjamin E, Geneviève M, Paolo A, Pierre D, Robert Y, Walter S, Hanz S, Carl S, Christoph D, Pierre E, Jean-Luc VL, Jacques D, Peter V, Andrian S. Endoscopic ultrasound elastography for evaluation of lymph nodes and pancreatic masses: a multicenter study. *World J Gastroenterol* 2009; **15**: 1587-1593 [PMID: 19340900]

80 **Janssen J**, Dietrich CF, Will U, Greiner L. Endosonographic elastography in the diagnosis of mediastinal lymph nodes. *Endoscopy* 2007; **39**: 952-957 [PMID: 18008203 DOI: 10.1055/s-2007-966946]

81 **Larsen MH**, Fristrup C, Hansen TP, Hovendal CP, Mortensen MB. Endoscopic ultrasound, endoscopic sonoelastography, and strain ratio evaluation of lymph nodes with histology as gold standard. *Endoscopy* 2012; **44**: 759-766 [PMID: 22752891 DOI: 10.1055/s-0032-1309817]

82 **Xu W**, Shi J, Zeng X, Li X, Xie WF, Guo J, Lin Y. EUS elastography for the differentiation of benign and malignant lymph nodes: a meta-analysis. *Gastrointest Endosc* 2011; **74**: 1001-1009; quiz 1115.e1-4 [PMID: 22032315 DOI: 10.1016/j.gie.2011.07.026]

83 **Knabe M**, Günter E, Ell C, Pech O. Can EUS elastography improve lymph node staging in esophageal cancer? *Surg Endosc* 2013; **27**: 1196-1202 [PMID: 23093233 DOI: 10.1007/s00464-012-2575-y]

84 **Itoh Y**, Itoh A, Kawashima H, Ohno E, Nakamura Y, Hiramatsu T, Sugimoto H, Sumi H, Hayashi D, Kuwahara T, Morishima T, Funasaka K, Nakamura M, Miyahara R, Ohmiya N, Katano Y, Ishigami M, Goto H, Hirooka Y. Quantitative analysis of diagnosing pancreatic fibrosis using EUS-elastography (comparison with surgical specimens). *J Gastroenterol* 2014; **49**: 1183-1192 [PMID: 24026103 DOI: 10.1007/s00535-013-0880-4]

85 **Janssen J**, Papavassiliou I. Effect of aging and diffuse chronic pancreatitis on pancreas elasticity evaluated using semiquantitative EUS elastography. *Ultraschall Med* 2014; **35**: 253-258 [PMID: 24327468 DOI: 10.1055/s-0033-1355767]

86 **Dietrich CF**, Hirche TO, Ott M, Ignee A. Real-time tissue elastography in the diagnosis of autoimmune pancreatitis. *Endoscopy* 2009; **41**: 718-720 [PMID: 19618344 DOI: 10.1055/s-0029-1214866]

87 **Dominguez-Muñoz JE**, Iglesias-Garcia J, Castiñeira Alvariño M, Luaces Regueira M, Lariño-Noia J. EUS elastography to predict pancreatic exocrine insufficiency in patients with chronic

pancreatitis. *Gastrointest Endosc* 2015; **81**: 136-142 [PMID: 25088920 DOI: 10.1016/j.gie.2014.06.040]
88 **Jenssen C**, Dietrich CF. Endoscopic ultrasound of gastrointestinal subepithelial lesions. *Ultraschall Med* 2008; **29**: 236-256; quiz 257-264 [PMID: 18516768 DOI: 10.1055/s-2008-1027388]
89 **Waage JE**, Leh S, Røsler C, Pfeffer F, Bach SP, Havre RF, Haldorsen IS, Ødegaard S, Baatrup G. Endorectal ultrasonography, strain elastography and MRI differentiation of rectal adenomas and adenocarcinomas. *Colorectal Dis* 2015; **17**: 124-131 [PMID: 25407010 DOI: 10.1111/codi.12845]
90 **Azuma M**, Kusano C, Gotoda T. Diagnostic potential of endoscopic ultrasonography-elastography for gastric submucosal tumors. *Dig Endosc* 2015; **27** Suppl 1: 23 [PMID: 25556630 DOI: 10.1111/den.12429]
91 **Allgayer H**, Ignee A, Zipse S, Crispin A, Dietrich CF. Endorectal ultrasound and real-time elastography in patients with fecal incontinence following anorectal surgery: a prospective comparison evaluating short- and long-term outcomes in irradiated and non-irradiated patients. *Z Gastroenterol* 2012; **50**: 1281-1286 [PMID: 23225555 DOI: 10.1055/s-0032-1313000]
92 **Rustemovic N**, Cukovic-Cavka S, Opacic M, Petrovecki M, Hrstic I, Radic D, Ostojic R, Pulanic R, Vucelic B. Endoscopic ultrasound elastography as a method for screening the patients with suspected primary sclerosing cholangitis. *Eur J Gastroenterol Hepatol* 2010; **22**: 748-753 [PMID: 19494783 DOI: 10.1097/MEG.0b013e32832d489f]
93 **Rustemovic N**, Opacic D, Ostojic Z, Opacic M, Ledinsky I, Višijić A, Ravić KG, Iveković H, Markoš P. Comparison of elastography methods in patients with pancreatic masses. *Endosc Ultrasound* 2014; **3**: S4 [PMID: 26425528]
94 **Opačić D**, Rustemović N, Kalauz M, Markoš P, Ostojić Z, Majerović M, Ledinsky I, Višnjić A, Krznarić J, Opačić M. Endoscopic ultrasound elastography strain histograms in the evaluation of patients with pancreatic masses. *World J Gastroenterol* 2015; **21**: 4014-4019 [PMID: 25852289 DOI: 10.3748/wjg.v21.i13.4014]
95 **Săftoiu A**, Vilmann P, Ciurea T, Popescu GL, Iordache A, Hassan H, Gorunescu F, Iordache S. Dynamic analysis of EUS used for the differentiation of benign and malignant lymph nodes. *Gastrointest Endosc* 2007; **66**: 291-300 [PMID: 17643702 DOI: 10.1016/j.gie.2006.12.039]
96 **Chapman CG**, Siddiqui UD. New Scopes, New Accessories, New Stents for Interventional Endoscopic Ultrasound. *Clin Endosc* 2016; **49**: 41-46 [PMID: 26855923 DOI: 10.5946/ce.2016.49.1.41]
97 **Kuiper T**, van den Broek FJ, van Eeden S, Fockens P, Dekker E. Feasibility and accuracy of confocal endomicroscopy in comparison with narrow-band imaging and chromoendoscopy for the differentiation of colorectal lesions. *Am J Gastroenterol* 2012; **107**: 543-550 [PMID: 22433922 DOI: 10.1038/ajg.2012.14]
98 **Neumann H**, Vieth M, Atreya R, Grauer M, Siebler J, Bernatik T, Neurath MF, Mudter J. Assessment of Crohn's disease activity by confocal laser endomicroscopy. *Inflamm Bowel Dis* 2012; **18**: 2261-2269 [PMID: 22344873 DOI: 10.1002/ibd.22907]
99 **van den Broek FJ**, van Es JA, van Eeden S, Stokkers PC, Ponsioen CY, Reitsma JB, Fockens P, Dekker E. Pilot study of probe-based confocal laser endomicroscopy during colonoscopic surveillance of patients with longstanding ulcerative colitis. *Endoscopy* 2011; **43**: 116-122 [PMID: 21165821 DOI: 10.1055/s-0030-1255954]
100 **Sharma P**, Meining AR, Coron E, Lightdale CJ, Wolfsen HC, Bansal A, Bajbouj M, Galmiche JP, Abrams JA, Rastogi A, Gupta N, Michalek JE, Lauwers GY, Wallace MB. Real-time increased detection of neoplastic tissue in Barrett's esophagus with probe-based confocal laser endomicroscopy: final results of an international multicenter, prospective, randomized, controlled trial. *Gastrointest Endosc* 2011; **74**: 465-472 [PMID: 21741642 DOI: 10.1016/j.gie.2011.04.004]
101 **Bhutani MS**, Koduru P, Joshi V, Karstensen JG, Saftoiu A, Vilmann P, Giovannini M. EUS-Guided Needle-Based Confocal Laser Endomicroscopy: A Novel Technique With Emerging Applications. *Gastroenterol Hepatol* (N Y) 2015; **11**: 235-240 [PMID: 27099595]
102 **Napoleon B**, Lemaistre AI, Pujol B, Caillol F, Lucidarme D, Bourdariat R, Morellon-Mialhe B, Fumex F, Lefort C, Lepilliez V, Palazzo L, Monges G, Poizat F, Giovannini M. In vivo characterization of pancreatic cystic lesions by needle-based confocal laser endomicroscopy (nCLE): proposition of a comprehensive nCLE classification confirmed by an external retrospective evaluation. *Surg Endosc* 2016; **30**: 2603-2612 [PMID: 26428198 DOI: 10.1007/s00464-015-4510-5]
103 **Napoléon B**, Lemaistre AI, Pujol B, Caillol F, Lucidarme D, Bourdariat R, Morellon-Mialhe B, Fumex F, Lefort C, Lepilliez V, Palazzo L, Monges G, Filoche B, Giovannini M. A novel approach to the diagnosis of pancreatic serous cystadenoma: needle-based confocal laser endomicroscopy. *Endoscopy* 2015; **47**: 26-32 [PMID: 25325684 DOI: 10.1055/s-0034-1390693]
104 **Karia K**, Waxman I, Konda VJ, Gress FG, Sethi A, Siddiqui UD, Sharaiha RZ, Kedia P, Jamal-Kabani A, Gaidhane M, Kahaleh M. Needle-based confocal endomicroscopy for pancreatic cysts: the current agreement in interpretation. *Gastrointest Endosc* 2016; **83**: 924-927 [PMID: 26382051 DOI: 10.1016/j.gie.2015.08.080]
105 **Jais B**, Rebours V, Malleo G, Salvia R, Fontana M, Maggino L, Bassi C, Manfredi R, Moran R, Lennon AM, Zaheer A, Wolfgang C, Hruban R, Marchegiani G, Fernández Del Castillo C, Brugge W, Ha Y, Kim MH, Oh D, Hirai I, Kimura W, Jang JY, Kim SW, Jung W, Kang H, Song SY, Kang CM, Lee WJ, Crippa S, Falconi M, Gomatos I, Neoptolemos J, Milanetto AC, Sperti C, Ricci C, Casadei R, Bissolati M, Balzano G, Frigerio I, Girelli R, Delhaye M, Bernier B, Wang H, Jang KT, Song DH, Huggett MT, Oppong KW, Pererva L, Kopchak KV, Del Chiaro M, Segersvard R, Lee LS, Conwell D, Osvaldt A, Campos V, Aguero Garcete G, Napoleon B, Matsumoto I, Shinzeki M, Bolado F, Fernandez JM, Keane MG, Pereira SP, Acuna IA, Vaquero EC, Angiolini MR, Zerbi A, Tang J, Leong RW, Faccinetto A, Morana G, Petrone MC, Arcidiacono PG, Moon JH, Choi HJ, Gill RS, Pavey D, Ouaïssi M, Sastre B, Spandre M, De Angelis CG, Rios-Vives MA, Concepcion-Martin M, Ikeura T, Okazaki K, Frulloni L, Messina O, Lévy P. Serous cystic neoplasm of the pancreas: a multinational study of 2622 patients under the auspices of the International Association of Pancreatology and European Pancreatic Club (European Study Group on Cystic Tumors of the Pancreas). *Gut* 2016; **65**: 305-312 [PMID: 26045140 DOI: 10.1136/gutjnl-2015-309638]
106 **Konda VJ**, Meining A, Jamil LH, Giovannini M, Hwang JH, Wallace MB, Chang KJ, Siddiqui UD, Hart J, Lo SK, Saunders MD, Aslanian HR, Wroblewski K, Waxman I. A pilot study of in vivo identification of pancreatic cystic neoplasms with needle-based confocal laser endomicroscopy under endosonographic guidance. *Endoscopy* 2013; **45**: 1006-1013 [PMID: 24163192 DOI: 10.1055/s-0033-1344714]
107 **Nakai Y**, Iwashita T, Park DH, Samarasena JB, Lee JG, Chang KJ. Diagnosis of pancreatic cysts: EUS-guided, through-the-needle confocal laser-induced endomicroscopy and cystoscopy trial: DETECT study. *Gastrointest Endosc* 2015; **81**: 1204-1214 [PMID: 25634486 DOI: 10.1016/j.gie.2014.10.025]
108 **Giovannini M**, Caillol F, Monges G, Poizat F, Lemaistre AI, Pujol B, Lucidarme D, Palazzo L, Napoléon B. Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy in solid pancreatic masses. *Endoscopy* 2016; **48**: 892-898 [PMID: 27576181 DOI: 10.1055/s-0042-112573]
109 **Kongkam P**, Pittayanon R, Sampatanukul P, Angsuwatcharakon P, Aniwan S, Prueksapanich P, Sriuranpong V, Navicharern P, Treeprasertsuk S, Kullavanijaya P, Rerknimitr R. Endoscopic ultrasound-guided needle-based confocal laser endomicroscopy for diagnosis of solid pancreatic lesions (ENES): a pilot study. *Endosc Int Open* 2016; **4**: E17-E23 [PMID: 26793780 DOI: 10.1055/s-0034-1393183]
110 **Karstensen J**, Cartana T, Pia K, Saftoiu A, Vilmann P. Endoscopic ultrasound-guided needle confocal laser endomicroscopy in pancreatic masses. *Endosc Ultrasound* 2014; **3**: S2-S3 [PMID: 26425525]

111 **Giovannini M**, Caillol F, Lemaistre A, Monges G, Napoleon B, Pujol B. Endoscopic ultrasound guided confocal microscopy: Atlas of cystic pancreatic lesions. *Endosc Ultrasound* 2014; **3**: S19-S21 [PMID: 26425522 DOI: 10.4103/2303-9027.129481]

112 **Benias PC**, D'Souza LS, Papafragkakis H, Kim J, Harshan M, Theise ND, Carr-Locke DL. Needle-based confocal endomicroscopy for evaluation of malignant lymph nodes - a feasibility study. *Endoscopy* 2016; **48**: 923-928 [PMID: 27434768 DOI: 10.1055/s-0042-109775]

113 **Nakai Y**, Shinoura S, Ahluwalia A, Tarnawski AS, Chang KJ. In vivo visualization of epidermal growth factor receptor and survivin expression in porcine pancreas using endoscopic ultrasound guided fine needle imaging with confocal laser-induced endomicroscopy. *J Physiol Pharmacol* 2012; **63**: 577-580 [PMID: 23388473]

114 **Samarasena JB**, Ahluwalia A, Shinoura S, Choi KD, Lee JG, Chang KJ, Tarnawski AS. In vivo imaging of porcine gastric enteric nervous system using confocal laser endomicroscopy & amp; molecular neuronal probe. *J Gastroenterol Hepatol* 2016; **31**: 802-807 [PMID: 26482711 DOI: 10.1111/jgh.13194]

115 **Robles-Medranda C**. Confocal endomicroscopy: Is it time to move on? *World J Gastrointest Endosc* 2016; **8**: 1-3 [PMID: 26788257 DOI: 10.4253/wjge.v8.i1.1]

P- Reviewer: Santoro GA, Slomiany BL, Tarnawski AS
S- Editor: Qi Y **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.42

World J Gastroenterol 2017 January 7; 23(1): 42-47
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Neutropenic enterocolitis

Fabio G Rodrigues, Giovanna Dasilva, Steven D Wexner

Fabio G Rodrigues, Giovanna Dasilva, Steven D Wexner, Department of Colorectal Surgery, Cleveland Clinic Florida, Weston, FL 33331, United States

Author contributions: Rodrigues FG, Dasilva G and Wexner SD contributed equally to this work including designing and performing the research, analyzing the data, and writing the paper.

Conflict-of-interest statement: The authors have no relevant financial disclosures.

Manuscript source: Invited manuscript

Correspondence to: Steven D Wexner, MD, PhD (Hon), Department of Colorectal Surgery, Cleveland Clinic Florida, 2950 Cleveland Clinic Blvd, Weston, FL 33331, United States. wexners@ccf.org
Telephone: +1-954-6596020

Received: June 8, 2016
Peer-review started: June 13, 2016
First decision: October 20, 2016
Revised: November 18, 2016
Accepted: December 8, 2016
Article in press: December 8, 2016
Published online: January 7, 2017

Abstract

Neutropenic colitis is a severe condition usually affecting immunocompromised patients. Its exact pathogenesis is not completely understood. The main elements in disease onset appear to be intestinal mucosal injury together with neutropenia and the weakened immune system of the afflicted patients. These initial conditions lead to intestinal edema, engorged vessels, and a disrupted mucosal surface, which becomes more vulnerable to bacterial intramural invasion. Chemotherapeutic agents can cause direct mucosal injury (mucositis) or can predispose to distension and necrosis, thereby altering intestinal motility. This article aims to review current concepts regarding neutropenic colitis' pathogenesis, diagnosis, and management.

Key words: Neutropenic enterocolitis; Neutropenic colitis; Immunocompromise; Intestinal mucosal injury; Neutropenia; Intestinal edema; Intramural invasion; Pathogenesis

Core tip: Neutropenic colitis is a severe condition usually affecting immunocompromised patients. Its exact pathogenesis is not completely understood. The main elements in disease onset appear to be intestinal mucosal injury together with neutropenia and the weakened immune system of the afflicted patients. These initial conditions lead to intestinal edema, engorged vessels, and a disrupted mucosal surface, which becomes more vulnerable to bacterial intramural invasion. Chemotherapeutic agents can cause direct mucosal injury or can predispose to distension and necrosis, thereby altering intestinal motility.

Rodrigues FG, Dasilva G, Wexner SD. Neutropenic enterocolitis. *World J Gastroenterol* 2017; 23(1): 42-47 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/42.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.42

INTRODUCTION

Neutropenic enterocolitis (NE) is also known as typhlitis, ileocecal syndrome, cecitis, or necrotizing enterocolitis. Despite the previous use of the term

"necrotizing enterocolitis" to describe NE cases, necrotizing enterocolitis is a different inflammatory illness seen in newborns and is beyond the scope of this review[1]. NE is a clinical entity initially described in leukemic pediatric patients. It has also been reported in adults with hematologic malignancies such as leukemia, lymphoma, multiple myeloma, aplastic anemia, and myelodysplastic syndromes, as well as other immunosuppressive causes such as AIDS, therapy for solid tumors, and organ transplant[2].

The true incidence of NE is unknown[2]. One systematic review published in 2005 suggested a pooled incidence of 5.6% in hospitalized adults with hematological malignancies, chemotherapy for solid tumors, and aplastic anemia[3]. The reported mortality also varies with rates as high as 50%[4].

NE was reported initially after the use of taxane drugs, but more recently an increasing number of chemotherapeutic drugs have been implicated[5]. Other drugs linked to NE include cytosine arabinoside, gemcitabine, vincristine, doxorubicin, gemcitabine, cyclophosphamide, 5-fluorouracil, leuvocorin, and daunorubicin. Immunosuppressive therapy for organ transplant, antibiotics, and sulfasalazine for the treatment of rheumatoid arthritis have also been considered causes of NE[6,7].

This review aims to assess current concepts regarding the pathogenesis, diagnosis, and management of neutropenic colitis. A search for the terms "neutropenic enterocolitis", "neutropenic colitis", "typhlitis", "ileocecal syndrome", "cecitis", and "necrotizing enterocolitis" was made in PubMed, exclusive to human studies and with no time limits.

PATHOGENESIS

The exact pathogenesis of NE is not completely understood. The main elements in disease onset appear to be intestinal mucosal injury together with neutropenia and the immunocompromised state of the afflicted patients. These initial conditions lead to intestinal edema, engorged vessels, and a disrupted mucosal surface, which becomes more vulnerable to bacterial intramural invasion. Chemotherapeutic agents can cause direct mucosal injury (mucositis) or can predispose to distension and necrosis, thereby altering intestinal motility[8,9]. Cytosine arabinoside (cytarabine) is a chemotherapeutic agent used to treat leukemia and lymphoma that is particularly associated with the development of NE. Among its adverse effects, gastrointestinal mucosal toxicity and ileus have been described[10,11].

Intestinal leukemic infiltration is another potential factor in the pathogenesis of NE, which may explain the presence of acute myelogenous leukemia presenting as NE before the onset of chemotherapy regimens[12,13]. However, some studies have not reported this leukemic infiltration after histologic evaluation[13-15]. Other histologic findings have included mucosal ulcers, intramural hemorrhage (usually associated with thrombocytopenia), and necrosis.

The cecum is always affected by NE and very often extends to the ileum. The ascending and transverse colon may also be involved. A case of diffuse colorectal inflammation following chemotherapy in a pediatric leukemic patient was reported[16]. This predilection by the cecum may be explained by its distensibility and limited blood supply[2].

Although a superimposed infection of the damaged mucosa in the neutropenic patient is not universally considered a diagnostic criterion, it definitively plays an important role in the pathogenesis of NE[3]. Gram-negative rods, gram-positive cocci, enterococci, fungi, and virus have been implicated as causes[8,17,18]. Bacterial translocation and bacteremia is also frequently seen in these patients. While some authors associate NE with infection by *Clostridium septicum*, this is not always implicated among the pathogens in other studies[19]. Sloas *et al*[20] reported NE in 24 leukemic children and found six different pathogens in eight patients with bacteremia (*Escherichia coli* in 3 patients, *Kleibsiella pneumoniae* in 2 patients, *Enterobacter taylorae, Morganella morganii,* and a *Streptococcus viridans* in 1 patient each). They also found *Clostriduim difficile* toxin in the stool of three of the 16 patients who were tested. Immunosuppression and the frequent use of antimicrobials in NE can alter normal flora and facilitate infection by less common agents[17]. Fungal infections can play an important role in NE. One systematic review of published case studies found a significantly lower mortality rate in patients receiving antifungal agents for the treatment of NE[21].

CLINICAL PRESENTATION

Patients with neutrophil counts < 500/μL are at increased risk for developing NE. Reports of neutrophil counts < 1000/μL) have also been published[22]. The most common symptoms of NE are abdominal pain, diarrhea, and fever[23-25]. Nausea, vomiting, and abdominal distension are also common symptoms. Abdominal pain can be localized in the lower right quadrant or can be more diffuse. Tenderness can be found on palpation. Abdominal compartment syndrome has been reported in a patient with NE presenting with abdominal distension and ascites[26].

Melena or hematochezia are generally less common forms of presentation[27]. One autopsy series reported a 35% lower gastrointestinal bleeding rate in pediatric patients and considered this to precede a terminal event[8]. Severe hemorrhage with hemodynamic instability have also been reported and these patients should undergo immediate interventional radiologic procedures (*i.e.*, angiography with embolization) in an attempt to avoid surgery[28,29]. Peritoneal signs, shock, and rapid clinical deterioration can be suggestive of

necrosis and bowel perforation.

Symptoms often appear within two weeks following the completion of chemotherapy and coincide with the low leukocyte count following chemotherapy[30]. Shamberger *et al*[31] found that NE occurred after induction chemotherapy in the majority of their patients (19/25 pediatric patients with NE). Wade *et al*[32] reported that the 22 patients in their study had been leukopenic for > 1 wk before the onset of abdominal pain and that all patients had an absolute count of < 500 cells/μL at some point during the leukopenia. Leukocyte count recovery after the onset of NE seems to be associated with survival[33]. Regarding NE after hematopoietic stem cell transplantation in children, Lee *et al*[34] considered NE to be a pre-engraftment phase complication (occurring before 30 days following transplantation) in their study of hematopoietic stem cell transplantation in children. Specifically, this is the period of marrow aplasia and pancytopenia. Recurrence can occur after resolution of the first episode.

DIAGNOSIS

Due to its nonspecific presentation, NE can mimic many other diagnoses. Differential diagnoses include pseudomembranous colitis, inflammatory bowel disease, appendicitis, ischemic colitis, and other infectious colitides.

Diagnosis generally involves the findings of fever, abdominal pain, neutropenia, and thickening of the abdominal wall (usually the cecum and ascending colon)[21,35]. In a study that included 40 pediatric patients, the clinical triad (fever, abdominal pain, and neutropenia) was present in 31 patients (78%). The remaining 9 patients (22%) had their diagnosis made after imaging exams (US/CT) in addition to 2/3 clinical features[36].

Abdominal plain X-rays can show a dilated atonic cecum and ascending colon filled with liquid or gas, signs of intramural gas, and small bowel dilation. However, this simple imaging technique has limited value due to its poor sensitivity and specificity[20,35]. Radiographic imaging can also show pneumoperitoneum in patients with suspected bowel perforation[37]. Ultrasonic examination is still an important tool in pediatric patients because it is inexpensive, readily available, and avoids radiation or radiopharmaceuticals[35]. Computed tomography (CT) is an attractive non-invasive option for diagnosis, with higher accuracy compared to plain radiography and ultrasound[20,38]. CT can delineate bowel wall thickening, a dilated cecum or other colonic segment, an inflammatory mass, pericolonic inflammation, and pneumatosis intestinalis. It can also help visualize other organs and a make differential diagnosis. Colonic wall thickening may suggest the need for surgical treatment and affect prognosis[21]. A study by Cartoni *et al*[35] reported a mortality rate of 60% due to NE in patients with colonic wall thickness of 10 mm compared to a mortality rate of 4.2% in patients with mural thickness of < 10 mm, seen on ultrasound.

Other imaging methods can be applied in the evaluation of NE. Barium enema is useful in showing torsion and edema of the cecum, but may potentially cause colonic perforation and septicemia[39-42]. Scintigraphic studies have shown uptake of radiopharmaceuticals in the lower right quadrant and suggest a diagnosis of NE[43]. Colonoscopy is rarely indicated in suspected NE because of cytopenia and the risk of perforation[2,36].

Laboratory findings are often nonspecific. Neutropenia and thrombocytopenia are frequent alterations and have a role in the pathogenesis of NE itself. Electrolyte imbalance and albumin loss is a frequent finding in patients receiving chemotherapy with cytosine-arabinoside. Fecal examination suggests that these patients have significant loss of potassium in the stool[10]. Blood and stool cultures can guide the therapy to specific agents.

MANAGEMENT

The lack of high-quality studies looking at therapeutic strategies makes it impossible for standardized recommendations in the management of patients with NE[2,3]. Initial reports of NE showed a preference for surgical treatment as the high mortality associated with NE led to a more aggressive treatment regimen[44]. A better understanding of the disease and higher success rates of conservative management have contributed to reserving surgery for complicated and more severe cases[44,45].

Conservative management consists of aggressive fluid resuscitation, correction of electrolyte imbalance, bowel rest, abdominal decompression, and broad-spectrum antibiotics. Correction of thrombocytopenia and clotting abnormalities can require blood component transfusion.

Patients who present with a recovery in the leukocyte count tend to have better outcomes[46]. Leukocyte transfusions and granulocyte-colony stimulation factors (G-CSF) have been applied to treat these patients. Although there are no randomized controlled studies regarding the use of G-CSF in neutropenic colitis, guidelines with recommendations have been proposed[47,48]. Patient-related factors such as profound neutropenia (absolute neutrophil < 100/mL), uncontrolled primary disease, pneumonia, hypotension, multiorgan dysfunction, and invasive fungal infection are possible indications for the use of G-CSF in NE[3].

Bowel rest is commonly used in cases of NE[2,3,20,24]. Parenteral nutrition can be used to maintain a nutritional source in patients who are at nutritional risk. Bowel rest can also mean intestinal villous atrophy and

Table 1 Antibiotics and dosages for empiric treatment of neutropenic enterocolitis

Antibiotics	Dosages
Adults with NE	
Monotherapy	
Piperacillin-tazobactam	3.375 g IV every 6 h
Imipenem-cillastin	500 mg IV every 6 h or 1 g IV every 6-8 h
Duotherapy	
Ceftazidime	1 g IV every 8-12 h
OR	OR
Cefepime	1 g IV every 8 h
Plus Metronidazole	1 g IV every 6 h
Children (1-12 yr of age) with NE	
Monotherapy	
Piperacillin-tazobactam	(> 9 mo and < 40 kg) 300 mg/kg per day IV divided every 8 h
Imipenem-cillastin	(> 3 mo) 60-100 mg/kg per day IV divided every 6 h maximum 2-4 g/d
Duotherapy	
Ceftazidime	90-150 mg/kg per day IV divided every 8 h - maximum 6 g/d or
OR	50 mg/kg IV every 8 h - maximum: 2 g/dose
Cefepime	
Plus Metronidazole	30 mg/kg per day IV divided every 6 h - maximum 4 g/d

Modified from Cloutier *et al*[44]. IV: Intravenous; NE: Neutropenic enterocolitis.

mucosal integrity breaching. Some authors consider the possibility of continuing gastrointestinal tract use (oral or enteral) in selected patients[3,14]. The use of glutamine as an immunonutrient is being studied in other patients receiving parenteral nutrition and the results may suggest its potential use in NE patients.

Prompt administration of antibiotics is essential in the treatment of NE patients. Antibiotics should cover gram-positive, gram-negative, and anaerobic pathogens. Coverage against enterococci should be added in the most critically ill patients. Specific local epidemiology and resistance patterns should guide the choice of antimicrobial agents.

An antibiotic treatment regimen usually starts with β-lactamic monotherapy or combined with aminoglycoside[44]. Other monotherapy agents such as cefepime, imipenem, and meropenem can be also used. In cases of patients with known or suspected resistant pathogens, combination regimens are preferred. Duotherapy, combing ceftazidime or cefepime with metronidazole is also an option[44]. For patients in which *Clostridium difficile* cannot be excluded, metronidazole or vancomycin should be added to the regimen[1]. Recommended drugs for adults and children are summarized in Table 1. Initial empiric coverage for fungal agents is not routinely recommended, but can be considered if the initial therapy does not show good response after 72 h[1].

Current indications for surgery in NE are evidence of intraperitoneal bowel perforation, uncontrolled bleeding after correction of cytopenia and clotting abnormalities, and the development of other surgical conditions (abscess, appendicitis). Perforated or necrotic bowel should be resected. Primary anastomosis is not recommended due to the impaired healing and immunossupression in these patients. Drainage of the necrotic region without resection seems to be insufficient[13,46].

CONCLUSION

NE should always be considered as a possible diagnosis in immunosuppressed patients, especially those receiving chemotherapy. NE is a life threatening condition and prompt aggressive treatment is warranted in these patients. Resolution of the disease will depend on recovery of leukocyte count and infection control. Conservative treatment is the recommended first step in treatment, with close monitoring of the patient in the event that surgical intervention is required.

REFERENCES

1 **Nesher L**, Rolston KV. Neutropenic enterocolitis, a growing concern in the era of widespread use of aggressive chemotherapy. *Clin Infect Dis* 2013; **56**: 711-717 [PMID: 23196957 DOI: 10.1093/cid/cis998]

2 **Davila ML**. Neutropenic enterocolitis. *Curr Opin Gastroenterol* 2006; **22**: 44-47 [PMID: 16319675 DOI: 10.1007/s11938-006-0043-2]

3 **Gorschlüter M**, Mey U, Strehl J, Ziske C, Schepke M, Schmidt-Wolf IG, Sauerbruch T, Glasmacher A. Neutropenic enterocolitis in adults: systematic analysis of evidence quality. *Eur J Haematol* 2005; **75**: 1-13 [PMID: 15946304 DOI: 10.1111/j.1600-0609.2005.00442.x]

4 **Ullery BW**, Pieracci FM, Rodney JR, Barie PS. Neutropenic enterocolitis. *Surg Infect* (Larchmt) 2009; **10**: 307-314 [PMID: 19566419 DOI: 10.1089/sur.2008.061]

5 **Mehdi I**, Al Bahrani B. Chemotherapy-induced neutropenic necrotizing enterocolitis: a review. *J Pak Med Assoc* 2012; **62**: 718-723 [PMID: 23866523]

6 **Bibbo C**, Barbieri RA, Deitch EA, Brolin RE. Neutropenic enterocolitis in a trauma patient during antibiotic therapy for osteomyelitis. *J Trauma* 2000; **49**: 760-763 [PMID: 11038099 DOI: 10.1097/00005373-200010000-00029]

7 **Chavakravarty K**, Scott DG, McCann BG. Fatal neutropenic enterocolitis associated with sulphasalazine theraphy for reumathoid arthritis. *Br J Rheumatol* 1992; **31**: 351-352 [DOI: 10.1093/rheumatology/31.5.351]

8 **Katz JA**, Wagner ML, Gresik MV. Typhlitis. An 18-year experience and postmortem review. *Cancer* 1990; **65**: 1041-1047 [DOI: 10.1002/1097-0142(19900215)65:4<1041::AID-CNCR2820650433>3.0.CO;2-A]

9 **Bavaro MF**. Neutropenic enterocolitis. *Curr Gastroenterol Rep* 2002; **4**: 297-301 [PMID: 12149175 DOI: 10.1007/s11894-002-0079-y]

10 **Slavin RE**, Dias MA, Saral R. Cytosine arabinoside induced gastrointestinal toxic alterations in sequential chemotherapeutic protocols: a clinical-pathologic study of 33 patients. *Cancer* 1978; **42**: 1747-1759 [PMID: 709532 DOI: 10.1002/1097-0142(197810)42::4<1747::AID-CNCR2820420413>3.0.CO;2-T]

11 **Stentoft J**. The toxicity of cytarabine. *Drug Saf* 1990; **5**: 7-27 [PMID: 2178634 DOI: 10.2165/00002018-199005010-00003]

12 **Ahsan N**, Sun CC, Di John D. Acute ileotyphlitis as presenting manifestation of acute myelogenous leukemia. *Am J Clin Pathol*

1988; **89**: 407-409 [PMID: 3162345 DOI: 10.1093/ajcp/89.3.407]
13 **Alt B**, Glass NR, Sollinger H. Neutropenic enterocolitis in adults. Review of the literature and assessment of surgical intervention. *Am J Surg* 1985; **149**: 405-408 [PMID: 3856402 DOI: 10.1016/S0002-9610(85)80119-7]
14 **Urbach DR**, Rotstein OD. Typhlitis. *Can J Surg* 1999; **42**: 415-419 [PMID: 10593241]
15 **D'Souza S**, Lindberg M. Typhlitis as a presenting manifestation of acute myelogenous leukemia. *South Med J* 2000; **93**: 218-220 [PMID: 10701793 DOI: 10.1097/00007611-200002000-00014]
16 **Ozgen U**, Uzüm I, Mizrak B, Saraç K. "Typhlitis" in rectum. *Pediatr Int* 2010; **52**: e32-e33 [PMID: 20158643 DOI: 10.1111/j.1442-200X.2009.02989.x]
17 **Buchheidt D**, Böhme A, Cornely OA, Fätkenheuer G, Fuhr HG, Heussel G, Junghanss C, Karthaus M, Kellner O, Kern WV, Schiel X, Sezer O, Südhoff T, Szelényi H. Diagnosis and treatment of documented infections in neutropenic patients--recommendations of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). *Ann Hematol* 2003; **82** Suppl 2: S127-S132 [PMID: 13680171 DOI: 10.1007/s00277-003-0766-2]
18 **Yuen KY**, Woo PC, Liang RH, Chiu EK, Chen FF, Wong SS, Lau YL, Ha SY, Peiris JS, Siau H, Chan TK. Clinical significance of alimentary tract microbes in bone marrow transplant recipients. *Diagn Microbiol Infect Dis* 1998; **30**: 75-81 [PMID: 9554172 DOI: 10.1016/S0732-8893(97)00213-7]
19 **Ettinghausen SE**. Collagenous colitis, eosinophilic colitis, and neutropenic colitis. *Surg Clin North Am* 1993; **73**: 993-1016 [PMID: 8378836 DOI: 10.1016/S0039-6109(16)46137-2]
20 **Sloas MM**, Flynn PM, Kaste SC, Patrick CC. Typhlitis in children with cancer: a 30-year experience. *Clin Infect Dis* 1993; **17**: 484-490 [PMID: 8218694 DOI: 10.1093/clinids/17.3.484]
21 **Cardona Zorrilla AF**, Reveiz Herault L, Casasbuenas A, Aponte DM, Ramos PL. Systematic review of case reports concerning adults suffering from neutropenic enterocolitis. *Clin Transl Oncol* 2006; **8**: 31-38 [PMID: 16632437 DOI: 10.1007/s12094-006-0092-y]
22 **Aksoy DY**, Tanriover MD, Uzun O, Zarakolu P, Ercis S, Ergüven S, Oto A, Kerimoglu U, Hayran M, Abbasoglu O. Diarrhea in neutropenic patients: a prospective cohort study with emphasis on neutropenic enterocolitis. *Ann Oncol* 2007; **18**: 183-189 [PMID: 17023562 DOI: 10.1093/annonc/mdl337]
23 **Shafey A**, Ethier MC, Traubici J, Naqvi A, Sung L. Incidence, risk factors, and outcomes of enteritis, typhlitis, and colitis in children with acute leukemia. *J Pediatr Hematol Oncol* 2013; **35**: 514-517 [PMID: 23823116 DOI: 10.1097/MPH.0b013e31829f3259]
24 **Hsu TF**, Huang HH, Yen DH, Kao WF, Chen JD, Wang LM, Lee CH. ED presentation of neutropenic enterocolitis in adult patients with acute leukemia. *Am J Emerg Med* 2004; **22**: 276-279 [PMID: 15258868 DOI: 10.1016/j.ajem.2004.02.014]
25 **McCarville MB**, Adelman CS, Li C, Xiong X, Furman WL, Razzouk BI, Pui CH, Sandlund JT. Typhlitis in childhood cancer. *Cancer* 2005; **104**: 380-387 [PMID: 15952190 DOI: 10.1002/cncr.21134]
26 **van Vliet M**, van der Hoeven HJ, van der Velden WJ. Abdominal compartment syndrome in neutropenic enterocolitis. *Br J Haematol* 2013; **160**: 273 [PMID: 23206290 DOI: 10.1111/bjh.12140]
27 **Cardona AF**, Combariza JF, Reveiz L, Ospina EG, Poveda CM, Ruiz CA, Ramos P, Aponte DM. [Clinical and microbiological characteristics of neutropenic enterocolitis in adults with blood cancer in the National Cancer Institute of Bogota D.C. (Colombia)]. *Enferm Infecc Microbiol Clin* 2004; **22**: 462-466 [PMID: 15482688 DOI: 10.1157/13066853]
28 **Meyerovitz MF**, Fellows KE. Typhlitis: a cause of gastrointestinal hemorrhage in children. *AJR Am J Roentgenol* 1984; **143**: 833-835 [PMID: 6332493 DOI: 10.2214/ajr.143.4.833]
29 **de Lijster MS**, Smets AM, van den Berg H, Reekers JA. Embolisation for caecal bleeding in a child with typhlitis. *Pediatr Radiol* 2015; **45**: 283-285 [PMID: 24917127 DOI: 10.1007/s00247-014-3059-0]
30 **Hegerova LT**, Swiecicki PL, Kumar SK. 35-year-old man with Fever and abdominal pain. *Mayo Clin Proc* 2013; **88**: 866-870 [PMID: 23910413 DOI: 10.1016/j.mayocp.2012.10.007]
31 **Shamberger RC**, Weinstein HJ, Delorey MJ, Levey RH. The medical and surgical management of typhlitis in children with acute nonlymphocytic (myelogenous) leukemia. *Cancer* 1986; **57**: 603-609 [PMID: 3484659 DOI: 10.1002/1097-0142(19860201)57::3<603::AID-CNCR2820570335>3.0.CO;2-K]
32 **Wade DS**, Nava HR, Douglass Jr HO. Neutropenic enterocolitis. Clinical diagnosis and treatment. *Cancer* 1992; **69**: 17-23 [DOI: 10.1002/1097-0142(19920101)69:1<17::AID-CNCR2820690106>3.0.CO;2-X]
33 **Wade DS**, Douglass H, Nava HR, Piedmonte M. Abdominal pain in neutropenic patients. *Arch Surg* 1990; **125**: 1119-1127 [PMID: 2400304 DOI: 10.1001/archsurg.1990.01410210045006]
34 **Lee JH**, Lim GY, Im SA, Chung NG, Hahn ST. Gastrointestinal complications following hematopoietic stem cell transplantation in children. *Korean J Radiol* 2008; **9**: 449-457 [PMID: 18838855 DOI: 10.3348/kjr.2008.9.5.449]
35 **Cartoni C**, Dragoni F, Micozzi A, Pescarmona E, Mecarocci S, Chirletti P, Petti MC, Meloni G, Mandelli F. Neutropenic enterocolitis in patients with acute leukemia: prognostic significance of bowel wall thickening detected by ultrasonography. *J Clin Oncol* 2001; **19**: 756-761 [PMID: 11157028]
36 **Mullassery D**, Bader A, Battersby AJ, Mohammad Z, Jones EL, Parmar C, Scott R, Pizer BL, Baillie CT. Diagnosis, incidence, and outcomes of suspected typhlitis in oncology patients--experience in a tertiary pediatric surgical center in the United Kingdom. *J Pediatr Surg* 2009; **44**: 381-385 [PMID: 19231539 DOI: 10.1016/j.jpedsurg.2008.10.094]
37 **Ramia Angel JM**, de la Plaza Llamas R, Veguillas Redondo P, Quiñones Sampedro JE, García-Parreño Jofré J. [Stomach perforation in a patient with an intragastric balloon]. *Cir Esp* 2010; **87**: 397-399 [PMID: 19735911 DOI: 10.1016/j.ciresp.2009.06.008]
38 **Kirkpatrick ID**, Greenberg HM. Gastrointestinal complications in the neutropenic patient: characterization and differentiation with abdominal CT. *Radiology* 2003; **226**: 668-674 [PMID: 12601214 DOI: 10.1148/radiol.2263011932]
39 **Archibald RB**, Nelson JA. Necrotizing enterocolitis in acute leukemia: radiographic findings. *Gastrointest Radiol* 1978; **3**: 63-65 [PMID: 276504 DOI: 10.1007/BF01887037]
40 **Abramson SJ**, Berdon WE, Baker DH. Childhood typhlitis: its increasing association with acute myelogenous leukemia. Report of five cases. *Radiology* 1983; **146**: 61-64 [PMID: 6571759 DOI: 10.1148/radiology.146.1.6571759]
41 **Richman LS**, Short WF, Cooper WM. Barium enema septicemia. Occurrence in a patient with leukemia. *JAMA* 1973; **226**: 62-63 [PMID: 4361133 DOI: 10.1001/jama.226.1.62]
42 **Khan JS**, Moran BJ. Iatrogenic perforation at colonic imaging. *Colorectal Dis* 2011; **13**: 481-493 [PMID: 20015266 DOI: 10.1111/j.1463-1318.2009.02147.x]
43 **Gandy W**, Greenberg BR. Successful medical management of neutropenic enterocolitis. *Cancer* 1983; **51**: 1551-1555 [DOI: 10.1002/1097-0142(19830415)51:8<1551::AID-CNCR2820510833>3.0.CO;2-L]
44 **Cloutier RL**. Neutropenic enterocolitis. *Emerg Med Clin North Am* 2009; **27**: 415-422 [PMID: 19646645 DOI: 10.1016/j.emc.2009.04.002]
45 **Gomez L**, Martino R, Rolston KV. Neutropenic enterocolitis: spectrum of the disease and comparison of definite and possible cases. *Clin Infect Dis* 1998; **27**: 695-699 [PMID: 9798018 DOI: 10.1086/514946]
46 **Moir CR**, Scudamore CH, Benny WB. Typhlitis: selective surgical management. *Am J Surg* 1986; **151**: 563-566 [PMID: 3458380 DOI: 10.1016/0002-9610(86)90547-7]
47 **Aapro MS**, Bohlius J, Cameron DA, Dal Lago L, Donnelly JP, Kearney N, Lyman GH, Pettengell R, Tjan-Heijnen VC, Walewski J, Weber DC, Zielinski C. 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours.

Eur J Cancer 2011; **47**: 8-32 [PMID: 21095116 DOI: 10.1016/j.ejca.2010.10.013]

48 **Crawford J**, Armitage J, Balducci L, Becker PS, Blayney DW, Cataland SR, Heaney ML, Hudock S, Kloth DD, Kuter DJ, Lyman GH, McMahon B, Rugo HS, Saad AA, Schwartzberg LS, Shayani S, Steensma DP, Talbott M, Vadhan-Raj S, Westervelt P, Westmoreland M, Dwyer M, Ho M. Myeloid growth factors. *J Natl Compr Canc Netw* 2013; **11**: 1266-1290 [PMID: 24142827]

P- Reviewer: Ashton JJ, Tarnawski AS, Zhou XY **S- Editor**: Yu J
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.48

World J Gastroenterol 2017 January 7; 23(1): 48-59
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Novel CagA ELISA exhibits enhanced sensitivity of *Helicobacter pylori* CagA antibody

Yuichi Matsuo, Yasutoshi Kido, Junko Akada, Seiji Shiota, Tran Thanh Binh, Tran Thi Huyen Trang, Ho DQ Dung, Pham Huu Tung, Tran Dinh Tri, Ngo P Minh Thuan, Le Quang Tam, Bui Chi Nam, Vu Van Khien, Yoshio Yamaoka

Yuichi Matsuo, Yasutoshi Kido, Junko Akada, Seiji Shiota, Tran Thanh Binh, Tran Thi Huyen Trang, Yoshio Yamaoka, Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu, Oita 879-5593, Japan

Seiji Shiota, Department of General Medicine, Oita University Faculty of Medicine, Yufu, Oita 879-5593, Japan

Tran Thanh Binh, Ho DQ Dung, Pham Huu Tung, Tran Dinh Tri, Ngo P Minh Thuan, Department of Endoscopy, Cho Ray Hospital, Hochiminh 800010, Vietnam

Tran Thi Huyen Trang, Department of Molecular Biology, 108 Military Central Hospital, Hanoi 100083, Vietnam

Le Quang Tam, Department of Endoscopy, Daklak Hospital, Daklak 55000, Vietnam

Bui Chi Nam, Department of Endoscopy, Lao Cai Hospital, Lao Cai 19000, Vietnam

Vu Van Khien, Department of Hepatogastroentrology, 108 Military Center Hospital, Hanoi 19000, Vietnam

Yoshio Yamaoka, Department of Medicine-Gastroenterology, Baylor College of Medicine and Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX 77030, United States

Author contributions: Matsuo Y, Kido Y, Akada J, Shiota S and Yamaoka Y conceived and designed the experiments; Matsuo Y, Kido Y, Akada J, Binh TT and Trang TTH performed the experiments; Binh TT, Dung HDQ, Tung PH, Tri TD, Thuan NPM, Tam LQ, Nam BC and Khien VV contributed to obtaining the samples; Matsuo Y, Kido Y, Akada J and Yamaoka Y contributed to analysis and interpretation; Matsuo Y, Kido Y, Akada J and Yamaoka Y drafted the manuscript.

Supported by the National Institutes of Health, No. DK62813; Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (in part), No. 25293104, No. 26640114 and No. 15H02657); the Japan Society for the Promotion of Science Institutional Program for Young Researcher Overseas Visits and the Strategic Funds for the Promotion of Science and Technology from the Japan Science and Technology Agency.

Institutional review board statement: Ethical approval was obtained from the Ethics Committees of Daklak Hospital and Lao Cai Hospital, Vietnam and the Oita University Faculty of Medicine, Japan.

Conflict-of-interest statement: No conflicts of interest exist.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Yamaoka Yoshio, MD, PhD, Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, Yufu, Oita 879-5593, Japan. yyamaoka@oita-u.ac.jp
Telephone: +81-97-5865740
Fax: +81-97-5865749

Received: August 15, 2016
Peer-review started: August 16, 2016
First decision: September 20, 2016
Revised: October 3, 2016
Accepted: October 27, 2016
Article in press: October 27, 2016
Published online: January 7, 2017

Abstract

AIM

To develop a novel *Helicobacter pylori* (*H. pylori*) CagA antibody enzyme-linked immunosorbent assay (ELISA) suitable for detecting serum anti-CagA antibodies with high sensitivity.

METHODS

Recombinant East Asian-type CagA protein was purified and immobilized for ELISA. Serum samples from 217 Vietnamese individuals (110 *H. pylori*-infected and 107 uninfected individuals) were applied. Conventional ELISA from Western-type CagA and our East Asian-type CagA ELISA were evaluated by comparing 38 subjects with the Western-type genotype and 72 subjects with the East Asian-type *cagA* genotype. Histological scores of the gastric mucosa were determined using the updated Sydney System to examine the relationship with anti-CagA antibody titers.

RESULTS

Recombinant 70-100 kDa fragments were immobilized on the ELISA plate. In ROC analysis, the area under the curve of our East Asian-type CagA ELISA was comparable to that of conventional CagA ELISA. The sensitivity of the two ELISAs differed depending on the *cagA* genotype. The sensitivity of East Asian-type CagA ELISA was higher for subjects infected with East Asian-type *cagA H. pylori* ($P < 0.001$), and the sensitivity of the conventional CagA ELISA tended to be higher for subjects infected with Western *cagA H. pylori* ($P = 0.056$). The titer of anti-CagA antibody tended to correlate with monocyte infiltration scores ($r = 0.25$, $P = 0.058$) and was inversely correlated with *H. pylori* density ($r = -0.26$, $P = 0.043$).

CONCLUSION

The novel ELISA is useful to detect anti-CagA antibodies in East Asian countries, and the titer may be a marker for predicting chronic gastritis.

Key words: *Helicobacter pylori*; *cagA* genotype; Anti-CagA antibody; Enzyme-linked immunosorbent assay; Inflammation; Gastritis

Core tip: We developed a novel East Asian-type CagA enzyme-linked immunosorbent assay (ELISA) to determine whether this method could detect CagA seropositivity with greater sensitivity in East Asian countries than the conventional anti-CagA antibody ELISA, which utilizes Western-type CagA as the antigen. Our findings revealed that conventional CagA ELISA underestimated CagA seropositivity in East Asian countries and the novel CagA ELISA could detect anti-CagA antibodies with higher sensitivity. In addition, the anti-CagA antibody titer tended to correlate with chronic inflammation in the stomach. Therefore, the titer of East Asian CagA ELISA may be a useful marker for predicting chronic inflammation in the gastric mucosa.

Matsuo Y, Kido Y, Akada J, Shiota S, Binh TT, Trang TTH, Dung HDQ, Tung PH, Tri TD, Thuan NPM, Tam LQ, Nam BC, Khien VV, Yamaoka Y. Novel CagA ELISA exhibits enhanced sensitivity of *Helicobacter pylori* CagA antibody. *World J Gastroenterol* 2017; 23(1): 48-59 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/48.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.48

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative microaerophilic bacterium, which is etiologically associated with various diseases, such as gastritis, peptic ulcer, mucosa associated lymphoid tissue (MALT) lymphoma, and gastric cancer. Although over half of the world's population is infected with *H. pylori*, the incidence of *H. pylori*-associated diseases varies geographically. These geographic differences in the incidence of gastric cancer can be explained, at least in part, by the presence of different types of *H. pylori* virulence factors, in particular cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), and outer inflammatory protein A (OipA)[1].

CagA, the major virulence factor, is delivered into gastric epithelial cells *via* the type IV secretion system of *H. pylori*[2]. Structural variants of CagA have been shown to alter bacterial virulence. The C-terminus of CagA possesses a variable number of tyrosine phosphorylation sites, which are located within the Glu-Pro-Ile-Tyr-Ala (EPIYA) motif[3]. EPIYA segments can be classified into four types according to the amino acid sequence surrounding the EPIYA motif. *H. pylori* found in Western countries possess Western-type CagA, which contains EPIYA-A, EPIYA-B, and EPIYA-C segments. In contrast, *H. pylori* in East Asian countries possess East Asian-type CagA, which contains EPIYA-A, EPIYA-B, and EPIYA-D segments[4,5]. These EPIYA motifs can exhibit varying numbers and configurations in the C-terminal end of CagA variants[6]. The EPIYA-D segment has been reported to bind more strongly to the proto-oncogenic SH2-domain-containing tyrosine phosphatase (SHP2) than the EPIYA-C segment, leading to hyper-stimulation of Ras-Erk signaling[7,8]. Therefore, the East Asian-type CagA is associated with greater virulence than the Western-type CagA owing to the structural variance of CagA.

CagA is also a highly antigenic protein[9,10]. Comprehensive epidemiological studies have reported on the relationship between CagA seropositivity and clinical outcomes in Western and East Asian countries[11-17]; however, the results are controversial. Huang *et al*[18] used meta-analysis to analyze the relationship between CagA seropositivity and gastric cancer and concluded that infection with *cagA*-positive *H. pylori* further increased the risk of gastric cancer

over that associated with *cagA*-negative *H. pylori* infection. Our previous meta-analysis also showed that CagA seropositivity was significantly associated with gastric cancer in East Asian countries[19]. However, the positive rate of CagA antibodies among *H. pylori*-infected Japanese individuals was relatively low (53.7% to 81.1%) although the majority of *H. pylori* strains in Japan possess an East Asian-type *cagA* gene[20,21]; the prevalence of *cagA* positive *H. pylori* was 95.0% to 95.5% in Vietnam[22,23] and 86.4% to 96.3% in Japan[24,25].

Therefore, we hypothesized that the commercially available CagA antibody enzyme-linked immunosorbent assay (ELISA), which uses Western-type CagA as the antigen, might underestimate serum CagA antibody levels in East Asian countries. In the present study we developed an East Asian-type CagA ELISA, which immobilizes East Asian-type recombinant CagA, and assessed the characteristics of two types of CagA based ELISA systems.

To examine differences in the performance of both types of CagA ELISA, we chose to use serum samples from Vietnamese individuals because *cagA* genotype prevalence is region-dependent in Vietnam. The predominant *cagA* genotype in the central region (Daklak province) is the Western-type *cagA* and in the northern region (Lao Cai province) is the East Asian-type *cagA*. Our results indicate that the accuracy of the two types of CagA ELISA is comparable for these Vietnamese samples. In addition, we examined the relationship between CagA antibody titer and the degree of inflammation in each individual.

MATERIALS AND METHODS

Volunteers for endoscopic survey

The endoscopic survey was conducted in nine rural areas in the Daklak and Lao Cai provinces, Vietnam, from July 2012 and April 2013. We travelled to these areas twice and spent several days each visit recruiting the volunteers. Ethical approval was obtained from the Ethics Committees of Daklak Hospital and Lao Cai Hospital, Vietnam and the Oita University Faculty of Medicine, Japan. Written informed consent was obtained from all participants prior to the study.

Four biopsy specimens (three from the antrum and one from the upper posterior wall of the corpus) were obtained during endoscopy. The antrum specimens were used for the rapid urease test, *H. pylori* culturing, and histological examination. The corpus specimen was used for histological examination. Blood samples were collected from all participants immediately following endoscopy.

Determination of H. pylori status

The rapid urease test, culturing test, histological tests confirmed by immunohistochemistry (IHC), and serum *H. pylori* antibody test were used to maximize the accuracy of the *H. pylori* infection diagnosis.

H. pylori was isolated using a standard culturing method[25]. The *H. pylori* total antibody titer in serum samples was measured by E-plate (Eiken Co. Ltd, Tokyo, Japan). CagA antibody titer in sera was measured using the CagA ELISA kit (Genesis Diagnostics Ltd, Ely, United Kingdom), which represented Western CagA ELISA in this study. Stomach biopsy specimens were also provided for histological testing as previously described[26].

In this study, *H. pylori*-infected status was defined as positive by *H. pylori* culturing. While, *H. pylori*-uninfected status was defined as all negative by *H. pylori* culturing, rapid urease test, serum *H. pylori* antibody, serum CagA antibody, and histopathological examination results.

Classification of cagA genotype

Genomic DNA was extracted from cultured *H. pylori* using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The *cagA* genotypes (Western-type or East Asian-type) were determined by PCR based direct sequencing of the C-terminal region containing the EPIYA segments, as previously described[24]. The *cagA* genotype was determined by evaluating the amino acid sequences of the EPIYA segments using MEGA6 software[27].

Expression of glutathione sulfate-transferase-fused recombinant CagA

The full length *cagA* gene was PCR amplified from the genomic DNA of a clinical *H. pylori* strain isolated from a Japanese volunteer with gastritis (Supplementary material). The 5′ terminus of the amplified fragment contained a *Sma*I recognition site and the 3′ terminus contained an *Xho* I recognition site. The 3.5-kb amplified *cagA* product was cloned into the *Sma*I and *Xho* I digested pGEX-6P-1 vector (GE Healthcare, Little Chalfont, United Kingdom) using T4 DNA ligase (TAKARA Inc) and the resulting plasmid, pGEX-CagA, was propagated in *Escherichia coli* DH5α competent cells (Merck Millipore, Darmstadt, Germany). pGEX-CagA was then transformed into the *E. coli* Rosetta Blue DE3 pLysS expression strain (Merck Millipore). These cells were then grown to an OD_{600} of 0.7 in Luria Bertani (LB) broth supplemented with ampicillin (100 μg/mL), chloramphenicol (40 μg/mL), and 0.2% (w/v) glucose at 37 ℃. Expression of the glutathione sulfate-transferase (GST)-fused recombinant CagA (rCagA) protein was induced by the addition of 0.4 mM (final concentration) of isopropyl β-D-1-thiogalactopyranoside (IPTG) for 2 h at 30 ℃; the cells were then harvested by centrifugation at 8000 × *g*, 4 ℃ for 10 min.

Purification of East Asian-type recombinant CagA

The harvested *E. coli* cells were suspended in a sonication buffer. The *E. coli* lysate was sonicated and then centrifuge to remove the unlysed cells. The clarified supernatant was collected and the

N-terminally tagged GST-rCagA was purified by GST-tag affinity chromatography, which utilizes the binding ability of GST to glutathione sepharose 4B resin (GE Healthcare). The resin was washed and the rCagA was eluted following the use of PreScission protease (GE Healthcare). The rCagA preparation buffer was exchanged to a phosphate buffer (pH 7.4) by ammonium sulfate precipitation. Each fraction was electrophoresed on sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and stained with Colloidal CBB stain (Bio-Rad). rCagA expression was confirmed by western blotting using anti-CagA Rabbit IgG (Austral Líneas Aéreas, Buenos Aires, Argentina) as the primary antibody and anti-Rabbit IgG conjugated alkaline phosphatase (Jackson Immuno Research Labs, West Grove PA, United States) as the secondary antibody.

Production of an East-Asian type CagA antibody ELISA

East Asian-type rCagA (0.1 μg/well) in phosphate buffer was immobilized on Maxi-sorp 96-well plates (Thermo Fisher Scientific, Massachusetts, United States). Human serum samples were reacted with the rCagA immobilized plate. Anti-CagA rabbit IgG (1 mg/mL; Austral Líneas Aéreas) was reacted concurrently to obtain a standard curve for calculating the amount of human IgG. The plate was washed and then anti-human IgG conjugated horse radish peroxidase (anti-human IgG-HRP; Jackson Immuno Research Labs) and anti-rabbit IgG conjugated horse radish peroxidase (anti-rabbit IgG-HRP; Jackson Immuno Research Labs) were added to the plate. After washing the plate, ELISA peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB; NACALAI TESQUE, Kyoto, Japan) was used for coupling and then the absorbance at 450 nm was measured. The amount of CagA antibody was calculated from the standard curve using anti-CagA rabbit IgG/anti rabbit IgG-HRP. The detailed protocol is described in supplementary material.

Chronic gastritis scoring

Biopsy specimens were stained with hematoxylin and eosin. The grade of neutrophil infiltration, mononuclear cell infiltration, atrophy, and intestinal metaplasia were scored in each specimen based on the updated Sydney System (0, none; 1, mild; 2, moderate; and 3, severe)[28].

Statistical analysis

All statistical analyses was performed using EZR software[29]. Receiver Operating Characteristic (ROC) analysis was used to define the cut off value for a novel CagA antibody ELISA. Discrete variables were tested using the χ^2 and McNemar's test. Spearman rank coefficients (r) were determined to evaluate the association between CagA antibody levels and the histological score. P value < 0.05 was considered statistically significant. The statistical analysis of this study were reviewed by Kido Y, Akada J, Yamaoka Y.

Table 1 *cagA* genotype and clinical outcome of subjects

H. pylori infection status and *cagA* genotype	Gastritis	Peptic ulcer	GERD	Total
H. pylori-infected	95	12	3	110
Western *cagA*	34	4	0	38
East Asian *cagA*	61	8	3	72
H. pylori-uninfected	98	3	6	107
Total	193	15	9	217

GERD: Gastroesophageal reflux disease; *H. pylori*: *Helicobacter pylori*.

RESULTS

Purification of East Asian-type rCagA and development of a novel East Asian-type CagA ELISA

To develop an East Asian CagA ELISA, we first cloned the *cagA* gene from a clinical *H. pylori* strain isolated from a Japanese gastritis patient and constructed a CagA expression vector. The EPIYA segments of *cagA* were confirmed as East Asian type *cagA*, ABD segments, by DNA sequencing (Gen Bank accession number LC158593).

The full length East Asian-type rCagA was expressed in *E. coli* as a GST-tag fusion protein (deduced molecular size of 160 kDa). GST-rCagA was expressed to a high level in the soluble fraction of the *E. coli* lysate following induction (Figure 1A, lane 2 *vs* lane 1, uninduced). The GST-rCagA was bound to glutathione beads and rCagA was collected by cleaving the tag during the elution step (Figure 1A, lane 5). Western blotting using an anti-CagA antibody showed that the size of the eluted rCagA was 75-100 kDa (Figure 1B, lane 3); full length rCagA (135 kDa) was estimated to constitute < 1% of the total protein based on CBB stained SDS-PAGE (Figure 1A, lane 5). Hence, the full-length CagA was cleaved into 75-100-kDa fragments. We used this purified rCagA protein for the development of a novel East-Asian CagA ELISA.

Evaluation of the East Asian-type CagA ELISA compared with Western-type CagA ELISA

The subjects in this study included 310 subjects in Vietnam. As described at methods, *H. pylori*-positive status was defined. Our selection criteria to determine infected and uninfected samples presumably reduce pseudo-positive and pseudo-negative facilitating the evaluation of the a novel ELISA method; 110 *H. pylori*-infected subjects and 107 *H. pylori*-uninfected subjects (217 serum samples in total) were used for the initial experiment. Endoscopic diagnoses showed that most volunteers had only gastritis and that some had peptic ulcers and gastroesophageal reflux disease (GERD) (Table 1). PCR-based direct sequencing was used to determine the *cagA* genotype of the 110 *H. pylori*-

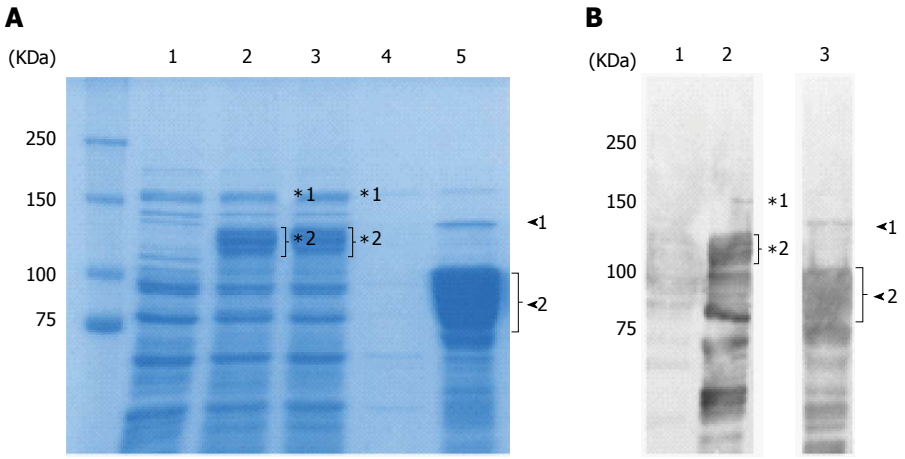


Figure 1 Purification of recombinant East Asian-type CagA. A: CBB-stained 8% SDS-PAGE gel; 20 μg of protein were loaded per well. Lane 1: Uninduced lysate; 2: 0.4 mmol/L IPTG induced lysate; 3: Flow through; 4: Wash; 5: Elution. In the elution fraction (Lane 5), the 135 kDa band (arrowhead 1) is the full length rCagA. The 75-100 kDa rCagA cleavage product (arrowhead 2) comprised approximately 50% of the elution fraction; B: Western blot analysis with anti-CagA antibody. Lane 1: Uninduced lysate; 2: 0.4 mmol/L IPTG induced lysate; 3: Elution. Following induction (Lane 2), the full length GST-fused rCagA (*1) was expressed. However, it was subsequently cleaved (*2). In the elution fraction (Lane 3), the amount of full length rCagA was low (arrowhead 1) and various smaller-sized bands (arrowhead 2) were confirmed as rCagA fragments.

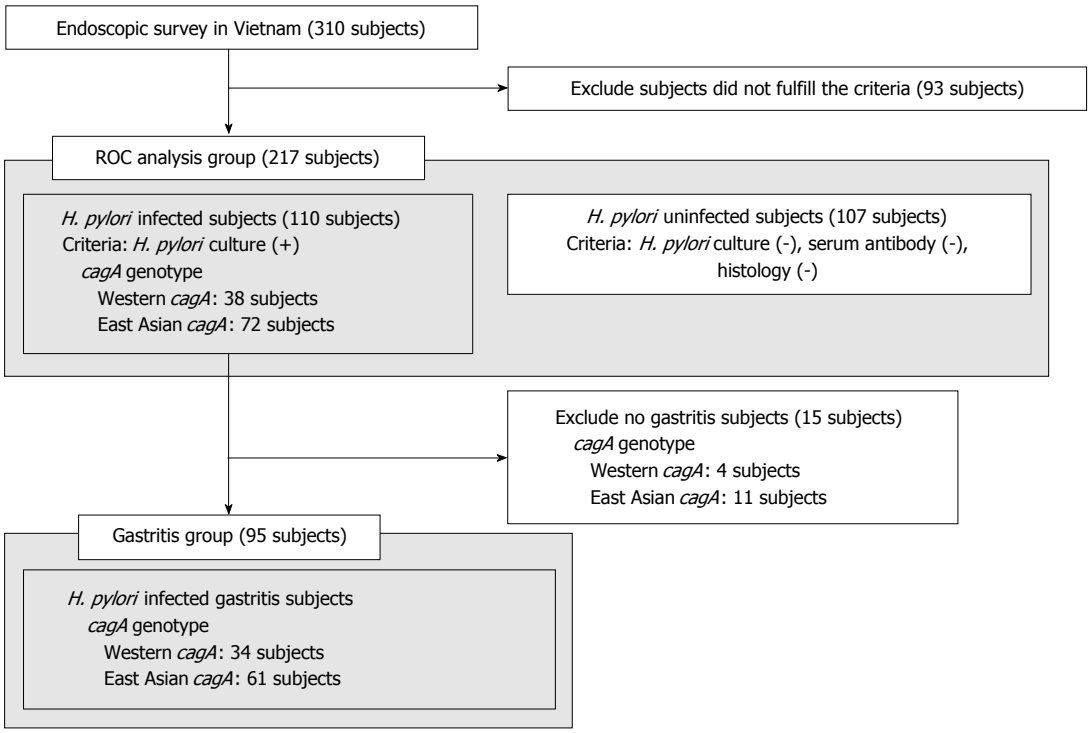


Figure 2 Subjects groups used in this study. ROC analysis was performed to confirm the accuracy of East Asian-type CagA ELISA for subjects in the ROC analysis group. The correlation between CagA antibody titer and histological score was compared between subjects infected with either Western-type or East Asian-type cagA *Helicobacter pylori* (*H. pylori*) in the gastritis group.

infected volunteers; 38 were infected with Western-type *cagA* and 72 with East Asian-type *cagA* (Figure 2, ROC analysis group).

Next, we compared our East Asian-type CagA ELISA with a commercially available CagA ELISA, which utilizes Western-type CagA. The CagA antibody titer was measured for the 110 *H. pylori*-infected and 107 uninfected subjects (Table 2). Western-type CagA ELISA showed that 103 uninfected subject serum samples (96%) had a low titer (0-5 U/mL). The serum titers of infected subjects demonstrated two peaks; the titer of 36 subjects was within the 0-5 U/mL range, while the other 17 showed had titers > 85 U/mL (Figure 3A). The 36 subjects in the 0-5 U/mL range included 33 subjects infected with *H. pylori* with East Asian-type *cagA* and only 3 with Western-type *cagA*. The 17 subjects with a titer > 85 U/mL consisted of 14 subjects infected with *H. pylori* with Western-type

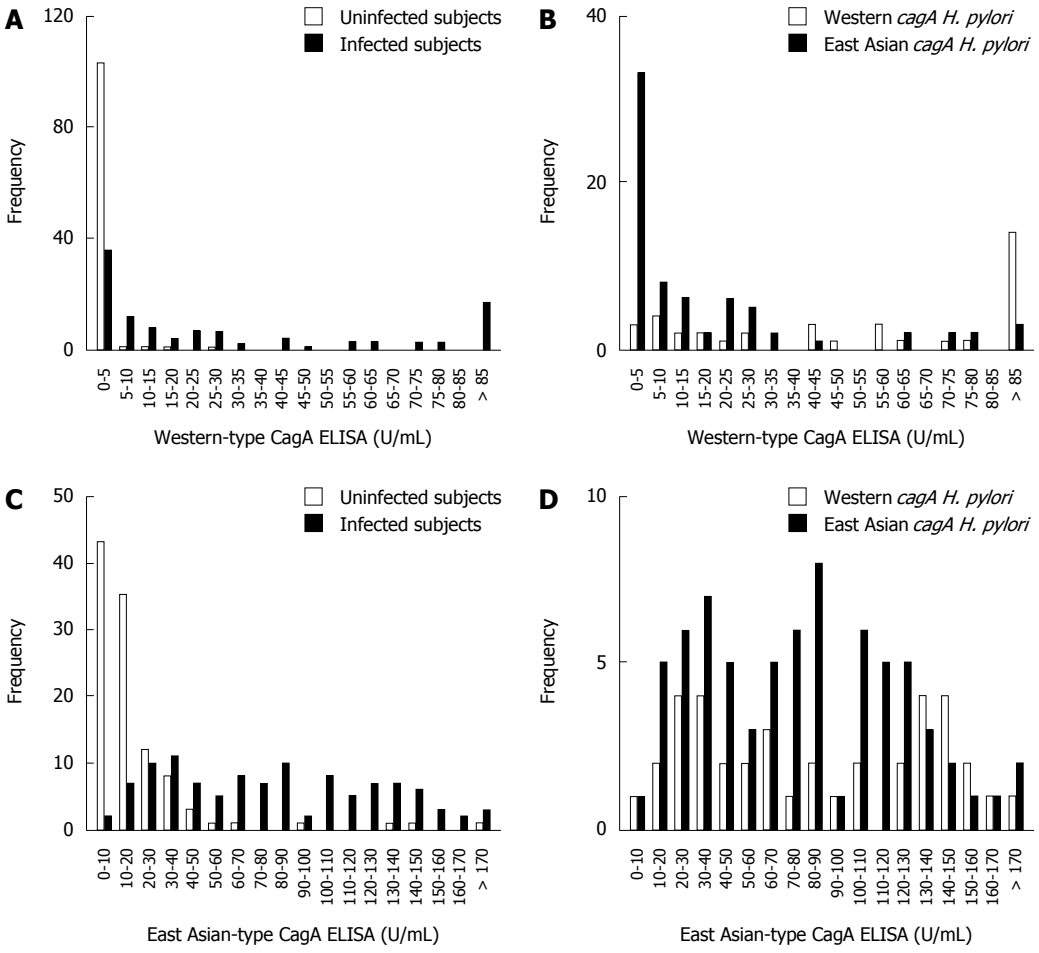


Figure 3 The distribution of CagA antibody ELISA titers is dependent on *Helicobacter pylori* infection status and *Helicobacter pylori* CagA-type. A: Histogram of Western-type CagA ELISA titers of *Helicobacter pylori* (*H. pylori*)-infected (gray bars) and uninfected subjects (white bars); B: Histogram of Western-type CagA ELISA titers of *H. pylori* infected subjects is dependent on *cagA* genotype; Western-type *cagA H. pylori* (white bars), East Asian-type *cagA H. pylori* (gray bars); C: Histogram of East Asian-type CagA ELISA titers of *H. pylori* infection and uninfected subjects. Bar coloring is the same as in (A); D: Histogram of East Asian-type CagA ELISA titers of *H. pylori* infected subjects is dependent on *cagA* genotype. Bar coloring is the same as in (B).

cagA and only three with East-Asian *cagA* (Figure 3B). Additionally, the mean antibody titer against Western-type CagA (78.8 ± 71.6 U/mL) was significantly higher than that against East Asian-type CagA (22.6 ± 40.7 U/mL; $P < 0.001$). These data indicate that Western-type CagA ELISA reacts less efficiently with the CagA antibody present in individuals infected with *H. pylori* possessing East Asian-type CagA.

In contrast, the East Asian-type CagA ELISA revealed that 101 (94%) uninfected volunteers had titers < 50 U/mL. The titers of the infected subjects ranged from 30-40 U/mL (Figure 3C). The mean ± SD CagA antibody titer was 85.9 ± 52.0 U/mL in subjects infected with Western-type *cagA H. pylori* and 79.1 ± 47.6 U/mL in subjects infected with East Asian-type *cagA H. pylori*; the differences were not statistically significant ($P = 0.50$; Figure 3D). These data show that the East Asian-type CagA ELISA reacted equally with CagA antibodies derived from subjects infected *H. pylori* with both types of CagA.

ROC analysis was used to evaluate the accuracy of East Asian-type CagA ELISA for all 217 subjects. The area under the curve (AUC) of the 110 *H. pylori* infected and 107 uninfected samples was nearly equal for both types of CagA ELISA: 0.91 for East Asian-type CagA ELISA and 0.87 for Western-type CagA ELISA (Table 3). These results indicate that the accuracy of East Asian-type CagA ELISA is comparable to that of Western-type CagA ELISA. Additionally, the positive cut off value of the East Asian-type CagA ELISA was determined as > 45 U/mL based on the ROC curve (Figure 4).

Characteristic differences between Western-type CagA and East Asian-type CagA ELISA

The CagA antibody titers of all subjects were independently measured using the two types of CagA ELISA and plotted separately as the following four groups: *H. pylori*-uninfected group, *H. pylori*-infected group, subjects infected with Western-type *cagA H. pylori* group, and subjects infected with East Asian-type *cagA H. pylori* group. The cut off value for the East Asian-type CagA ELISA was determined to be 45 U/mL based on the ROC curve and that of Western-

Table 2 Subject *Helicobacter pylori*-infection status

H. pylori status and *cagA* genotype	*H. pylori* infection status		*cagA* genotype	
	Infected	Uninfected	Western	East Asian
ROC analysis group				
n	110	107	38	72
Male	63 (57%)[1]	46 (43%)[1]	17 (45%)	46 (64%)
Age (yr)				
mean ± SD	40.1 ± 12.9	38.5 ± 12.8	38.6 ± 10.8	41.0 ± 13.8
Range	18-76	19-78	18-69	21-76
Gastritis subjects group				
n			34	61
Male			19 (56%)	38 (62%)
Age (yr)				
mean ± SD			37.5 ± 8.5	39.3 ± 13.0
Range			22-60	20-70

[1]Statistically significant between *Helicobacter pylori* (*H. pylori*) infected and uninfected status ($P < 0.05$).

Table 3 Association between *cagA* genotype and the accuracy of CagA antibody ELISA

Group name *cagA* genotype	*n*	Western-type CagA ELISA (cut off value: 6.25 U/mL)				East Asian-type CagA ELISA (cut off value: 45.0 U/mL)			
		Sens.	Sp.	AUC	95%CI	Sens.	Sp.	AUC	95%CI
Western and East Asian	110	62.7	97.2	0.87	0.82-0.92	70.9	93.5	0.91	0.86-0.95
Western	38	84.2	97.2	0.92	0.83-0.99	65.8	93.5	0.91	0.86-0.96
East Asian	72	51.4[1]	97.2	0.85	0.79-0.91	73.6[1]	93.5	0.90	0.85-0.95

[1]Statistically significant between Western-type CagA ELISA and East Asian-type CagA ELISA ($P < 0.001$). *n*: The number of *Helicobacter pylori* (*H. pylori*) infected subjects; 107 *H. pylori* uninfected subjects were added for ROC analysis. Sens.: Sensitivity; Sp.: Specificity; AUC: Area under the curve.

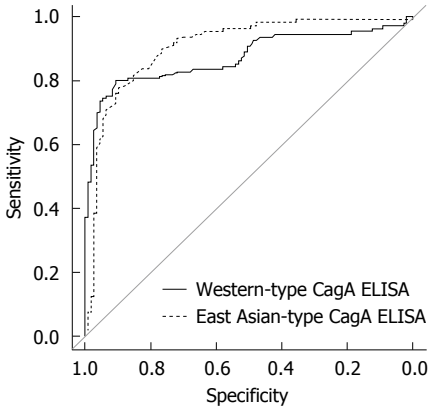


Figure 4 ROC curve analysis of Western-type and East Asian-type CagA ELISA. The ROC curves of both types of CagA ELISA were analyzed using all 217 serum samples of the ROC analysis subjects group (Figure 2). The area under the curve (AUC) of the commercial Western-type CagA antibody ELISA was 0.87 and that of East Asian CagA antibody ELISA was 0.91. No significant difference was apparent.

type CagA ELISA was used as 6.25 U/mL according to the manufacturer's instruction.

Of the 107 *H. pylori*-uninfected subjects, 104 subjects (97.2%) were negative by Western-type CagA ELISA and 100 were negative (93.5%) by East Asian-type CagA ELISA (Figure 5A). This indicates that the specificity of East Asian-type CagA ELISA is sufficiently high and similar to that of Western-type CagA ELISA. Of the 110 *H. pylori* infected subjects, 69 (62.7%) were identified as positive by Western-type CagA ELISA and 78 (70.9%) by East Asian-type CagA ELISA (Figure 5B). The accuracy of each of the ELISA results was further examined using the *cagA* genotype sub-group; of the 38 subjects infected with Western-type *cagA H. pylori*, 32 (84.2%) were identified as positive by Western-type CagA ELISA and 25 (65.8%) by East Asian-type CagA ELISA. In these 32 subjects, nine subjects were identified as positive by Western-type CagA ELISA, but negative by East Asian-type CagA ELISA (Figure 5C). Of the 72 subjects infected with East Asian-type *cagA H. pylori*, 53 (73.6%) were found to be positive by the East Asian-type CagA ELISA, and 37 (51.4%) were found to be positive by the Western-type CagA ELISA. Of these 53 subjects, 19 were identified as positive by the East Asian-type CagA ELISA, but negative by the Western-type CagA ELISA (Figure 5D).

Table 3 summarizes the sensitivity and specificity of both types of ELISA. The sensitivity of Western-type CagA ELISA tended to be higher than that of East Asian-type CagA ELISA for the sub-group of subjects infected with Western *cagA H. pylori* ($P = 0.065$; Table 3). Similarly, the sensitivity of East Asian-type CagA ELISA was higher than that of Western-type CagA ELISA for the sub-group of subjects infected with East Asian-type *cagA H. pylori* ($P < 0.001$; Table 3).

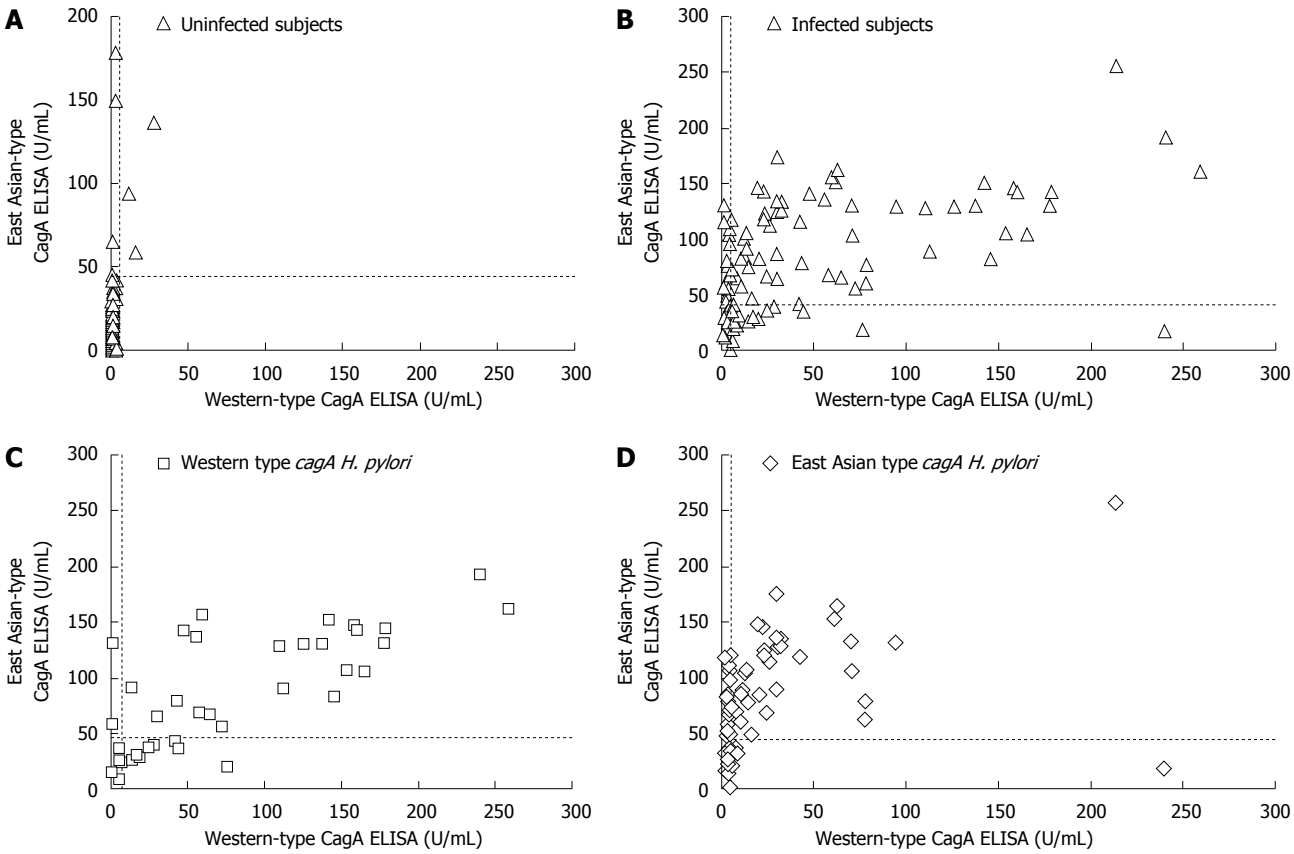


Figure 5 Relationship between the two types of CagA antibody ELISA titers. Dotted lines indicate the cut off values (6.25 U/mL for Western-type CagA ELISA and 45.0 U/mL for East Asian-type CagA ELISA). A: Scatter plot of uninfected subjects. Of the 107 *Helicobacter pylori* (*H. pylori*) uninfected subjects, 104 subjects (97.2%) were negative by Western-type CagA ELISA and 100 subjects (93.5%) by East Asian-type CagA ELISA; B: Scatter plots of infected subjects. Of the 110 *H. pylori* infected subjects, 69 (62.7%) subjects were positive by Western-type CagA ELISA and 78 (70.9%) by East Asian-type CagA ELISA; C: Scatter plot of Western-type cagA *H. pylori* infected subjects. Of the 38 subjects infected with western-type cagA *H. pylori*, 32 subjects (84.2%) were positive by Western-type CagA ELISA and 25 subjects (65.8%) were positive by East Asian-type CagA ELISA; D: Scatter plot of East Asian-type cagA *H. pylori* infected subjects. Of the 72 subjects infected with East Asian-type cagA *H. pylori*, 53 subjects (73.6%) were positive by East Asian-type CagA ELISA and 37 subjects (51.4%) were positive by Western-type CagA ELISA.

Relationship between CagA antibody titer and histological gastritis scores

The relationship between lower CagA seropositivity and clinical outcomes in East Asian countries has not yet been clarified, even when almost all of the examined individuals have been infected with *cagA*-positive *H. pylori*. The novel East Asian-type CagA ELISA presented in this study had higher sensitivity for East Asian-type *cagA H. pylori*-infected subjects than Western-type CagA ELISA. Thus, East Asian-type CagA ELISA was used to investigate the relationship between the CagA antibody titer and histological scores of gastritis in subjects infected with either East Asian-type or Western-type *cagA H. pylori.*

Ulcer and GERD subjects were excluded from the *H. pylori* infected subjects of ROC analysis group and all gastritis subjects were included in the gastritis group (Figure 2). A total of 95 volunteers, including 34 subjects infected with Western-type *cagA H. pylori* and 61 infected with East Asian-type *cagA H. pylori,* were used for this analysis (Table 2). The correlation between CagA antibody titer and histological score was examined using the Spearman rank coefficients test. Histological scores were evaluated using the updated Sydney System for the classification and grading of gastritis. The East-Asian CagA antibody titers of gastritis subjects infected with East Asian-type *cagA H. pylori* (measured by East Asian-type CagA ELISA) tended to correlate with the monocyte infiltration at the stomach antrum scores (r = 0.25, P = 0.058; Figure 6A). The monocyte score included monocytes as well as lymphocytes and plasma cells. Furthermore, the East-Asian CagA antibody titers were inversely correlated with *H. pylori* density in the antrum (r = -0.26, P = 0.043; Figure 6B). In contrast, no correlation was apparent for Western-CagA antibody titers (measured by Western-type CagA ELISA) among gastritis subjects infected with Western-type *cagA H. pylori* (Figure 6C and D).

DISCUSSION

In this study, we developed a novel East-Asian CagA antibody ELISA and compared it with commercial Western-type CagA ELISA using subjects infected with either East Asian-type or Western-type *cagA H.*

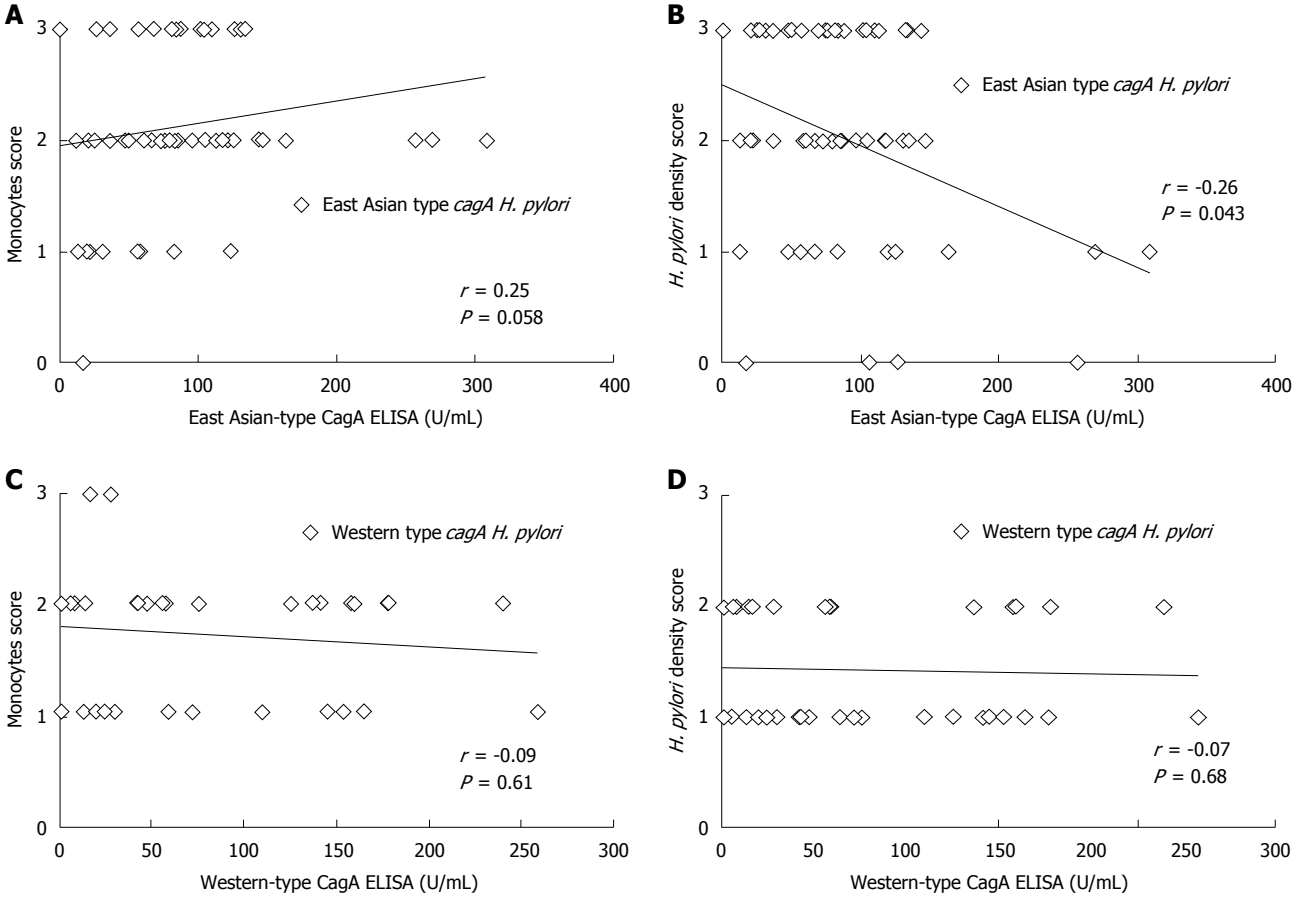


Figure 6 Correlation between CagA antibody ELISA titer and histological score. A: Relationship between East Asian-type CagA ELISA titer and histological monocytes score. Among East Asian-type *cagA* possessed *Helicobacter pylori* (*H. pylori*) infected subjects diagnosed as gastritis (Gastritis group in Figure 1), East Asian-type CagA antibody titers tended to positive correlate with the infiltration of monocytes at antrum in stomach (r = 0.25, P = 0.058); B: Relationship between East Asian-type CagA ELISA titer and histological *H. pylori* density score. East Asian-type CagA antibody titers were negative correlated with the *H. pylori* density at an antrum in stomach (r = -0.26, P = 0.043); C: Relationship between Western-type CagA ELISA titer and histological monocytes score. There is no correlation (P = 0.61); D: No correlation was apparent for Western-type CagA ELISA titer and histological *H. pylori* density (P = 0.68).

pylori. The sensitivity of the East Asian-type CagA ELISA was higher than that of the Western-type CagA ELISA for the subgroup of individuals infected with East Asian-type *cagA H. pylori*, while the sensitivity of the Western-type CagA ELISA tended to be higher than that of the East Asian-type CagA ELISA for the subgroup of individuals infected with Western-type *cagA H. pylori*. These results indicate that Western-type CagA ELISA might not be suitable for application in East Asian countries and that East Asian-type CagA ELISA could be useful for detecting the East Asian-type CagA antibody with high sensitivity.

During the development of the East Asian-type CagA ELISA, we noted that full length rCagA seemed to be unstable and was cleaved into 75-100 kDa fragments. This instability of CagA has been previously demonstrated in both *in vivo* and *in vitro* studies[30-32]. CagA was cleaved into 100 kDa and 35 kDa fragments *in vivo*, with the N-terminus of CagA present in 100 kDa fragment. Although the C-terminus of CagA was cleaved during protein purification, our novel East Asian-type CagA ELISA still exhibited greater sensitivity for East Asian-type CagA. This suggests that the 75-100 kDa East Asian-type CagA fragment still contains East Asian-type specific epitopes.

The CagA antibody epitope seems to depend on *cagA* genotype. Klimovich *et al*[33] reported that the majority of *H. pylori*-positive serum samples reacted with rCagAfr.2 antigen, which was located in the middle section of the Western-type CagA fragment. Furthermore, the middle section of East Asian-type CagA has been found to be highly antigenic in serum samples from *H. pylori*-positive Japanese children[34]. Of the subjects infected with East Asian-type *cagA H. pylori* in the present study, 19 were identified as positive by the East Asian-type CagA ELISA, but negative by the Western-type CagA ELISA. In addition, the anti-CagA antibody titer of the CagA ELISA was independent of CagA EPIYA variant motifs, although the number of minor variant EPIYA motifs was very small. These results suggest that high-antigenicity regions specific to East Asian CagA are present in regions without EPIYA motifs. Moreover, the CagA antibody epitope may depend on the *cagA* genotype. Therefore, the novel East Asian-type CagA ELISA should be used in order to increase the sensitivity of

CagA seropositivity detection in East Asian countries.

East Asian-type CagA antibody titer positively correlated with monocyte infiltration and negatively with *H. pylori* density in the antrum. These results are consistent with the fact that the atrophic mucosa cannot be colonized easily by *H. pylori* and that anti-*H. pylori* antibody titer decreases in a time-dependent manner[35-38]. Furthermore, the half-life of the CagA antibody is greater than that of the anti-*H. pylori* antibody[39-41]. These findings are consistent with the negative correlation between CagA antibody titer and *H. pylori* density in the stomach, which reflects the progression of atrophy at the stomach mucosa. Previous studies have demonstrated the increased antigenicity of East Asian-type CagA. Miura *et al*[42] showed that transgenic mice expressing East Asian-type CagA developed tumors more frequently than those expressing Western-type CagA; and Satomi *et al*[43] reported that patients infected with *H. pylori* carrying the East Asian-type CagA were associated with severe gastric atrophy and gastric cancer in Japan. Therefore, the correlation between anti-CagA antibody titer and histological score in this study may be a reflection of severe inflammation derived from the virulence of CagA.

In conclusion, the novel East Asian-type CagA ELISA developed in this study should be used concomitantly with Western-type CagA ELISA in order to increase the sensitivity of CagA seropositivity detection in East Asian countries. Moreover, subjects with higher CagA antibody titers could be classified into higher risk population to cause gastric cancer. The findings gained of this study may help us to further understand the potential marker of predicting *H. pylori* pathogenicity. Recently, it was reported that human genetic factor determines the antigen epitope and reduced the risk of severe gastric disease[44]. To fully appreciate the immunity to CagA and its interaction with the human genetic factor will need to reveal the relationship CagA antibody and clinical outcomes. In addition, further epidemiological studies are required to confirm the accuracy of East-Asian CagA ELISA and to determine a more reliable cut off value for the global use of this method.

ACKNOWLEDGMENTS

We wish to thank Ms. Miyuki Matsuda, Ms. Ayaka Takahashi, and Ms. Yoko Kudo for their excellent technical assistance.

COMMENTS

Background

The *cagA* genotype is known to be responsible for the pathogenicity of *Helicobacter pylori* (*H. pylori*) as well as the geographic differences associated with the incidence of gastric cancer. The *cagA* genotype was classified according to the amino acid sequence of the surrounding EPIYA motifs. Most *H. pylori* strains isolated in East Asian countries possess the *cagA* gene; the predominant *cagA* genotype is the East Asian-type, which causes more severe inflammation than the Western-type. However, the relationship between CagA seropositivity and clinical outcomes is controversial.

Research frontiers

Many researchers have reported the relationships between CagA antibody titers and clinical outcomes using conventional CagA ELISA, which was immobilized Western-type CagA. However, the positive rate of CagA antibodies among *H. pylori*-infected Japanese individuals was relatively low, although the majority of *H. pylori* strains in Japan possess an East Asian-type *cagA* gene. Therefore, conventional CagA ELISA may underestimate the seropositivity of anti-CagA antibody detection. Further studies are needed to support this hypothesis.

Innovations and breakthroughs

In present study, the authors developed a novel East Asian-type CagA ELISA to detect anti-CagA antibodies with higher sensitivity in East Asian countries and then demonstrated that conventional CagA ELISA underestimated the seropositivity of anti-CagA antibody detection for subjects infected with East Asian *cagA H. pylori*. CagA has sequence variations in different populations around the world, and CagA ELISAs specific for a geographic location may improve diagnoses.

Applications

The novel East Asian-type CagA ELISA developed in this study could be used concomitantly with the Western-type CagA ELISA in order to increase the sensitivity of CagA seropositivity detection in East Asian countries. This may be useful in South Asian countries in which *H. pylori* harbor East Asian-type or Western-type CagA. Moreover, the titer of East Asian CagA ELISA was correlated with the activity of chronic inflammation in the gastric mucosa. Thus, individuals with higher anti-CagA antibody titers could be classified as having a higher risk of gastric cancer.

Terminology

Receiver operating characteristic (ROC) curve is used to evaluate the discrimination ability of the diagnostic tool for the target disease. The accuracy of the tool is evaluated from the area under the curve, and the cut-off value is determined based on the ROC curve. The sensitivity and specificity of the tool depend on the cut-off value.

Peer-review

These researchers determined the presence and the levels of anti-CagA antibodies in two groups of patients, one infected by *H. pylori* strains with East Asian-type CagA, the other one with Western type CagA. The gold standard for the *H. pylori* infectious status was endoscopy with rapid urease test, biopsy culture, histological tests confirmed by immunohistochemistry and detection of serum antibodies to whole *H. pylori* antigens. They observed that ELISA using East Asian-type CagA had greater sensitivity with patients infected by strains expressing East Asian CagA; in addition, the levels of anti-CagA antibodies in these patients tended to correlate with histologic chronic inflammation score. This study is important, since it partly solve a main problem of *H. pylori* and CagA serology, sensitivity. The manuscript is written in good English and is very clear.

REFERENCES

1 **Posselt G**, Backert S, Wessler S. The functional interplay of Helicobacter pylori factors with gastric epithelial cells induces a multi-step process in pathogenesis. *Cell Commun Signal* 2013; **11**: 77 [PMID: 24099599 DOI: 10.1186/1478-811X-11-77]

2 **Fischer W**. Assembly and molecular mode of action of the Helicobacter pylori Cag type IV secretion apparatus. *FEBS J* 2011; **278**: 1203-1212 [PMID: 21352490 DOI: 10.1111/j.1742-4658.2011.08036.x]

3 **Mueller D**, Tegtmeyer N, Brandt S, Yamaoka Y, De Poire E, Sgouras D, Wessler S, Torres J, Smolka A, Backert S. c-Src and c-Abl kinases control hierarchic phosphorylation and function of the CagA effector protein in Western and East Asian Helicobacter

pylori strains. *J Clin Invest* 2012; **122**: 1553-1566 [PMID: 22378042 DOI: 10.1172/JCI61143]
4 **Hatakeyama M**. Oncogenic mechanisms of the Helicobacter pylori CagA protein. *Nat Rev Cancer* 2004; **4**: 688-694 [PMID: 15343275 DOI: 10.1038/nrc1433]
5 **Hatakeyama M**. Anthropological and clinical implications for the structural diversity of the Helicobacter pylori CagA oncoprotein. *Cancer Sci* 2011; **102**: 36-43 [PMID: 20942897 DOI: 10.1111/j.1349-7006.2010.01743.x]
6 **Backert S**, Tegtmeyer N, Selbach M. The versatility of Helicobacter pylori CagA effector protein functions: The master key hypothesis. *Helicobacter* 2010; **15**: 163-176 [PMID: 20557357 DOI: 10.1111/j.1523-5378.2010.00759.x]
7 **Chan RJ**, Feng GS. PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. *Blood* 2007; **109**: 862-867 [PMID: 17053061 DOI: 10.1182/blood-2006-07-028829]
8 **Matozaki T**, Murata Y, Saito Y, Okazawa H, Ohnishi H. Protein tyrosine phosphatase SHP-2: a proto-oncogene product that promotes Ras activation. *Cancer Sci* 2009; **100**: 1786-1793 [PMID: 19622105 DOI: 10.1111/j.1349-7006.2009.01257.x]
9 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795 [PMID: 8516329]
10 **Tummuru MK**, Cover TL, Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of Helicobacter pylori: evidence of linkage to cytotoxin production. *Infect Immun* 1993; **61**: 1799-1809 [PMID: 8478069]
11 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115 [PMID: 7743510]
12 **Kuipers EJ**, Pérez-Pérez GI, Meuwissen SG, Blaser MJ. Helicobacter pylori and atrophic gastritis: importance of the cagA status. *J Natl Cancer Inst* 1995; **87**: 1777-1780 [PMID: 7473834]
13 **Nomura AM**, Lee J, Stemmermann GN, Nomura RY, Perez-Perez GI, Blaser MJ. Helicobacter pylori CagA seropositivity and gastric carcinoma risk in a Japanese American population. *J Infect Dis* 2002; **186**: 1138-1144 [PMID: 12355365 DOI: 10.1086/343808]
14 **Parsonnet J**, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. *Gut* 1997; **40**: 297-301 [PMID: 9135515]
15 **Zhao Z**, Li Y, Liu S, Fu W. Serum Helicobacter pylori CagA antibody may not be used as a tumor marker for diagnosing gastric cancer in east Asian countries. *Tumour Biol* 2014; **35**: 12217-12224 [PMID: 25168369 DOI: 10.1007/s13277-014-2530-8]
16 **Yahav J**, Fradkin A, Weisselberg B, Diver-Haver A, Shmuely H, Jonas A. Relevance of CagA positivity to clinical course of Helicobacter pylori infection in children. *J Clin Microbiol* 2000; **38**: 3534-3537 [PMID: 11015359]
17 **Kato S**, Sugiyama T, Kudo M, Ohnuma K, Ozawa K, Iinuma K, Asaka M, Blaser MJ. CagA antibodies in Japanese children with nodular gastritis or peptic ulcer disease. *J Clin Microbiol* 2000; **38**: 68-70 [PMID: 10618065]
18 **Huang JQ**, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003; **125**: 1636-1644 [PMID: 14724815]
19 **Shiota S**, Matsunari O, Watada M, Yamaoka Y. Serum Helicobacter pylori CagA antibody as a biomarker for gastric cancer in east-Asian countries. *Future Microbiol* 2010; **5**: 1885-1893 [PMID: 21155667 DOI: 10.2217/fmb.10.135]
20 **Shimoyama T**, Fukuda S, Tanaka M, Mikami T, Munakata A, Crabtree JE. CagA seropositivity associated with development of gastric cancer in a Japanese population. *J Clin Pathol* 1998; **51**: 225-228 [PMID: 9659265]
21 **Yamaoka Y**, Kodama T, Kashima K, Graham DY. Antibody against Helicobacter pylori CagA and VacA and the risk for gastric cancer. *J Clin Pathol* 1999; **52**: 215-218 [PMID: 10450182]
22 **Nguyen TL**, Uchida T, Tsukamoto Y, Trinh DT, Ta L, Mai BH, Le SH, Thai KD, Ho DD, Hoang HH, Matsuhisa T, Okimoto T, Kodama M, Murakami K, Fujioka T, Yamaoka Y, Moriyama M. Helicobacter pylori infection and gastroduodenal diseases in Vietnam: a cross-sectional, hospital-based study. *BMC Gastroenterol* 2010; **10**: 114 [PMID: 20920280 DOI: 10.1186/1471-230X-10-114]
23 **Truong BX**, Mai VT, Tanaka H, Ly le T, Thong TM, Hai HH, Van Long D, Furumatsu K, Yoshida M, Kutsumi H, Azuma T. Diverse characteristics of the CagA gene of Helicobacter pylori strains collected from patients from southern vietnam with gastric cancer and peptic ulcer. *J Clin Microbiol* 2009; **47**: 4021-4028 [PMID: 19846630 DOI: 10.1128/JCM.00504-09]
24 **Matsunari O**, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, Fujioka T, Kinjo F, Yamaoka Y. Association between Helicobacter pylori virulence factors and gastroduodenal diseases in Okinawa, Japan. *J Clin Microbiol* 2012; **50**: 876-883 [PMID: 22189111 DOI: 10.1128/JCM.05562-11]
25 **Yamaoka Y**, Kodama T, Kita M, Imanishi J, Kashima K, Graham DY. Relationship of vacA genotypes of Helicobacter pylori to cagA status, cytotoxin production, and clinical outcome. *Helicobacter* 1998; **3**: 241-253 [PMID: 9844065]
26 **Uchida T**, Miftahussurur M, Pittayanon R, Vilaichone RK, Wisedopas N, Ratanachu-Ek T, Kishida T, Moriyama M, Yamaoka Y, Mahachai V. Helicobacter pylori Infection in Thailand: A Nationwide Study of the CagA Phenotype. *PLoS One* 2015; **10**: e0136775 [PMID: 26355839 DOI: 10.1371/journal.pone.0136775]
27 **Tamura K**, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 2013; **30**: 2725-2729 [PMID: 24132122 DOI: 10.1093/molbev/mst197]
28 **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 [PMID: 8827022]
29 **Kanda Y**. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* 2013; **48**: 452-458 [PMID: 23208313 DOI: 10.1038/bmt.2012.244]
30 **Moese S**, Selbach M, Zimny-Arndt U, Jungblut PR, Meyer TF, Backert S. Identification of a tyrosine-phosphorylated 35 kDa carboxy-terminal fragment (p35CagA) of the Helicobacter pylori CagA protein in phagocytic cells: processing or breakage? *Proteomics* 2001; **1**: 618-629 [PMID: 11681214 DOI: 10.1002/1615-9861(200104)1:4<618::AID-PROT618>3.0.CO;2-C]
31 **Jiménez-Soto LF**, Kutter S, Sewald X, Ertl C, Weiss E, Kapp U, Rohde M, Pirch T, Jung K, Retta SF, Terradot L, Fischer W, Haas R. Helicobacter pylori type IV secretion apparatus exploits beta1 integrin in a novel RGD-independent manner. *PLoS Pathog* 2009; **5**: e1000684 [PMID: 19997503 DOI: 10.1371/journal.ppat.1000684]
32 **Backert S**, Moese S, Selbach M, Brinkmann V, Meyer TF. Phosphorylation of tyrosine 972 of the Helicobacter pylori CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. *Mol Microbiol* 2001; **42**: 631-644 [PMID: 11722731]
33 **Klimovich AV**, Samoylovich MP, Gryazeva IV, Terekhina LA, Suvorov AN, Klimovich VB. Development of immunoreagents for diagnostics of CagA-positive Helicobacter pylori infections. *Helicobacter* 2010; **15**: 193-200 [PMID: 20557360 DOI: 10.1111/j.1523-5378.2010.00754.x]
34 **Akada J**, Okuda M, Hiramoto N, Kitagawa T, Zhang X, Kamei S, Ito A, Nakamura M, Uchida T, Hiwatani T, Fukuda Y, Nakazawa T, Kuramitsu Y, Nakamura K. Proteomic characterization of Helicobacter pylori CagA antigen recognized by child serum antibodies and its epitope mapping by peptide array. *PLoS One* 2014; **9**: e104611 [PMID: 25141238 DOI: 10.1371/journal.pone.0104611]
35 **Dinis-Ribeiro M**, Areia M, de Vries AC, Marcos-Pinto R,

Monteiro-Soares M, O'Connor A, Pereira C, Pimentel-Nunes P, Correia R, Ensari A, Dumonceau JM, Machado JC, Macedo G, Malfertheiner P, Matysiak-Budnik T, Megraud F, Miki K, O' Morain C, Peek RM, Ponchon T, Ristimaki A, Rembacken B, Carneiro F, Kuipers EJ. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy* 2012; **44**: 74-94 [PMID: 22198778 DOI: 10.1055/s-0031-1291491]

36 **Maeda S**, Yoshida H, Ogura K, Yamaji Y, Ikenoue T, Mitsushima T, Tagawa H, Kawaguchi R, Mori K, Mafune Ki, Kawabe T, Shiratori Y, Omata M. Assessment of gastric carcinoma risk associated with Helicobacter pylori may vary depending on the antigen used: CagA specific enzyme-linked immunoadsorbent assay (ELISA) versus commercially available H. pylori ELISAs. *Cancer* 2000; **88**: 1530-1535 [PMID: 10738209]

37 **Kreuning J**, Lindeman J, Biemond I, Lamers CB. Relation between IgG and IgA antibody titres against Helicobacter pylori in serum and severity of gastritis in asymptomatic subjects. *J Clin Pathol* 1994; **47**: 227-231 [PMID: 8163693]

38 **Zagari RM**, Pozzato P, Martuzzi C, Fuccio L, Martinelli G, Roda E, Bazzoli F. 13C-urea breath test to assess Helicobacter pylori bacterial load. *Helicobacter* 2005; **10**: 615-619 [PMID: 16302988 DOI: 10.1111/j.1523-5378.2005.00358.x]

39 **Yanaoka K**, Oka M, Mukoubayashi C, Yoshimura N, Enomoto S, Iguchi M, Magari H, Utsunomiya H, Tamai H, Arii K, Ohata H, Fujishiro M, Takeshita T, Mohara O, Ichinose M. Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 838-845 [PMID: 18398025 DOI: 10.1158/1055-9965.EPI-07-2762]

40 **Kokkola A**, Kosunen TU, Puolakkainen P, Sipponen P, Harkonen M, Laxen F, Virtamo J, Haapiainen R, Rautelin H. Spontaneous disappearance of Helicobacter pylori antibodies in patients with advanced atrophic corpus gastritis. *APMIS* 2003; **111**: 619-624 [PMID: 12969017]

41 **Karnes WE**, Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SW, Walsh JH. Positive serum antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. *Gastroenterology* 1991; **101**: 167-174 [PMID: 2044906]

42 **Miura M**, Ohnishi N, Tanaka S, Yanagiya K, Hatakeyama M. Differential oncogenic potential of geographically distinct Helicobacter pylori CagA isoforms in mice. *Int J Cancer* 2009; **125**: 2497-2504 [PMID: 19588494 DOI: 10.1002/ijc.24740]

43 **Satomi S**, Yamakawa A, Matsunaga S, Masaki R, Inagaki T, Okuda T, Suto H, Ito Y, Yamazaki Y, Kuriyama M, Keida Y, Kutsumi H, Azuma T. Relationship between the diversity of the cagA gene of Helicobacter pylori and gastric cancer in Okinawa, Japan. *J Gastroenterol* 2006; **41**: 668-673 [PMID: 16933004 DOI: 10.1007/s00535-006-1838-6]

44 **Chen L**, Li B, Yang WC, He JL, Li NY, Hu J, He YF, Yu S, Zhao Z, Luo P, Zhang JY, Li HB, Zeng M, Lu DS, Li BS, Guo H, Yang SM, Guo G, Mao XH, Chen W, Wu C, Zou QM. A dominant CD4(+) T-cell response to Helicobacter pylori reduces risk for gastric disease in humans. *Gastroenterology* 2013; **144**: 591-600 [PMID: 23232294 DOI: 10.1053/j.gastro.2012.12.002]

P- Reviewer: Ananthakrishnan N, Figura N, Garcia-Olmo D
S- Editor: Yu J **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.60

World J Gastroenterol 2017 January 7; 23(1): 60-75
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier

Da Zhou, Qin Pan, Feng-Zhi Xin, Rui-Nan Zhang, Chong-Xin He, Guang-Yu Chen, Chang Liu, Yuan-Wen Chen, Jian-Gao Fan

Da Zhou, Qin Pan, Feng-Zhi Xin, Rui-Nan Zhang, Chong-Xin He, Yuan-Wen Chen, Jian-Gao Fan, Department of Gastroenterology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China

Guang-Yu Chen, Clinical Epidemiology Center, Shanghai Jiaotong University School of Medicine, Shanghai 200092, China

Chang Liu, Department of Medical Microbiology and Parasitology, Institutes of Medical Sciences, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Author contributions: Zhou D and Pan Q are performed the majority of experiments; Zhou D and Chen GY analyzed the data; Xin FZ, Zhang RN, He CX and Liu C are participated in treatment of animals; Chen YW and Fan JG designed and coordinated the research; Zhou D and Fan JG wrote the paper.

Supported by the State Key Development Program for Basic Research of China, No. 2012CB517501; National Natural Science Foundation of China, No. 81070322, No. 81270491, No. 81470840 and No. 31400001; and 100 Talents Program, No. XBR2011007h.

Institutional review board statement: The study was reviewed and approved by the Xinhua Hospital Ethics Committee Affiliated to Shanghai Jiaotong University School of Medicine.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Xinhua hospital affiliated to Shanghai Jiao Tong University School of Medicine.

Conflict-of-interest statement: The authors declare no competing financial interests.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at fattyliver2004@126.com. Participants gave informed consent for data sharing.

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Jian-Gao Fan, Professor, Director, Department of Gastroenterology, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 1665 Kongjiang Road, Shanghai 200092, China. fattyliver2004@126.com
Telephone: +86-21-25077340
Fax: +86-21- 63846590

Received: September 12, 2016
Peer-review started: September 12, 2016
First decision: October 20, 2016
Revised: October 25, 2016
Accepted: November 15, 2016
Article in press: November 16, 2016
Published online: January 7, 2017

Abstract

AIM

To investigate whether gut microbiota metabolite sodium butyrate (NaB) is an effective substance for attenuating non-alcoholic fatty liver disease (NAFLD) and the internal mechanisms.

METHODS

Male C57BL/6J mice were divided into three groups,

normal control were fed standard chow and model group were fed a high-fat diet (HFD) for 16 wk, the intervention group were fed HFD for 16 wk and treated with NaB for 8 wk. Gut microbiota from each group were detected at baseline and at 16 wk, liver histology were evaluated and gastrointestinal barrier indicator such as zonula occluden-1 (ZO-1) were detected by immunohistochemistry and realtime-PCR, further serum or liver endotoxin were determined by ELISA and inflammation- or metabolism-associated genes were quantified by real-time PCR.

RESULTS

NaB corrected the HFD-induced gut microbiota imbalance in mice, while it considerably elevated the abundances of the beneficial bacteria *Christensenellaceae*, *Blautia* and *Lactobacillus*. These bacteria can produce butyric acid in what seems like a virtuous circle. And butyrate restored HFD induced intestinal mucosa damage, increased the expression of ZO-1 in small intestine, further decreased the levels of gut endotoxin in serum and liver compared with HF group. Endotoxin-associated genes such as TLR4 and Myd88, pro-inflammation genes such as MCP-1, TNF-α, IL-1, IL-2, IL-6 and IFN-γ in liver or epididymal fat were obviously downregulated after NaB intervention. Liver inflammation and fat accumulation were ameliorated, the levels of TG and cholesterol in liver were decreased after NaB intervention, NAS score was significantly decreased, metabolic indices such as FBG and HOMA-IR and liver function indicators ALT and AST were improved compared with HF group.

CONCLUSION

NaB may restore the dysbiosis of gut microbiota to attenuate steatohepatitis, which is suggested to be a potential gut microbiota modulator and therapeutic substance for NAFLD.

Key words: Non-alcoholic fatty liver disease; Sodium butyrate; Gut microbiota; Gastrointestinal barrier; Endotoxin

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a global epidemic metabolic health crisis that lacks effective therapeutic strategies. We found that NaB could correct the high-fat diet (HFD)-induced gut microbiota imbalance in mice, while it considerably elevated the abundances of the beneficial bacteria. These bacteria can produce butyric acid in what seems like a virtuous circle. And butyrate restored HFD induced intestinal mucosa damage, improved tight junction structure, reduced gut endotoxin into liver, leading to attenuate HFD induced liver inflammation and lipid accumulation, which may be a potential gut microbiota modulator and therapeutic substance for NAFLD.

Zhou D, Pan Q, Xin FZ, Zhang RN, He CX, Chen GY, Liu C, Chen YW, Fan JG. Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J Gastroenterol* 2017; 23(1): 60-75 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/60.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.60

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an emerging public health problem with an increasing incidence and prevalence globally[1,2]. NAFLD is a clinical-histological syndrome that is characterized histopathologically by predominantly macrovesicular steatosis with varying amounts of inflammation, cytological ballooning and fibrosis, and it is associated with significant morbidity and mortality. Few diagnostic and therapeutic strategies for patients with NAFLD are established[3].

The gut microbiota plays a pivotal role in the development and progression of NAFLD, although the underlying mechanisms remain largely uninvestigated[4-6]. Recent evidence has revealed that not only the gut microbiota themselves but also the bacterial metabolites are important for regulating the body's life activities and metabolism. These include short-chain fatty acids (SCFAs)[7], which have fewer than 7 carbon atoms and are mainly produced by the fermentation of gut microbiota. SCFAs have been suggested to play a key role in ameliorating obesity, hypertension, and dyslipidemia.

The SCFA butyrate has multiple beneficial effects in mammals[8], such as regulation of the secretion of gut hormones[9], inhibition of the production of pro-inflammatory factors[10,11], and even inhibition of the growth of pernicious bacteria in the gut[12] as well as its beneficial role for barrier function in the gastrointestinal tract[13], which is great associated with pathogenetic mechanism of NAFLD development. However, many apparently contradictory results demonstrate the complexity of the interactions among the gut microbiota, butyrate concentration and host energy metabolism[14], the further interactions among NAFLD, gut microbiota and its metabolites still need more investigations to clarify[15].

We hypothesized that sodium butyrate (NaB) supplementation alone would attenuate high-fat diet (HFD)-induced steatohepatitis in mice *via* modulation of gut microbiota. Our results showed that NaB treatment protected mice against HFD-induced liver fat accumulation and inflammation, improved gut microbiota dysbiosis induced by HFD and attenuated gut microbiota-derived endotoxin-induced liver injury. Based on these findings, we propose that NaB may play an important role in relieving steatohepatitis

and may be a useful therapeutic approach in the management of NAFLD.

MATERIALS AND METHODS

Animal experiments

Specified pathogen-free (SPF) male C57BL/6 mice (SLAC laboratory animal co., LTD, Shanghai, China) were housed in a controlled environment (23 ℃, 12 h daylight cycle, lights off at 18.00 h). The mice were acclimatized for 7 d after arrival with free access to water and a standard chow diet. The mice were then assigned randomly to three groups: control, model (HF) and intervention group (HF + NaB) (n = 15/group, 5 mice per cage). Mice from the control group were fed a standard diet. The HF group and HF + NaB group were fed a high-lard-fat and high-cholesterol diet (88% standard diet, 10% lard and 2% cholesterol). Body weight and food consumption were recorded weekly. Eight weeks after initiation of the experimental diets, we sacrificed 3 randomly selected mice from every group to assess liver damage. The intervention group was underwent daily intragastric administration with NaB at 200 mg/kg body weight (Sigma-Aldrich, United States), while the HF group received the same amount of normal saline once per day for 8 wk. After 8 wk of intervention, the mice were fasted for 12 h and blood or tissue samples were collected. All animals were euthanized by pentobarbital sodium for tissue collection.

All animal experiments were approved by the Institutional Animal Care and Use Committee of Xinhua hospital affiliated to Shanghai Jiao Tong University School of Medicine and were conducted in accordance with the National Research Council Guide for Care and Use of Laboratory Animals.

Fecal sample collection from mice

Fecal samples were collected immediately upon defecation from each mouse at baseline and at 16 wk and stored at -80 ℃. Fecal DNA was extracted from fecal samples using the E.Z.N.A Soil DNA Kit (Omega Bio-tek, Norcross, GA, United States) according to the manufacturer's protocols. The V4-V5 region of the bacteria 16S ribosomal RNA gene was amplified by PCR. Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, United States). Purified amplicons were pooled at equimolar concentrations and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to standard protocols. Processing of the sequencing data and bioinformatics analysis were conducted by Majorbio in Shanghai[16]. These sequences were clustered into operational taxonomic units (OTUs) with a 97% sequence identity using mothur (furthest neighbor method) and chopseq (Majorbio). Rarefaction analysis was performed using mothur and plot-rarefaction (Majorbio). From these analyses, the Shannon diversities and Chao1 richness estimations were calculated using mothur. The unweighted UniFrac distance was used to quantify differences in community composition. Principal component analysis (PCA)[17] and nonmetric multidimensional scaling (NMDS) diagrams[18] were generated using the R package vegan to demonstrate the clustering of different samples. The hierarchical cluster analysis was performed using MVSP 3.1 software (Majorbio)[19].

Serum assays

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose in plasma were then measured using an automated analyzer (Sysmex CHEMIX-180, Japan). Insulin (Rat/Mouse Insulin ELISA Kit, Merck-Millipore) in serum were measured by enzyme-linked immunosorbent assay. Mouse endotoxin concentrations in serum and liver homogenate were measured by enzyme-linked immunosorbent assay (Mouse ET ELISA Kit, Trust Specialty Zeal). Samples and standards were processed according to the manufacturer's instructions.

Tissue histological analysis

Liver tissue was fixed in 4% paraformaldehyde, frozen in O.C.T, or snap-frozen in liquid nitrogen and stored at -80 ℃. The small intestine was either fixed in 4% paraformaldehyde or snap-frozen in liquid nitrogen and stored at -80 ℃. The small intestinal morphometric analysis was performed in 30 villi from each animal. The height of the villi comprises from extension of the crypt-villus junction up to top of the villi. The images were captured by an optical microscope (Leica DMI3000B, United States). The image analysis software Image-Pro Plus version 4.5.0.29 (Media Cybernetics, Silver Spring, MD, United States) was used for morphometric measurements in recorded images through the determination of villus height in μm. Epididymal fat was snap-frozen in liquid nitrogen and stored at -80 ℃. Paraformaldehyde-fixed paraffin sections of the liver and small intestine were stained with hematoxylin-eosin for pathological analysis or Masson's trichrome for fibrosis, and the nonalcoholic fatty liver activity score (NAS) was assessed. Frozen sections were stained with Oil Red O to detect lipids. For zonula occluden-1 (ZO-1) (Abcam, United States) staining, paraffin-embedded sections were used. Horseradish peroxidase-conjugated secondary antibody was applied, and the reaction was visualized using 3,3'-diaminobenzidine tetrahydrochloride.

Triglyceride and cholesterol evaluation in liver

Intrahepatic triglycerides (TGs) and cholesterol were measured using a triglyceride assay kit or cholesterol assay kit (Applygen Technologies Inc., Beijing,

China). Samples and standards were then processed according to the manufacturer's instructions. The final concentrations of triglycerides and cholesterol were corrected for protein content.

Real-time quantitative polymerase chain reaction

Total RNA was extracted from liver and small intestine using TRIzol (D9108B, Takara, Dalian, China) and reverse-transcribed into cDNA using PrimeScript RT master mix (RR036A, Takara, Dalian, China). Real-time quantitative polymerase chain reaction (qPCR) was performed with an Applied Biosystems 7500 Real-time PCR system using the SYBR Premix Ex Taq (Tli RNase H Plus) (RR420A, Takara, Dalian, China). Primers of the target genes were synthesized by Sangon Biotech (Shanghai, China). The primer sequences are listed in Supplementary Table 1. The primer specificity was confirmed by dissociation curves using 7500 system SDS software. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (B661304, Sangon Biotech) was used as the internal control.

Statistical analysis

Data are expressed as the means ± SEM. Comparisons were performed using one-way analysis of variance (ANOVA) in GraphPad Prism 5. Post-hoc Student-Newman-Keuls analyses were performed when > 2 groups were present. $P < 0.05$ was considered statistically significant. The statistical methods of this study were reviewed by GuangYu Chen from Clinical Epidemiology Center, Shanghai Jiaotong University.

RESULTS

NaB treatment improved liver indices and metabolism in HFD-fed mice

After 16 wk of the HF diet, all of the mice in the HF group gained much more body weight than those in the control group. The liver specimens were larger and paler in the HF group than the control group (Figure 1A). However, the HF + NaB group displayed a significantly improved whole body and liver profile than the HF group (Figure 1A).

The HF group displayed significantly higher liver and epididymal fat indices, elevated fasting blood glucose, a higher homeostasis model assessment of insulin resistance (HOMA-IR), and a lower insulin sensitivity index (ISI) than the control group (Figure 1). The NaB intervention attenuated HF diet-induced weight gain without reductions in energy intake, accompanied by opposite changes in HOMA-IR, ISI, fasting blood glucose, and especially liver indices (resulting in a 22% reduction, equivalent to the control group) (Figure 1). However, the NaB intervention had no effect on the epididymal fat index or insulin (Figure 1). Both ALT and AST, the specific markers of liver function, were significantly increased in the HF group compared with those in the control and were significantly decreased by the NaB intervention compared with those in the HF group (Figure 1).

Effects of NaB intervention on the tight junction of the small intestine and morphometry of the villi

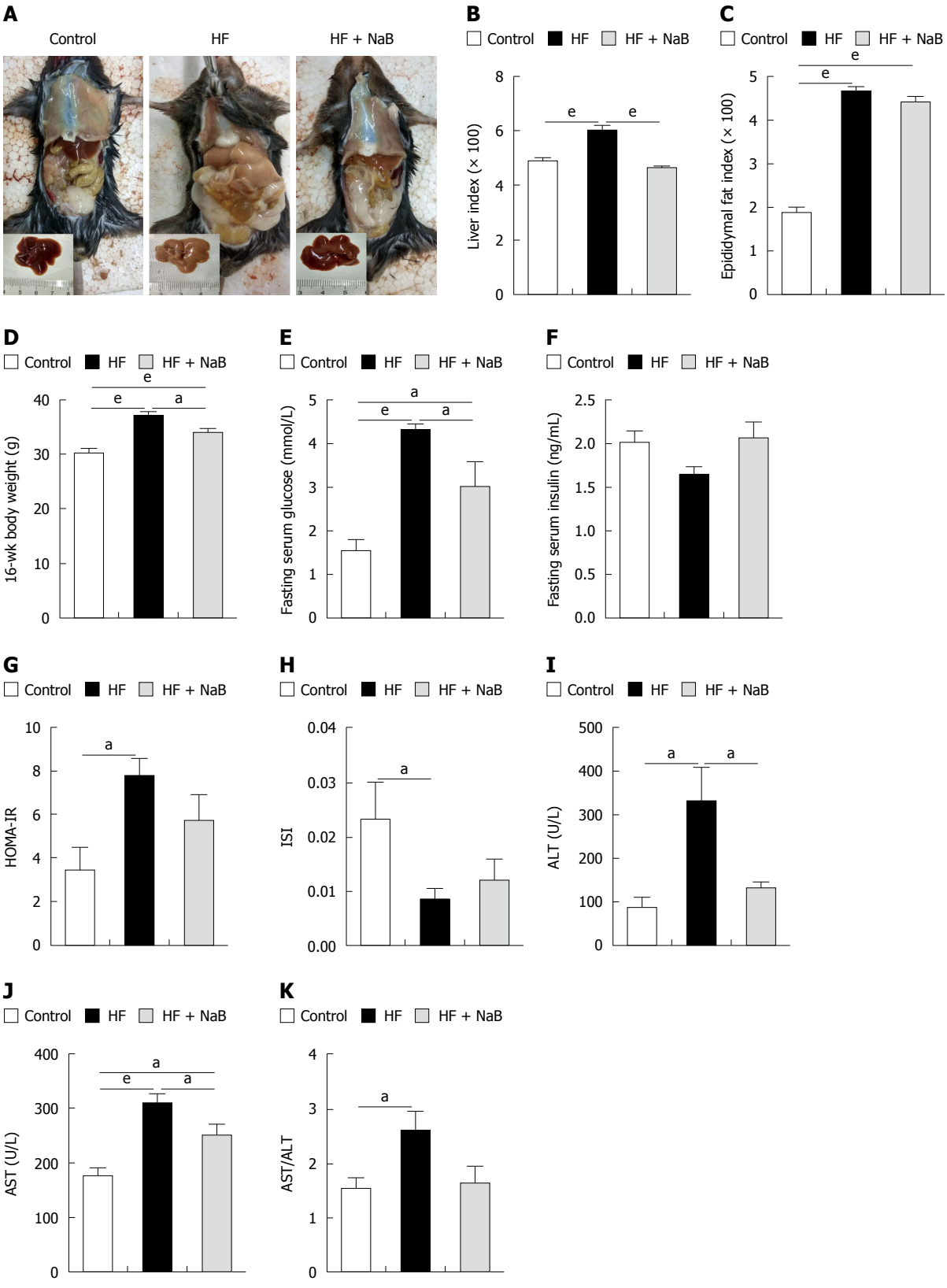
Integrated tight junctions of the small intestine are associated with systemic inflammation. HE staining of the small intestine revealed greater damage to the intestinal mucosa in the HF group than the control group, which was repaired to a certain degree by NaB intervention (Figure 2A). The HF group displayed a reduction of the villus height of the small intestine of 20%, compared to the control group, while NaB intervention significantly attenuated the reduction of the villus height induced by HFD (Figure 2B). Immunohistochemistry for ZO-1, a tight junction marker, revealed that ZO-1 was more abundant after NaB intervention than in the HF group (Figure 2C). Next, we measured the mRNA levels of ZO-1 in the small intestine. Although there was no significant difference between the control and the HF group, ZO-1 expression was significantly increased in the HF + NaB group compared with either the control or the HF group (Figure 2D).

Gut microbiota variations in response to diet or NaB intervention

To elucidate the effects of diet and NaB on the composition of the microbiota, we conducted Illumina MiSeq sequencing of bacterial 16S rRNA at baseline and at 16 wk after treatment. The quality of the sequencing, which included microbial richness, biodiversity, and rarefaction curves, met the requirements for subsequent analysis.

Sixteen weeks of HFD feeding induced significant changes in the gut microbial community at the phylum level compared with the control, with increased abundances of Bacteroidetes (63.1% *vs* 52.9%) and decreased abundances of Actinobacteria (0.04% *vs* 0.15%), Tenericutes (0.09% *vs* 1.42%) and Firmicutes (35.8% *vs* 44.6%). In addition, the ratio of Firmicutes to Bacteroidetes was lower in the HF group compared with the control (0.572 *vs* 0.851). However, NaB intervention mitigated the HFD-induced decrease in Actinobacteria and Tenericutes, enhanced the HFD-induced decreases in Firmicutes and the ratio of Firmicutes to Bacteroidetes, and enhanced the HFD-induced increases in Bacteroidetes and Proteobacteria (Supplementary Table 2).

At the genus level, *Alistipes*, *Christensenellaceae_uncultured*, *Enterorhabdus*, *Lactobacillus*, *Parabacteroides*, *Parasutterella*, *Rikenella*, *Ruminococcaceae_incertae_sedis*, *Ruminococcaceae_unclassified*, and *Ruminococcaceae_uncultured* were reduced in the HF group compared with the control (Figure 3). NaB intervention reversed the HFD-induced changes in *Christensenellaceae_uncultured, Parabacteroides, Parasutterella* and *Lactobacillus* (Figure 3). Furthermore, NaB treatment significantly increased *Blautia* compared with both the HF group and


A
Control
HF
HF + NaB
B
Control HF HF + NaB
Liver index (× 100)
C
Control HF HF + NaB
Epididymal fat index (× 100)
D
Control HF HF + NaB
16-wk body weight (g)
E
Control HF HF + NaB
Fasting serum glucose (mmol/L)
F
Control HF HF + NaB
Fasting serum insulin (ng/mL)
G
Control HF HF + NaB
HOMA-IR
H
Control HF HF + NaB
ISI
I
Control HF HF + NaB
ALT (U/L)
J
Control HF HF + NaB
AST (U/L)
K
Control HF HF + NaB
AST/ALT

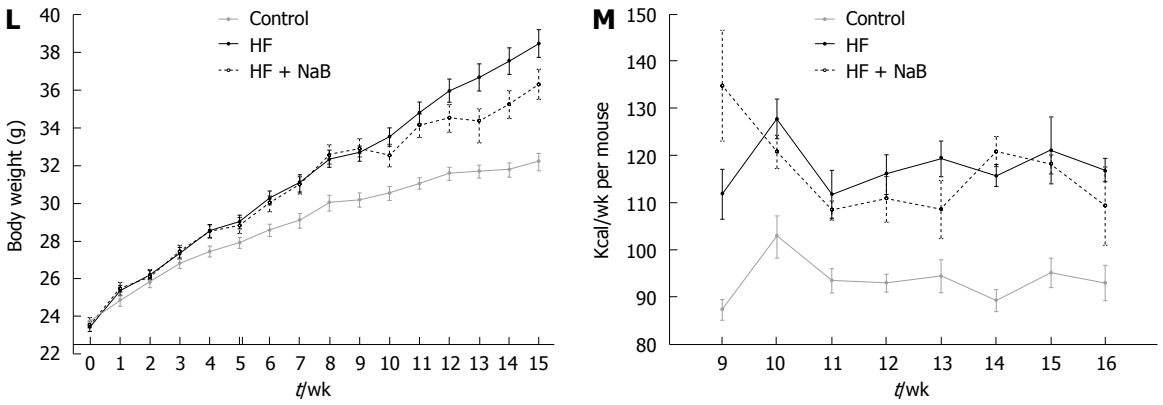


Figure 1 Sodium butyrate attenuates high-fat diet-induced obesity, liver injury and metabolic disturbance. A: Gross appearances of mice in the control, HF and HF + NaB groups; B: Liver index = liver weight/body weight × 100; C: Epididymal fat index = epididymal fat weight/body weight × 100; D: Body weight at 16 wk; E-H: Fasting serum glucose, fasting serum insulin, HOMA-IR and ISI of the three groups; I-K: Liver aminotransferases represent liver function; L: Body weight changes; M: Energy intake per mouse per week. Data represent means ± SEM. (n = 12 mice per group), $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{e}P < 0.001$.

the control group (Figure 3).

The NMDS analysis based on the Bray-Curtis distance clearly separated the 16 wk-HF group from the 16 wk-control, with the 16 wk-HF + NaB group positioned between them (Figure 4A). Thus, NaB intervention shifted the overall composition of the HFD-disrupted gut microbiota toward that of the control mice. PCA based on the OTU abundance was performed to provide an overview of the gut microbiota composition of 6 animal groups at baseline and at the end of the trial. There were no detectable differences in microbiota composition among the different groups before the intervention (Figure 4B). PC1, accounting for 68.24% of the total variance, predominantly reflected age-related changes in the composition of the gut microbiota because PC1 clearly separated samples obtained at baseline from those obtained at 16 wk PC2, accounting for 14.48% of the total variance, separated the 16 wk-control from the 16 wk-HF and 16 wk-HF + NaB groups, indicating that PC2 reflects the effect of diet (Figure 4B). Furthermore, the hierarchical cluster analysis showed that the 1 w-control, 1 wk-HF, 1 wk-HF + NaB, 16 wk-control and 16 wk-HF + NaB communities grouped together and then clustered in order with the 16 wk-HF communities (Figure 4C), which was consistent with the results of both the NMDS and the PCA analysis.

Effects of NaB on inflammation and the metabolism of epididymal fat

To evaluate fat inflammation, mRNA expression of MCP-1 was detected in epididymal fat in the HF group, and the results showed that it was almost 3 times higher than that in the control. The mRNA levels of TNF-α were 1.5 times higher in the HF group than in the control. NaB intervention significantly reduced MCP-1 and TNF-α expression to the level of the control (Figure 5A). In addition, we found that both PPAR-α (Peroxisome Proliferator Activated Receptor-α) and PPAR-γ expression, either in the HF or the HF + NaB group, were significantly reduced compared with the control (Figure 5B).

NaB ameliorated inflammation and fat accumulation in the liver of HFD-fed mice

After 16 wk, the end point, the HF group had higher NAS (5.67 ± 0.225 *vs* 0.20 ± 0.145 and 4.42 ± 0.358), steatosis (3.00 *vs* 0 and 2.17 ± 0.112), inflammation (0.75 ± 0.217 *vs* 0.06 ± 0.066 and 0.50 ± 0.150), and ballooning scores than the control and HF + NaB groups (1.92 ± 0.083 *vs* 0.13 ± 0.091 and 1.75 ± 0.131) (Table 1). Although HE staining directly demonstrated increased fat accumulation in the liver in the HF group compared with the control or HF + NaB groups, oil red O staining provided a much more indicative view of the fat accumulation (Figure 6A). As confirmation, intrahepatic TGs were 4 times and almost 1.33 times higher in the HF group and HF + NaB group, respectively (Figure 6B). Intrahepatic cholesterol was 10 times and almost 3.33 higher in the HF group and HF + NaB group (Figure 6C). Masson staining was conducted to assess liver fibrosis, but no positive results were found, which indicated that the liver had not progressed to fibrosis at 16 wk (Figure 6D). Although collagen deposition and liver fibrosis were not detected by the naked eye, fibrosis-associated gene expression was significantly enhanced in the liver in the HF group, indicating a tendency toward future fibrosis. Further, both serum and liver endotoxin in HF group were increased compared with control, while significantly decreased after NaB intervention (Figure 6E and F). TGF-β1, α-SMA, Smad7, and Smad2 were increased > 200% by HFD feeding. Notably, NaB intervention reduced these mRNAs to the levels observed in the control group (Figure 6G).

Pro-inflammatory cytokine mRNA expression was widely enhanced in the liver in the HF group, such as

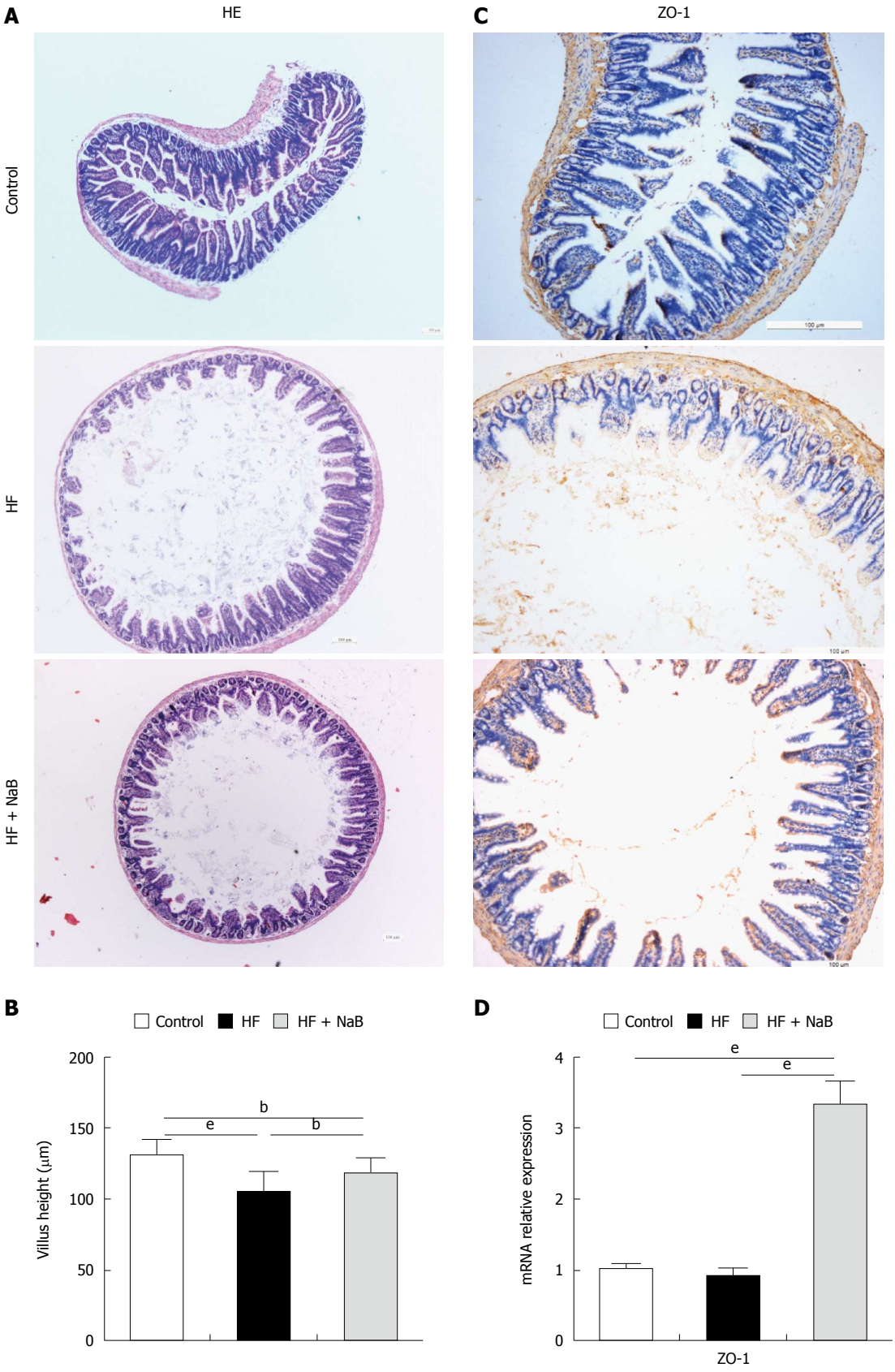
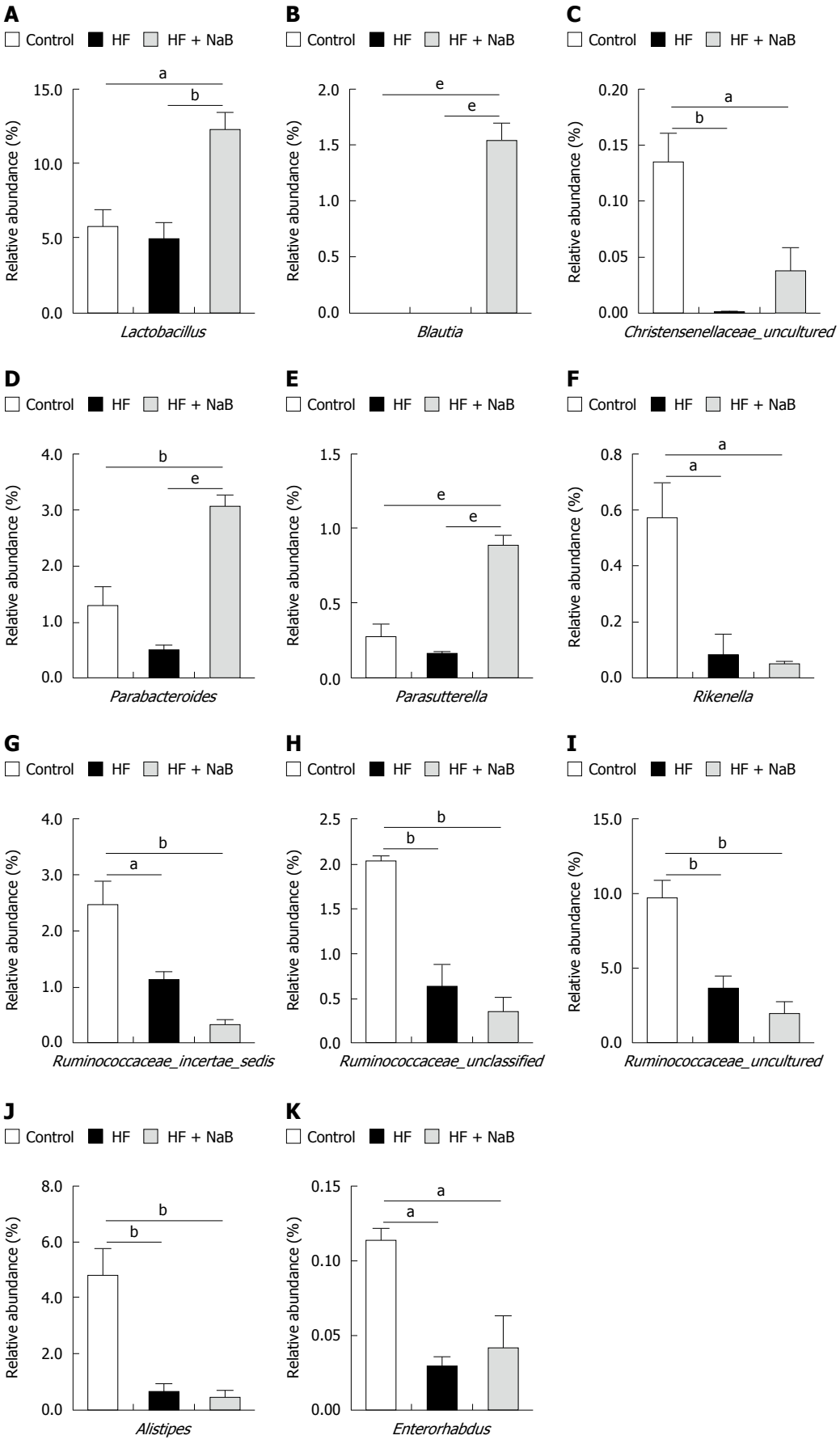


Figure 2 Beneficial effects of sodium butyrate on the small intestine. A: HE staining of the small intestine showing that NaB ameliorated HFD-induced mucosal damage; B: Morphometric analysis of villus; C: Immunohistochemistry for ZO-1 showing that NaB increased ZO-1 expression, indicating improvement of the tight junctions of the small intestine; D: ZO-1 mRNA expression in the small intestine. Data represent means ± SEM (n = 12 mice per group), $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{e}P < 0.001$.


A
Control HF HF + NaB
Relative abundance (%)
15.0
10.0
5.0
0.0
a
b
Lactobacillus
B
Control HF HF + NaB
Relative abundance (%)
2.0
1.5
1.0
0.5
0.0
e
e
Blautia
C
Control HF HF + NaB
Relative abundance (%)
0.20
0.15
0.10
0.05
0.00
a
b
Christensenellaceae_uncultured
D
Control HF HF + NaB
Relative abundance (%)
4.0
3.0
2.0
1.0
0.0
b
e
Parabacteroides
E
Control HF HF + NaB
Relative abundance (%)
1.5
1.0
0.5
0.0
e
e
Parasutterella
F
Control HF HF + NaB
Relative abundance (%)
0.8
0.6
0.4
0.2
0.0
a
a
Rikenella
G
Control HF HF + NaB
Relative abundance (%)
4.0
3.0
2.0
1.0
0.0
b
a
Ruminococcaceae_incertae_sedis
H
Control HF HF + NaB
Relative abundance (%)
2.5
2.0
1.5
1.0
0.5
0.0
b
b
Ruminococcaceae_unclassified
I
Control HF HF + NaB
Relative abundance (%)
15.0
10.0
5.0
0.0
b
b
Ruminococcaceae_uncultured
J
Control HF HF + NaB
Relative abundance (%)
8.0
6.0
4.0
2.0
0.0
b
b
Alistipes
K
Control HF HF + NaB
Relative abundance (%)
0.15
0.10
0.05
0.00
a
a
Enterorhabdus

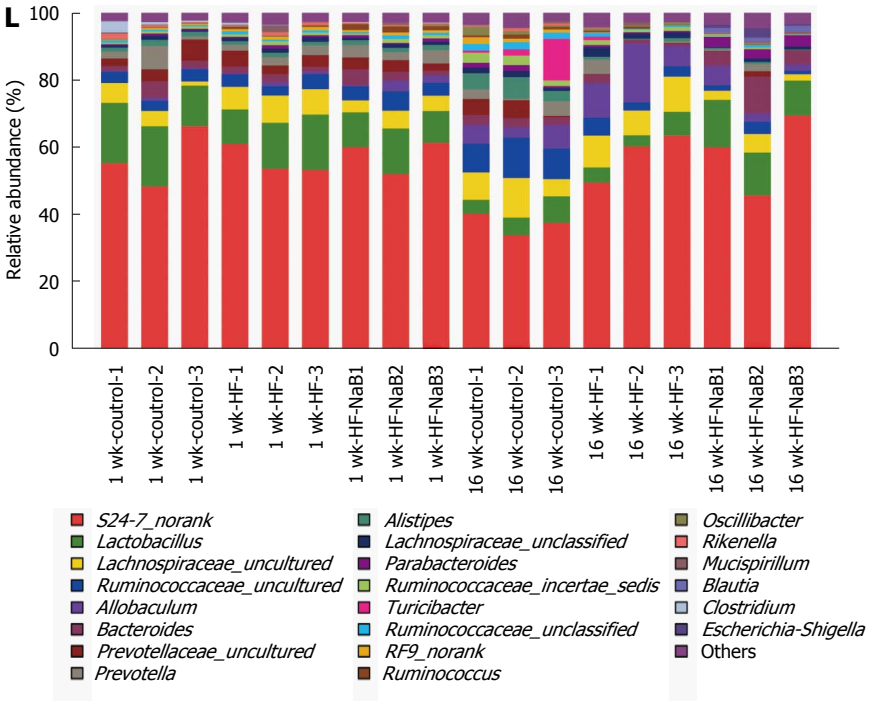


Figure 3 Influences of sodium butyrate on gut microbiota at the genus level. A-K: *Alistipes*, *Christensenellaceae_uncultured*, *Enterorhabdus*, *Lactobacillus*, *Parabacteroides*, *Parasutterella*, *Rikenella*, *Ruminococcaceae_incertae_sedis*, *Ruminococcaceae_unclassified*, and *Ruminococcaceae_uncultured* in the HF group were lower than the control. NaB intervention reversed the changes in *Christensenellaceae_uncultured*, *Parabacteroides*, *Parasutterella* and *Lactobacillus*. NaB treatment significantly increased Blautia; L: Relative read abundance of different bacterial genera within the different communities. Sequences that could not be classified into any known group were assigned as "norank". Data represent means ± SEM. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{e}P < 0.001$.

Table 1 Liver nonalcoholic fatty liver activity score including steatosis, ballooning, and lobular inflammation

Group	*n*	Steatosis	Ballooning	Lobular inflammation	NAS
Control	12	-	0.13 ± 0.091	0.06 ± 0.066	0.20 ± 0.145
HF	12	3.00[b]	1.92 ± 0.083[b]	0.75 ± 0.217[b]	5.67 ± 0.225[b]
HF + NaB	12	2.17 ± 0.112[b,c]	1.75 ± 0.131[b]	0.50 ± 0.150[b]	4.42 ± 0.358[b,d]

$^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* HF. NAS: Nonalcoholic fatty liver activity score.

MCP-1, TNF-α, IL-1, IL-2, IL-6 and IFN-γ; endotoxin-associated TLR4 and Myd88 were significantly increased compared with the control group, while anti-inflammatory cytokines, such as IL-4, IL-10, were also unexpectedly enhanced in the liver in the HF group (Figure 6H-J). In contrast, these pro-inflammatory cytokine and endotoxin-associated gene mRNAs were all significantly reduced, and anti-inflammatory cytokine mRNAs were enhanced by NaB intervention compared with HF alone (Figure 6H-J). These results indicated that NaB promoted the maintenance of liver homeostasis.

To investigate lipid metabolism in the liver, we measured two vital lipid-associated transcription factors, PPAR-α and PPAR-γ. PPAR-α was significantly increased in the HF group compared with the control but was similar between the HF + NaB group and the control. PPAR-γ was similar between the HF and control groups but was significantly increased in the HF + NaB group compared with either the control or the HF group (Figure 6K).

DISCUSSION

NAFLD is the most common cause of liver disease worldwide, and its prevalence has increased in parallel with that of obesity. In recent years, the importance of the gut microbiota for health has been widely acknowledged. Accumulating data support the pivotal role of the gut microbiota or its metabolites in NAFLD development and progression[5]. We speculated that therapeutic targeting of the gut microbiota or its metabolites may be applied for the treatment of NAFLD. The new findings of this study are that NaB intervention significantly improved the gut microbiota in HFD-fed mice, enhanced intestinal mucosal barrier then reduced gut endotoxin induced systemic inflammation, finally attenuated liver histological damage induced by HFD.

Previous study[20] found a greater proportion of Ruminococcaceae in healthy subjects compared with patients with NASH. The physiological function of Ruminococcaceae is to produce SCFAs, including butyrate[21-23], and another study revealed that the

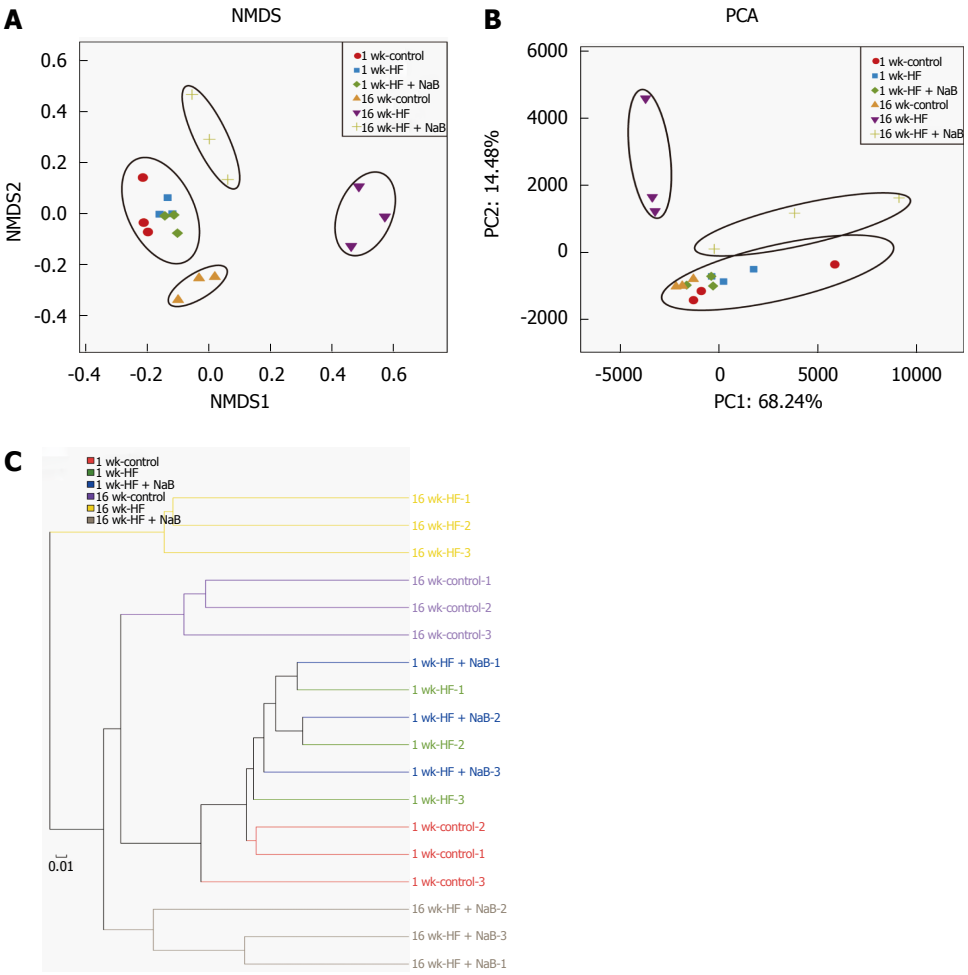


Figure 4 Effects of sodium butyrate on the overall structure of the gut microbiota. A: Nonmetric multidimensional scaling (NMDS) showing the difference in bacterial communities according to the Bray-Curtis distance; B: Scatter plot of the principal component analysis (PCA) score showing the similarity of the 18 bacterial communities based on the Unifrac distance. Principal components (PCs) 1 and 2 explained 68.24% and 14.48% of the variance, respectively; C: Hierarchical cluster analysis showing that the 1 wk groups and 16 wk-control communities grouped together, and then clustered in order with the 16 wk-HF + NaB groups, finally clustering with the 16 wk-HF groups.

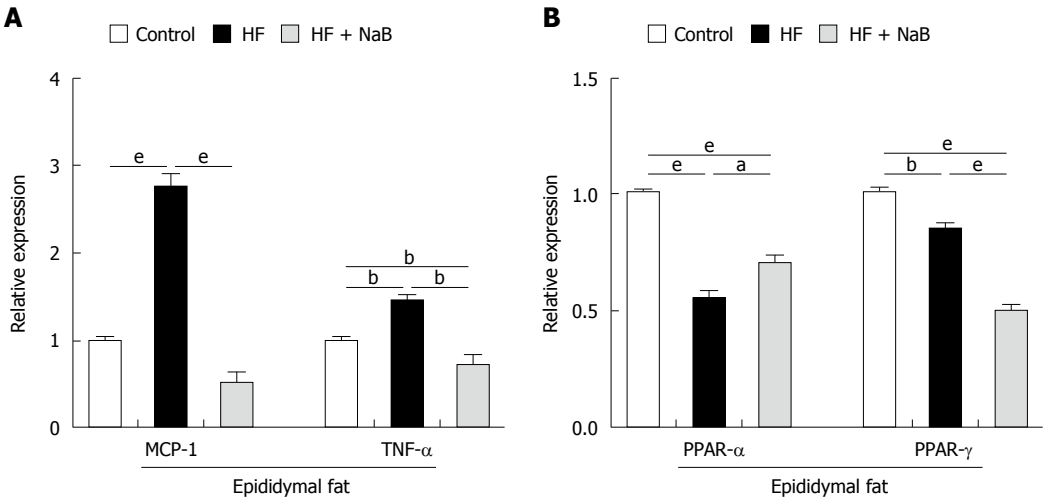
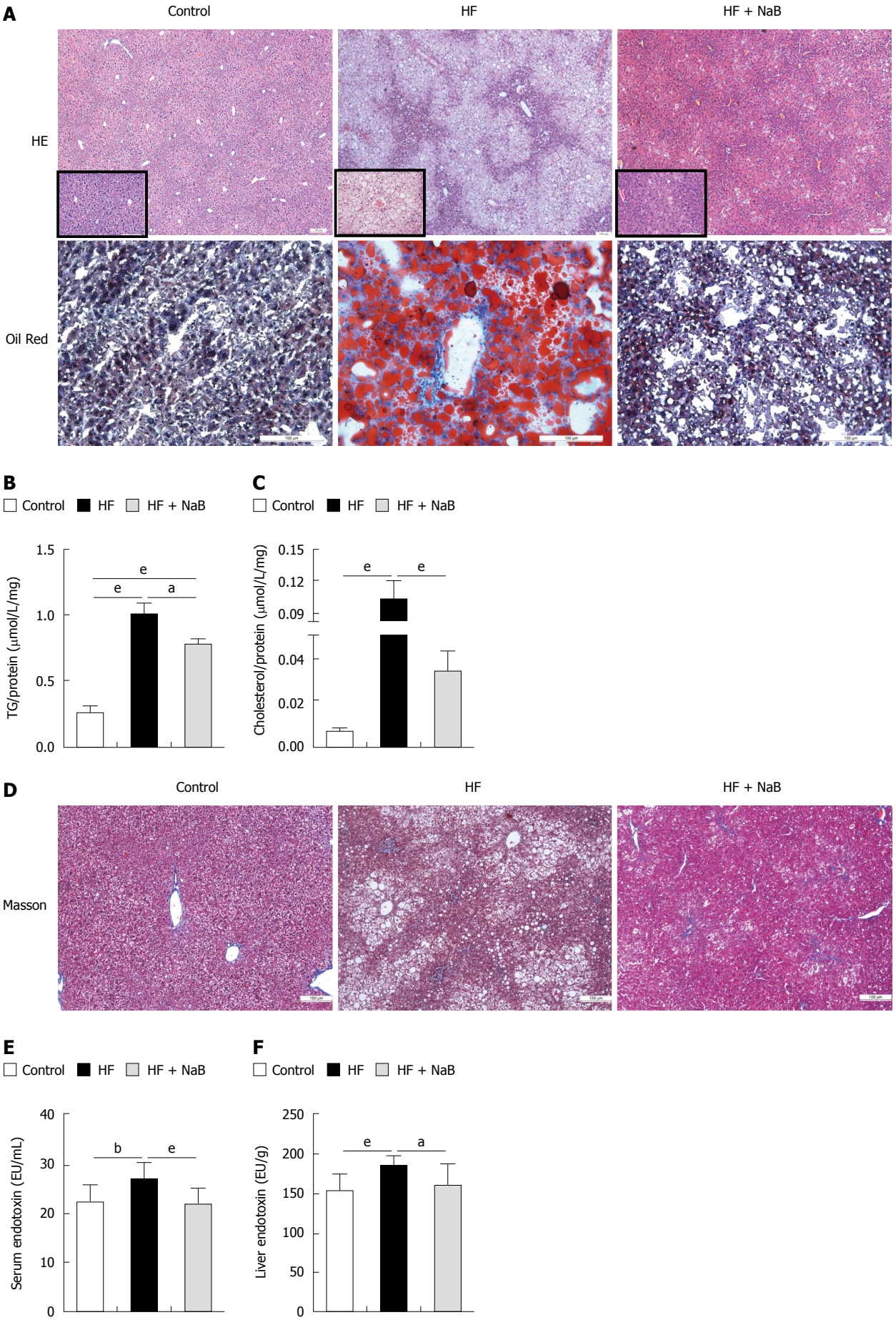


Figure 5 Sodium butyrate improves inflammation and lipid metabolism in epididymal fat. A: Gene expression levels of MCP-1 and TNF-α in epididymal fat; B: Gene expression levels of PPAR-α and PPAR-γ in epididymal fat. Data represent means ± SEM (n = 12 mice per group), $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{e}P < 0.001$.


A
Control
HF
HF + NaB
HE
Oil Red
B
Control
HF
HF + NaB
TG/protein (μmol/L/mg)
1.5
1.0
0.5
0.0
e
e
a
C
Control
HF
HF + NaB
Cholesterol/protein (μmol/L/mg)
0.15
0.12
0.09
0.04
0.02
0.00
e
e
D
Control
HF
HF + NaB
Masson
E
Control
HF
HF + NaB
Serum endotoxin (EU/mL)
40
30
20
10
0
b
e
F
Control
HF
HF + NaB
Liver endotoxin (EU/g)
250
200
150
100
50
0
e
a

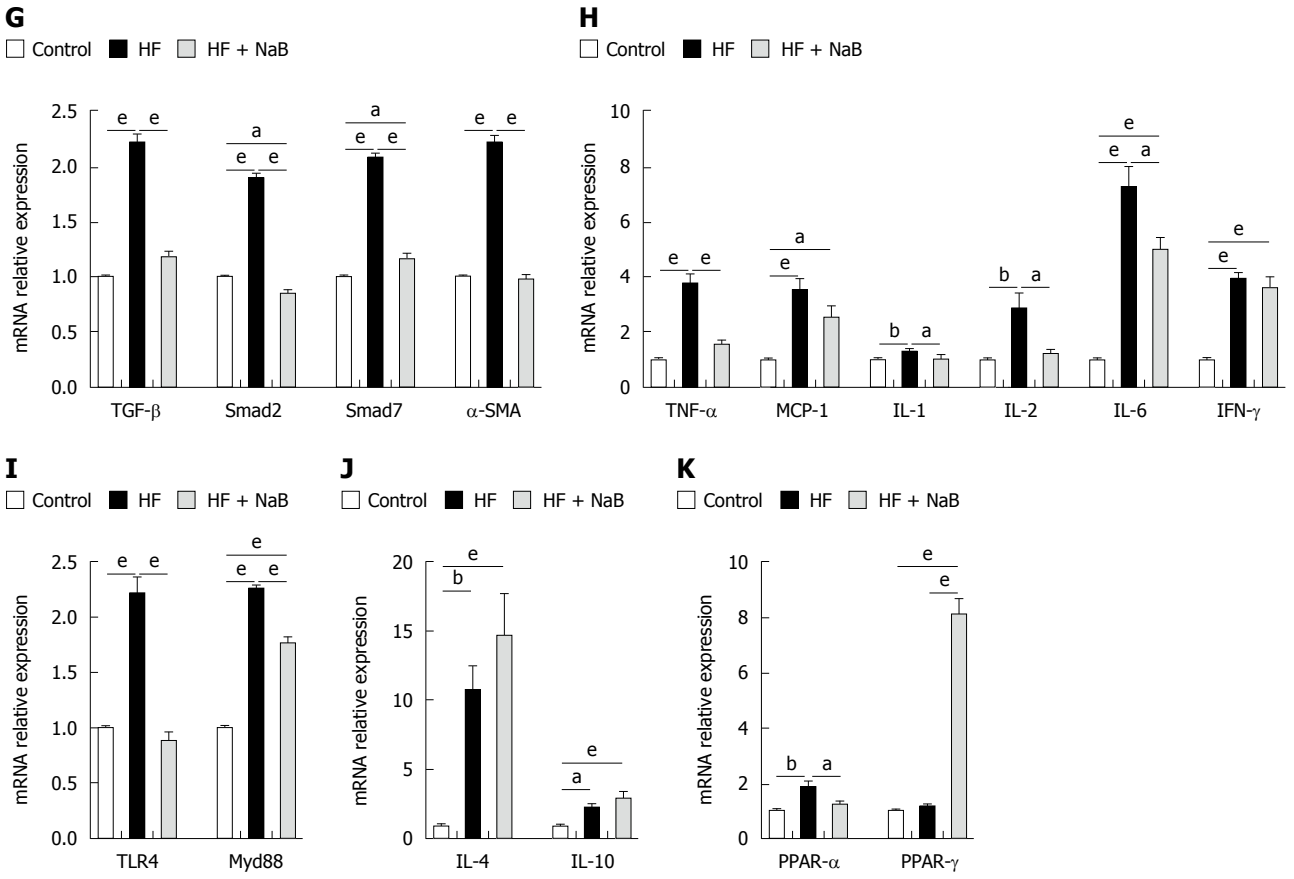


Figure 6 Sodium butyrate improves inflammation and lipid metabolism in liver. A: HE and oil red O staining; B: TG concentration; C: Cholesterol concentration; D: Masson staining; E, F: The levels of serum and liver endotoxin; G: Fibrosis-associated gene expression of TGF-β, Smad2, Smad7 and α-SMA; H: Pro-inflammation-associated gene expression; I: Endotoxin-associated gene expressions; J: Anti-inflammation-associated gene expression; K: Lipid metabolism-associated PPAR-α and PPAR-γ gene expression. Gene expression levels are expressed as values relative to the control group. Data represent means ± SEM (n = 12 mice per group), $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{e}P < 0.001$.

butyrate concentration in feces in HFD-fed mice was significantly lower than that in the control[24]. These findings were consistent with two studies conducted in diabetes patients who lacked butyrate-producing bacteria compared with the control[25,26]. SCFAs exert multiple beneficial effects on mammalian metabolism[6,7]. Dietary administration of SCFAs protected mice against diet-induced obesity and insulin resistance, and another study found a significant reduction of butyrate-producing bacteria in feces with lower rather than bacterial gene counts[27]. One clinical study showed that propionate stimulated the release of gut hormones from human colonic cells and that inulin-propionate ester supplementation significantly reduced weight gain, intra-abdominal adipose tissue distribution, and intrahepatocellular lipid content and prevented the deterioration in insulin sensitivity[28]. And previous studies have demonstrated beneficial effects of butyrate on animal models of steatohepatitis[29,30]. But there are also many apparently contradictory results[14]. Some studies have shown that obese *ob/ob* mice and obese human subjects have increased amounts of cecal and fecal SCFAs[31,32].

Our investigation revealed that NaB intervention effectively decreased HFD-induced weight gain and serum glucose despite no reductions in energy intake, which was consistent with the findings of Henagan *et al*[33]. Both HOMA-IR and ISI decreased but not significantly in response to NaB treatment compared with HF alone. The serum insulin and epididymal fat index were not altered by NaB intervention. These findings are not completely concordant with the study conducted by Gao, although the methodological differences between our study and theirs should not be ignored[34]. A recent cytology study revealed that butyrate enhanced adipogenesis and lipid accumulation in adipocytes, reduced lipolysis, and induced adiponectin expression, resulting in the activation of downstream target genes such as AMPK[8]. This cell-level research may explain why butyrate had no effect on the epididymal fat index. The decrease in body weight may be associated with the ability of butyrate to promote energy expenditure and induce mitochondrial activity[34]. Furthermore, PPAR-α mainly acts on fatty acid oxidation, whereas PPAR-γ regulates lipid homeostasis and insulin sensitivity, and PPAR-γ agonist can improve liver histology[35], both of the two nuclear factors were obviously changed after NaB

intervention which suggested that NaB triggered lipid metabolism of the body.

The gut microbiota is intimately related to healthy function of intestinal mucosal barrier which exhibits an inseparable relationship with metabolic health, including NAFLD[5]. We performed assays to detect any influence of NaB on the gut microbiota. According to the NMDS and PCA results, NaB treatment reversed the changes in the overall gut microbiota composition induced by HFD, resulting in a pattern more similar to the control. This phenomenon reflected the integrally bifidogenic effect of NaB. Recent studies on the gut microbiota suggest that it is better to focus on the genus or species level than the phylum level to investigate connections between gut microbiota and diseases[36]. Our study revealed that HFD induced dysbiosis of the gut microbiota by decreasing the proportions of *Alistipes*, *Christensenellaceae* and *Lactobacillus*, all of which are linked to a healthy state[36]. NaB treatment significantly increased the abundance of *Christensenellaceae_uncultured*, *Blautia* and *Lactobacillus*. *Christensenellaceae* is related to a low body mass index in humans and reduced weight gain in mice and is more likely to reduce body weight[37]. *Lactobacillus* is a probiotic bacterium with numerous beneficial effects on body metabolism and human health, including NAFLD[38,39]. *Lactobacillus* produces lactate, which can increase butyrate production in feces[27], and it increases butyrate uptake in intestinal epithelial cells, which is essential for intracellular effects such as the promotion of gut hormone secretion and colonic mucosal integrity, as well as the inhibition of inflammation[40]. *Blautia*, another genus that is affected by NaB, is a beneficial bacterium that is negatively correlated with metabolic syndromes. Its main biological function is to produce butyrate[23]. *Blautia* contributes to an increase in the butyrate concentration in feces, which may further enhance intestinal health. These bifidogenic effects may be partially attributed to the NaB-induced decrease in the gut pH to a more suitable level for the growth of beneficial but not harmful bacteria[41], another study suggested that butyrate could enhances antibacterial effects *via* immunity regulation[42]. Thus, these effects of NaB seem to form a virtuous circle, promoting beneficial effects on the body.

Our study demonstrated that HF diet disrupted gut microbiota then further impaired intestinal mucosal barrier, visually, NaB repaired the damage to the intestinal mucosa and strengthened the intestinal tight junctions. Previous studies indicated that microbial butyrate may contribute to the restoration of the tight junction barrier *via* up-regulating the protein level of ZO-1, which was attributed to its histone deacetylase inhibition[13,43]. It decreased the intestinal permeability to reduce the escape of pathogen-associated molecular patterns into the blood; thus, we comfirmed that no matter serum or liver endotoxin derived from gut microbiota was significantly decreased compared with HF group, Toll-like receptor 4 (TLR4), a receptor of lipopolysaccharide (LPS) or endotoxin, and its downstream protein Myd88 were significantly decreased in the liver after NaB treatment. TLR activation leads to the translocation of NF-κB into the nucleus and the induction of pro-inflammatory gene transcription, such as TNF-α, IL-1β, and IL-6[44], which is a classic pathway participated in the progression of NAFLD[5]. This may represent a pivotal LPS-associated mechanism to attenuate liver inflammation after NaB intervention. These beneficial effects were greatly associated with the improvement of the intrahepatic environment, such as, many pro- inflammatory factors in the liver (MCP-1, TNF-α, IL-1, IL-2, IL-6, IFN-γ) and epididymal fat (MCP-1, TNF-α), were as expected decreased and anti-inflammatory factors in the liver (IL-4, IL-10) were significantly increased after NaB treatment. The changes of these immune factors were partially inseparable from the immunoregulation of NaB *via* inhibiting histone acetylation enzymes or G protein-coupled receptors pathway[10-12,45-49], helping correct an unbalanced physiological environment induced by HFD, and significantly improved liver histology by attenuating inflammation and fat accumulation.

Our study also had some limitations. First, the role of butyrate in maintaining intestinal homeostasis is undoubted which is confirmed by our study[13,50], meanwhile, gut microbiota has an indispensable role in the human body[51,52], and the challenge is to find out whether these changes in gut microbiota composition are the cause or the consequence of disorder[53]. Further researches on this aspect may uncover the precise relationship between gut microbiota and NAFLD. Second, this study was conducted in an animal model. Further studies should be performed in human subjects.

In conclusion, we demonstrated the application of NaB to attenuate HFD-induced steatohepatitis in mice. NaB beneficially regulated the gut microbiota and enhanced gastrointestinal health to improve whole body metabolism. This study was limited to animals, and whether it will work on clinical populations remains a significant challenge, however, the unpleasant taste and odor of NaB make it extremely difficult to administrate orally[29]. Thus, new formulations of butyrate with a better palatability, which can be easily administered orally, are needed. Overall, it opens potential avenues for intervention strategies for the treatment of NAFLD. Urgent attention should be devoted to the gut microbiota and its metabolites, which could produce novel therapeutic targets.

COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) is an emerging public health problem with an increasing incidence that lacks effective therapeutic strategies. Previous data demonstrates that the gut microbiota and its metabolites such as butyrate play a pivotal role in the development and progression of NAFLD, however, the

underlying mechanisms and interactions among NAFLD, gut microbiota and its metabolites still need more investigations to clarify.

Research frontiers

Sodium butyrate (NaB) is one of gut metabolite which exerts multiple beneficial effects on mammalian metabolism. The research hotspot lies in that NaB could significantly improve the overall structure of gut microbiota, correct the high-fat diet (HFD)-induced gut microbiota dysbiosis in mice, meanwhile it considerably elevated the abundances of the beneficial bacteria. These bacteria can produce butyric acid in what seems like a virtuous circle.

Innovations and breakthroughs

To elucidate the effects of HFD and NaB on the composition of the microbiota and further clarify the effect of gut metabolite sodium butyrate (NaB) on NAFLD, Illumina MiSeq sequencing of bacterial 16S rRNA from gut microbiota was conducted and analysed, and further metabolism indices, liver and small intestine histologies were evaluated. Inflammation- or metabolism-associated genes in the liver and epididymal fat tissue were detected, furthermore, the serum and intrahepatic levels of endotoxin, intrahepatic triglyceride and cholesterol were measured. This study found that NaB significantly corrected the gut microbiota dysbiosis induced by HFD, restored intestinal mucosa damage, improved tight junction structure, finally reduced gut endotoxin into liver and attenuated HFD induced steatohepatitis.

Applications

The results suggested that gut metabolite NaB had the ability to reverse HFD-induced dysbiosis of gut microbiota and finally attenuated HFD-induced steatohepatitis which indicated the potential therapeutic approach in the treatment of NAFLD.

Terminology

Short chain fatty acids, also referred to as volatile fatty acids, are mainly produced by the fermentation of gut microbiota and have fewer than 6 carbon atoms, including formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid and isovaleric acid.

Peer-review

The study of Zhou *et al* is an interesting study describing sodium butyrate attenuation of high-fat diet-induced steatohepatitis in mice. The authors investigated its mechanisms and concluded that the effects were possibly by improving gut microbiota and gastrointestinal barrier.

REFERENCES

1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

2 **Wang FS**, Fan JG, Zhang Z, Gao B, Wang H-Y. The global burden of liver disease: The major impact of China. *Hepatology* 2014; **60**: 2099-2108 [DOI: 10.1002/hep.27406]

3 **Rinella ME**. Nonalcoholic Fatty Liver Disease. *Jama* 2015; **31**: 2263 [DOI: 10.1001/jama.2015.5370]

4 **Boursier J**, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016; **63**: 764-775 [PMID: 26600078 DOI: 10.1002/hep.28356]

5 **Gkolfakis P**, Dimitriadis G, Triantafyllou K. Gut microbiota and non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int* 2015; **14**: 572-581 [PMID: 26663004]

6 **Tremaroli V**, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012; **489**: 242-249 [PMID: 22972297 DOI: 10.1038/nature11552]

7 **Hartstra AV**, Bouter KE, Bäckhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. *Diabetes Care* 2015; **38**: 159-165 [PMID: 25538312 DOI: 10.2337/dc14-0769]

8 **Yan H**, Ajuwon KM. Mechanism of Butyrate Stimulation of Triglyceride Storage and Adipokine Expression during Adipogenic Differentiation of Porcine Stromovascular Cells. *PLoS One* 2015; **10**: e0145940 [PMID: 26713737 DOI: 10.1371/journal.pone.0145940]

9 **Clarke G**, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: Gut microbiota: the neglected endocrine organ. *Mol Endocrinol* 2014; **28**: 1221-1238 [PMID: 24892638 DOI: 10.1210/me.2014-1108]

10 **Park JS**, Lee EJ, Lee JC, Kim WK, Kim HS. Anti-inflammatory effects of short chain fatty acids in IFN-gamma-stimulated RAW 264.7 murine macrophage cells: involvement of NF-kappaB and ERK signaling pathways. *Int Immunopharmacol* 2007; **7**: 70-77 [PMID: 17161819]

11 **Zhang WH**, Jiang Y, Zhu QF, Gao F, Dai SF, Chen J, Zhou GH. Sodium butyrate maintains growth performance by regulating the immune response in broiler chickens. *Br Poult Sci* 2011; **52**: 292-301 [PMID: 21732874 DOI: 10.1080/00071668.2011.578121]

12 **Wen ZS**, Lu JJ, Zou XT. Effects of sodium buty-rate on the intestinal morphology and DNA-binding activity of intestinal nuclear factor-kappa B in wean-ling pigs. *Dongwu Yixue Jinzhan* 2012; **11**: 814-821

13 **Plöger S**, Stumpff F, Penner GB, Schulzke JD, Gäbel G, Martens H, Shen Z, Günzel D, Aschenbach JR. Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Ann N Y Acad Sci* 2012; **1258**: 52-59 [PMID: 22731715 DOI: 10.1111/j.1749-6632.2012.06553.x]

14 **den Besten G**, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res* 2013; **54**: 2325-2340 [PMID: 23821742 DOI: 10.1194/jlr.R036012]

15 **Fan JG**, Jia JD, Li YM, Wang BY, Lu LG, Shi JP, Chan LY. Guidelines for the diagnosis and management of nonalcoholic fatty liver disease: update 2010: (published in Chinese on Chinese Journal of Hepatology 2010; 18: 163-166). *J Dig Dis* 2011; **12**: 38-44 [PMID: 21276207 DOI: 10.1111/j.1751-2980.2010.00476.x]

16 **Schloss PD**, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009; **75**: 7537-7541 [PMID: 19801464 DOI: 10.1128/AEM.01541-09]

17 **Wang Y**, Sheng HF, He Y, Wu JY, Jiang YX, Tam NF, Zhou HW. Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags. *Appl Environ Microbiol* 2012; **78**: 8264-8271 [PMID: 23001654 DOI: 10.1128/AEM.01821-12]

18 **Noval Rivas M**, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, Chehoud C, Kuczynski J, DeSantis T, Warrington J, Hyde ER, Petrosino JF, Gerber GK, Bry L, Oettgen HC, Mazmanian SK, Chatila TA. A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis. *J Allergy Clin Immunol* 2013; **131**: 201-212 [PMID: 23201093 DOI: 10.1016/j.jaci.2012.10.026]

19 **Jiang XT**, Peng X, Deng GH, Sheng HF, Wang Y, Zhou HW, Tam NF. Illumina sequencing of 16S rRNA tag revealed spatial variations of bacterial communities in a mangrove wetland. *Microb Ecol* 2013; **66**: 96-104 [PMID: 23649297 DOI: 10.1007/s00248-013-0238-8]

20 **Raman M**, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, Bailey J, Myers RP, Rioux KP. Fecal Microbiome and Volatile Organic Compound Metabolome in Obese Humans With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2013; **11**: 868-875.e863 [DOI: 10.1016/j.cgh.2013.02.015]

21 **De Filippo C**, Cavalieri D, Di Paola M, Ramazzotti M, Poullet

JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010; **107**: 14691-14696 [PMID: 20679230 DOI: 10.1073/pnas.1005963107]

22 **Zhang J**, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, Wang G, Wang F, Xu J, Cao H, Xu H, Lv Q, Zhong Z, Chen Y, Qimuge S, Menghe B, Zheng Y, Zhao L, Chen W, Zhang H. A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. *ISME J* 2015; **9**: 1979-1990 [PMID: 25647347 DOI: 10.1038/ismej.2015.11]

23 **Berni Canani R**, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, Calignano A, Khan AA, Gilbert JA, Nagler CR. Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME J* 2016; **10**: 742-750 [PMID: 26394008 DOI: 10.1038/ismej.2015.151]

24 **Wang J**, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van-Hylckama Vlieg JE, Strissel K, Zhao L, Obin M, Shen J. Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. *ISME J* 2015; **9**: 1-15 [PMID: 24936764 DOI: 10.1038/ismej.2014.99]

25 **Karlsson FH**, Tremaroli V, Nookaew I, Bergström G, Behre CJ, Fagerberg B, Nielsen J, Bäckhed F. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013; **498**: 99-103 [PMID: 23719380 DOI: 10.1038/nature12198]

26 **Qin J**, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**: 55-60 [PMID: 23023125 DOI: 10.1038/nature11450]

27 **Le Chatelier E**, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541-546 [PMID: 23985870 DOI: 10.1038/nature12506]

28 **Chambers ES**, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SE, MacDougall K, Preston T, Tedford C, Finlayson GS, Blundell JE, Bell JD, Thomas EL, Mt-Isa S, Ashby D, Gibson GR, Kolida S, Dhillo WS, Bloom SR, Morley W, Clegg S, Frost G. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* 2015; **64**: 1744-1754 [PMID: 25500202]

29 **Mattace Raso G**, Simeoli R, Russo R, Iacono A, Santoro A, Paciello O, Ferrante MC, Canani RB, Calignano A, Meli R. Effects of sodium butyrate and its synthetic amide derivative on liver inflammation and glucose tolerance in an animal model of steatosis induced by high fat diet. *PLoS One* 2013; **8**: e68626 [PMID: 23861927 DOI: 10.1371/journal.pone.0068626]

30 **Jin CJ**, Sellmann C, Engstler AJ, Ziegenhardt D, Bergheim I. Supplementation of sodium butyrate protects mice from the development of non-alcoholic steatohepatitis (NASH). *Br J Nutr* 2015; **114**: 1745-1755 [PMID: 26450277 DOI: 10.1017/S0007114515003621]

31 **Nieuwdorp M**, Gilijamse PW, Pai N, Kaplan LM. Role of the microbiome in energy regulation and metabolism. *Gastroenterology* 2014; **146**: 1525-1533 [PMID: 24560870 DOI: 10.1053/j.gastro.2014.02.008]

32 **Schwiertz A,** Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. Microbiota and SCFA in Lean and Overweight Healthy Subjects. *Obesity* 2010; **18**: 190-195 [DOI: 10.1038/oby.2009.167]

33 **Henagan TM**, Stefanska B, Fang Z, Navard AM, Ye J, Lenard NR, Devarshi PP. Sodium butyrate epigenetically modulates high-fat diet-induced skeletal muscle mitochondrial adaptation, obesity and insulin resistance through nucleosome positioning. *British J Pharmacol* 2015; **172**: 2782-2798 [DOI: 10.1111/bph.13058]

34 **Gao Z**, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* 2009; **58**: 1509-1517 [PMID: 19366864 DOI: 10.2337/db08-1637]

35 **Gastaldelli A**, Harrison SA, Belfort-Aguilar R, Hardies LJ, Balas B, Schenker S, Cusi K. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. *Hepatology* 2009; **50**: 1087-1093 [PMID: 19670459 DOI: 10.1002/hep.23116]

36 **Stenman LK**, Burcelin R, Lahtinen S. Establishing a causal link between gut microbes, body weight gain and glucose metabolism in humans - towards treatment with probiotics. *Benef Microbes* 2015; Epub ahead of print: 1-12 [PMID: 26565087 DOI: 10.3920/bm2015.0069]

37 **Goodrich JK**, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. Human genetics shape the gut microbiome. *Cell* 2014; **159**: 789-799 [PMID: 25417156 DOI: 10.1016/j.cell.2014.09.053]

38 **Nova E**, Pérez de Heredia F, Gómez-Martínez S, Marcos A. The Role of Probiotics on the Microbiota: Effect on Obesity. *Nutr Clin Pract* 2016; **31**: 387-400 [PMID: 26869611 DOI: 10.1177/0884533615620350]

39 **Mishra AK**, Dubey V, Ghosh AR. Obesity: An overview of possible role(s) of gut hormones, lipid sensing and gut microbiota. *Metabolism* 2016; **65**: 48-65 [PMID: 26683796 DOI: 10.1016/j.metabol.2015.10.008]

40 **Kumar A**, Alrefai WA, Borthakur A, Dudeja PK. Lactobacillus acidophilus counteracts enteropathogenic E. coli-induced inhibition of butyrate uptake in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G602-G607 [PMID: 26272259 DOI: 10.1152/ajpgi.00186.2015]

41 **Guilloteau P**, Martin L, Eeckhaut V, Ducatelle R, Zabielski R, Van Immerseel F. From the gut to the peripheral tissues: the multiple effects of butyrate. *Nutr Res Rev* 2010; **23**: 366-384 [PMID: 20937167 DOI: 10.1017/s0954422410000247]

42 **Fernando MR**, Saxena A, Reyes JL, McKay DM. Butyrate enhances antibacterial effects while suppressing other features of alternative activation in IL-4-induced macrophages. *Am J Physiol Gastrointest Liver Physiol* 2016; **310**: G822-G831 [PMID: 27012776 DOI: 10.1152/ajpgi.00440.2015]

43 **Bordin M**, D'Atri F, Guillemot L, Citi S. Histone deacetylase inhibitors up-regulate the expression of tight junction proteins. *Mol Cancer Res* 2004; **2**: 692-701 [PMID: 15634758]

44 **Zhang G**, Ghosh S. Molecular mechanisms of NF-kappaB activation induced by bacterial lipopolysaccharide through Toll-like receptors. *J Endotoxin Res* 2000; **6**: 453-457 [PMID: 11521070]

45 **Kim CH**, Park J, Kim M. Gut microbiota-derived short-chain Fatty acids, T cells, and inflammation. *Immune Netw* 2014; **14**: 277-288 [PMID: 25550694 DOI: 10.4110/in.2014.14.6.277]

46 **Hooper LV**, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012; **336**: 1268-1273 [PMID: 22674334 DOI: 10.1126/science.1223490]

47 **Park J**, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, Kim CH. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* 2015; **8**: 80-93 [PMID: 24917457 DOI: 10.1038/mi.2014.44]

48 **Millard A**, Mertes P, Ittelet D, Villard F, Jeannesson P, Bernard J. Butyrate affects differentiation, maturation and function of human monocyte-derived dendritic cells and macrophages. *Clin Exper Immunol* 2002; **130**: 245-255

49 **Vinolo MA**, Ferguson GJ, Kulkarni S, Damoulakis G, Anderson K, Bohlooly-Y M, Stephens L, Hawkins PT, Curi R. SCFAs induce

mouse neutrophil chemotaxis through the GPR43 receptor. *PLoS One* 2011; **6**: e21205 [PMID: 21698257]

50 **Leonel AJ**, Alvarez-Leite JI. Butyrate: implications for intestinal function. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 474-479 [PMID: 22797568 DOI: 10.1097/MCO.0b013e32835665fa]

51 **O'Hara AM**, Shanahan F. The gut flora as a forgotten organ. *EMBO reports* 2006; **7**: 688-693 [DOI: 10.1038/sj.embor.7400731]

52 **Sommer F**, Bäckhed F. The gut microbiota--masters of host development and physiology. *Nat Rev Microbiol* 2013; **11**: 227-238 [PMID: 23435359 DOI: 10.1038/nrmicro2974]

53 **Rivière A**, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and Butyrate-Producing Colon Bacteria: Importance and Strategies for Their Stimulation in the Human Gut. *Frontiers Microbiol* 2016; **7** [DOI: 10.3389/fmicb.2016.00979]

P- Reviewer: Cardoso CRL **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.76

World J Gastroenterol 2017 January 7; 23(1): 76-86
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

miRNA-133a-UCP2 pathway regulates inflammatory bowel disease progress by influencing inflammation, oxidative stress and energy metabolism

Xi Jin, Dong Chen, Ruo-Heng Zheng, Hong Zhang, Yi-Peng Chen, Zun Xiang

Xi Jin, Hong Zhang, Yi-Peng Chen, Zun Xiang, Department of Gastroenterology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

Dong Chen, Department of Colorectal Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

Ruo-Heng Zheng, Department of Clinical Medicine, Hangzhou Medical College, Hangzhou 310053, Zhejiang Province, China

Author contributions: Jin X and Chen D contributed equally as co-first authors; Jin X and Chen D performed the majority of experiments; Zheng RH, Zhang H and Chen YP performed the qRT-PCR, Western blot, RNAi, and data analysis and participated in the establishment of the animal model; Xiang Z designed the study and wrote the manuscript.

Supported by National Natural Science Foundation of China, No. 81370008 and No. 81000169; and Natural Science Foundation of Zhejiang Province, No. R2110159, No. LY15H030006 and No. LY16H030003.

Institutional review board statement: This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Institutional animal care and use committee statement: The protocol on animal was approved by the institutional review board of the First Affiliated Hospital of Zhejiang University.

Conflict-of-interest statement: There are no conflicts of interest or commercial financial support for this manuscript. The funding sources had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data sharing statement: There is no other date elsewhere.

Manuscript source: Invited manuscript

Correspondence to: Zun Xiang, PhD, Department of Gastroenterology, The First Affiliated Hospital, School of Medicine, Zhejiang University, No. 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. jxfl007@zju.edu.cn
Telephone: +86-571-87236611
Fax: +86-571-87236611

Received: September 5, 2016
Peer-review started: September 6, 2016
First decision: September 28, 2016
Revised: October 9, 2016
Accepted: November 15, 2016
Article in press: November 16, 2016
Published online: January 7, 2017

Abstract

AIM

To investigate the role of the miR-133a-UCP2 pathway in the pathogenesis of inflammatory bowel disease (IBD) and to explore the potential downstream mechanisms with respect to inflammation, oxidative stress and energy metabolism.

METHODS

C57BL/6 mice were fed dextran sulfate sodium (DSS) liquid for 7 consecutive days, followed by the administration of saline to the DSS group, UCP2 siRNA to the UCP2 group and a miR-133a mimic to the miR-133a group on days 8 and 11. Body weight, stool consistency

and rectal bleeding were recorded daily, and these composed the disease activity index (DAI) score for the assessment of disease severity. After cervical dislocation was performed on day 14, the length of the colon in each mouse was measured, and colonic tissue was collected for further study, which included the following: haematoxylin and eosin staining, UCP2 and miR-133a detection by immunohistochemical staining, western blot and quantitative real-time PCR, measurement of apoptosis by TUNEL assay, and the assessment of inflammation (TNF-α, IL-1β, IL-6 and MCP1), oxidative stress (H_2O_2 and MDA) and metabolic parameters (ATP) by ELISA and colorimetric methods.

RESULTS

An animal model of IBD was successfully established, as shown by an increased DAI score, shortened colon length and specific pathologic changes, along with significantly increased UCP2 and decreased miR-133a levels. Compared with the DSS group, the severity of IBD was alleviated in the UCP2 and the miR-133a groups after successful UCP2 knockdown and miR-133a overexpression. The extent of apoptosis, as well as the levels of TNF-α, IL-1β, MDA and ATP, were significantly increased in both the UCP2 and miR-133a groups compared with the DSS group.

CONCLUSION

The miR-133a-UCP2 pathway participates in IBD by altering downstream inflammation, oxidative stress and markers of energy metabolism, which provides novel clues and potential therapeutic targets for IBD.

Key words: miR-133a; Mitochondrial uncoupling protein 2; Inflammatory bowel disease; Dextran sulfate sodium

Core tip: The pathogenesis of inflammatory bowel disease (IBD) is unclear, but increasing evidence supports the involvement of epigenetic regulation such as the formation of miRNA-mRNA pairs. In this study, we investigated the role of the miR-133a-UCP2 pathway in the pathogenesis of IBD in a well-established mouse model. We found that the severity of IBD was alleviated after the UCP2 and miR-133a levels were antagonized and that the underlying mechanism may involve changes in inflammation, oxidative stress and energy metabolism. Our data provide novel clues and potential therapeutic targets for IBD.

Jin X, Chen D, Zheng RH, Zhang H, Chen YP, Xiang Z. miRNA-133a-UCP2 pathway regulates inflammatory bowel disease progress by influencing inflammation, oxidative stress and energy metabolism. *World J Gastroenterol* 2017; 23(1): 76-86 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/76.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.76

INTRODUCTION

Inflammatory bowel disease (IBD) is characterized by chronic, unspecific inflammation of the gastrointestinal tract and may be classified into two major subtypes: Crohn's disease (CD) and ulcerative colitis (UC)[1]. Although the prevalence of IBD is high in western countries, a rapid rise in IBD has been observed in Asia[2], where its prevalence has reached approximately 11.6×10^5 for UC and 1.4×10^5 for CD in China[3]. Although a combination of genetic, environmental, infectious and immunologic factors is considered to be involved in the pathogenesis of IBD, the underlying mechanism is still unclear[4]. Moreover, the effectiveness of routine therapy is discouraging, and aetiology-directed therapy is rare[5]. Therefore, an in-depth investigation of IBD would help reveal the pathogenesis of IBD and provide a novel theoretical base for innovative treatments.

MicroRNAs (miRNAs) are a family of non-coding RNAs that are 19-25 nucleotides (nt) long. They are processed from double-stranded hairpin precursors, which are 70-100 nt in length, by the RNaseIII family member Dicer. Dicer is endogenously expressed in the cytoplasm as part of the RNA-induced silencing complex[6]. miRNAs recognize the 3' untranslated region of target mRNAs with imperfect complementarity, which leads to translational repression in mammals and mRNA cleavage in plants[7]. With the development of high-throughput methods such as microarray and deep sequencing, miRNA profiling has been widely applied to determine molecular biomarkers in tumours[8] and other diseases such as nonalcoholic fatty liver disease (NAFLD)[9,10]. Additionally, miRNA-mRNA pathways have been revealed to participate in various diseases[11]. These features make miRNAs attractive research targets.

The effect and underlying mechanism of miRNAs in the pathogenesis and progression of IBD have become popular research topics. With the use of microarray screening and bioinformatics approaches, researchers have revealed specific tissue[12] and serum[13] miRNA profiles of IBD, which have provided researchers with novel diagnostic biomarkers. Further functional studies have reported the regulation of IBD inflammation by miR-146a[14] and miR-132[15] through the hedgehog and cholinergic pathways. Moreover, the involvement of the miR-224-p21 pathway was found to participate in the early pathogenesis of IBD[16]. However, compared with the large amount of miRNA data from microarray analyses, few miRNA-mRNA pathways have been reported in IBD, which is why this topic is worth more intensive study in the future.

We previously summarized the effect of uncoupling protein (UCP), which belongs to a specific mitochondrial inner membrane protein family with the capacity to uncouple oxidative phosphorylation in NAFLD[17]; we also revealed the effect of liver-specific UCP in

conditions of oxidative stress[18]. Since oxidative stress has been demonstrated to be strongly associated with IBD[19] and previous studies have identified the association between the genetic polymorphism 866G/A of UCP2[20] and IBD, we proposed an important role for UCP2 (the most common and widely studied UCP) in IBD. More importantly, previous studies confirmed the direct regulation of UCP2 by miR-133a[21,22], which highlights the possible effect of the miR-133a-UCP2 pathway in IBD and why this pathway has become the research focus of this study.

MATERIALS AND METHODS

Ethics statement

This study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocol was approved by the institutional review board of the First Affiliated Hospital of Zhejiang University.

Establishment of an animal model of IBD

A total of 50 female C57BL/6 mice aged 8-12 wk were purchased from Shilek Lab Animal (Shanghai, China). All mice received food and water *ad libitum* and were maintained on a 12/12-h light/dark cycle at 25 ℃ for 7 d as part of adaptive feeding. Thereafter, the first 10 mice were randomly divided into two groups to establish an animal model of IBD. Under a common basic diet, the control (n = 5) group received normal liquid, while the DSS (n = 5) group received 3% Dextran Sulfate Sodium (DSS) liquid (MP Biomedicals, Shanghai, China) instead of normal liquid for 7 consecutive days. The body weight, stool consistency and rectal bleeding of each mouse were recorded daily; these constituted the DAI (range from 0 to 12), which was used to assess the clinical severity of colitis, as previously described[23]. After 7 d of feeding, all mice were sacrificed by cervical dislocation, after which the colonic tissue was collected for haematoxylin and eosin (HE) staining and for the detection of the UCP2 and miR-133a levels.

After the animal model of IBD was verified, the remaining mice were further randomly divided into four groups as follows: control (n = 10), DSS (n = 10), UCP2 (n = 10) and miR-133a (n = 10), and the diet of mice in the UCP2 and the miR-133a groups was the same as that of mice in the DSS group. After 7 consecutive days of feeding, an intravenous injection of 100-200 μL saline was given to the control and DSS groups; similarly, UCP2 siRNA was given to mice in the UCP2 group and miR-133a mimics were given to mice in the miR-133a group on days 8 and 11 with the final dose of 33 μg according to the manufacturer's instruction, respectively. There was no anesthesia before intravenous injection. All mice were sacrificed by cervical dislocation on day 14, after which the colonic tissue was collected for HE staining and for the detection of inflammation, oxidative indicators and energy-related markers.

RNA interference and gene over expression

Specific siRNA against the UCP2 gene and a scrambled siRNA, which was used as a negative control, were synthesized at and purchased from GenePharma (Shanghai, China). The mouse miR-133aRNA mimic was also designed at and purchased from GenePharma. The sequence of the negative control (5'-3') was sense-UUCUCCGA ACGUGUCACGUTT and antisense-ACGUGACACGUUCGGAGAATT; the sequence of the miR133a mimic (5'-3') was sense-UUUGGUCCCCUUCAACCAG CUG and antisense-GCUGGUUGAAGGGGACCAAAUU; the sequence of the UCP2 siRNA(5'-3') was sense-GGAAAGGGACUUCUCCCAATT and antisense-UUGGGAGAAGUCCCUUUCCTT. The administration of the siRNA and RNA mimic was performed through intravenous injection using an *in vivo* transfection reagent (Auckland, New Zealand) according to the method of Entranster.

ELISA and TUNEL staining

The mouse colonic tissue was routinely homogenized and harvested for the detection of TNF-α, IL-1β, IL-6 and MCP1 by ELISA according to the manufacturer's instructions (Neobioscience, Shenzhen, China). The level of apoptosis in the colonic tissue was also routinely detected by TUNEL staining according to the manufacturer's instructions (Roche, Shanghai, China), as previously reported[24]. Specifically, the apoptotic cells were dyed and were observed under an Olympus microscope. Typically, 10 visual fields were selected and 100 cells within each field were counted, where the apoptosis index = (apoptosis cell/total cell) × 100%.

Detection of oxidative stress and energy-related markers

The tissues were routinely assessed for the oxidative stress-related markers H_2O_2 and MDA by a colorimetric method using an OD value of 405 nm at 37 ℃ and by a thiobarbituric acid method using an OD value of 532 nm at 95 ℃, respectively. The level of the major energy product ATP was also routinely measured using a colorimetric method and an OD value of 636 nm at 37 ℃. All these experiments were performed according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Immunohistochemical staining

For immunohistochemical staining, the colon tissues collected from the mice were sequentially fixed in 10% formaldehyde solution for at least 24 h, processed and paraffin embedded, cut into 4-μm-thick sections, and mounted onto glass slides. Each slide was deparaffinized and rehydrated in xylene and graded alcohol solutions, respectively, which was followed by antigen retrieval in Citrate Antigen Retrieval solution at 126 ℃ for 40 min. The slides were then washed in PBS and blocked with goat serum for 10 to 30 min

and incubated overnight at 4 ℃ with diluted anti-UCP2 antibody. After three washes in PBS, the slides were incubated with goat-anti-rabbit IgG for 20 min, which was followed by reaction with diaminobenzidine and counterstaining with haematoxylin. The slides were finally mounted in neutral gum for semiquantitative analysis by microscopy. Negative control slides were incubated with PBS instead of the primary antibody.

Western blot and quantitative real-time PCR

For a more accurate analysis, the UCP2 protein level was further quantified by Western blot with a primary mouse polyclonal antibody raised against UCP2 (Abcam, ab77363) and an ECL chemiluminescence kit (Santa Cruz, United States). Normalization was performed by blotting the same samples with a mouse anti-β-actin antibody. For miR-133a quantitative analysis, 2 μg of retrieved total RNA was reverse transcribed using stem-loop antisense primer mix and AMV transcriptase (TaKaRa, Dalian, China). Real-time PCR was routinely performed in an MX3000p real time PCR system (Stratagene, United States). U6 snRNA and GAPDH were amplified as normalization controls, and the relative amount of miR-133a compared with U6 and the relative amount of UCP2 compared with GAPDH were calculated using the equation $2^{-\Delta CT}$, where $\Delta CT = C_{TmiRNA/UCP2} - C_{Tu6/GAPDH}$. The detailed primer sequences are shown in Supplementary Figure 1.

Statistical analysis

Statistical analyses were performed using SPSS version 16 (Chicago, IL, United States). Data are presented as the mean ± standard deviation when normally distributed or as the median if the distribution was skewed. Differences between two groups were analysed using Student's *t*-test or the Mann-Whitney *U* test; differences among four groups were analysed with One-Way Anova.

RESULTS

Altered UCP2 and miR-133a levels in a successfully established IBD mouse model

After the C57BL/6 mice were fed 3% DSS for 7 consecutive days, a representative IBD mouse model was successfully established (Figure 1). Generally, after the first 3 d of DSS feeding, the water and food intake and body weight of mice in the DSS group were slightly increased, while on the 4th day, which was the turning point, the above-mentioned markers had steadily decreased. In addition, the total colon length was also significantly shortened in the DSS group compared with the control group. Finally, HE staining of the colon tissue showed intact colonic mucosa and regularly arranged colonic glands in the control group, whereas destroyed colonic glands, mucosal ulceration and inflammatory cell infiltration were observed in the DSS group. The average DAI for the DSS group was 11.67 on day 7. As shown in Figure 2, we also identified significantly increased UCP2 mRNA and protein levels through qRT-PCR and by a combination of western blot and immunohistochemical staining of colon tissue from mice in the DSS group. Furthermore, a significantly decreased miR-133a level was also detected in the colon tissue of mice in the DSS group by quantitative real-time (qRT)-PCR.

IBD phenotype change after successful alteration of the UCP2 and miR-133a levels

Compared with the DSS group, the severity of IBD was alleviated in the UCP2 and miR-133a groups after successful UCP2 down regulation and miR-133a over expression. Specifically, on day 7, the mice in the DSS, UCP2 and miR-133a groups all experienced significant weight loss, diarrhoea and rectal bleeding. However, as shown in Figure 3A, compared with the continuing weight loss and rectal bleeding in the DSS group, rectal bleeding ceased in the mice in the UCP2 and miR-133a groups on days 10 and 14, respectively. Mice in these two groups also experienced similar significant weight gain by day 14, although they did not recover to the level of the control group. A decrease in the DAI score was achieved (8, 6.8 and 7.2 for the DSS, UCP2 and miR-133a groups, respectively) on day 14, although the differences were not statistically significant. Moreover, compared with the DSS group, the colon tissue from mice in the UCP2 and miR-133a groups demonstrated intact tissue structure, well-organized glands as well as rare mucosal oedema, congestion and inflammatory cell infiltration. In addition, H&E staining revealed that disease alleviation was achieved in the miR-133a group, but the degree of alleviation was less than that in the UCP2 group (Figure 3B). Taken together, the targeting of both UCP2 and miR-133a was able to reduce the severity of IBD, but the targeting of UCP2 was more effective. More intriguingly, compared with the DSS group, the UCP2 level was decreased in the miR-133a group, in which the level of miR133a was up regulated, which indirectly supports the regulatory role of miR-133a on UCP2 (Figure 3C).

Changes in markers of apoptosis and inflammation in the different groups

To explore the potential underlying mechanism of IBD alleviation, we further investigated the changes in apoptosis and other common inflammation-associated markers. We found a significantly increased level of apoptosis in the DSS group compared with the control group, as shown by TUNEL staining. Further UCP2 down regulation and miR-133a over expression could significantly inhibit apoptosis, as indicated by the notable decrease in the brown colour of the cells in Figure 4A. Furthermore, as shown in Figure 4B, the levels of inflammation-associated markers TNF-α, IL-1β, IL-6 and MCP1 were significantly increased in the DSS group compared with the control group. Additionally, although all four inflammation-associated markers

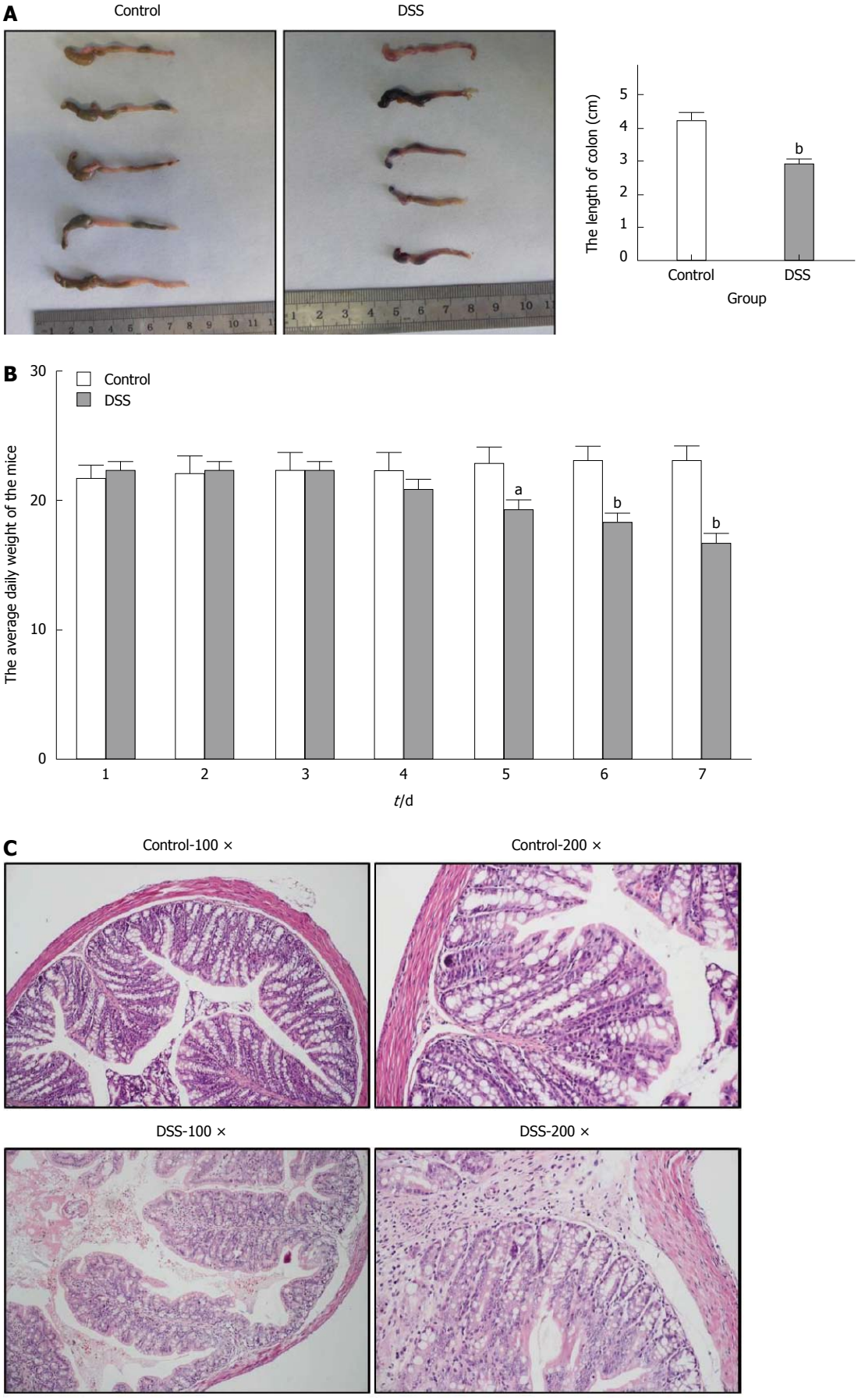


Figure 1 Successful establishment of an inflammatory bowel disease mouse model. A: The overall colon length was significantly shortened in the dextran sulfate sodium (DSS) group; B: The average weight of the mice began to decrease significantly from Day 4 in the DSS group; C: Haematoxylin and eosin staining showed damage to the colon glands, mucosal ulceration and inflammatory cell infiltration in the DSS group; different magnifications are shown. $^{a}P < 0.05$; $^{b}P < 0.01$.

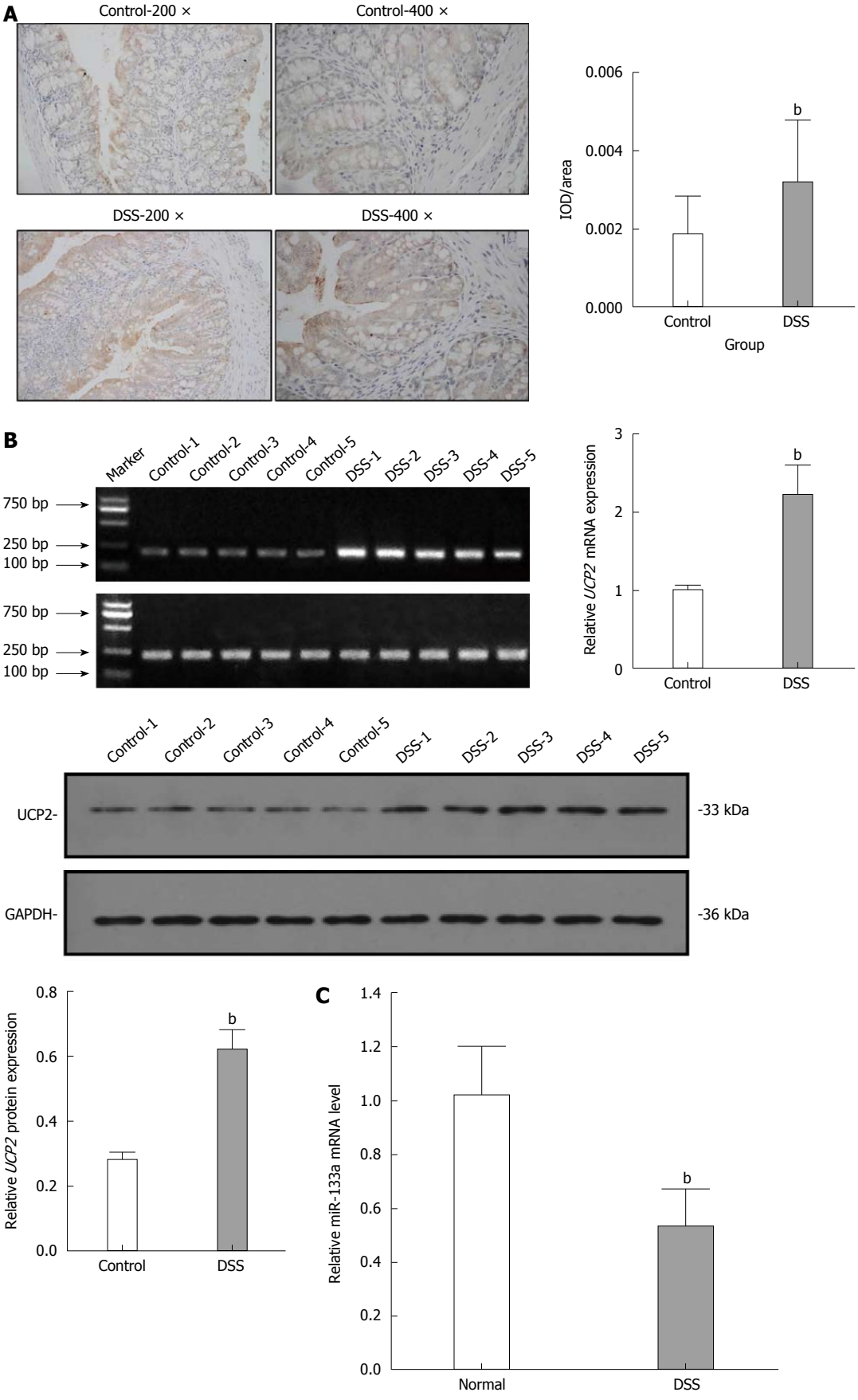


Figure 2 Significantly increased UCP2 and decreased miR-133a levels in the dextran sulfate sodium group. A: Immunohistochemical staining (upper panel) and Western blot (lower panel) showed increased UCP2 expression in the cytoplasm of colons from mice in the dextran sulfate sodium (DSS) group; B: qRT-PCR showed significantly increased UCP2 mRNA in the DSS group; C: qRT-PCR showed significantly decreased miR-133a mRNA in the DSS group. $^{b}P < 0.01$.

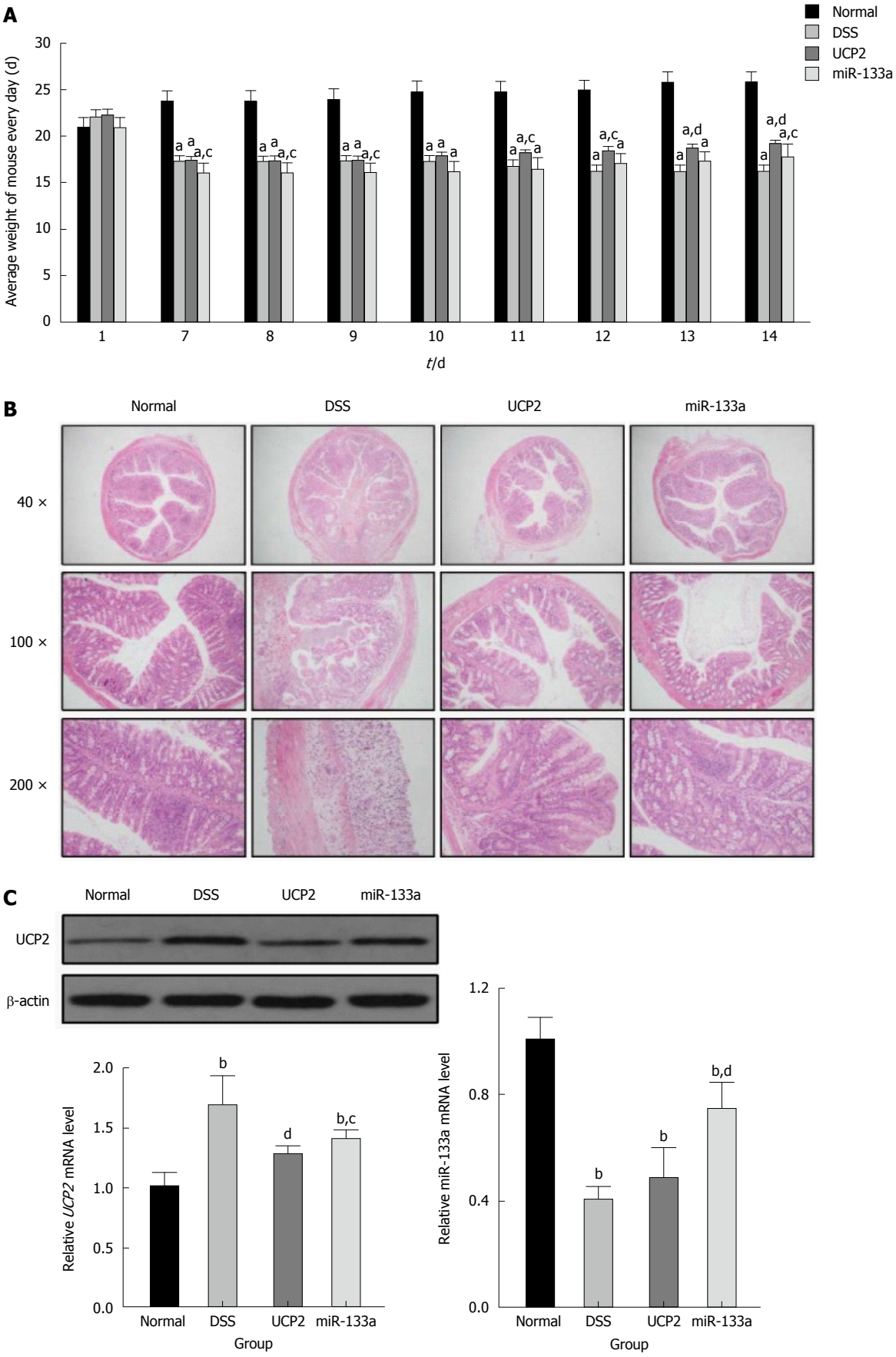


Figure 3 Inflammatory bowel disease phenotype change after the successful alteration of the UCP2 and miR-133a levels. A: Changes in the weight of mice in the control, dextran sulfate sodium (DSS), UCP2 and miR-133a groups between days 7 and 14; B: Alleviation of IBD after the alteration of the UCP2 and miR-133a levels, as demonstrated by HE staining; C: Successful UCP2 down regulation and miR-133a up regulation in the UCP2 and miR-133a groups, as shown by Western blot and qRT-PCR, respectively. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* DSS.

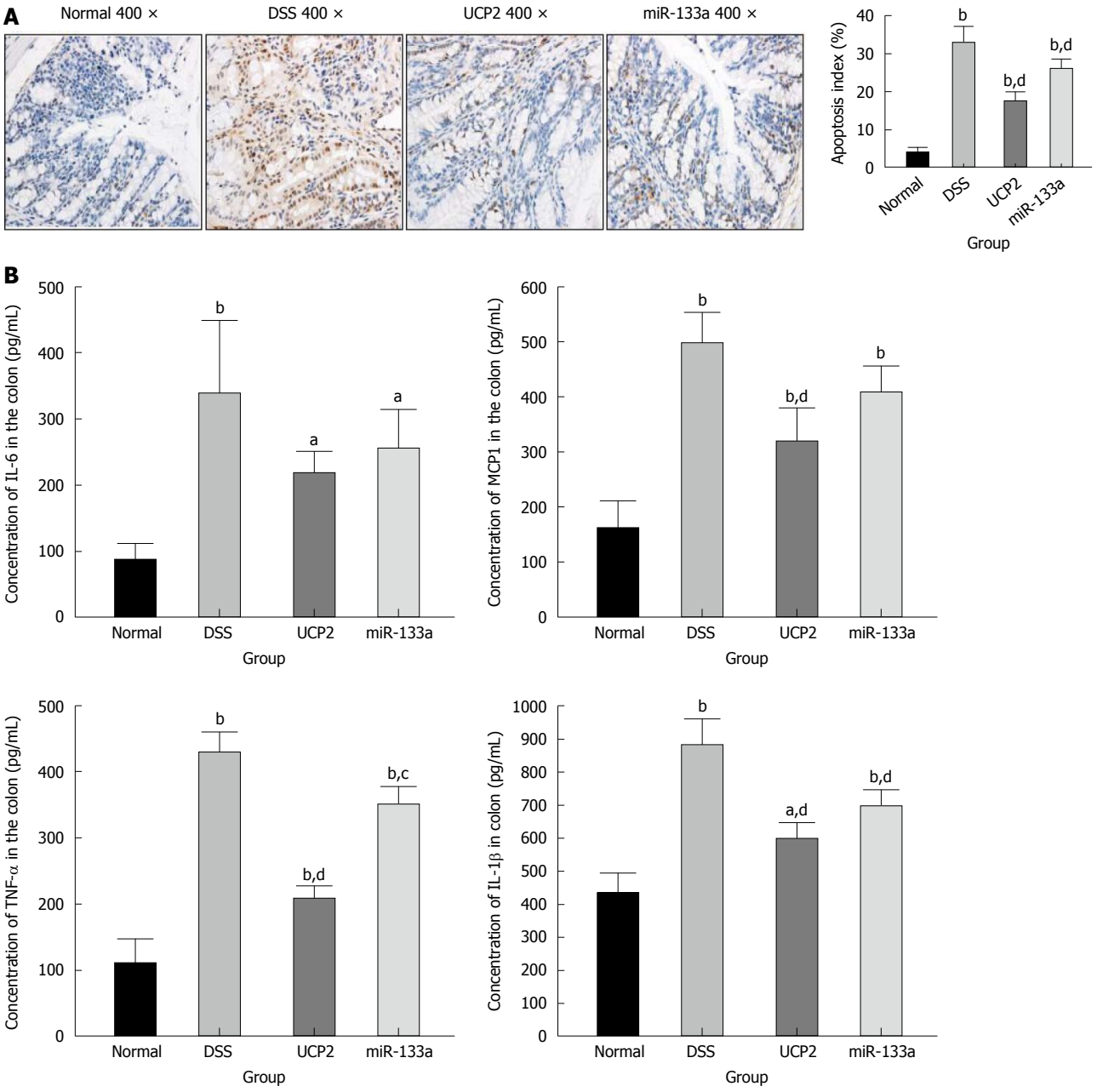


Figure 4 Changes in markers of apoptosis and inflammation in the dextran sulfate sodium, UCP2 and miR-133a groups. A: Changes in apoptosis in the different groups, as shown by dTUNEL staining (brown colour in the cell indicates apoptosis); B: Changes in the levels of IL-6, MCP1, TNF-α and IL-1β in the different groups. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* DSS. DSS: Dextran sulfate sodium.

showed a tendency to decrease, only the differences in the levels of TNF-α, IL-1β, and MCP1 in the UCP2 group and the levels of TNF-α and IL-1β in the miR-133a group reached statistical significance compared with the corresponding levels in the DSS group. Moreover, compared with the DSS group, the degree of declination of all markers was larger in the UCP2 group than in the miR-133a group, which indicates a greater therapeutic effect of UCP2 over miR-133a.

Changes in oxidative stress and energy-related markers in the different groups

Oxidative stress-related markers include MDA and H_2O_2, while ATP is a vital representative of energy metabolism. In this study, we identified a significantly increased MDA level and decreased H_2O_2 and ATP levels in the DSS group compared with the control group. Nevertheless, after UCP2 down regulation or miR-133a over expression, the H_2O_2 and ATP levels were significantly increased, while the MDA level was significantly decreased compared with the DSS group. This may be attributed to the diminished uncoupling of oxidative phosphorylation as a result of the decreased UCP2 level (Figure 5).

DISCUSSION

Recently, IBD has become a global health problem

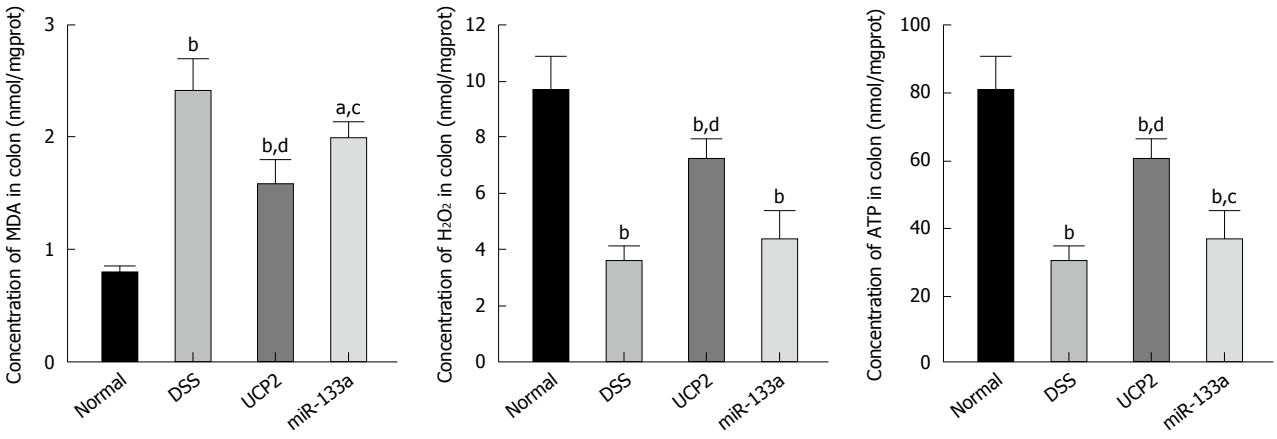


Figure 5 Changes in oxidative stress- and energy-related markers in the dextran sulfate sodium, UCP2 and miR-133a groups. The levels of MDA (left panel), H_2O_2 (middle panel) and ATP (right panel) were tested by a colorimetric method. $^aP < 0.05$, $^bP < 0.01$ *vs* control; $^cP < 0.05$, $^dP < 0.01$ *vs* DSS. DSS: Dextran sulfate sodium.

as its prevalence has gradually increased, which has placed a heavy burden on the economy and has increased the suffering of patients. However, the unclear aetiology of IBD has made effective therapy a huge challenge. Therefore, the exploration of the potential underlying mechanism of IBD is of great clinical importance. The effect of miRNA, which is an important type of non-coding RNA, in IBD was summarized in 2016[25], while novel serum miRNA signatures used in the diagnosis of IBD were also proposed in mice[26] and humans[27]. Additionally, an interesting study established an association between miRNA polymorphisms and the risk of IBD[28]. All these studies support the importance of miRNA in IBD.

It is well acknowledged that UCPs play a pivotal role in various diseases due to their ability to influence energy metabolism, peroxidation and inflammation through the uncoupling of oxidative phosphorylation. A previous study found increased UCP2 expression in colon cancer and identified an association between the level of UCP2 and cancer metastasis[29]. Currently, the effects of specific miRNAs in the pathogenesis of IBD have not been frequently reported, and the role of miRNA-mRNA pathways in IBD has been reported even less frequently. After a literature review, we only found the following: the miR-346-TNF-α pathway influences Vitamin D metabolism[30], the miR-193a-3p-PepT1 pathway reduces intestinal inflammation[31], and the miR-132/miR-223-FOXO3a pathway increases the production of inflammatory cytokines[32]. Intriguingly, the effect of miR-133a in colonic epithelial cells and in experimental colitis was reported[33]. Considering the important role of UCP2 and the direct regulation of UCP2 by miR-133a[21,22,34], it is theoretically reasonable to explore the role of the miR-133a-UCP2 pathway in the pathogenesis of IBD.

In this study, we firstly reported increased UCP2 and decreased miR-133a levels in a successfully established animal model of IBD, which indicates the potential role of miR-133a and UCP2 in IBD. This result was in accordance with the result of a previous study, which supports the protective role of UCP2 in DSS-induced colitis[35]. Secondly, we found an inverse association between UCP2 and miR-133a, and more importantly, miR133a over expression could significantly decrease the UCP2 level. This finding indirectly verified the regulation of UCP2 by miR-133a at the post transcriptional level. Thirdly, with the use of RNA interference and gene over expression, we found that antagonizing both the UCP2 and miR-133a level could alleviate the severity of IBD.

To investigate the potential underlying mechanism and downstream targets of the miR-133a-UCP2 pathway, we selected typical inflammation-, oxidative- and energy metabolism-associated markers. Briefly, TNF-α, IL-1β, IL-6 and MCP1 are classical markers of inflammation with broad applications. TNF-α overproduction has been associated with mucosal damage in IBD, while anti-TNF-α therapy with infliximab is considered the representative biological therapy for IBD[36]. IL-1β and IL-6 belong to the interleukin family, and the immune response they mediate has been tightly linked with IBD progression[37]. MCP1 functions as a monocyte chemoattractant, and its A2518G polymorphism is related to IBD risk[38]. With respect to oxidative stress, MDA is an important product of lipid peroxidation, and its diagnostic value in CD has been reported[39]. H_2O_2 is mainly produced during the process of oxidative phosphorylation and is regarded as a universal mediator of the inflammatory process in the colon[40]. Finally, ATP is a basic energy form used in various pathophysiological processes, and its impaired production due to mitochondrial dysfunction has been identified in the intestines of patients with IBD[41]. Taken together, the results showed that increased apoptosis, inflammation, oxidative stress and ATP depletion were involved as downstream effectors of the miR-133a-UCP2 pathway.

This study has several limitations that should be acknowledged. First, whether the miR-133a-UCP2 pathway in an animal model could be generalized to humans requires further study. Second, it is better

to use UCP2 knockout mice rather than RNAi to obtain more convincing data of the effects of UCP2 in IBD. Third, since novel studies have advocated the "miRNA sponge" effect of circRNA[42], the existence of circRNA as a regulator of miR-133a warrants more in depth studies. Fourth, although it is well known that increased UCP2 can enhance the uncoupling of oxidative phosphorylation, which results in a decrease in ATP and an increase in H_2O_2, the detailed mechanism of the miR-133a-UCP2 pathway on other inflammatory- and oxidative stress-associated markers is still unclear and requires further investigation. Finally, as distinct representatives of oxidative stress, H_2O_2 and MDA demonstrated inverse changes in their levels in the IBD animal model, which calls for further studies of the underlying mechanisms.

Overall, our study revealed the role of the miR-133a-UCP2 pathway in IBD and the potential downstream effectors, including inflammation-, oxidative stress- and energy metabolism-associated markers, which provide new clues in the pathogenesis of IBD and potential targets for disease therapy.

COMMENTS

Background

Inflammatory bowel disease (IBD) has been considered as a chronic and relapsing inflammatory disease that could affect any part of the intestine, with major two subtypes as Crohn's disease and ulcerative colitis. The prevalence of IBD is increasing in developing and developed countries, making it a global health care problem and an interesting research area.

Research frontiers

The mechanism of IBD remains vague; the involvement of genetic predisposition, immune response and environmental factors has been advocated. Currently, the effect of microRNA (miRNA) and uncoupling protein was intensively investigated for their respective effect in epigenomic regulation and uncoupling energy metabolism.

Innovations and breakthroughs

However, the specific miRNA-UCP pathway in the pathogenesis of IBD has not been reported hitherto. In this study, the authors investigated the role of the miR-133a-UCP2 pathway in the pathogenesis of IBD and to explore the potential downstream mechanisms with respect to inflammation, oxidative stress and energy metabolism.

Applications

The miR-133a-UCP2 pathway participates in IBD by altering downstream inflammation, oxidative stress and markers of energy metabolism, which provides novel clues and potential therapeutic targets for IBD.

Terminology

miRNA belongs to a family of non-coding RNAs that are 19-25 nucleotides (nt) long. They are processed from double-stranded hairpin precursors, which are 70-100 nt in length, by the RNaseIII family member Dicer.

Peer-review

The present study shows an investigation of the miR-133a-UCP2 pathway in the pathogenesis of IBD. For this used an adequate methodology and experimental conditions. With the found results in this study the author concluded that miR-133a-UCP2 pathway participates of IBD influencing the inflammatory process and this pathway could be a potential therapeutic targets for IBD. The manuscript is clear and well written.

REFERENCES

1 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078 [PMID: 19923578 DOI: 10.1056/NEJMra0804647]

2 **Engel MA**, Khalil M, Neurath MF. Highlights in inflammatory bowel disease--from bench to bedside. *Clin Chem Lab Med* 2012; **50**: 1229-1235 [PMID: 23024984]

3 **Ouyang Q**, Hu PJ, Qian JM, Zheng JJ, Hu RW. Consensus on the management of inflammatory bowel disease in China in 2007. *J Dig Dis* 2008; **9**: 52-62 [PMID: 18251795 DOI: 10.1111/j.1443-9573.2007.00320.x]

4 **Zhang YZ**, Li YY. Inflammatory bowel disease: pathogenesis. *World J Gastroenterol* 2014; **20**: 91-99 [PMID: 24415861 DOI: 10.3748/wjg.v20.i1.91]

5 **Mao R**, Hu PJ. The Future of IBD Therapy: Where Are We and Where Should We Go Next? *Dig Dis* 2016; **34**: 175-179 [PMID: 26982457 DOI: 10.1159/000443135]

6 **Lau NC**, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. *Science* 2001; **294**: 858-862 [PMID: 11679671 DOI: 10.1126/science.1065062]

7 **Ambros V**. The functions of animal microRNAs. *Nature* 2004; **431**: 350-355 [PMID: 15372042 DOI: 10.1038/nature02871]

8 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1997]

9 **Jin X**, Chen YP, Kong M, Zheng L, Yang YD, Li YM. Transition from hepatic steatosis to steatohepatitis: unique microRNA patterns and potential downstream functions and pathways. *J Gastroenterol Hepatol* 2012; **27**: 331-340 [PMID: 21793903 DOI: 10.1111/j.1440-1746.2011.06864.x]

10 **Jin X**, Ye YF, Chen SH, Yu CH, Liu J, Li YM. MicroRNA expression pattern in different stages of nonalcoholic fatty liver disease. *Dig Liver Dis* 2009; **41**: 289-297 [PMID: 18922750 DOI: 10.1016/j.dld.2008.08.008]

11 **Moreno-Moya JM**, Vilella F, Simón C. MicroRNA: key gene expression regulators. *Fertil Steril* 2014; **101**: 1516-1523 [PMID: 24314918 DOI: 10.1016/j.fertnstert.2013.10.042]

12 **Clark PM**, Dawany N, Dampier W, Byers SW, Pestell RG, Tozeren A. Bioinformatics analysis reveals transcriptome and microRNA signatures and drug repositioning targets for IBD and other autoimmune diseases. *Inflamm Bowel Dis* 2012; **18**: 2315-2333 [PMID: 22488912 DOI: 10.1002/ibd.22958]

13 **Paraskevi A**, Theodoropoulos G, Papaconstantinou I, Mantzaris G, Nikiteas N, Gazouli M. Circulating MicroRNA in inflammatory bowel disease. *J Crohns Colitis* 2012; **6**: 900-904 [PMID: 22386737 DOI: 10.1016/j.crohns.2012.02.006]

14 **Ghorpade DS**, Sinha AY, Holla S, Singh V, Balaji KN. NOD2-nitric oxide-responsive microRNA-146a activates Sonic hedgehog signaling to orchestrate inflammatory responses in murine model of inflammatory bowel disease. *J Biol Chem* 2013; **288**: 33037-33048 [PMID: 24092752 DOI: 10.1074/jbc.M113.492496]

15 **Maharshak N**, Shenhar-Tsarfaty S, Aroyo N, Orpaz N, Guberman I, Canaani J, Halpern Z, Dotan I, Berliner S, Soreq H. MicroRNA-132 modulates cholinergic signaling and inflammation in human inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 1346-1353 [PMID: 23598815 DOI: 10.1097/MIB.0b013e318281f47d]

16 **Olaru AV**, Yamanaka S, Vazquez C, Mori Y, Cheng Y, Abraham JM, Bayless TM, Harpaz N, Selaru FM, Meltzer SJ. MicroRNA-224 negatively regulates p21 expression during late neoplastic progression in inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 471-480 [PMID: 23399735 DOI: 10.1097/MIB.0b013e31827e78eb]

17 **Jin X**, Xiang Z, Chen YP, Ma KF, Ye YF, Li YM. Uncoupling protein and nonalcoholic fatty liver disease. *Chin Med J* (Engl) 2013; **126**: 3151-3155 [PMID: 23981628]

18 **Jin X**, Yang YD, Chen K, Lv ZY, Zheng L, Liu YP, Chen SH, Yu CH, Jiang XY, Zhang CY, Li YM. HDMCP uncouples yeast mitochondrial respiration and alleviates steatosis in L02 and hepG2 cells by decreasing ATP and H2O2 levels: a novel mechanism for NAFLD. *J Hepatol* 2009; **50**: 1019-1028 [PMID: 19303656 DOI: 10.1016/j.jhep.2008.10.034]

19 **Zhu H**, Li YR. Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence. *Exp Biol Med* (Maywood) 2012; **237**: 474-480 [PMID: 22442342 DOI: 10.1258/ebm.2011.011358]

20 **Yu X**, Wieczorek S, Franke A, Yin H, Pierer M, Sina C, Karlsen TH, Boberg KM, Bergquist A, Kunz M, Witte T, Gross WL, Epplen JT, Alarcón-Riquelme ME, Schreiber S, Ibrahim SM. Association of UCP2 -866 G/A polymorphism with chronic inflammatory diseases. *Genes Immun* 2009; **10**: 601-605 [PMID: 19387457 DOI: 10.1038/gene.2009.29]

21 **Bandyopadhyay S**, Lane T, Venugopal R, Parthasarathy PT, Cho Y, Galam L, Lockey R, Kolliputi N. MicroRNA-133a-1 regulates inflammasome activation through uncoupling protein-2. *Biochem Biophys Res Commun* 2013; **439**: 407-412 [PMID: 23988448 DOI: 10.1016/j.bbrc.2013.08.056]

22 **Chen X**, Wang K, Chen J, Guo J, Yin Y, Cai X, Guo X, Wang G, Yang R, Zhu L, Zhang Y, Wang J, Xiang Y, Weng C, Zen K, Zhang J, Zhang CY. In vitro evidence suggests that miR-133a-mediated regulation of uncoupling protein 2 (UCP2) is an indispensable step in myogenic differentiation. *J Biol Chem* 2009; **284**: 5362-5369 [PMID: 19073597 DOI: 10.1074/jbc.M807523200]

23 **Fitzpatrick LR**, Wang J, Le T. In vitro and in vivo effects of gliotoxin, a fungal metabolite: efficacy against dextran sodium sulfate-induced colitis in rats. *Dig Dis Sci* 2000; **45**: 2327-2336 [PMID: 11258552]

24 **Yamada S**, Koyama T, Noguchi H, Ueda Y, Kitsuyama R, Shimizu H, Tanimoto A, Wang KY, Nawata A, Nakayama T, Sasaguri Y, Satoh T. Marine hydroquinone zonarol prevents inflammation and apoptosis in dextran sulfate sodium-induced mice ulcerative colitis. *PLoS One* 2014; **9**: e113509 [PMID: 25409433 DOI: 10.1371/journal.pone.0113509]

25 **Soubières AA**, Poullis A. Emerging Biomarkers for the Diagnosis and Monitoring of Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2016; **22**: 2016-2022 [PMID: 27416044 DOI: 10.1097/mib.0000000000000836]

26 **Viennois E**, Zhao Y, Zhang M, Han M, Merlin D. O-016 YI Longitudinal Study of Circulating miRNA Biomarkers in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22** Suppl 1: S6 [PMID: 26849733 DOI: 10.1097/01.mib.0000480113.11943.30]

27 **Hübenthal M**, Hemmrich-Stanisak G, Degenhardt F, Szymczak S, Du Z, Elsharawy A, Keller A, Schreiber S, Franke A. Sparse Modeling Reveals miRNA Signatures for Diagnostics of Inflammatory Bowel Disease. *PLoS One* 2015; **10**: e0140155 [PMID: 26466382 DOI: 10.1371/journal.pone.0140155]

28 **Zhu M**, Li D, Jin M, Li M. Association between microRNA polymorphisms and the risk of inflammatory bowel disease. *Mol Med Rep* 2016; **13**: 5297-5308 [PMID: 27109937 DOI: 10.3892/mmr.2016.5157]

29 **Kuai XY**, Ji ZY, Zhang HJ. Mitochondrial uncoupling protein 2 expression in colon cancer and its clinical significance. *World J Gastroenterol* 2010; **16**: 5773-5778 [PMID: 21128330]

30 **Chen Y**, Du J, Zhang Z, Liu T, Shi Y, Ge X, Li YC. MicroRNA-346 mediates tumor necrosis factor α-induced downregulation of gut epithelial vitamin D receptor in inflammatory bowel diseases. *Inflamm Bowel Dis* 2014; **20**: 1910-1918 [PMID: 25192497 DOI: 10.1097/mib.0000000000000158]

31 **Dai X**, Chen X, Chen Q, Shi L, Liang H, Zhou Z, Liu Q, Pang W, Hou D, Wang C, Zen K, Yuan Y, Zhang CY, Xia L. MicroRNA-193a-3p Reduces Intestinal Inflammation in Response to Microbiota via Down-regulation of Colonic PepT1. *J Biol Chem* 2015; **290**: 16099-16115 [PMID: 25931122 DOI: 10.1074/jbc.M115.659318]

32 **Kim HY**, Kwon HY, Ha Thi HT, Lee HJ, Kim GI, Hahm KB, Hong S. MicroRNA-132 and microRNA-223 control positive feedback circuit by regulating FOXO3a in inflammatory bowel disease. *J Gastroenterol Hepatol* 2016; **31**: 1727-1735 [PMID: 26878986 DOI: 10.1111/jgh.13321]

33 **Law IK**, Pothoulakis C. MicroRNA-133α regulates neurotensin-associated colonic inflammation in colonic epithelial cells and experimental colitis. *RNA Dis* 2015; **2**: pii: e472 [PMID: 26005712 DOI: 10.14800/rd.472]

34 **Yuan Y**, Yao YF, Hu SN, Gao J, Zhang LL. MiR-133a Is Functionally Involved in Doxorubicin-Resistance in Breast Cancer Cells MCF-7 via Its Regulation of the Expression of Uncoupling Protein 2. *PLoS One* 2015; **10**: e0129843 [PMID: 26107945 DOI: 10.1371/journal.pone.0129843]

35 **Zhang H**, Kuai XY, Yu P, Lin L, Shi R. Protective role of uncoupling protein-2 against dextran sodium sulfate-induced colitis. *J Gastroenterol Hepatol* 2012; **27**: 603-608 [PMID: 21871020 DOI: 10.1111/j.1440-1746.2011.06879.x]

36 **Komaki Y**, Komaki F, Sakuraba A, Cohen R. Approach to Optimize Anti-TNF-α Therapy in Patients With IBD. *Curr Treat Options Gastroenterol* 2016; **14**: 83-90 [PMID: 26872815 DOI: 10.1007/s11938-016-0079-x]

37 **Magyari L**, Kovesdi E, Sarlos P, Javorhazy A, Sumegi K, Melegh B. Interleukin and interleukin receptor gene polymorphisms in inflammatory bowel diseases susceptibility. *World J Gastroenterol* 2014; **20**: 3208-3222 [PMID: 24695754 DOI: 10.3748/wjg.v20.i12.3208]

38 **Li YW**, Yang CQ, Xiao YL, Li J, Xie CX, Zhang SH, Yu Q, Wang HL, Lu WM, Chen MH. The -A2518G polymorphism in the MCP-1 gene and inflammatory bowel disease risk: A meta-analysis. *J Dig Dis* 2015; **16**: 177-185 [PMID: 25620350 DOI: 10.1111/1751-2980.12232]

39 **Boehm D**, Krzystek-Korpacka M, Neubauer K, Matusiewicz M, Paradowski L, Gamian A. Lipid peroxidation markers in Crohn's disease: the associations and diagnostic value. *Clin Chem Lab Med* 2012; **50**: 1359-1366 [PMID: 22868800 DOI: 10.1515/cclm-2011-0817]

40 **Te Velde AA**, Pronk I, de Kort F, Stokkers PC. Glutathione peroxidase 2 and aquaporin 8 as new markers for colonic inflammation in experimental colitis and inflammatory bowel diseases: an important role for H2O2? *Eur J Gastroenterol Hepatol* 2008; **20**: 555-560 [PMID: 18467915 DOI: 10.1097/MEG.0b013e3282f45751]

41 **Novak EA**, Mollen KP. Mitochondrial dysfunction in inflammatory bowel disease. *Front Cell Dev Biol* 2015; **3**: 62 [PMID: 26484345 DOI: 10.3389/fcell.2015.00062]

42 **Wilusz JE**, Sharp PA. Molecular biology. A circuitous route to noncoding RNA. *Science* 2013; **340**: 440-441 [PMID: 23620042 DOI: 10.1126/science.1238522]

P- Reviewer: Coelho T, Lakatos PL, Witaicenis A **S- Editor**: Yu J
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.87

World J Gastroenterol 2017 January 7; 23(1): 87-92
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Hydrogen sulfide attenuates gastric mucosal injury induced by restraint water-immersion stress *via* activation of K_{ATP} channel and NF-κB dependent pathway

Hong-Zhao Sun, Shan Zheng, Kai Lu, Feng-Tian Hou, Jie-Xue Bi, Xue-Lian Liu, Shan-Shan Wang

Hong-Zhao Sun, Shan Zheng, Kai Lu, Feng-Tian Hou, Jie-Xue Bi, Xue-Lian Liu, Shan-Shan Wang, College of Life Science, Qi Lu Normal University, Zhangqiu 250200, Jinan, Shandong Province, China

Author contributions: Sun HZ and Zheng S contributed equally to this work; Sun HZ conceived and designed the experiments; Zheng S, Lu K, Hou FT, Bi JX, Liu XL and Wang SS performed the experiments; Sun HZ and Zheng S analyzed the data; Sun HZ contributed reagents/materials/analysis tools; Sun HZ wrote the paper.

Supported by Natural Science Foundation of Shandong Province, No. ZR2015CL016 and No. ZR2011CL012; and Colleges and Universities of Shandong Province Science and Technology Plan Projects, No. J11LC17.

Institutional review board statement: The study was reviewed and approved by the Qi Lu Normal University Institutional Review Board.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Qi Lu Normal University (IACUC protocol No. 14276).

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Hong-Zhao Sun, Associate Professor, College of Life Science, Qi Lu Normal University, No. 2, Wenbo Road, Zhangqiu 250200, Jinan, Shandong Province, China. sunhongzhao18@126.com
Telephone: +86-531-66778211
Fax: +86-531-66778037

Received: August 16, 2016
Peer-review started: August 16, 2016
First decision: October 11, 2016
Revised: October 19, 2016
Accepted: November 15, 2016
Article in press: November 16, 2016
Published online: January 7, 2017

Abstract

AIM

To explore the effect of hydrogen sulfide (H_2S) on restraint water-immersion stress (RWIS)-induced gastric lesions in rats and the influence of adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway on such an effect.

METHODS

Male Wistar rats were randomly divided into a control group, a physiological saline (PS) group, a sodium hydrosulfide (NaHS) group, a glibenclamide (Gl) group, Gl plus NaHS group, a pyrrolidine dithiocarbamate (PDTC) group, and a PDTC plus NaHS group. Gastric mucosal injury was induced by RWIS for 3 h in rats, and gastric mucosal damage was analyzed after that. The PS, NaHS (100 μmol/kg body weight), Gl (100 μmol/kg body weight), Gl (100 μmol/kg or 150 μmol/kg body weight) plus NaHS (100 μmol/kg body weight), PDTC (100 μmol/kg body weight), and PDTC (100 μmol/kg body weight) plus NaHS (100 μmol/kg body

weight) were respectively injected intravenously before RWIS.

RESULTS

RWIS induced serious gastric lesions in the rats in the PS pretreatment group. The pretreatment of NaHS (a H_2S donor) significantly reduced the damage induced by RWIS. The gastric protective effect of the NaHS during RWIS was attenuated by PDTC, an NF-κB inhibitor, and also by glibenclamide, an ATP-sensitive potassium channel blocker, in a dose-dependent manner.

CONCLUSION

These results suggest that exogenous H_2S plays a protective role against RWIS injury in rats, possibly through modulation of K_{ATP} channel opening and the NF-κB dependent pathway.

Key words: Hydrogen sulfide; Nuclear factor kappa B; Gastric mucosal injury; Restraint water-immersion stress; Adenosine triphosphate-sensitive potassium

Core tip: In this study, the authors demonstrate that exogenous hydrogen sulfide plays a protective role against restraint water-immersion stress injury in rats possibly through modulation of adenosine triphosphate-sensitive potassium channel opening and the nuclear factor kappa-light-chain-enhancer of activated B cells dependent pathway.

Sun HZ, Zheng S, Lu K, Hou FT, Bi JX, Liu XL, Wang SS. Hydrogen sulfide attenuates gastric mucosal injury induced by restraint water-immersion stress *via* activation of K_{ATP} channel and NF-κB dependent pathway. *World J Gastroenterol* 2017; 23(1): 87-92 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/87.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.87

INTRODUCTION

Restraint water-immersion stress (RWIS), considered to be a mixture of physical and psychological stress, can induce anxiety, hypothermia, and severe gastric dysfunction including gastric hypercontractility, gastric acid hypersecretion, and gastric mucosal lesions within a few hours[1-4]. This model is used to study the mechanism of gastric mucosal lesions induced by stress and filter drugs in clinical trials. As is well known, not only is gastric stress ulcera common complication in patients with clinical critical disease, but also the number of primary gastric stress ulcers seen continues to increase with the fierce competition and pressure common in modern society. Thus, clarifying the mechanism that causes gastric mucosal lesions as a result of RWIS and looking for ways to reduce these lesions are very important to prevent and cure gastric stress damage in the clinical setting.

Recent studies suggest that hydrogen sulfide (H_2S) is the third gaseous mediator in mammals after nitric oxide (NO) and carbon monoxide (CO) and that it regulates a range of physiological and pathological processes in the nervous system, cardiovascular system, respiratory system, and digestive system, and regulates metabolism and immunity, *etc*[5-11].

Recent studies on rats suggest that H_2S can protect the gastric mucosa, possibly through mechanisms that involve anti-oxidant and anti-inflammatory actions[12], but the effect of H_2S on gastric mucosa damage induced by RWIS still needs further research. Previous reports have shown that H_2S regulates a range of physiological and pathological processes involving K_{ATP} channels. Hydrogen sulfide has been shown to protect gastric epithelial cells from ischemia-reperfusion injury by Keap1 S-sulfhydration, mitogen-activated protein kinase (MAPK) dependent anti-apoptosis, and the NF-κB dependent anti-inflammation pathway[13]. Therefore, in this study, we evaluated the effect of H_2S on RWIS-induced gastric lesions in rats and the influence of K_{ATP} channels and the NF-κB dependent pathway on this effect.

MATERIALS AND METHODS

Animal and drug preparation

Experiments were performed on male Wistar rats (220-280 g) purchased from the Experimental Animal Center of Shandong University. Animals were maintained in a temperature-controlled environment with a 12-h light/dark cycle. They were allowed free access to food and water for one week. Prior to the experiments, the animals were fasted for 24 h but allowed free access to water. All procedures performed were according to the guidelines of the International Association for the Study of Pain[14] and were approved by the Experimental Animal Ethical Association in Qi Lu Normal University.

Chemicals used and their sources were as follows[15]: sodium hydrosulfide (NaHS, 100 μmol/kg body weight), glibenclamide (Gl, 100 or 150 μmol/kg body weight), and pyrrolidine dithiocarbamate (PDTC, 100 μmol/kg body weight), purchased from Sigma (Saint Louis, MO, United States). NaHS and PDTC were dissolved in 0.9% saline, but Gl was dissolved in dimethyl sulfoxide. All chemicals were injected intraperitoneally (IP) before inducing RWIS.

Experimental group and protocol

The rats were randomly divided into 7 groups with 13 rats per group: (1) in the control group, the rats were not stressed under otherwise identical conditions; (2) in the physiological saline (PS) group, the rats were given RWIS for 3 h after pretreatment with IP injection of PS; (3) in the NaHS group, the rats were given RWIS for 3 h after pretreatment with IP injection of

Figure 1 Representative of the degree of gastric mucosal damage induced by restraint water-immersion stress. A: Representative of the degree of gastric mucosal damage in the control group; B: Representative of the degree of gastric mucosal damage in the physiological saline group; C: Representative of the degree of gastric mucosal damage in the NaHS group.

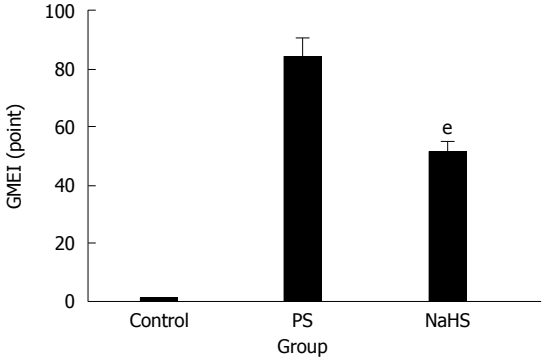


Figure 2 Gastric mucosal erosion index of the control, physiological saline and NaHS groups. $^{e}P < 0.001$. GMEI: Gastric mucosal erosion index; PS: Physiological saline.

NaHS (100 μmol/kg body weight); (4) in the Gl group, the rats were given RWIS for 3 h after pretreatment with IP injection of Gl (100 μmol/kg body weight); (5) in the Gl plus NaHS group, the rats were given RWIS for 3 h after pretreatment with IP injection of Gl (100 or 150 μmol/kg body weight) and NaHS; (6) in the PDTC group, the rats were given RWIS for 3 h after pretreatment with IP injection of PDTC (100 μmol/kg body weight); and (7) in the PDTC plus NaHS group, the rats were given RWIS for 3 h after pretreatment with IP injection of PDTC (100 μmol/kg body weight) and NaHS.

In the RWIS groups, after light ether anesthesia, the four limbs of each rat were gently bound on a wooden board securely using medical adhesive tape. After the rats were conscious, they were vertically immersed in cold water (21 ℃ ± 1 ℃) to the level of the xiphoid for 3 h. All of the experiments were terminated by a bolus IP injection of sodium pentobarbital (100 mg/kg body weight). Then the abdomen of each rat was opened, and the stomach was removed and fixed with 1% formalin. The gastric lesions were examined with a light microscope, and a scoring system was used to assess the gastric mucosal erosion index (GMEI) of each rat[16]. Scores were given according to the length of lesions: ≤ 1 mm = 1 point, 1 to ≤ 2 mm = 2 points, and so on. The score was multiplied by 2 when the damage was more than 1 mm in width. The cumulative scores of all lesions in a rat served as the GMEI of that rat.

Statistical analysis

All values were analyzed using SPSS13.0 software (SPSS Inc.) and presented as mean ± SE. Statistical analysis was performed by the Student *t*-test. Significance was accepted at the level of $P < 0.05$.

RESULTS

Effect of NaHS on RWIS-induced gastric mucosal injury

The mucosal surface of the control group was smooth, and no significant abnormality in the gastric mucosa was observed under the light microscope (Figure 1A). However, significant hemorrhage and edema and several erosions of varying depths and sizes were observed on the surface of the mucosa of the RWIS groups (Figure 1B and C). The GMEI was 1.54 ± 0.27 points in the control group, 84.38 ± 6.34 points in the PS group (compared with the control group, $P < 0.001$), and 51.23 ± 4.08 points in the NaHS group (compared with the control group, $P < 0.001$) (Figure 2).

Compared with the PS group, the injury area and the extent of mucosal damage significantly decreased in the NaHS group (Figure 1B and C). The GMEI in the NaHS group was obviously lower than that in the PS group (51.23 ± 4.08 points *vs* 84.38 ± 6.34 points, $P < 0.001$) (Figure 2).

Gl prevented the protective effect of NaHS on RWIS-induced gastric mucosal injury in a dose-dependent manner

The gastric protective effect of NaHS during RWIS was abolished by Gl, an ATP-sensitive potassium channel K_{ATP} blocker (Figure 3). Under the light microscope, the GMEIs in the Gl (100 μmol/kg body weight) plus NaHS group and the Gl (150 μmol/kg body weight) plus NaHS group were much higher than those in the NaHS

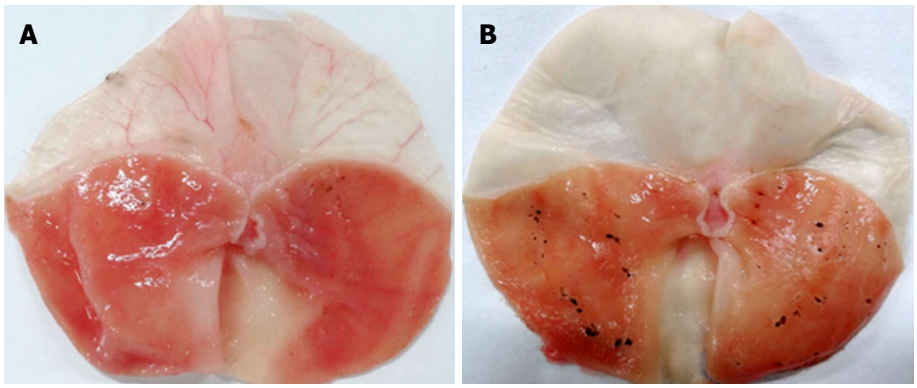


Figure 3 Representative of the degree of gastric mucosal damage in the NaHS group (A) and the glibenclamide + NaHS group (B).

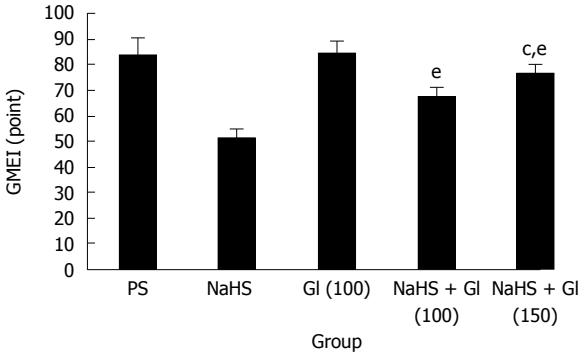


Figure 4 Gastric mucosal erosion index in the NaHS group and the glibenclamide + NaHS group. Gl + NaHS group *vs* the NaHS group, $^{e}P < 0.001$; Gl (100 μmol/kg body weight) plus NaHS group *vs* the Gl (150 μmol/kg body weight) + NaHS group, $^{c}P < 0.05$. GMEI: Gastric mucosal erosion index; PS: Physiological saline; Gl: Glibenclamide.

group (67.92 ± 4.63 points and 76.92 ± 4.71 points *vs* 51.23 ± 4.08 points, $P < 0.001$). The GMEI in the Gl (100 μmol/kg body weight) plus NaHS group was lower than that in the Gl (150 μmol/kg body weight) plus NaHS group ($P < 0.05$) (Figure 4). These results suggest that Gl prevented the protective effect of NaHS on RWIS-induced gastric mucosal injury in a dose-dependent manner.

PDTC weakened the protective effect of NaHS on RWIS-induced gastric mucosal injury

The gastric protective effect of NaHS during RWIS was weakened by PDTC, an NF-κB inhibitor (Figure 5). Under the light microscope, the GMEI in the PDTC (100 μmol/kg body weight) plus NaHS group was higher than that in the NaHS group (65.00 ± 4.01 points *vs* 51.23 ± 4.08 points, $P < 0.001$) (Figure 6). These results suggest that PDTC weakened the protective effect of NaHS on RWIS-induced gastric mucosal injury.

DISCUSSION

In this study, significant hemorrhage and edema and several erosions of varying depths and sizes were observed on the surface of the mucosa in the RWIS groups. The occurrence of RWIS-induced gastric mucosal erosion is possibly related to a number of factors, including excessive production of oxygen free radicals in the mucosa[12,17], leukocyte infiltration[18], decreased release of nitric oxide[19], gastric hypercontractility, gastric acid hypersecretion, and gastric mucosa ischemia caused by a reduction in the gastric mucosal blood flow.

H_2S is formed in mammalian cells by the activity of two pyridoxal phosphate-dependent enzymes: cystathionine-γ-1yase and cystathionine-β-synthase[20]. NaHS, as a H_2S donor, dissociates in vivo into sodium ions and sulfhydryl group ions, and the latter bind with hydrogen ions to generate H_2S. Thus, H_2S and NaHS are in dynamic equilibrium[21]. Previous work has demonstrated that H_2S has anti-inflammatory and antioxidant activities[22]. The gastroprotective effect of endogenous H_2S against gastric ischemia-reperfusion injury may be mediated by enhancing the anti-oxidative capacity through increasing glutathione and superoxide dismutase to reduce free radical production[21]. In this study, NaHS significantly attenuates gastric mucosal injury induced by restraint water-immersion stress. We surmise that the mechanism is possibly through anti-oxidant and anti-inflammatory actions.

Previous reports showed that ATP-sensitive potassium K_{ATP} channels regulate a range of physiological and pathological processes. Dawe *et al*[23] found that H_2S in the hypothalamus decreases blood pressure and heart rate by a K_{ATP} channel-dependent mechanism in freely moving rats. Data support the hypothesis that endogenous H_2S produces cardiovascular inhibition functions in the nucleus of solitary tract, mainly mediated by K_{ATP} channel regulation or/and glutamate receptors[24]. Exogenous H_2S plays a protective role against gastric ischemia-reperfusion injury in rats possibly through modulation of K_{ATP} channel opening[25]. Here, we have shown that Gl, an ATP-sensitive potassium channel blocker, reversed the protective effect of NaHS on RWIS-induced gastric damage in a dose-dependent manner. These results suggest that H_2S plays a protective role against gastric RWIS injury in rats, possibly through

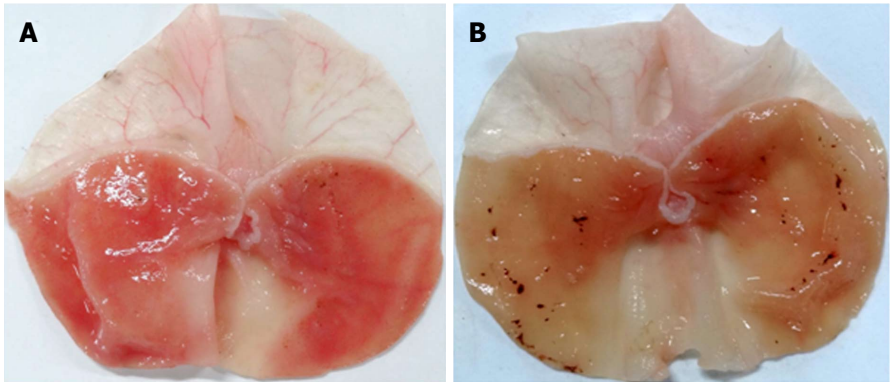


Figure 5 Representative of the degree of gastric mucosal damage in the NaHS group (A) and pyrrolidine dithiocarbamate + NaHS group (B).

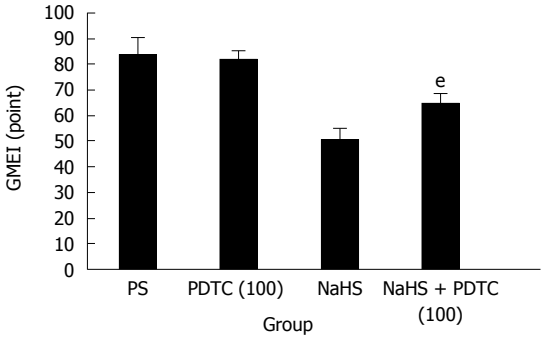


Figure 6 Gastric mucosal erosion index in the NaHS group and PDTC + NaHS group. $^{e}P < 0.001$. GMEI: Gastric mucosal erosion index; PDTC: Pyrrolidine dithiocarbamate; PS: Physiological saline.

modulation of K_{ATP} channel opening mechanisms. Data have demonstrated that the H_2S-induced relaxation of mesenteric artery beds was mediated by ATP-sensitive K^+ (K_{ATP}) channel activity in vascular smooth muscle cells[26]. Therefore, we speculate that on the one hand, H_2S, by opening K_{ATP} channels, relaxes gastric mucosal blood vessels and increases gastric mucosal blood flow. This reduces the damage caused by RWIS by accelerating the removal of harmful substances. On the other hand, H_2S, by opening the K_{ATP} channels, increases the K^+ efflux, which attenuates RWIS-induced gastric mucosal injury by hyperpolarizing the oxyntic cell membrane to reduce gastric acid secretion.

H_2S is a small gas molecule, which can freely pass through a variety of biological membranes, target a wide range, which may affect multiple signaling pathways such as mitogen-activated protein kinase (MAPK) signaling pathways, NF-κB signal through-road, and phosphoinositide 3-kinase (PI3K) and its downstream molecules, serine/threonine protein kinase AKT (PI3K/AKT)[27,28]. Hydrogen sulfide protected gastric epithelial cells from ischemia-reperfusion injury by Keap1 s-sulfhydration, MAPK dependent anti-apoptosis, and the NF-κB dependent anti-inflammation pathway[13]. Our study results show that PDTC, an NF-κB inhibitor, reversed the protective effect of NaHS on RWIS-induced gastric damage, which suggests that H_2S plays a protective role against gastric RWIS injury in rats, possibly through an NF-κB dependent anti-inflammation mechanism.

In conclusion, the results of this study suggest that H_2S plays a protective role against RWIS-induced gastric mucosal injury in rats, possibly through modulation of K_{ATP} channel opening and through the NF-κB dependent pathway.

COMMENTS

Background

Recent studies suggest that hydrogen sulfide (H_2S) is the third gaseous mediator in mammals after nitric oxide and carbon monoxide and that it modulates a range of physiological and pathological processes. H_2S has been found throughout the gastrointestinal tract, but little is known about the effect of H_2S on restraint water-immersion stress (RWIS)-induced gastric lesions in rats and the influence of adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels and the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway on such an effect.

Research frontiers

Previous reports have shown that H_2S is a small gas molecule, which may not only regulate a range of physiological and pathological processes involving K_{ATP} channels, but also affect multiple signaling pathways, such as mitogen-activated protein kinase signaling pathways, NF-κB signal through-road, and phosphoinositide 3-kinase and its downstream molecules serine/threonine protein kinase. In this study, the authors demonstrate that exogenous H_2S plays a protective role against RWIS injury in rats possibly through modulation of K_{ATP} channel opening and the NF-κB dependent pathway.

Innovations and breakthroughs

This is the first study to report that exogenous H_2S plays a protective role against RWIS injury in rats possibly through modulation of K_{ATP} channel opening and the NF-κB dependent pathway.

Applications

This study may provide a future strategy for therapeutic intervention in case of stress gastric lesions by helping understand the mechanism of action of H_2S on RWIS-induced gastric lesions.

Terminology

In the gastrointestinal tract, cystathionine-β-synthase and cystathionine-γ-lyase are mainly responsible for endogenous H_2S synthesis. H_2S is involved in gastric motility, gastric acid secretion, and gastric mucosal injury.

Peer-review

This is a well written and planned study demonstrating the protective effects of H_2S in gastric stress lesions in rats. The protective effects of H_2S seem to arise from modulation of K_{ATP} channel and the NF-κB dependent pathway.

REFERENCES

1 **Mersereau WA**, Hinckley EJ. Hypothermia-induced gastric hypercontracility in the genesis of the restraint ulcer. *Can J Surg* 1981; **24**: 622-625 [PMID: 7326627]

2 **Arai I**, Muramatsu M, Aihara H. Body temperature dependency of gastric regional blood flow, acid secretion and ulcer formation in restraint and water-immersion stressed rats. *Jpn J Pharmacol* 1986; **40**: 501-504 [PMID: 3735801 DOI: 10.1254/jjp.40.501]

3 **Ai HB**, Zhang ZD. Studies on the mechanism of gastric mucosal injury induced by water-immersion stress in rats. *Sheng Li Xue Bao* 1990; **42**: 496-502 [PMID: 1703664]

4 **Ephgrave KS**, Cullen JJ, Broadhurst K, Kleiman-Wexler R, Shirazi SS, Schulze-Delrieu K. Gastric contractions, secretions and injury in cold restraint. *Neurogastroenterol Motil* 1997; **9**: 187-192 [PMID: 9347475 DOI: 10.1046/j.1365-2982.1997.d01-42.x]

5 **Geng B**, Cui Y, Zhao J, Yu F, Zhu Y, Xu G, Zhang Z, Tang C, Du J. Hydrogen sulfide downregulates the aortic L-arginine/nitric oxide pathway in rats. *Am J Physiol Regul Integr Comp Physiol* 2007; **293**: R1608-R1618 [PMID: 17634203 DOI: 10.1152/ajpregu.00207.2006]

6 **Kimura Y**, Goto Y, Kimura H. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxid Redox Signal* 2010; **12**: 1-13 [PMID: 19852698 DOI: 10.1089/ars.2008.2282]

7 **Sowmya S**, Swathi Y, Yeo AL, Shoon ML, Moore PK, Bhatia M. Hydrogen sulfide: regulatory role on blood pressure in hyperhomocysteinemia. *Vascul Pharmacol* 2010; **53**: 138-143 [PMID: 20685250 DOI: 10.1016/j.vph.2010.05.004]

8 **Tamizhselvi R**, Moore PK, Bhatia M. Inhibition of hydrogen sulfide synthesis attenuates chemokine production and protects mice against acute pancreatitis and associated lung injury. *Pancreas* 2008; **36**: e24-e31 [PMID: 18437075 DOI: 10.1097/MPA.0b013e31816857bb]

9 **Webb GD**, Lim LH, Oh VM, Yeo SB, Cheong YP, Ali MY, El Oakley R, Lee CN, Wong PS, Caleb MG, Salto-Tellez M, Bhatia M, Chan ES, Taylor EA, Moore PK. Contractile and vasorelaxant effects of hydrogen sulfide and its biosynthesis in the human internal mammary artery. *J Pharmacol Exp Ther* 2008; **324**: 876-882 [PMID: 18029544 DOI: 10.1124/jpet.107.133538]

10 **Zhang H**, Zhi L, Moochhala SM, Moore PK, Bhatia M. Endogenous hydrogen sulfide regulates leukocyte trafficking in cecal ligation and puncture-induced sepsis. *J Leukoc Biol* 2007; **82**: 894-905 [PMID: 17599903 DOI: 10.1189/jlb.0407237]

11 **Fu Z**, Liu X, Geng B, Fang L, Tang C. Hydrogen sulfide protects rat lung from ischemia-reperfusion injury. *Life Sci* 2008; **82**: 1196-1202 [PMID: 18482739 DOI: 10.1016/j.lfs.2008.04.005]

12 **Aboubakr EM**, Taye A, El-Moselhy MA, Hassan MK. Protective effect of hydrogen sulfide against cold restraint stress-induced gastric mucosal injury in rats. *Arch Pharm Res* 2013; **36**: 1507-1515 [PMID: 23812778 DOI: 10.1007/s12272-013-0194-3]

13 **Guo C**, Liang F, Shah Masood W, Yan X. Hydrogen sulfide protected gastric epithelial cell from ischemia/reperfusion injury by Keap1 s-sulfhydration, MAPK dependent anti-apoptosis and NF-κB dependent anti-inflammation pathway. *Eur J Pharmacol* 2014; **725**: 70-78 [PMID: 24444438 DOI: 10.1016/j.ejphar.2014.01.009]

14 **Zimmermann M**. Ethical considerations in relation to pain in animal experimentation. *Acta Physiol Scand* 1986; **128**: 221-233 [PMID: 11649998]

15 **Fiorucci S**, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, Orlandi S, Zanardo R, Renga B, Di Sante M, Morelli A, Cirino G, Wallace JL. Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. *Gastroenterology* 2005; **129**: 1210-1224 [PMID: 16230075 DOI: 10.1053/j.gastro.2005.07.060]

16 **Guth PH**, Aures D, Paulsen G. Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prostaglandin, cimetidine, and probanthine. *Gastroenterology* 1979; **76**: 88-93 [PMID: 361495]

17 **Kwiecień S**, Brzozowski T, Konturek SJ. Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. *J Physiol Pharmacol* 2002; **53**: 39-50 [PMID: 11939718]

18 **Andrews FJ**, Malcontenti-Wilson C, O'Brien PE. Polymorphonuclear leukocyte infiltration into gastric mucosa after ischemia-reperfusion. *Am J Physiol* 1994; **266**: G48-G54 [PMID: 8304457]

19 **Wada K**, Kamisaki Y, Ohkura T, Kanda G, Nakamoto K, Kishimoto Y, Ashida K, Itoh T. Direct measurement of nitric oxide release in gastric mucosa during ischemia-reperfusion in rats. *Am J Physiol* 1998; **274**: G465-G471 [PMID: 9530146]

20 **Moore PK**, Bhatia M, Moochhala S. Hydrogen sulfide: from the smell of the past to the mediator of the future? *Trends Pharmacol Sci* 2003; **24**: 609-611 [PMID: 14654297 DOI: 10.1016/j.tips.2003.10.007]

21 **Cui J**, Liu L, Zou J, Qiao W, Liu H, Qi Y, Yan C. Protective effect of endogenous hydrogen sulfide against oxidative stress in gastric ischemia-reperfusion injury. *Exp Ther Med* 2013; **5**: 689-694 [PMID: 23403765 DOI: 10.3892/etm.2012.870]

22 **Mard SA**, Neisi N, Solgi G, Hassanpour M, Darbor M, Maleki M. Gastroprotective effect of NaHS against mucosal lesions induced by ischemia-reperfusion injury in rat. *Dig Dis Sci* 2012; **57**: 1496-1503 [PMID: 22271414 DOI: 10.1007/s10620-012-2051-5]

23 **Dawe GS**, Han SP, Bian JS, Moore PK. Hydrogen sulphide in the hypothalamus causes an ATP-sensitive K+ channel-dependent decrease in blood pressure in freely moving rats. *Neuroscience* 2008; **152**: 169-177 [PMID: 18201837 DOI: 10.1016/j.neuroscience.2007.12.008]

24 **Qiao W**, Yang L, Li XY, Cao N, Wang WZ, Chai C, Lu Y. The cardiovascular inhibition functions of hydrogen sulfide within the nucleus tractus solitarii are mediated by the activation of KATP channels and glutamate receptors mechanisms. *Pharmazie* 2011; **66**: 287-292 [PMID: 21612157]

25 **Zou JH**, Qiao WL, Wang GM, Ma HJ, Qi YJ, Sun H, Yan CD. [Exogenous hydrogen sulfide attenuates gastric ischemia-reperfusion injury via activation of K(ATP) channel]. *Sheng Li Xue Bao* 2012; **64**: 27-32 [PMID: 22348957]

26 **Cheng Y**, Ndisang JF, Tang G, Cao K, Wang R. Hydrogen sulfide-induced relaxation of resistance mesenteric artery beds of rats. *Am J Physiol Heart Circ Physiol* 2004; **287**: H2316-H2323 [PMID: 15191893 DOI: 10.1152/ajpheart.00331.2004]

27 **Calvert JW**, Coetzee WA, Lefer DJ. Novel insights into hydrogen sulfide--mediated cytoprotection. *Antioxid Redox Signal* 2010; **12**: 1203-1217 [PMID: 19769484 DOI: 10.1089/ars.2009.2882]

28 **Bos EM**, Snijder PM, Jekel H, Weij M, Leemans JC, van Dijk MC, Hillebrands JL, Lisman T, van Goor H, Leuvenink HG. Beneficial effects of gaseous hydrogen sulfide in hepatic ischemia/reperfusion injury. *Transpl Int* 2012; **25**: 897-908 [PMID: 22716165 DOI: 10.1111/j.1432-2277.2012.01514.x]

P- Reviewer: Kim JS **S- Editor**: Yu J
L- Editor: A **E- Editor**: Liu WX

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.93

World J Gastroenterol 2017 January 7; 23(1): 93-102
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Role of mast cell-miR-490-5p in irritable bowel syndrome

Hai-Xia Ren, Fa-Can Zhang, He-Sheng Luo, Guo Zhang, Lie-Xin Liang

Hai-Xia Ren, He-Sheng Luo, Department of Gastroenterology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China

Fa-Can Zhang, Guo Zhang, Lie-Xin Liang, Department of Gastroenterology, Renmin Hospital of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Author contributions: Zhang FC, Luo HS, Zhang G and Liang LX designed this research; Ren HX performed the research; Ren HX, Zhang FC and Luo HS analyzed the data; Ren HX wrote the paper.

Supported by the National Natural Science Foundation of China, No. 81160053 (to Zhang FC).

Institutional review board statement: The study was reviewed and approved by the People's Hospital of Wuhan University and People's Hospital of Guangxi Zhuang Autonomous Region Institutional Review Board.

Conflict-of-interest statement: We declare that we have no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: He-Sheng Luo, MD, Professor, Department of Gastroenterology, Renmin Hospital of Wuhan University, No. 238, Jiefang Road, Wuchang District, Wuhan 430060, Hubei Province, China. xhnk@163.com
Telephone: +86-27-97718831
Fax: +86-27-97718831

Received: July 13, 2016
Peer-review started: July 17, 2016
First decision: August 19, 2016
Revised: September 5, 2016
Accepted: September 14, 2016
Article in press: September 14, 2016
Published online: January 7, 2017

Abstract

AIM

To determine the functional role of miR-490-5p in mast cell proliferation and apoptosis, and in the mast cell tryptase/PAR-2 signal pathway.

METHODS

The 3rd generation of lentivirus vector systems containing enhanced green fluorescent protein (EGFP) (Ruisai Inc., Shanghai, China), which acts as a reporter gene was used to construct the mmu-miR-490-5p lentivirus expression vector pEGFP-antagomiR-490-5p, and the lentivirus vector pEGFP-negative was used as a negative control. The stably transfected mast cell line p815 was then constructed. GFP positive cells were successfully transfected cells. We determined the expression of miR-490-5p in p815 mast cells before and after transfection using quantitative real-time PCR (qRT-PCR). In addition, after transduction with the lentivirus vectors, the role of miR-490-5p in mast cell proliferation and apoptosis was investigated using the CCK-8 assay and flow cytometry, respectively. The mRNA levels of tryptase and PAR-2 were detected by qRT-PCR and the protein levels were detected by Western blot.

RESULTS

The inhibition of miR-490-5p expression promoted apoptosis and inhibited proliferation of p815 mast cells. The mRNA levels of tryptase and PAR-2 were significantly increased after transfection compared

with the control group, tryptase (P = 0.721, normal *vs* null; P = 0.001, siRNA *vs* normal; P = 0.002, siRNA *vs* null) and PAR-2 (P = 0.027, siRNA *vs* null; P = 0.353, normal *vs* null; P = 0.105, siRNA *vs* normal). The protein levels of tryptase and PAR2 were slightly higher in the siRNA group than those in the control group, but the difference was not statistically significant (P > 0.05).

CONCLUSION

miR-490-5p plays a vital role in the pathogenesis of irritable bowel syndrome by affecting mast cell proliferation and apoptosis; with down-regulation of miR-490-5p, the mRNA level of mast cell tryptase and PAR-2 increased, and the protein level increased, but the difference was not statistically significant.

Key words: miR-490-5p; Mast cell tryptase; PAR-2; Irritable bowel syndrome

Core tip: Mast cells and mast cell degranulation play important roles in the pathophysiology of irritable bowel syndrome. miRNA is a class of important endogenous single-stranded non-coding RNA, and plays an essential regulatory role in complex biological systems without protein translation. In the present study, we aimed to determine the role of miR-490-5p in regulating mast cell proliferation and apoptosis, the expression of mast cell tryptase and PAR-2, and thus predict the role of miR-490-5p in the pathogenesis of irritable bowel syndrome.

Ren HX, Zhang FC, Luo HS, Zhang G, Liang LX. Role of mast cell-miR-490-5p in irritable bowel syndrome. *World J Gastroenterol* 2017; 23(1): 93-102 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/93.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.93

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal (GI) disorders. The cause of IBS is unknown and seems to be multifactorial[1-4]. Several mechanisms including visceral hypersensitivity, motility disorder, infection, and psychiatric factors, have been suggested as possible etiologic links to the development of IBS[5,6]. In addition, there is increasing evidence of a genetic contribution in IBS such as gene polymorphism[7-9] and dysregulated microRNA (miRNA) expression[10-14]. Mast cells and mast cell degranulation function as a bridge in the neuro-immuno-endocrine system in IBS[15]. Tryptase is one of the most important proteins in mast cell degranulation and it exerts its effects mainly through the tryptase-PAR-2 signal pathway[16]. However, considering the complexity of the expression and function of tryptase, there are obvious limitations in the single analysis of the signal pathway. Multiple factors are involved in the regulation of the above signaling pathway. Among these factors, intracellular concentration of Ca^{2+}, calmodulin, DAG/PKC and Rho GTPase have been noted to affect mast cell degranulation[17,18]. miRNA is a an important endogenous single-stranded non-coding RNA, which plays an essential regulatory role in complex biological systems without protein translation. Mature miRNA down-regulates gene expression by binding to the 3'-UTR region of the target gene and causes translational inhibition or mRNA degradation ultimately moderating the protein expression level. To date, multiple dysregulated microRNA expression has been reported in IBS. For example, miRNA-29a affects intestinal membrane permeability through its regulation of the glutamate-ammonia ligase gene and miRNA-510 plays an important role in the regulation of 5-HT3E expression[19,20]. Previously, we detected an elevation of miR-490-5p in diarrhea predominant IBS (IBS-D) patients using high-throughput microarray, but miR-490-5p has not been investigated in IBS. TargetScan was used to predict the target gene of miR-490-5p, and we noted the following: *CABP5*: encoding calcium binding protein5; *CAMK1D*: encoding calcium/calmodulin-dependent protein kinase ID; *CASP3*: encoding caspase 3, apoptosis-related cysteine peptidase; TNFSF18: encoding tumor necrosis factor (ligand) superfamily, member 18; *BOP1*: encoding proliferation-associated protein. Of these, both calcium and calmodulin-dependent protein kinase were highly correlated with mast cell degranulation[17,18]. Caspase, apoptosis-related cysteine peptidase and proliferation associated protein have been reported to be involved in cell proliferation and apoptosis in other diseases[21]. However, there are no studies available concerning the relationship between these factors and IBS. By comprehensively analyzing the results of gene microarray and bioinformatics, we focused our studies on the expression and functional role of miR-490-5p in mast cells and the mast cell/tryptase/PAR-2 signaling pathway, and then to predict whether and how miR-490-5p was involved in the pathogenesis of IBS. The results of this study may provide a theoretical basis for further study of biomarkers for the diagnosis and new treatment of IBS.

MATERIALS AND METHODS

Cell line

The p815 mast cell line was purchased from Shanghai Cell Biochemical Institute, China Academy of Science (Shanghai, China). This cell line is a suitable transfection host. P815 cells phagocytose latex beads, but not zymosan or BCG. They do not function in antibody-dependent cell-mediated cytotoxicity. Growth of these cells is not inhibited by dextran sulfate,

lipopolysaccharide (LPS) or tuberculin purified protein derivative (PPD). The cells were also found to be negative for the ectromelia virus (mousepox).

Reagents

Mast cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), phosphate-buffered saline (PBS), trypsin, penicillin/streptomycin combination, miR-490-5p-siRNA vector, siRNA negative lentiviral vector, blasticidin, RIPA lysate [50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton X-100 (v/v)], protease inhibitor cocktail, 1 mmol/L sodium vanadate and 10 mmol/L NaF. DNA marker, agarose, EB substitute, Trizol and cDNA reverse transcription kit were obtained from Takara. Polymerase chain reaction (PCR) primers were synthesized by Sangon Biotechnology (Shanghai, China). SuperReal PreMix Plus was purchased from Tiangen Biotechnology (Beijing, China). Mouse anti-GAPDH monoclonal antibody for western blot was obtained from Cell Signaling Technology (Danvers, MA, United States). Anti-PAR-2 monoclonal antibody for western blot was obtained from Abcam (Shanghai, China). Antitryptase clonal antibody for western blot was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, United States).

Screening for target miRNA

Based on the results of a previous high-throughput microarray and bioinformatics analysis, TargetScan analysis (http://www.targetscan.org) was used to predict the target gene of the miRNA. A GO enrichment analysis and a KEGG pathway analysis of the target gene were subsequently performed. Based on the results of the bioinformatics analysis, we searched miRDB and miRBase to query the specific features of target genes of interest. Our selection criteria for the target miRNA were as follows: (1) it is abnormally expressed in IBS-D patients[22]; (2) the biological function of the target gene should be related to mast cell degranulation, proliferation and apoptosis or the signal transduction pathway or visceral sensitivity or neurotransmitter-release associated protein or intracellular material transportation-associated protein, as the products of these target genes may correlate highly with the pathogenesis of IBS[23]; and (3) it exists both in human and mouse mast cells. Finally, we choose miR-490-5p as our target miRNA.

miR-490-5p RNA interference in mast cells

The 3rd generation of a lentivirus vector system containing an expression vector and 3 packaging auxiliary plasmids was used to construct the miR-490-5p recombinant silencing vector. The expression vector contained the basic components of HIV 5 'LTR and 3' LTR as well as other auxiliary components, CMV promoter, blasticidin markers and enhanced green fluorescent protein (EGFP). Packaging auxiliary plasmid contained pGag-Pol, PRev and pVSVG. According to the mouse mmu-miR-490-5p (MIMAT0017261) gene sequence in the miBase database (5'-CCAUGGAUCUCCAGGUGGGU-3'), a pair of oligonucleotide chains containing the miR-490-5p sequence and the reverse complementary miR-145 sequence, which can form an shRNA precursor sequence containing a stem-loop structure after annealing, were designed. (Top strand: 5'-TGCTGACCCACCTGGAGATCCATGGGTTTTGGCCACTGACTGACCCATGGATCCAGGTGGGT-3' Bottom strand: 5'-CCTGACCCACCTGGATCCATGGGTCAGTCAGTGGCCAAAACCCATGGATCTCCAGGTGGGTC-3'). The shRNA was then connected with the linearized miRNA vector, the product was used to transform *Escherichia coli* DH5α, and the recombinant plasmids were extracted and analyzed by sequencing, and named pcDNA6.2-EGFP-mmu-490-5p, which was then used as a template to amplify the following primers:

(Lenti-Asc1-F: 5'-TACTGGCGCGCCGCCACCATGGTGAGCAAGGGCGAGGA-3';

Lenti-Pme1-R: 5'-ACTAGTTTAAACTGCGGCCAGATCTGGGC-3').

The pLV-shRNA lentiviral expression vector was generated *via* T4 DNA ligase, and an unrelated negative control sequence was established in the same manner. The above protocol was completed by Shanghai RS Biotechnology Co., Ltd (Shanghai, China).

HEK-293T cells (5×10^4; ATCC, Maryland, United States) were seeded in a 6-well cell culture plate in DMEM and incubated at 37 ℃ with 5% CO_2 for 24 h. When the cells reached 50%-70% confluence, pLV-shRNA plasmids and auxiliary plasmids were co-transfected into them using Lipo3000. After 48 h of transfection, the cell supernatant was collected for titer determination. Gradient dilution of the supernatant was performed using PBS at ratios of 10^{-1}-10^{-6}. Three wells were used for each gradient, and 50 μL of lentiviral diluent was placed in each well for infection. After 48 h of infection, we recorded the number of infected fluorescent cells at the dilution gradient where the ratio of green fluorescent protein $(GFP)^+$ cells was approximately 20%, and mean values were calculated. Lentiviral titers were calculated according to the following formula (BT = TU/mL): TU/μL = (P × N/100 × V) × 1/DF (P = number of GFP^+ cells, N = 10^5, V = volume of lentiviral diluent = 50 μL, DF = dilution factor).

Construction of the stably transfected cell line

Growing mast cells were seeded in 24-well plates (30000 cells per well). After adherence and the cell density reached 50%, the cells were transduced with recombinant lentivirus vectors at a multiplicity of infection (MOI) of 60. The cells were maintained at 37 ℃ in a humidified atmosphere of 5% CO_2 for 24 h, and the medium was then changed. After 48 h of

Table 1 Primers for mmu-miR-490-5p and U6

mmu-miR-490-5p	Forward	TGGCGGCCATGGATCTCCAG
	RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCAC
	Reverse	ATCCAGTGCAGGGTCCGAGG
U6	Forward	CGCTTCACGAATTTGCGTGTGTCAT
	Reverse	GCTTCGGCAGCACATATACTAAAAT

Table 2 Primers for real-time polymerase chain reaction

Gene	Forward/reverse primer	PCR product, bp	Gene bank No.
Tryptase	GCCTCTCCCACCTCCTTATC/GGTATTTCCAGCACACAGCA	145	NM_010781.3
PAR-2	AGTTCTCTGCGTCCATCCTC/GGGTGTTTCTTCTTCGTTCG	139	NM_007974.4
U6	CGCTTCACGAATTTGCGTGTGTCAT/ATCCAGTGCAGGGTCCGAGG	106	NM-0012042741

transfection, the transfection efficiency was assessed by inverted fluorescence microscopy. Thereafter, positively transfected cells were selected by adding blasticidin to the medium at the minimal effective dose of 6 μg/mL (the minimal effective dose was defined as the dose at which most of the cells died within 7 d). The efficiency of GFP expression was detected by flow cytometry. When the expression efficiency of GFP was more than 85%, the dose of blasticidin was replaced by half of the minimal effective dose.

Detection of the miR-490-5p level after silencing

Total RNA was extracted both from normal cells and stably transfected cells using Trizol (Roche, Mannheim, Germany) according to the manufacturer's instructions. Complementary DNA (cDNA) templates were synthesized using the PrimeScript™ RT reagent Kit (Takara, Dalian, China) after the concentration and purity of total RNA were measured by NanoDrop. The expression level of miR-490-5p was measured by qRT-PCR. The primers used in RT-PCR are listed in Table 1.

Cell proliferation assay

The cell counting kit-8 (CCK-8) assay was used to examine cell proliferation. A standard curve was constructed, and the cells were then washed three times with PBS, resuspended in PBS, counted, and diluted 1:2 geometrically. The cells were seeded in 96-well plates at different final concentrations (3×10^4, 6×10^4, 12×10^4, 24×10^4, 48×10^4 per mL) in assay medium and incubated at 37 ℃ in a 5% CO_2 incubator for approximately 2-4 h. Next, 10 μL of CCK-8 was added to each well, and the cells were incubated at 37 ℃. At different time points (1 h, 2 h, 3 h, 4 h), the absorbance at 490 nm was read on a GF-M3000 microplate reader (Gaomicaihong Analysis Instrument Company, Shandong, China). The absorbance was used as the Y-axis, and the cell number was used as the X-axis to draw a standard curve and the optimal number of cells and the time of detection were determined. In addition, using the standard curve, we assessed whether CCK-8 was suitable for determining the level of proliferation of mast cells. In the second step, we washed, resuspended, and counted the cells as described above. The cells were then seeded in 96-well plates at a final concentration of 3×10^4 per mL in assay medium and incubated at 37 ℃ in a 5% CO_2 incubator. At different time points (24 h, 48 h, 72 h), 10 μL of CCK-8 was added to each well, and the cells were incubated at 37 ℃ for another 4 h. The absorbance at 490 nm was read on a microplate reader.

Annexin V-PE/7-AAD double-staining flow cytometry for the detection of apoptosis

Cell apoptosis was detected by flow cytometry (FCM) and analyzed by CellQuest software (Becton Dickinson, Bedford, MA, United States). The transfected and normal cells were collected, washed three times with cool PBS, and then resuspended in PBS. 1×10^6 cells were then processed for labeling with Annexin V/7AAD according to the PE Annexin V apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, United States).

Real-time polymerase chain reaction

Real-time PCR was used to detect the mRNA level of tryptase and PAR-2 in p815 mast cells after transfection. Total RNA was extracted from both normal cells and stably transfected cells using Trizol. Total RNA was transcribed using the reverse transcription kit after measuring the concentration of total RNA with Nano drop. Total RNA was quantified to 1 μg. Information regarding the primers of tryptase, PAR-2 and the endogenous control U6 are listed in Table 2. The PCR samples were prepared in a volume of 20 μL, containing 2 μL of cDNA diluted 1:5 with PCR grade water, 10.4 μL of SYBR Green Supermix, and 300 nmol/L each of the forward and reverse primers. The PCR conditions consisted of preliminary denaturation at 95 ℃ for 15 min, followed by 40 cycles at 95 ℃ for 10 s, and 60 ℃ for 32 s. Melting curve analysis was performed to confirm the specificity and the integrity of the PCR products by the presence of a single peak. Products were subjected to agarose

gel electrophoresis. Expression levels of mRNA were quantified by calculating threshold cycle values compared with the U6 endogenous control using 2-DDCt.

Western blot

Total protein was extracted from p815 mast cells and lysed in RIPA lysate [50 mmol/L Tris (pH 7.4), 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton X-100 (v/v), 1 × protease inhibitor cocktail, 1 mmol/L sodium vanadate, and 10 mmol/L NaF]. Equal amounts of total protein were separated by SDS-PAGE with a 12% resolving layer and a 4% stacking layer and the proteins were transferred to PVDF membranes at 300 mA for 1.5 h. After blocking in 5% nonfat milk diluted with TBS-T for 1 h at room temperature with shaking, the membranes were incubated with primary antibodies (working dilution of tryptase 1:300; PAR-2 1:400) with gentle shaking overnight at 4 ℃, washed with TBS-T (3 times for 5 min each time) and then incubated with species-appropriate secondary antibodies for 2 h at 37 ℃. After washing in TBS-T (3 times for 5 min each time), the membranes were incubated using the ECL Developer Kit in a dark room. The efficiency of protein loading and transfer was assessed by reprobing the membranes with an anti-GAPDH antibody. The density of each band was analyzed using Image J software. Expression of the target protein band was compared with that of the corresponding control band.

Statistical analysis

The experimental data are represented by the mean ± SD. SPSS 20.0 was used for data analysis. One-way analysis of variance was used for analysis. Spearman analysis was used for correlation analysis. A P value less than 0.05 was considered statistically significant.

RESULTS

Transfection efficiency and miR-490-5p level after silencing

The transfection rate was measured by flow cytometry and the results showed that the efficiency was more than 85% in both the siRNA group and null group. We performed qRT-PCR to confirm the expression of miR-490-5p in p815 mast cells after silencing. As shown in Figure 1, the level of miR-490-5p in the siRNA group was significantly decreased compared with the empty vector group ($P < 0.05$).

Proliferation and apoptosis

From the results of the standard curve, we found that when the number of cells varied between 3000 and 48000, there was a linear correlation between the number of cells and OD value. Therefore, CCK-8 was suitable for determining the level of proliferation of mast cells. In the proliferation test, the results showed that inhibition of miR-490-5P expression significantly reduced cell viability compared with the control group ($P < 0.05$). In the apoptosis test, the rate of apoptosis was significantly higher following the inhibition of miR-490-5p expression than in the control group ($P < 0.05$), as shown in Figure 2.

mRNA levels of tryptase and PAR-2

The mRNA levels of tryptase ($P = 0.721$ normal *vs* null, $P = 0.001$ siRNA *vs* normal, $P = 0.002$ siRNA *vs* null) and PAR-2 ($P = 0.027$ siRNA *vs* null, $P = 0.353$ normal *vs* null, $P = 0.105$ siRNA *vs* normal) were higher in the siRNA group than in the normal and null groups. In addition, the difference between the groups was statistically significant, and all the real-time PCR products from the three groups migrated as expected (Figures 3 and 4).

Protein levels of tryptase and PAR-2

The expression results of the siRNA group were compared with normal and null controls. The density of each band was compared with the corresponding control band and normalized to the GAPDH gene. Elevated expression of tryptase and PAR-2 was found in the siRNA group compared with both the normal and null group, but there were no significant differences between the siRNA group and the controls ($P > 0.05$) (Figure 5).

DISCUSSION

In this study, we found that miR-490-5p promotes mast cell proliferation and resistance to apoptosis. Inhibition of miR-490-5p expression significantly increased the mRNA level of mast cell tryptase and PAR-2, but the protein level of tryptase and PAR-2 increased slightly, and a statistically significant difference between the siRNA and the control groups was not observed.

miRNAs are an abundant class of 20-22-nt non-coding single-stranded RNA and play significant roles in various physiological and pathological processes[24,25]. Many miRNAs have been shown to be associated with IBS. We previously detected an elevation of miR-490-5p in IBS-D patients. miR-490-5p is a member of the miR-490 family. The expression and functional role of miR-490-5p has also been reported in other diseases. It is involved in cell proliferation, apoptosis and the regulation of signaling pathways in different ways. In the study by Shiqi Li, miR-490-5p was found to be a novel tumor suppressor of bladder cancer cell proliferation by targeting c-Fos[26,27]. In renal cancer, miR-490-5p was confirmed to directly bind to the 3'UTR of PIK3CA mRNA and reduce the expression of PIK3CA at both the mRNA and protein level, which further inhibited the PIK3/Akt signaling pathway[28]. While, the role of miRNA-490-5p in the development and progression of IBS has not been

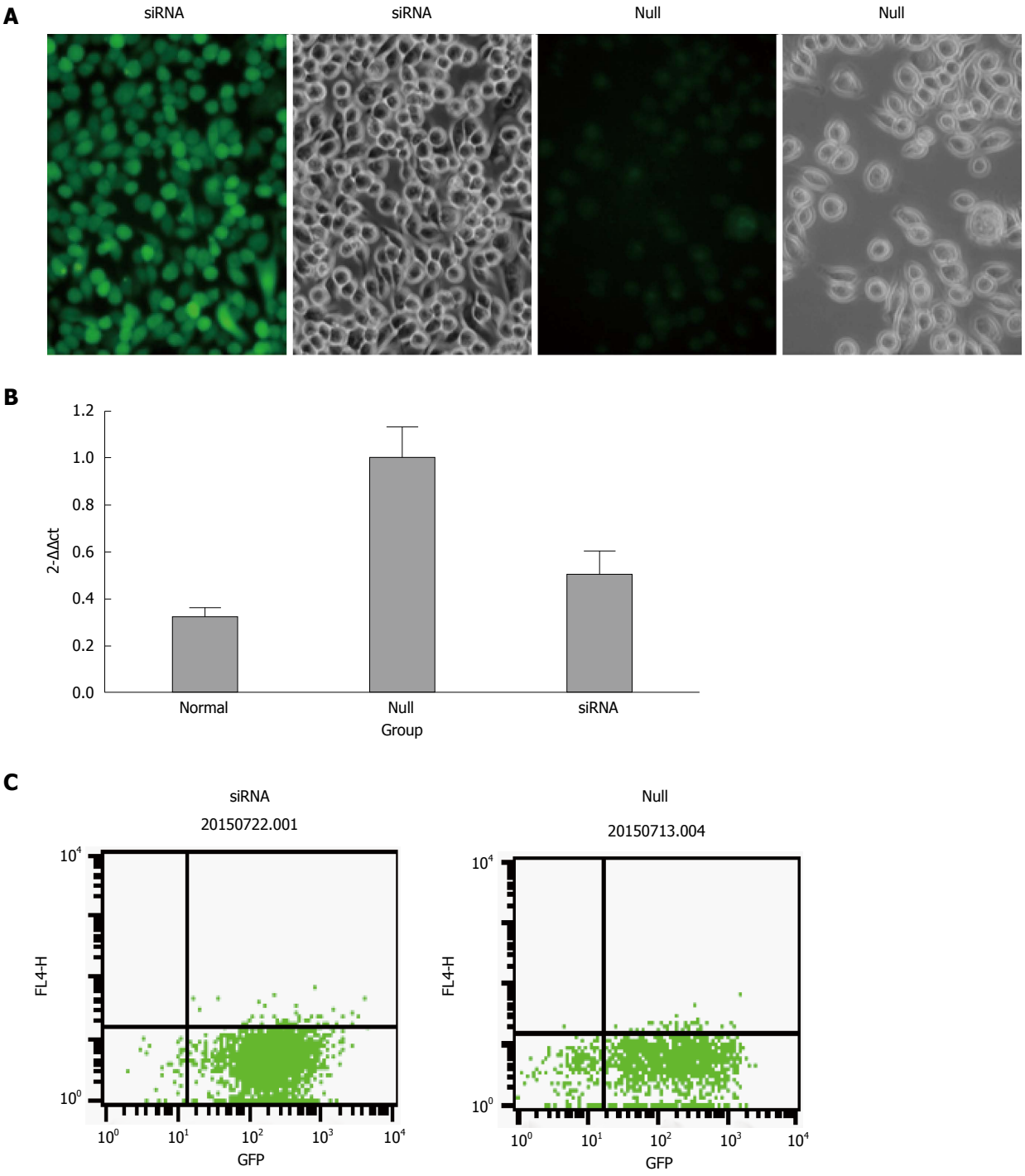


Figure 1 Transduction efficiency and the expression of miR-490-5P after transduction. A: Renderings of mast cells after lentivirus transfection, the transduction rate in the siRNA group was almost 96%; the transduction rate in the null group was almost 86%; B: The relative expression of miR-490-5p after transduction, which was significantly decreased in the siRNA group compared with the null group; C: Mast cell transfection efficiency detected by flow cytometry, the efficiency in each group was more than 85%.

reported. Its target genes include *CABP5*: encoding calcium binding protein 5; *CAMK1D*: encoding calcium/calmodulin-dependent protein kinase ID; *CASP3*: encoding caspase 3, apoptosis-related cysteine peptidase; TNFSF18: encoding tumor necrosis factor (ligand) superfamily, member 18; *BOP1*: encoding proliferation-associated protein. Of these, both calcium and calmodulin-dependent protein kinase were highly correlated with mast cell degranulation[17,18]. Caspase, apoptosis-related cysteine peptidase and proliferation associated protein have been reported to be involved in cell proliferation and apoptosis. In the present study, we found that inhibition of miR-490-5p significantly reduced mast cell proliferation and promoted apoptosis. Furthermore, the level of miR-490-5p was highly correlated with the mRNA level of mast cell tryptase and PAR-2. However, why the increase in PAR-2 and tryptase mRNA did not lead to

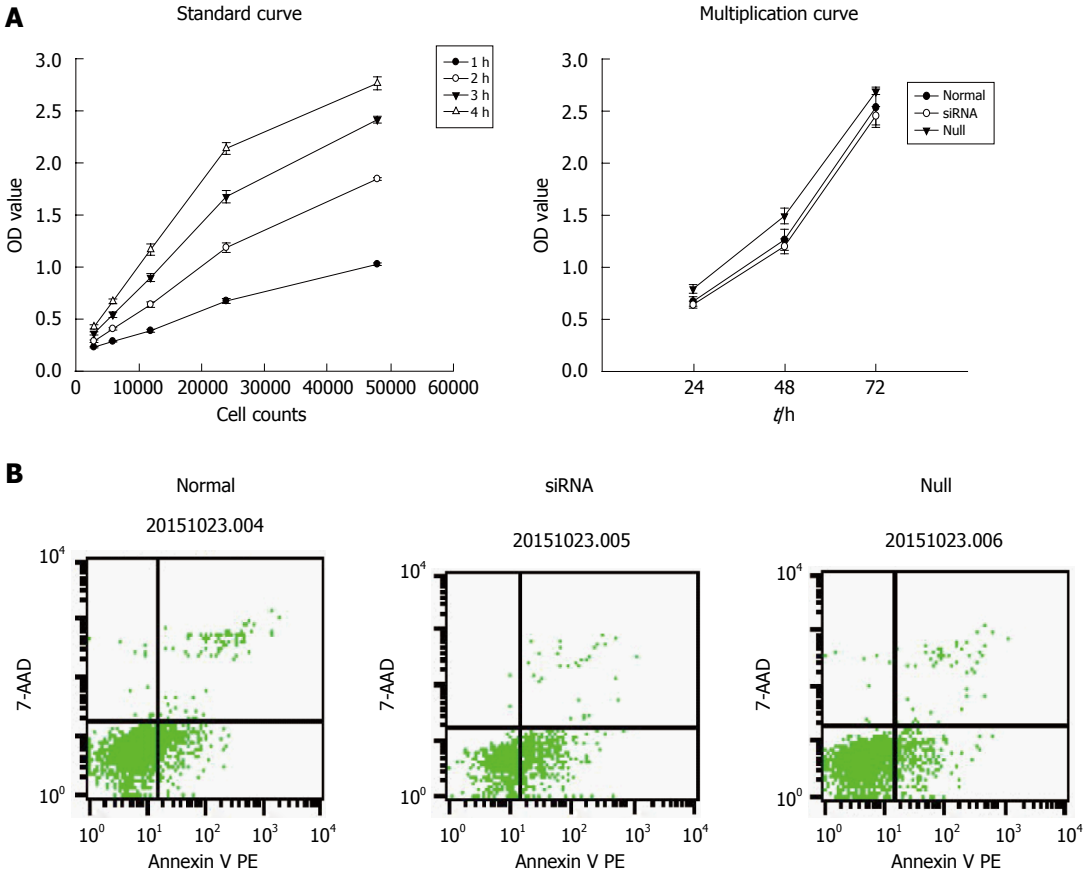


Figure 2 Effect of miR-490-5p on proliferation and apoptosis of p815 mast cells. A: Effect of miR-490-5p on proliferation of p815 mast cells, cell viability in the siRNA group was significantly reduced compared to the normal and null group ($P < 0.05$), there was no significant difference between the normal and null group; B: Mast cell apoptosis after transfection, in the normal group the mean apoptotic index was 15.86%, in the siRNA group the mean apoptotic index was 41.19%, in the null group the mean apoptotic index was 11.84% ($n = 3$).

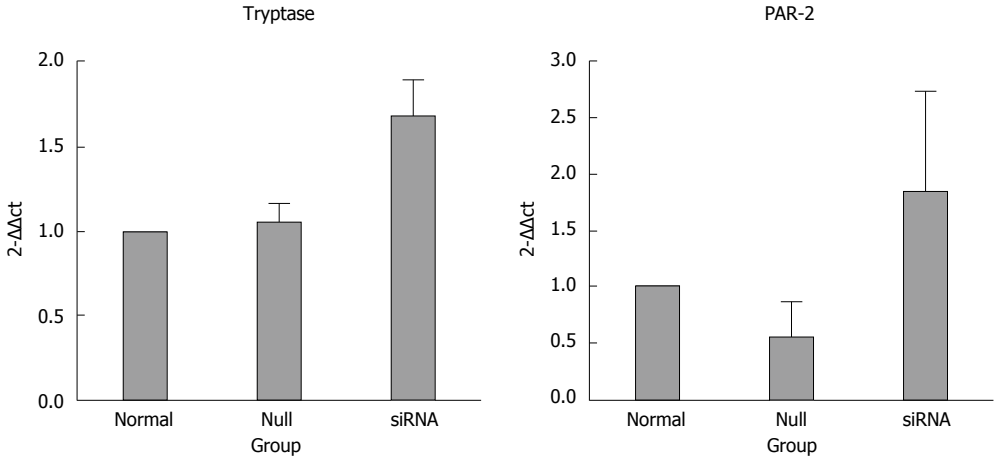


Figure 3 Quantification of tryptase and protease-activated receptor 2 mRNA after normalization to the housekeeping gene U6. Significant elevation of tryptase and PAR-2 expression was observed in the siRNA group compared with the normal and null group ($P < 0.05$), and no difference was observed between the normal and null group. The fold change in mRNA expression was calculated relative to the endogenous U6 control using 2-ΔΔCt. The data are expressed as the mean ± SEM.

a significant elevation in PAR-protein and tryptase-protein remains unknown. Possible reasons are as follows: Firstly, some other factors may be involved in the post-transcriptional regulation or the protein translation process. Secondly, the role of miR-490-5p is mainly up-regulating the function of tryptase/PAR-2 rather than affecting their expression. Thirdly, mast cells are merely normal cells in the resting state, and lack activating factors; however, in IBS-D patients, mast cells are activated. This is the first time that the role of miR-490-5p in IBS has been demonstrated, and information on the biological role of miRNA in IBS

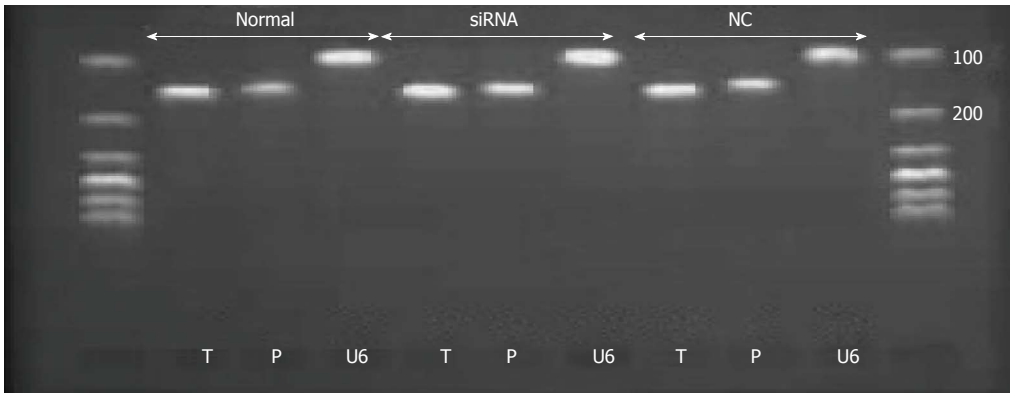


Figure 4 Polymerase chain reaction products were subjected to agarose gel electrophoresis. All real-time-PCR products from the three groups migrated as expected. T: Tryptase 145 bp; P: Protease-activated receptor 2; 139 bp; U6: 106 bp.

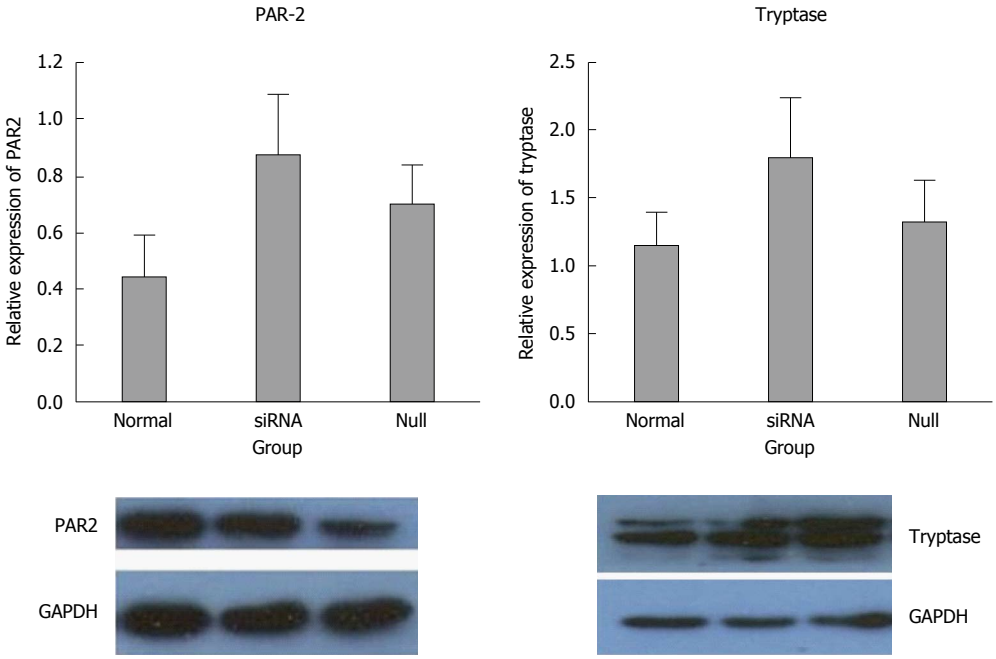


Figure 5 Expression of tryptase and protease-activated receptor 2 in p815 mast cells after silencing miR-490-5p. The density of each group band was compared with the corresponding control band and normalized to the *GAPDH* gene. The level of PAR-2 increased in the siRNA group, but no significant difference was observed between the siRNA group and the controls ($P > 0.05$). The level of tryptase increased slightly in the siRNA group, but no significant difference was observed between the siRNA group and the controls ($P > 0.05$).

which could be used for reference was limited. Previous studies have reported that the number of intestinal mucosal mast cells increased in IBS patients and the mast cell tryptase/PAR-2 signal pathway played an important role in the pathogenesis of IBS. Thus, any factors associated with the above process are important. In summary, although problems occurred in our study, which require further investigation, we can conclude that mast cell miR-490-5p may participate in the occurrence and development of IBS by regulating the proliferation and apoptosis of mast cells; however, its effect on the mast cell tryptase/PAR-2 signal pathway is complex. The limitations in this study were that mir-490-5p was highly correlated with mast cell proliferation and apoptosis, but the specific target gene through which miR-490-5p plays a crucial role in IBS remains unknown. Furthermore, the elevation of tryptase and PAR-2 mRNA did not lead to an increase in protein level, which should be explored. The next step is to screen out the target gene of miR-490-5p involved in the regulation of the above biological process using bioinformatics analysis, selectively knockout the target gene, and then determine the level of mast cell tryptase and PAR-2. This may provide a new target for the treatment of IBS.

ACKNOWLEDGMENTS

The authors thank Dr. Wei Jiao, Dr. Fei Liu, Dr. Jiao Lan and Rui-Ping Xiao from Scientific Research Center of the People's Hospital of Guangxi Zhuang Autonomous Region for their valuable technological assistance on

our project.

COMMENTS

Background

Previous studies have reported that mast cells and mast cell degranulation are involved in the pathogenesis of irritable bowel syndrome (IBS). Tryptase is an important component of mast cell degranulation which leads to visceral hypersensitivity in IBS patients by activation of PAR-2. The authors previous studies showed that IBS patients demonstrated dysregulation of miR-490-5p whose target gene was highly correlated with visceral sensitivity or neurotransmitter release associated protein, or intracellular material transportation associated protein. Thus, we predicted that miR-490-5p may be indirectly involved in the regulation of the mast cell-tryptase-PAR-2 signaling pathway.

Research frontiers

A growing number of studies have reported that different miRNAs are involved in the regulation of proliferation and differentiation of digestive tract epithelial cells and smooth muscle cells. Recent research reported that miRNA plays an important role in the differentiation, proliferation and functional regulation of bone marrow mast cells in the inflammatory response. Another study reported that miR-29a was highly correlated with increased intestinal permeability in IBS-D patients. Thus, the authors can conclude that the regulation of miRNA may be closely related to the pathogenesis of IBS.

Innovations and breakthroughs

The authors proved, for the first time, that miR-490-5p may be directly or indirectly involved in mast cell proliferation and apoptosis and the mast cell/tryptase/PAR-2 signaling pathway may play an important role in IBS-D.

Applications

miRNA-490-5p may be a new biomarker for the diagnosis of IBS and a new target for therapy.

Terminology

RNA interference (RNAi), a fundamental biological process by which cells regulate gene expression, acts through complementary base-pairing with target mRNA and retrieves cellular RNases which in turn degrade mRNA transcripts. RNAi is now routinely used to evaluate gene function both *in vitro* and *in vivo* and many innovative screens reported the use of RNAi to investigate potential drug targets.

Peer-review

The author supported that miR-490-5p may directly or indirectly involved in the pathogenesis of IBS. It was the first research focusing on the miR-490-5p and IBS and it really could improve our understanding of IBS to some extent.

REFERENCES

1 **Liang WJ**, Zhang G, Luo HS, Liang LX, Huang D, Zhang FC. Tryptase and Protease-Activated Receptor 2 Expression Levels in Irritable Bowel Syndrome. *Gut Liver* 2016; **10**: 382-390 [PMID: 26446924 DOI: 10.5009/gnl14319]

2 **La JH**, Kim TW, Sung TS, Kim HJ, Kim JY, Yang IS. Role of mucosal mast cells in visceral hypersensitivity in a rat model of irritable bowel syndrome. *J Vet Sci* 2004; **5**: 319-324 [PMID: 15613815]

3 **Park JH.** Dysregulated MicroRNA Expression in Irritable Bowel Syndrome. *J Neurogastroenterol Motil* 2016; **22**: 166-167 [PMID: 27032543 DOI: 10.5056/jnm16044]

4 **Cenac N**, Andrews CN, Holzhausen M, Chapman K, Cottrell G, Andrade-Gordon P, Steinhoff M, Barbara G, Beck P, Bunnett NW, Sharkey KA, Ferraz JG, Shaffer E, Vergnolle N. Role for protease activity in visceral pain in irritable bowel syndrome. *J Clin Invest* 2007; **117**: 636-647 [PMID: 17304351 DOI: 10.1172/JCI29255]

5 **Chey WD**, Kurlander J, Eswaran S. Irritable bowel syndrome: a clinical review. *JAMA* 2015; **313**: 949-958 [PMID: 25734736 DOI: 10.1001/jama.2015.0954]

6 **Lacy BE**. Diagnosis and treatment of diarrhea-predominant irritable bowel syndrome. *Int J Gen Med* 2016; **9**: 7-17 [PMID: 26929659 DOI: 10.2147/IJGM.S93698]

7 **Zhang ZF**, Duan ZJ, Wang LX, Yang D, Zhao G, Zhang L. The serotonin transporter gene polymorphism (5-HTTLPR) and irritable bowel syndrome: a meta-analysis of 25 studies. *BMC Gastroenterol* 2014; **14**: 23 [PMID: 24512255 DOI: 10.1186/1471-230X-14-23]

8 **Qin SY**, Jiang HX, Lu DH, Zhou Y. Association of interleukin-10 polymorphisms with risk of irritable bowel syndrome: a meta-analysis. *World J Gastroenterol* 2013; **19**: 9472-9480 [PMID: 24409078 DOI: 10.3748/wjg.v19.i48.9472]

9 **Bashashati M**, Rezaei N, Bashashati H, Shafieyoun A, Daryani NE, Sharkey KA, Storr M. Cytokine gene polymorphisms are associated with irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol Motil* 2012; **24**: 1102-e566 [PMID: 22897390 DOI: 10.1111/j.1365-2982.2012.01990.x]

10 **Mansour MA**, Sabbah NA, Mansour SA, Ibrahim AM. MicroRNA-199b expression level and coliform count in irritable bowel syndrome. *IUBMB Life* 2016; **68**: 335-342 [PMID: 27015896 DOI: 10.1002/iub.1495]

11 **Liao XJ**, Mao WM, Wang Q, Yang GG, Wu WJ, Shao SX. MicroRNA-24 inhibits serotonin reuptake transporter expression and aggravates irritable bowel syndrome. *Biochem Biophys Res Commun* 2016; **469**: 288-293 [PMID: 26631964 DOI: 10.1016/j.bbrc.2015.11.102]

12 **Zhou Q**, Costinean S, Croce CM, Brasier AR, Merwat S, Larson SA, Basra S, Verne GN. MicroRNA 29 targets nuclear factor-κB-repressing factor and Claudin 1 to increase intestinal permeability. *Gastroenterology* 2015; **148**: 158-169.e8 [PMID: 25277410 DOI: 10.1053/j.gastro.2014.09.037]

13 **Andersen HH**, Duroux M, Gazerani P. MicroRNAs as modulators and biomarkers of inflammatory and neuropathic pain conditions. *Neurobiol Dis* 2014; **71**: 159-168 [PMID: 25119878 DOI: 10.1016/j.nbd.2014.08.003]

14 **Fourie NH**, Peace RM, Abey SK, Sherwin LB, Rahim-Williams B, Smyser PA, Wiley JW, Henderson WA. Elevated circulating miR-150 and miR-342-3p in patients with irritable bowel syndrome. *Exp Mol Pathol* 2014; **96**: 422-425 [PMID: 24768587 DOI: 10.1016/j.yexmp.2014.04.009]

15 **Wouters MM**, Vicario M, Santos J. The role of mast cells in functional GI disorders. *Gut* 2016; **65**: 155-168 [PMID: 26194403 DOI: 10.1136/gutjnl-2015-309151]

16 **Mayer EA**, Bradesi S, Chang L, Spiegel BM, Bueller JA, Naliboff BD. Functional GI disorders: from animal models to drug development. *Gut* 2008; **57**: 384-404 [PMID: 17965064 DOI: 10.1136/gut.2006.101675]

17 **Chakravarty N**, Nielsen EH. Histamine release from saponin-permeabilized rat mast cells by calcium. *Agents Actions* 1986; **18**: 65-67 [PMID: 2425595]

18 **Aridor M**, Sagi-Eisenberg R. Neomycin is a potent secretagogue of mast cells that directly activates a GTP-binding protein involved in exocytosis. *J Cell Biol* 1990; **111**: 2885-2891 [PMID: 1702786]

19 **Kapeller J**, Houghton LA, Mönnikes H, Walstab J, Möller D, Bönisch H, Burwinkel B, Autschbach F, Funke B, Lasitschka F, Gassler N, Fischer C, Whorwell PJ, Atkinson W, Fell C, Büchner KJ, Schmidtmann M, van der Voort I, Wisser AS, Berg T, Rappold G, Niesler B. First evidence for an association of a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. *Hum Mol Genet* 2008; **17**: 2967-2977 [PMID: 18614545 DOI: 10.1093/hmg/ddn195]

20 **Zhou Q**, Souba WW, Croce CM, Verne GN. MicroRNA-29a regulates intestinal membrane permeability in patients with irritable bowel syndrome. *Gut* 2010; **59**: 775-784 [PMID: 19951903 DOI: 10.1136/gut.2009.181834]

21 **Bi X**, Cao Y, Chen R, Liu C, Chen J, Min D. MicroRNA-184

Promotes Proliferation and Inhibits Apoptosis in HaCaT Cells: An In Vitro Study. *Med Sci Monit* 2016; **22**: 3056-3061 [PMID: 27571235]
22 **Guo XW**. The microRNAs profiles in the colonic mucosa of IBS patients. *Guangxi Yike Daxue Xuebao*, 2014
23 **Deiteren A**, de Wit A, van der Linden L, De Man JG, Pelckmans PA, De Winter BY. Irritable bowel syndrome and visceral hypersensitivity: risk factors and pathophysiological mechanisms. *Acta Gastroenterol Belg* 2016; **79**: 29-38 [PMID: 26852761]
24 **Filipowicz W**, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? *Nat Rev Genet* 2008; **9**: 102-114 [PMID: 18197166 DOI: 10.1038/nrg2290]
25 **Stefani G**, Slack FJ. Small non-coding RNAs in animal development. *Nat Rev Mol Cell Biol* 2008; **9**: 219-230 [PMID: 18270516 DOI: 10.1038/nrm2347]
26 **Li S**, Xu X, Xu X, Hu Z, Wu J, Zhu Y, Chen H, Mao Y, Lin Y, Luo J, Zheng X, Xie L. MicroRNA-490-5p inhibits proliferation of bladder cancer by targeting c-Fos. *Biochem Biophys Res Commun* 2013; **441**: 976-981 [PMID: 24220339 DOI: 10.1016/j.bbrc.2013.11.006]
27 **Lan G**, Yang L, Xie X, Peng L, Wang Y. MicroRNA-490-5p is a novel tumor suppressor targeting c-FOS in human bladder cancer. *Arch Med Sci* 2015; **11**: 561-569 [PMID: 26170849 DOI: 10.5114/aoms.2015.52359]
28 **Chen K**, Zeng J, Tang K, Xiao H, Hu J, Huang C, Yao W, Yu G, Xiao W, Guan W, Guo X, Xu H, Ye Z. miR-490-5p suppresses tumour growth in renal cell carcinoma through targeting PIK3CA. *Biol Cell* 2016; **108**: 41-50 [PMID: 26559013 DOI: 10.1111/boc.201500033]

P- Reviewer: Raju J, Zhao HT **S- Editor**: Qi Y
L- Editor: Webster JR **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.103

World J Gastroenterol 2017 January 7; 23(1): 103-109
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Case Control Study

High risk of temporomandibular disorder in irritable bowel syndrome: Is there a correlation with greater illness severity?

Serena Gallotta, Vincenzo Bruno, Santo Catapano, Nicola Mobilio, Carolina Ciacci, Paola Iovino

Serena Gallotta, Carolina Ciacci, Paola Iovino, Functional GI Disorders Center at Gastrointestinal Unit, AOU S. Giovanni di Dio e Ruggi d'Aragona, Department of Medicine and Surgery, University of Salerno, 84084 Salerno, Italy

Vincenzo Bruno, Santo Catapano, Nicola Mobilio, Dental School, University of Ferrara, 44121 Ferrara, Italy

Author contributions: Gallotta S, Bruno V, Catapano S, Mobilio N, Ciacci C and Iovino P designed the study and interpreted the data; Gallotta S, Bruno V, Ciacci C and Iovino P participated in the acquisition of the data; Gallotta S drafted the initial manuscript; Bruno V, Catapano S, Mobilio N, Ciacci C and Iovino P revised the article critically for important intellectual content.

Institutional review board statement: The study was reviewed and approved by the S. Giovanni di Dio e Ruggi D'aragona Ethical Committee of Salerno.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Paola Iovino, Professore Associato, Functional GI Disorders Center at Gastrointestinal Unit, AOU S. Giovanni di Dio e Ruggi d'Aragona, Department of Medicine and Surgery, University of Salerno, Via S Allende, Baronissi, 84084 Salerno, Italy. piovino@unisa.it

Telephone: +39-89-965030
Fax: +39-81-19335031

Received: September 21, 2016
Peer-review started: September 22, 2016
First decision: October 28, 2016
Revised: November 12, 2016
Accepted: December 8, 2016
Article in press: December 8, 2016
Published online: January 7, 2017

Abstract

AIM

To investigate the prevalence and the risk of temporomandibular disorders (TMDs) in patients with irritable bowel syndrome (IBS) (including each subtype: constipation, diarrhoea, and mixed) compared to the general population.

METHODS

Between January 2014 and December 2015 we enrolled consecutively adult patients diagnosed with IBS at the outpatient clinic of the University of Salerno and healthy controls (HC) without IBS. At enrollment, we analyzed all patients for the presence of TMDs according to the Research Diagnostic Criteria for TMD.

RESULTS

We enrolled 91 IBS patients (23 IBS-D, 30 IBS-C and 38 IBS-M) and 57 HC in the study. We found a higher risk of having TMD (OR = 3.41, 95%CI: 1.66-7.01) compared to the HC. The risk of having TMD was independent of IBS-subtype. Multiple regression analysis showed that facial pain was positively related to abdominal pain and higher level of depression.

CONCLUSION

IBS patients had a more than three times greater risk of TMD compared to HC. The risk of having TMD

was similar in different IBS subtypes. IBS patients that also fulfilled criteria for TMD seem to share along with chronic facial and abdominal pain a significant co-occurrence with psychiatric disorders and female preponderance.

Key words: Temporomandibular disorders; Irritable bowel syndrome; Chronic pain; Facial pain; Abdominal pain; Irritable bowel syndrome severity score symptoms; Irritable bowel syndrome predominant diarrhea; Irritable bowel syndrome predominant constipation; Irritable bowel syndrome mixed

Core tip: Temporomandibular disorders (TMD) seem to occur more frequently in patients with irritable bowel syndrome (IBS). In this study we analyzed all patients for the presence of TMD according to the Research Diagnostic Criteria for Temporomandibular Disorders. Based on our results, IBS patients had a more than three times greater risk of TMD compared to healthy controls. The risk of having TMD was similar in different IBS subtypes. IBS patients that also fulfilled criteria for TMD seem to share along with chronic facial and abdominal pain a significant co-occurrence with psychiatric disorders and female preponderance.

Gallotta S, Bruno V, Catapano S, Mobilio N, Ciacci C, Iovino P. High risk of temporomandibular disorder in irritable bowel syndrome: Is there a correlation with greater illness severity? *World J Gastroenterol* 2017; 23(1): 103-109 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/103.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.103

INTRODUCTION

Irritable bowel syndrome (IBS) is a common chronic functional disorder of the lower gastrointestinal tract characterized by chronic pain or discomfort in the abdomen associated with altered bowel habits[1].

Patients with IBS often complain of a variety of other GI (*i.e.,* dyspepsia) and non-GI symptoms (*i.e.,* migraine headaches, fatigue, sleep problems, dizziness, depression, anxiety, irritability, lower urinary tract symptoms and many IBS patients meet diagnostic criteria for other functional disorders[2], such as fibromyalgia (FM)[3], interstitial cystitis/painful bladder syndrome (IC/PBS)[4], migraine[5], temporomandibular disorders (TMDs). However, the association between IBS and TMDs is still relatively unexplored[6,7].

TMDs are a heterogeneous group of diseases of the stomatognathic system that involve the temporomandibular joint (TMJ), masticatory muscles and their related structures[8]. The cardinal symptoms are pain in the TMJ and/or masticatory muscles, joint sounds and alterations in mandibular movement.

The prevalence of TMD is recognized to be between 5% to 12%[9] and this data has been confirmed also in an Italian population[10]. However, only 7% to 15% with TMDs, ask for medical help, mainly women of reproductive ages[11].

The classification of TMDs is controversial. A diagnostic model was proposed in 1992, known as the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)[12]. Since its publication, the RDC/TMD has been widely used in epidemiological, clinical and experimental studies[13]. Two diagnostic axes are contemplated: axis Ⅰ establishes a diagnosis based on clinical variables, while axis Ⅱ allows the assessment of mandibular function, psychological status and level of TMD-related psychosocial disability.

Previous cross-sectional research has suggested an association between TMD and IBS, however these studies have methodological limitations due to a self-reported TMD diagnosis or old criteria to diagnose IBS as well as the small number of studied subjects[14-16].

In 2013 Sanders *et al*[17] showed that in a group of 74 subjects with IBS symptoms the incidence of first onset of TMD was 3 times higher, compared to the group of 2632 subjects without IBS symptoms. In 2015 another study showed that the number of comorbidities, IBS included, is positively associated with TMD pain duration and intensity, whereas among all comorbidities only the presence of specific conditions, such as migraine and chronic fatigue syndrome, is associated with an increase in TMD intensity and duration[5].

Interestingly, patients with IBS and another functional disorder, in comparison with patients with only IBS, have more severe IBS symptoms, a higher rate of psychological comorbidity such as depression, anxiety and somatization, greater impairment of quality of life, and more illness-related work absenteeism[18,19].

To our knowledge, the association between IBS and TMD taking into account the influence of IBS subtypes and illness severity has still not been sufficiently investigated. Increased awareness of the overlap between IBS and TMD likely will result in improved diagnoses and more effective management of both diseases[20]. Patients with IBS symptoms often are treated within a narrow gastrointestinal paradigm while clinicians ignore coexisting pain conditions, resulting in treatment failure and perpetuation of the problem.

The aims of the study were to evaluate, firstly, the risk of TMD determined on the basis of the RDC/TMD in IBS patients diagnosed by the Rome Ⅲ criteria, secondly the possible association between TMD and IBS subtype or severity. Finally, we aimed to observe any association between facial pain and abdominal pain.

Table 1 Chronic pain grade classification

Grade 0	
Low disability	No TMD pain in prior 6 mo
Grade Ⅰ	
Low intensity	Characteristic pain intensity < 50, and less than 3 disability points
Grade Ⅱ	
High intensity	Characteristic pain intensity ⩾ 50, and less than 3 disability points
High disability	
Grade Ⅲ	
Moderately limiting	3 to 4 disability points, regardless of characteristic pain intensity
Grade Ⅳ	
Severely limiting	5 to 6 disability points regardless of characteristic pain intensity

MATERIALS AND METHODS

IBS patients (aged 18-65 years) were consecutively recruited for this study from an outpatient clinic of the University of Salerno devoted only to functional bowel disorders and run weekly by gastroenterologists trained in the field of functional bowel disorders (FBD). All patients attending this clinic were referred from general practitioners and/or other gastrointestinal clinics. At the same time, a dentist V.B. observed all patients recruited to this study.

For the purposes of the study, we recruited also healthy controls (HC) among the patients' friends and the hospital staff, if scoring negative for IBS and with regular bowel habit similar for age and gender to IBS patients. Enrolment was done between January 2014 and December 2015. All subjects gave their written informed consent and the Ethical Committee of the University of Salerno approved the study protocol.

The diagnosis of IBS was made based on the Rome Ⅲ criteria, together with the exclusion of any organic disease, with complete physical examination, blood tests, flexible sigmoidoscopy and additional tests when indicated. Four different patterns of IBS resulted from the predominant bowel symptom: (1) diarrhoea predominant (IBS-D); (2) constipation predominant (IBS-C); (3) mixed IBS (IBS-M); and (4) unsubtyped IBS[21]. The IBS severity score was obtained using the severity index (IBS-SSS)[22]. This is a validated scoring system using easily obtained variables: current abdominal pain by visual analog scale (VAS) of 0-100 and frequency of abdominal pain; current abdominal distension by VAS of 0-100; the degree of satisfaction of evacuative behavior by VAS 0-100; the degree of IBS interference with normal work and social activities by VAS 0-100. IBS patients were then divided into three severity groups: mild (75 to 175), moderate (175-300), and severe (> 300).

According to the Italian version of the RDC/TMD criteria (Available from: http://www.rdc-tmdinternational.org. Accessed (1 December 2013) all subjects from the two groups were investigated for TMD. The following elements were considered in the assessment of TMD: each patient's responses to the validated subjective-symptoms questionnaire, an interview that included the patient's detailed medical history, and the results of a physical examination. The history questionnaire included questions about prior trauma to the head and neck, awareness of bruxism, various diurnal and nocturnal parafunctional habits, muscle fatigue, locking, clicking, or crepitation in the temporomandibular joint during mandibular function, impaired mandibular movement, pain during mandibular function or while at rest, and prior treatment for TMD.

The clinical examination of RDC/TMD axis Ⅰ consisted of the evaluation of masticatory muscles (temporal, masseter, medial, and lateral pterygoid), the temporomandibular joint, the range of mandibular movement in opening, lateral, and protrusive excursions, and temporomandibular joint sounds. Manual palpation has been reported as the technique most commonly used to assess muscle pain; thus the examination of masticatory muscles was performed via bilateral manual palpation.

Instead, the RDC/TMD axis Ⅱ has permitted the severity of chronic pain to be rated by means of the Graded Chronic Pain Scale (GCPS). The GCPS comprises the assessment of seven items assessed: 3 items assessing the intensity of pain on a 10 point scale, including the current pain; 3 items assessing the impact of pain on a 10 point scale; and one item regarding the number of days of inability due to facial pain. The scoring criteria are simple to use and allow the categorization of pain into five levels of pain-related impairment (Table 1).

Furthermore, the RDC/TMD axis Ⅱ has dealt with the assessment of depression and somatization levels by means of the depression and somatization scales of the Symptom Checklist 90R (SCL-90R)[23]. A total of 31 items were included in the axis Ⅱ, belonging either to the Depression and Vegetative Symptom Scale or to the Somatization Scale[24]. The mean scale score is calculated by simply adding the scores of the single item together. This allows patients to be rated as having normal, moderate or severe levels of impairment regarding depression and nonspecific physical symptoms (Table 2).

Statistical analysis

Data are presented as mean ± SE, unless otherwise indicated. $P < 0.05$ was considered statistically significant. χ^2 test and analysis of variance (ANOVA) were used to compare categorical and continuous data, respectively. The risk in IBS patients of having TMD compared to HC was analysed using a binary logistic regression. We then performed a subgroup analysis to test the risk of having TMD according to each IBS subgroup (IBS-D, IBS-C and IBS-M) using univariate logistic models. Two multivariate linear regression

Table 2 Depression and somatization classification

Classification	Normal	Moderate	Severe
Depression	< 0.535	⩾ 0.535, ⩽ 1.105	> 1.105
Nonspecific physical symptoms (including the painful symptoms)	< 0.500	⩾ 0.500, ⩽ 1.000	> 1.000
Nonspecific physical symptoms (excluding the painful symptoms)	< 0.428	⩾ 0.428, ⩽ 0.857	> 0.857

analyses were performed to analyze the relationships between illness severity of TMD and IBS taking into account age, sex, IBS severity, IBS subtypes and depression. The SPSS for Windows version 12.0 statistical package (SPSS, Chicago, Ill., United States) was used for the data analysis.

RESULTS

IBS Patients

None of the eligible patients refused to participate in the study. We enrolled 91 consecutive IBS patients (71 females, mean age 36.6 ± 1.4 years) and 57 healthy controls (HC) (37 females, mean age 34.2 ± 1.7 years) in the study. Gender and age distribution were not significantly different among groups (χ^2 test, P = 0.08 and Student t test, P = 0.3).

IBS patients were classified as follow: 23 (25.3%) as IBS-D, 30 (33.0%) as IBS-C and 38 (41.8%) as IBS-M. On the basis of IBS-SSS, in our IBS population we had 11(12.1%) mild IBS, 41 (45.1%) moderate and 39 (42.9%) severe IBS. The severity score was 120.0 ± 7.0 in mild IBS patients, 247.9 ± 6.8 in moderate IBS patients, and 361.3 ± 6.9 in severe IBS patients.

Risk of TMD in IBS compared to HC

According to the RDC/TMD classification criteria, the IBS group had a greater than three times risk of having TMD (OR = 3.41, 95%CI: 1.66-7.01), compared to HC. In fact, TMD was diagnosed in 50 (54.9%) IBS patients and 15 (26.3%) HC (χ^2 test, P = 0.001).

IBS patients compared to HC had significantly more facial pain (37.4% *vs* 19.3%, P = 0.02), TMJ locking (13.2 *vs* 3.5, P = 0.05) and TMJ clicking (41.8 *vs* 17.5, P = 0.002). TMJ crepitation was higher although it does not reach statistical significance (20.9 *vs* 10.5, P = 0.10).

From the RDC/TMD axis Ⅱ the severity of chronic pain rated by means of the GCPS in IBS was grade Ⅰ low intensity in 22.0%, grade Ⅱ in 12.1% and grade Ⅲ in 2.2%. IBS patients compared to HC had a significantly higher score of depression (0.95 ± 0.6 *vs* 0.62 ± 0.7, P = 0.001), of nonspecific physical symptoms (including the painful symptoms) (1.28 ± 0.1 *vs* 0.6 ± 0.06, P < 0.001) and of nonspecific physical symptoms (excluding the painful symptoms) (1.1 ± 0.08 *vs* 0.5 ± 0.07, P < 0.001).

Relationship between TMD and the type and severity of IBS

The risk of having TMD was similar in all three IBS subtypes, around three times higher than HC [IBS-D *vs* HC OR = 3.05 (95%CI: 1.11-8.37), IBS-C *vs* HC = 3.66 (1.44-9.30), IBS-M *vs* HC 3.46 (1.44-8.25)]. The respective prevalence was IBS-D 52.2% - IBS-C 56.7% - IBS-M 55.3%.

Looking at the severity of IBS, there was not a significant higher IBS-SSS score in IBS patients with or without TMD (281.2 ± 12.5 *vs* 261.7 ± 17.1, P = 0.35).

There was a significant positive correlation between the severity of chronic pain rated by means of the GCPS and the IBS-SSS (R = 0.17, P = 0.04). When multiple linear regression analysis was performed the GCPS was significantly related only to gender independent of age, IBS subtypes and IBS-SSS and depression (Table 3).

Multiple linear regression analysis showed that the current facial pain scoring from 0 to 10 was significantly related to the current abdominal pain (0-10 VAS) and to higher level of depression independent of gender, age, IBS subtypes and IBS-SSS (Table 4).

DISCUSSION

Our results showed that IBS patients had a TMD risk more than three times greater than HC. This increased risk is independent of any specific IBS subtype. Facial pain was positively related to abdominal pain and higher level of depression.

Studies on the association between TMD and IBS are scarce. In 1998 Korszun *et al*[14] reported the presence of IBS in 46% of 39 patients who self-reported TMD diagnosis. In 2000 Aaron *et al*[15] showed that IBS was present in 64% of 25 TMD patients, however diagnosis of IBS was made using the Manning criteria. In 2001, Jones *et al*[16] presented at DDW an abstract in which the presence of self-reported TMD diagnosis was 16% in 270 patients with IBS. Recently, in 2013 Sanders *et al*[17] in the multi-site OPPERA project performed a continuing prospective cohort study purposefully designed to investigate the etiology of first-onset TMD and variation in its genetic, biological and psychosocial determinants. Applying a rigorous methodology together with the large size of the study they demonstrated that incidence of first onset TMD was 3 times higher in people with IBS on enrolment as in people without IBS. IBS predicted first-onset TMD after adjustment for demographic characteristics and pain disorders; however their effects were rendered statistically non significant in the presence of other overlapping conditions. In this study the assessment of irritable bowel syndrome (IBS) symptoms was based on Rome Ⅲ criteria using two questions (52 and 53) that asked about bowel movements and the experience of discomfort or pain

Table 3 Multiple regression analysis; the chronic pain grade scale by age, gender, depression, irritable bowel syndrome-symptom severity scale and irritable bowel syndrome subtypes

	Unstandardized coefficients		Standardized coefficients	*t*	Significance	95%CI	
	β	SE	*β*			Lower	Upper
Constant	-0.220	0.530		-0.414	0.680	-1.274	0.834
Age	-0.007	0.007	-0.121	-1.124	0.264	-0.020	0.006
Gender	0.436	0.204	0.229	2.132	0.036	0.029	0.842
Depression	0.352	0.184	0.201	1.911	0.059	-0.014	0.718
IBS-SSS	-0.034	0.124	-0.290	-0.274	0.785	-0.280	0.213
IBS subtypes	0.032	0.109	0.031	0.292	0.771	-0.185	0.249

Dependent variable: Chronic pain grade scale. IBS: Irritable bowel syndrome.

Table 4 Multiple regression analysis; facial pain by age, gender, abdominal pain, depression, irritable bowel syndrome-symptom severity scale and irritable bowel syndrome subtypes

	Unstandardized coefficients		Standardized coefficients	*t*	Significance	95%CI	
	β	SE	*β*			Lower	Upper
Costant	-0.891	0.720		-1.239	0.217	-2.314	0.531
Age	-0.001	0.012	-0.008	-0.104	0.917	-0.024	0.022
gender	0.292	0.338	0.071	0.866	0.388	-0.375	0.960
Abdominal pain VAS	0.220	0.083	0.396	2.644	0.009	0.056	0.384
IBS-SSS	-0.485	0.274	-0.329	-1.770	0.079	-1.026	0.057
Depression classification	0.473	0.189	0.205	2.501	0.014	0.099	0.848
Subtypes di IBS e HC	0.204	0.200	0.130	1.019	0.310	-0.191	0.598

Dependent variable: facial pain VAS (0-10). VAS: Visual analog scale; IBS: Irritable bowel syndrome; HC: Healthy controls.

in the abdomen that lasted at least one day a week during the previous three weeks[17].

Compared to the previous literature, our study has taken into account several factors. Firstly, our population was enrolled in a tertiary outpatients clinic devoted only to functional bowel disorders and run weekly by gastroenterologists trained in the field of functional bowel disorders (FBD) together with a dentist. The presence of TMD in this population of patients with IBS and HC was evaluated according to the RDC/TMD criteria that are worldwide-accepted criteria for the diagnosis of these disorders. Therefore, our study results could be more generalizable for IBS patients seeking treatment. Secondly, to give strength to our study, the diagnosis of IBS and its different subtypes was based on a standardized questionnaire according to Rome Ⅲ criteria and not on the patients' subjective evaluation of their bowel habits. Moreover, we took into account the severity of IBS demonstrating a weak but significant correlation between the chronic grade of TMD pain and IBS severity according to Francis *et al*[22]; however, in the regression analysis this correlation was lost taking into account other factors. In fact, women resulted significantly related to the chronic grade of TMD confirmed that gender is a risk factor in TMD[11]. Finally, facial pain that is common in patients with TMD is strongly and positively related to abdominal pain and higher level of depression. We inquired about current facial and abdominal pain to avoid a recall bias. Chronic abdominal pain is the predominant feature in IBS. In fact the newest Rome Ⅳ criteria published last May eliminated the term discomfort from the diagnostic criteria and focused only on recurrent abdominal pain[25].

Furthermore, psychological status has been assessed and controlled for all subjects using RDC/TMD axis Ⅱ by means of the 31 items belonging either to the Depression and Vegetative Symptom Scale or to the Somatization Scale[24].

There are some limitations in this study. First, the small number of IBS patients could have affected our results especially in the analyses of the IBS-SSS association with TMD. However, facial pain is significantly correlated to higher abdominal pain and depression. In 2011 the Rome foundation working team stated that the physiological factors contributing to severity have both visceral and central nervous system contributions. As severity increases, the central nervous system provides a greater contribution and this is manifest by its co-association with psychosocial distress and comorbidities that increased symptom reporting and maladaptive coping[26].

Moreover, based on the current design, we were unable to investigate on the pathophysiological link between IBS and TMD. For instance, patients with both IBS and TMD are not only more pain sensitive[27] but also demonstrate reduced pain inhibition, possibly because of dysfunction of endogenous pain inhibition

systems in accordance with the theory of a generalized upregulation of pain processing in chronic pain conditions[28].

This may predispose to the development of other chronic pain syndromes (*i.e.*, IBS leading to TMD) or may lead to the transition from a localized pain disorder to a widespread pain disorder[29].

In addition, psychological symptoms were shown to contribute to these sensory dysfunctions and may be involved in pain modulation processes that are related to chronic pain.

These findings could be clinically relevant, suggesting that in order to improve IBS symptoms other existing comorbidities need to be assessed, probably in interdisciplinary clinics hosting multiple health professions.

In addition, studies about the treatment of these co-morbid conditions have shown that a simultaneous therapeutic approach to multiple diseases is more effective than the separate treatment of each[20]. Thus a multidisciplinary therapy should be encouraged. Likewise, future research elucidating neurobehavioral processes underlining chronic pain is welcomed.

COMMENTS

Background

Irritable bowel syndrome (IBS) is a functional intestinal disorder characterized by chronic pain or discomfort in the abdomen associated with altered bowel habits. IBS commonly overlap with other comorbid pain conditions. The association between IBS and temporomandibular disorders (TMDs) remains largely unexplored.

Research frontiers

It has been recently highlighted by Rome Ⅳ criteria that a variety of other GI (*i.e.,* dyspepsia) and non-GI symptoms (*i.e.,* migraine headaches, fibromyalgia, interstitial cystitis, dyspareunia) are frequently present in IBS patients; the presence of these concomitant symptoms lends further support to the diagnosis and should be considered for a better management of these patients.

Innovations and breakthroughs

These results showed that IBS patients had a higher risk of having TMD compared to Healthy Controls and demonstrated that IBS patients that fulfilled criteria for TMD share along with chronic facial and abdominal pain a significant co-occurrence with psychiatric disorders and female preponderance. Compared to similar studies in previous literature, this study evaluated the presence of TMD according to the RDC/TMD criteria that are worldwide-accepted criteria for the diagnosis of these disorders together with a diagnosis of IBS and its different subtypes based on a standardized questionnaire according to Rome Ⅲ criteria and not on the patients' subjective evaluation of their bowel habits. Moreover, the authors inquired about current facial and abdominal pain to avoid a recall bias. Chronic abdominal pain is the predominant feature in IBS. In fact, the newest Rome Ⅳ criteria published last May eliminated the term discomfort from the diagnostic criteria focused only on recurrent abdominal pain.

Applications

IBS patients could be better treated through patient-centered management that takes into account other coexisting comorbitidies. A simultaneous therapeutic approach to multiple diseases is more effective than the separate treatment of each. Thus a multidisciplinary therapy should be encouraged.

Terminology

TMDs are a heterogeneous group of diseases of the stomatognathic system that involve the temporomandibular joint (TMJ), masticatory muscles and their related structures. The cardinal symptoms are pain in the TMJ and/or masticatory muscles, joint sounds and alterations in mandibular movement.

Peer-review

Serena Gallota and co-workers aimed to investigate the prevalence and the risk of TMDs in patients with IBS, included in a prospective study. They showed that IBS patients had a higher risk of having TMD compared to healthy controls and demonstrated that in this study group IBS patients that fulfilled also criteria for TMD share along with chronic facial and abdominal pain a significant co-occurrence with psychiatric disorders and female preponderance.

REFERENCES

1 **Rome Foundation**. Guidelines--Rome III Diagnostic Criteria for Functional Gastrointestinal Disorders. *J Gastrointestin Liver Dis* 2006; **15**: 307-312 [PMID: 17203570]

2 **Whitehead WE**, Palsson O, Jones KR. Systematic review of the comorbidity of irritable bowel syndrome with other disorders: what are the causes and implications? *Gastroenterology* 2002; **122**: 1140-1156 [PMID: 11910364 DOI: 10.1053/gast.2002.32392]

3 **Lubrano E**, Iovino P, Tremolaterra F, Parsons WJ, Ciacci C, Mazzacca G. Fibromyalgia in patients with irritable bowel syndrome. An association with the severity of the intestinal disorder. *Int J Colorectal Dis* 2001; **16**: 211-215 [PMID: 11515679 DOI: 10.1007/s003840100299]

4 **Whorwell PJ**, McCallum M, Creed FH, Roberts CT. Non-colonic features of irritable bowel syndrome. *Gut* 1986; **27**: 37-40 [PMID: 3949235 DOI: 10.1136/gut.27.1.37]

5 **Dahan H**, Shir Y, Velly A, Allison P. Specific and number of comorbidities are associated with increased levels of temporomandibular pain intensity and duration. *J Headache Pain* 2015; **16**: 528 [PMID: 26002637 DOI: 10.1186/s10194-015-0528-2]

6 **Grossi ML**, Goldberg MB, Locker D, Tenenbaum HC. Irritable bowel syndrome patients versus responding and nonresponding temporomandibular disorder patients: a neuropsychologic profile comparative study. *Int J Prosthodont* 2008; **21**: 201-209 [PMID: 18548956]

7 **Traub RJ**, Cao DY, Karpowicz J, Pandya S, Ji Y, Dorsey SG, Dessem D. A clinically relevant animal model of temporomandibular disorder and irritable bowel syndrome comorbidity. *J Pain* 2014; **15**: 956-966 [PMID: 24981128 DOI: 10.1016/j.jpain.2014.06.008]

8 **Schiffman E**, Ohrbach R, Truelove E, Look J, Anderson G, Goulet JP, List T, Svensson P, Gonzalez Y, Lobbezoo F, Michelotti A, Brooks SL, Ceusters W, Drangsholt M, Ettlin D, Gaul C, Goldberg LJ, Haythornthwaite JA, Hollender L, Jensen R, John MT, De Laat A, de Leeuw R, Maixner W, van der Meulen M, Murray GM, Nixdorf DR, Palla S, Petersson A, Pionchon P, Smith B, Visscher CM, Zakrzewska J, Dworkin SF; International RDC/TMD Consortium Network, International association for Dental Research; Orofacial Pain Special Interest Group, International Association for the Study of Pain. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache* 2014; **28**: 6-27 [PMID: 24482784 DOI: 10.11607/jop.1151]

9 **LeResche L**. Epidemiology of Orofacial Pain: Prevalence, Incidence, and Risk Factors. In: Sessle BJ Lavigne GJ, Dubner R, Lund JP, editors. Orofacial Pain. From Basic Science to Clinical Management second ed. Chicago (IL): Quintessence Publishing Co., 2009: 13

10 **Mobilio N**, Casetta I, Cesnik E, Catapano S. Prevalence of self-reported symptoms related to temporomandibular disorders in an Italian population. *J Oral Rehabil* 2011; **38**: 884-890 [PMID: 21595739 DOI: 10.1111/j.1365-2842.2011.02228.x]

11 **Dworkin SF**, Huggins KH, LeResche L, Von Korff M, Howard J, Truelove E, Sommers E. Epidemiology of signs and symptoms in temporomandibular disorders: clinical signs in cases and controls. *J Am Dent Assoc* 1990; **120**: 273-281 [PMID: 2312947 DOI:

10.14219/jada.archive.1990.0043]
12 **Dworkin SF**, LeResche L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. *J Craniomandib Disord* 1992; **6**: 301-355 [PMID: 1298767]
13 **Jerjes W**, Upile T, Abbas S, Kafas P, Vourvachis M, Rob J, Mc Carthy E, Angouridakis N, Hopper C. Muscle disorders and dentition-related aspects in temporomandibular disorders: controversies in the most commonly used treatment modalities. *Int Arch Med* 2008; **1**: 23 [PMID: 18973654 DOI: 10.1186/1755-7682-1-23]
14 **Korszun A**, Papadopoulos E, Demitrack M, Engleberg C, Crofford L. The relationship between temporomandibular disorders and stress-associated syndromes. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; **86**: 416-420 [PMID: 9798224 DOI: 10.1016/S1079-2104(98)90366-3]
15 **Aaron LA**, Burke MM, Buchwald D. Overlapping conditions among patients with chronic fatigue syndrome, fibromyalgia, and temporomandibular disorder. *Arch Intern Med* 2000; **160**: 221-227 [PMID: 10647761 DOI: 10.1001/archinte.160.2.221]
16 **Jones KR**, Palsson OS, Levy RL, Feld AD, Longstreth GF, Bradshaw BH, Drossman DA, Whitehead WE. Comorbid disorders and symptoms in irritable bowel syndrome (IBS) compared to other gastroenterology patients. *Gastroenterology* 2001; **120** (suppl 1): A66 [DOI: 10.1016/S0016-5085(08)80328-9]
17 **Sanders AE**, Slade GD, Bair E, Fillingim RB, Knott C, Dubner R, Greenspan JD, Maixner W, Ohrbach R. General health status and incidence of first-onset temporomandibular disorder: the OPPERA prospective cohort study. *J Pain* 2013; **14**: T51-T62 [PMID: 24275223 DOI: 10.1016/j.jpain.2013.06.001]
18 **Sperber AD**, Dekel R. Irritable Bowel Syndrome and Co-morbid Gastrointestinal and Extra-gastrointestinal Functional Syndromes. *J Neurogastroenterol Motil* 2010; **16**: 113-119 [PMID: 20535341 DOI: 10.5056/jnm.2010.16.2.113]
19 **Riedl A**, Schmidtmann M, Stengel A, Goebel M, Wisser AS, Klapp BF, Mönnikes H. Somatic comorbidities of irritable bowel syndrome: a systematic analysis. *J Psychosom Res* 2008; **64**: 573-582 [PMID: 18501257 DOI: 10.1016/j.jpsychores.2008.02.021]
20 **Velly AM**, Fricton J. The impact of comorbid conditions on treatment of temporomandibular disorders. *J Am Dent Assoc* 2011; **142**: 170-172 [PMID: 21282683 DOI: 10.14219/jada.archive.2011.0060]
21 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491 [PMID: 16678561 DOI: 10.1053/j.gastro.2005.11.061]
22 **Francis CY**, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther* 1997; **11**: 395-402 [PMID: 9146781 DOI: 10.1046/j.1365-2036.1997.142318000.x]
23 **Prunas A**, Sarno I, Preti E, Madeddu F, Perugini M. Psychometric properties of the Italian version of the SCL-90-R: a study on a large community sample. *Eur Psychiatry* 2012; **27**: 591-597 [PMID: 21334861 DOI: 10.1016/j.eurpsy.2010.12.006]
24 **Manfredini D**, Borella L, Favero L, Ferronato G, Guarda-Nardini L. Chronic pain severity and depression/somatization levels in TMD patients. *Int J Prosthodont* 2010; **23**: 529-534 [PMID: 21209988]
25 **Mearin F**, Lacy BE, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel Disorders. *Gastroenterology* 2016; Epub ahead of print [PMID: 27144627 DOI: 10.1053/j.gastro.2016.02.031]
26 **Drossman DA**, Chang L, Bellamy N, Gallo-Torres HE, Lembo A, Mearin F, Norton NJ, Whorwell P. Severity in irritable bowel syndrome: a Rome Foundation Working Team report. *Am J Gastroenterol* 2011; **106**: 1749-1759; quiz 1760 [PMID: 21747417 DOI: 10.1038/ajg.2011.201]
27 **Greenspan JD**, Slade GD, Bair E, Dubner R, Fillingim RB, Ohrbach R, Knott C, Mulkey F, Rothwell R, Maixner W. Pain sensitivity risk factors for chronic TMD: descriptive data and empirically identified domains from the OPPERA case control study. *J Pain* 2011; **12**: T61-T74 [PMID: 22074753 DOI: 10.1016/j.jpain.2011.08.006]
28 **King CD**, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL. Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. *Pain* 2009; **143**: 172-178 [PMID: 19278784 DOI: 10.1016/j.pain.2008.12.027]
29 **Piché M**, Bouin M, Arsenault M, Poitras P, Rainville P. Decreased pain inhibition in irritable bowel syndrome depends on altered descending modulation and higher-order brain processes. *Neuroscience* 2011; **195**: 166-175 [PMID: 21889972 DOI: 10.1016/j.neuroscience.2011.08.040]

P- Reviewer: Carroccio A, Lakatos PL **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.110

World J Gastroenterol 2017 January 7; 23(1): 110-120
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Expression of trefoil factors and TWIST1 in colorectal cancer and their correlation with metastatic potential and prognosis

Akram Yusup, Bailikezi Huji, Cheng Fang, Fei Wang, Tuerxunjiang Dadihan, Hai-Jiang Wang, Halmurat Upur

Akram Yusup, Bailikezi Huji, Hai-Jiang Wang, Department of Gastrointestinal Surgery and Record Room, Affiliated Tumor Hospital, Xin Jiang Medical University, Urumqi 830011, Xinjiang Uygur Autonomous Region, China

Cheng Fang, Fei Wang, Department of Gastrointestinal Surgery, Xi Jing Digestive Disease Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China

Tuerxunjiang Dadihan, Department of Human Anatomy of Preclinical College, Xinjiang Medical University, Urumqi 830011, Xinjiang Uygur Autonomous Region, China

Halmurat Upur, Central Laboratory, Xinjiang Medical University, Urumqi 830054, Xinjiang Uygur Autonomous Region, China

Author contributions: Yusup A, Wang HJ and Upur H conceived and designed the study; Yusup A wrote the first draft of the manuscript; Huji B assisted with the collection of the clinicopathological materials and the overall statistics and analysis of the data; Fang C, Wang F and Dadihan T helped perform experiments such as immunohistochemistry, qRT-PCR and Western blot; all authors read and approved the final manuscript.

Supported by the Natural Science Fund of the Xinjiang Uygur Autonomous Region, No. 2014211C111.

Institutional review board statement: The study design and procedures described below were approved by the Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University (No. W-201401).

Informed consent statement: All patients provided informed written consent prior to their participation.

Conflict-of-interest statement: No conflict of interest exists in the submission of this manuscript.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Halmurat Upur, PhD, Central Laboratory, Xinjiang Medical University, 393 Xinyi Road, Urumqi 830054, Xinjiang Uygur Autonomous Region, China. bilik0731@163.com
Telephone: +86-991-4365765
Fax:+86-991-4365231

Received: August 27, 2016
Peer-review started: August 29, 2016
First decision: September 28, 2016
Revised: October 19, 2016
Accepted: December 2, 2016
Article in press: December 2, 2016
Published online: January 7, 2017

Abstract

AIM

To detect the expression of trefoil factors (TFFs) and TWIST1 in colorectal cancer (CRC) and analyze their correlation with metastasis and survival.

METHODS

This study examined the expression of TFF1, TFF3 and TWIST1 in a total of 75 tumor samples, 47 matched normal samples (15 cm from the lesion margin), 30 metastatic lymph nodes, and 10 liver metastatic cancer samples from patients with CRC. The relationship was then analyzed between the protein expression and different clinical records. TFF1, TFF3, TWIST1,E-

cadherin, vimentin and β-catenin mRNA and protein expression levels were measured in colon cancer cell lines with different metastatic potentials (HIEC, HT29, SW620, and LoVo cells), and the correlation of the expression levels with epithelial-mesenchymal transition (EMT) was discussed.

RESULTS

It was found that 66.7% (50/75), 78.7% (59/75) and 54.7% (41/75) of tumor tissue samples exhibited positive staining for TFF1, TFF3 and TWIST1 and so did 27.3% (13/47), 100% (47/47) and 17% (8/47) of adjacent normal colorectal tissues. Compared with adjacent normal tissues, significant differences were found in the expression of all three proteins in different cancerous tissues ($P < 0.05$). Higher expression of TFF3 and TWIST1 was significantly correlated with lymph node metastasis ($P = 0.034$, $P = 0.000$), advanced stage ($P = 0.031$, $P = 0.003$), and poorer survival ($P = 0.042$ for the TFF3 group, $P = 0.003$ for the TWIST1 group). The expression of TFF3 and TWIST1 in cancer cell lines was higher than that in HIEC (a normal human intestinal epithelial cell line)($P < 0.05$), and the expression intensity demonstrated a tendency to rise with increased metastatic potential both at the protein and mRNA levels. However, TFF1 expression demonstrated the opposite tendency. It was also observed that the expression of E-cadherin and β-catenin tended to decrease while that of vimentin, TWIST1 and Snail tended to rise with the increase in metastatic potential.

CONCLUSION

The expression of TFF3 and TWIST1 might be associated with the survival of patients with CRC after curative resection and might be pivotal predictors of disease progression. TFF3 may be correlated to the invasiveness of CRC.

Key words: Colorectal cancer; Trefoil factors; TWIST1; Survival; Metastasis

Core tip: The expression of trefoil factors (TFFs) and TWIST1 in colorectal cancer (CRC) and their roles in metastasis and survival are unclear. This study involved the preliminary examination of the expression of TFF1, TFF3 and TWIST1 in CRC tissues and different metastasis samples from patients and cell lines. This study also analyzed the relationship between the expression of these proteins and metastatic potential and survival. It can be concluded that the expression of TFF3 and TWIST1 in CRC might be associated with patient survival after curative resection and may play an active role in disease progression. Finally, TFF3 may be correlated to the invasiveness of the CRC.

Yusup A, Huji B, Fang C, Wang F, Dadihan T, Wang HJ, Upur H. Expression of trefoil factors and TWIST1 in colorectal cancer and their correlation with metastatic potential and prognosis. *World J Gastroenterol* 2017; 23(1): 110-120 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/110.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.110

INTRODUCTION

Colorectal cancer is one of the most common cancers and a principal cause of death worldwide. The morbidity and mortality rates of CRC continue to increase, but the etiology is still not clear. In 2012, 1361000 new cases of CRC were reported globally, of which 253000 (accounting for 18.6%) were in China; notably, CRC has become the third most common malignancy in China[1]. Approximately one quarter of patients have metastases when CRC is initially diagnosed and nearly half ultimately develop metastases; thus, the 5-year survival rate is less than 10%[2-3]. In order to improve the prognosis of CRC, the most important considerations are the selection of high-risk patients with the help of the biomarkers and the intervention in the metastases.

The trefoil factors (TFFs) are three-loop structures that function as secretory peptides. They contain a highly conserved motif of cysteine disulfide bonds, which endows them with significant functional stability[4-6]. TFF1 is predominantly expressed in the stomach and colon, TFF2 expression is primarily localized in the stomach, and TFF3 expression is commonly observed in the intestine[7,8]. Although TFFs have been shown to protect the gastrointestinal tract against mucosal damage[9,10], recent evidence from experimental and clinical studies indicates a pivotal role of TFFs in oncogenic transformation, growth, and metastasis of human solid tumors[11-19]. TFF1, also called "estrogen-inducible pS2 protein", can be isolated from human breast cancer and has been determined to have prognostic significance in breast cancer patients[20]. TFF3 has indicated both pro-invasive and pro-angiogenic activities in cells derived from several common human solid tumors[15,17,21-25]. TFF1 and TFF3 have been found to be co-expressed particularly in tumors of the human mammary gland[12,26], where they each regulate the expression of the other in a positive feedback loop[27]. Moreover, serum levels of TFFs in patients with several cancers have been reported as useful biomarkers for the prediction of the presence of cancer[28-30]. The exact role of TFFs in the progression of CRC and their prognostic value have not yet been extensively expounded. Despite that TFFs are primarily secreted by the epithelium of the gastrointestinal tract, there are no any consensus about if TFFs are oncogenic or anti-oncogenic[31].

TWIST1 is a highly conserved basic helix-loop-helix (bHLH) protein and transcription factor[32]. It is involved in embryonic development in vertebrates through its regulation of epithelial-mesenchymal transition (EMT) during neural crest migration[33,34]. The EMT program is

Table 1 TFF1, TFF3, and TWIST1 expression in different colorectal cancer tissues *n* (%)

Histology	*n*	TFF1				TFF3				TWIST1			
		-	+	++	+++	-	+	++	+++	-	+	++	+++
CRC	75	25 (41)	19 (35.8)	23 (67.6)	8 (57.1)	16 (72.7)	34 (55.7)	21 (38.9)	4 (16)	34 (34.7)	18 (56.3)	16 (64)	7 (100)
MNT	47	34 (55.7)	12 (22.6)	1 (2.9)	0	0	8 (13.1)	22 (40.7)	17 (68)	39 (41.9)	7 (21.9)	1 (4.3)	0
MLN	30	2 (3.3)	17 (32.1)	8 (23.5)	3 (21.4)	6 (27.3)	14 (23)	8 (14.8)	2 (8)	20 (21.7)	5 (15.6)	5 (21.7)	0
Liver M	10	0	5 (9.4)	2 (5.9)	3 (21.4)	0	5 (8.2)	3 (5.6)	2 (8.0)	5 (5.4)	2 (6.3)	3 (13)	0
Overall		$P = 0.000$				$P = 0.000$				$P = 0.000$			

CRC: Colorectal cancer; MNT: Matched normal tissue; MLN: Metastatic lymph node; Liver M: Liver metastasis; TFF: Trefoil factor.

also activated in a wide variety of cancer cells as they leave the primary tumor and colonize distant organs and form metastases[35,36]. TWIST1 overexpression induces EMT and E-cadherin repression, suggesting that TWIST1 promotes metastasis by inducing EMT[37,38]. Zhu *et al*[39] reported that TFF3 continuously up-regulates the expression of TWIST1 in HT29 cell line, which indicates the possible involvement of TFF3 in the process of EMT.

In this study, we detected the expression of TFF1, TFF3 and TWIST1 in different CRC tissues and cell lines with different metastatic potential to identify the relationship between the protein expression and metastatic potential and survival of CRC.

MATERIALS AND METHODS

This study included a total of 75 tumor samples, 47 matched normal controls (tissue located 15 cm away from the lesion margin), 30 metastatic lymph nodes, and 10 liver metastatic cancer samples from patients with CRC. Among the 75 patients studied, 48 (64%) were men and 27 (36%) were women with a median age of 56 years (range, 24 to 86 years). Fifteen (20%) cases had stage Ⅰ disease, 27 (36%) had stage Ⅱ disease, 27 (36%) had stage Ⅲ disease, and 6 (8%) had stage Ⅳ disease (Table 1). CRC tissues were obtained from treatment-naive patients who underwent surgery at the Fourth Military Medical University Hospital of Xi Jing, between 2007 and 2008. The clinicopathologic variables and survival data were obtained from the medical records, and the disease stages of the patients were classified according to the 2010 UICC colorectal cancer TNM staging system. The data were analyzed anonymously and reported. No patients received any interventions, and personal information was not revealed.

Immunohistochemistry

Immunohistochemical staining was performed using the streptavidin-biotin peroxidase complex with a commercially available SP-kit (Beijing ZhongshanJinqiao Biotechnology Limited Company, China, SP-9000). A rabbit anti-human TFF1 monoclonal antibody (Abcam-Epitomics, United States, 2801-1 dilution: 1:100), a rabbit anti-human TFF3 monoclonal antibody (Abcam-Epitomics, United States, 2816-1 dilution: 1:200), and a mouse anti-human TWIST1 monoclonal antibody (Abcam, United States, ab135180 dilution: 1:100) were used. As a negative control, PBS was used in place of the primary antibody. After deparaffinization and rehydration, the sections were heated in a microwave oven for 10 min at 100 ℃ in 10 mmol/L citrate buffer (pH 6.0). The sections were then incubated sequentially with fresh 3% hydrogen peroxide in PBS, 10% normal goat serum, primary antibody, biotinylated goat anti-rabbit IgG, and streptavidin-peroxidase; the slides were washed with PBS three times before each step. The visualization of the sites of peroxidase binding was achieved with diaminobenzidine. The sections were counterstained with hematoxylin. All slides were interpreted by two independent observers in a blinded fashion.

Evaluation of immunostaining

The immunohistochemistry results were evaluated according to a histoscore that combines the intensity of the immunoreaction with the scope of the positive staining area. The intensity (I) of staining was scored as 0 (no staining), 1 (weak), 2 (moderate), or 3 (strong). The density (D) of staining was scored as 1 (less than 10%), 2 (10%-50%), 3 (50%-80%), or 4 (80%-100%) according to the percentage of positively stained regions in relation to the total cancer area. The final immunohistochemical staining scores (0-12) for each tumor were the product of I × D. Samples with I × D $\leqslant$ 4 were considered weakly positive; those with I × D $\geqslant$ 4 were considered strongly positive.

Cell culture

All cell culture media were purchased from HyClone, Thermo. Three human colon carcinoma cell lines with different invasive potentials (HT29, SW620, and LoVo) and an immortalized human epithelial cell line (HIEC) were gifts from the laboratory of Xi Jing, Digestive Disease Hospital (Fourth Military Medical University). All cell lines were maintained in RPMI 1640 growth medium supplemented with 10% heat-inactivated fetal bovine serum, 1% penicillin, and 1% streptomycin. HIEC cells were also supplemented with human insulin (0.1 U/mL).

Western blot assay

The four colorectal cancer cell lines were harvested

separately in ice-cold PBS. Total protein was extracted, separated by SDS-PAGE, and transferred onto PVDF membranes. The membranes were blocked with 5% (w/v) dried skimmed milk powder in Tris-buffered saline (blocking solution) for 1 h at room temperature. Then, the membranes were probed with primary antibodies against TFF1 (1/500 dilution), TFF3 (1/300 dilution), and TWIST1 (1/500) in blocking solution overnight at 4 ℃. After a TBST wash, the membranes were incubated with the corresponding secondary antibody (1/2000 dilution) in blocking solution for 1 h at room temperature. Immunoreactive proteins were detected using enhanced chemiluminescence.

Quantitative real-time RT-PCR assays

The mRNA levels of target genes in colon cancer cell lines were compared with those in the immortalized human normal intestinal epithelial cell line (HIEC). Quantitative real-time RT-PCR (qRT-PCR) was performed as previously described[14]; Brilliant SYBR Green QRT-PCR Master Mix as part of a 2-Step kit (Stratagene, La Jolla, CA, United States) was used. The PCR amplification was performed using a Bio-Red Real Time PCR system. The primers used for qRT-PCR were as follows: GAPDH forward (F): 5′-AGCCTTCTCCATGGTGGTGAA-3′, GAPDH reverse (R): 5′-ATCACCATCTTCCAGGAGCGA-3′; TFF1 (F): 5′-AATAAGGGCTGCTGTTTCG-3′, TFF1 (R): 5′-ACTCCTCTTCTGGAGGGAC-3′; TFF3 (F): 5′-CTGCTGCTTTGACTCCAGGAT-3′, TFF3 (R): 5′-CAGCTGGAGGTGCCTCAGAA-3′; TWIST1 (F): 5′-CATGTCCGCGTCCCACTAG-3′, TWIST1-(R): 5′-TGTCCATTTTCTCCTTCTCTGG-3′; E-cadherin (F): 5′-GAGTGCCAACTGGACCATTCAGTA-3′, E-cadherin (R): 5′-AGTCACCCACCTCTAAGGCCATC-3′; vimentin (F): 5′-CAGGCAAAGCAGGAGTCCAC-3′; vimentin (R): 5′-GCAGCTTCAACGGCAAAGTTC-3′; β-catenin (F): 5′-TGAGTGTCATGAAGTGCACAGGAG-3′, β-catenin (R): 5′-AACAGGCTGATGGTGCCAGAG-3′; snail (F): 5′-CGCGCTCTTTCCTCGTCA-3′, snail (R): 5′-TCCCAGATGAGCATTGGCAG-3′. The Ct (threshold cycle) values of the target gene amplifications were normalized to those of the GAPDH control. All reactions were performed in triplicate in a 25-μL reaction volume. The PCR amplification program consisted of 30 s of an initial denaturation at 95 ℃ followed by 40 cycles of PCR at 95 ℃ for 5 s, and then 60 ℃ for 30 s. Standard curves were drawn and the relative amount of target gene mRNA was normalized to that of GAPDH. Specificity was verified by melt curve analysis. The comparative CT method was used to calculate the relative quantification of gene expression.

RESULTS

Expression of TFFs and TWIST1 in normal, primary tumor, and metastatic tissues

The positive expression of TFF1 and TFF3 was found in the cytoplasm and cytomembrane (Figure 1A), while TWIST1 staining was observed in both the cytoplasm and nuclei of the cells (Figure 1A). TFF1 was mainly expressed in CRC cells with varying intensity and was only occasionally expressed or was negative in matched normal tissues (Figure 1B). TFF3 was distributed diffusely and was expressed as fine granules in most goblet cells in normal tissues, but its expression was decreased in the cytoplasm of CRC cells (Figure 1A and B). We found selective expression of TWIST1 in cancerous tissues but scarcely in their normal counterparts (Figure 1A and B).

Among the 50 tissues that were positive for TFF1, 19 demonstrated weak staining (38%), 23 demonstrated moderate staining (46%), and 8 demonstrated strong staining (16%) (Table 1). Among the 59 patients with TFF3-positive cancer, 34 showed weak staining (57.6%), 21 showed moderate staining (35.6%), and 4 showed strong staining (6.8%) (data not shown). Of the 41 patients with TWIST1-positive cancer, 18 showed weak staining (43.9%), 16 showed moderate staining (39%), and 7 showed strong staining (17.1%) (Table 1).

Results from the assessment of TFF1, TFF3 and TWIST1 expression in primary tumor tissues, metastatic lymph nodes, and liver metastatic tissues are presented in Table 1. It was shown that 66.7% (50/75), 78.7% (59/75), and 54.7% (41/75) of samples exhibited positive staining for TFF1, TFF3 and TWIST1, respectively. In addition, 27.3% (13/47), 100% (47/47), and 17% (8/47) of normal tissues adjacent to colorectal CRC tissues showed positive staining for TFF1, TFF3 and TWIST1, respectively. Compared with normal mucosal tissues, primary CRC tissues expressed significantly higher levels of TFF1 and TWIST1 but a lower level of TFF3 (Table 1). No significant difference was observed in TFF3 or TWIST1 expression between primary CRC tissues and metastatic lymph nodes or liver metastatic tissues (P = 0.879, P = 0.105 for TFF3 and P = 0.08, P = 0.780 for TWIST1). However, the disparity in the expression of TFF1 between metastatic lymph nodes and primary colorectal cancer was statistically significant (P = 0.01, P = 0.03) (Table 2). The correlation analysis between the expression of TFF1 and TFF3 proteins also showed a negative correlation in CRC (P = 0.007, r = -0.312; Table 3). We also found that the expression levels of TFF3 were stronger in mucinous and signet-ring cell cancers than in other cancer types, which might illustrate that the more mucus that is secreted, the stronger the TFF3 expression.

Expression of TFF1, TFF3 and TWIST1 in relation to clinicopathological features

The results illustrating a correlation between TFF1, TFF3, and TWIST1 expression and clinicopathological variables are presented in Table 4. Higher expression of TFF3 and TWIST1 was significantly correlated with

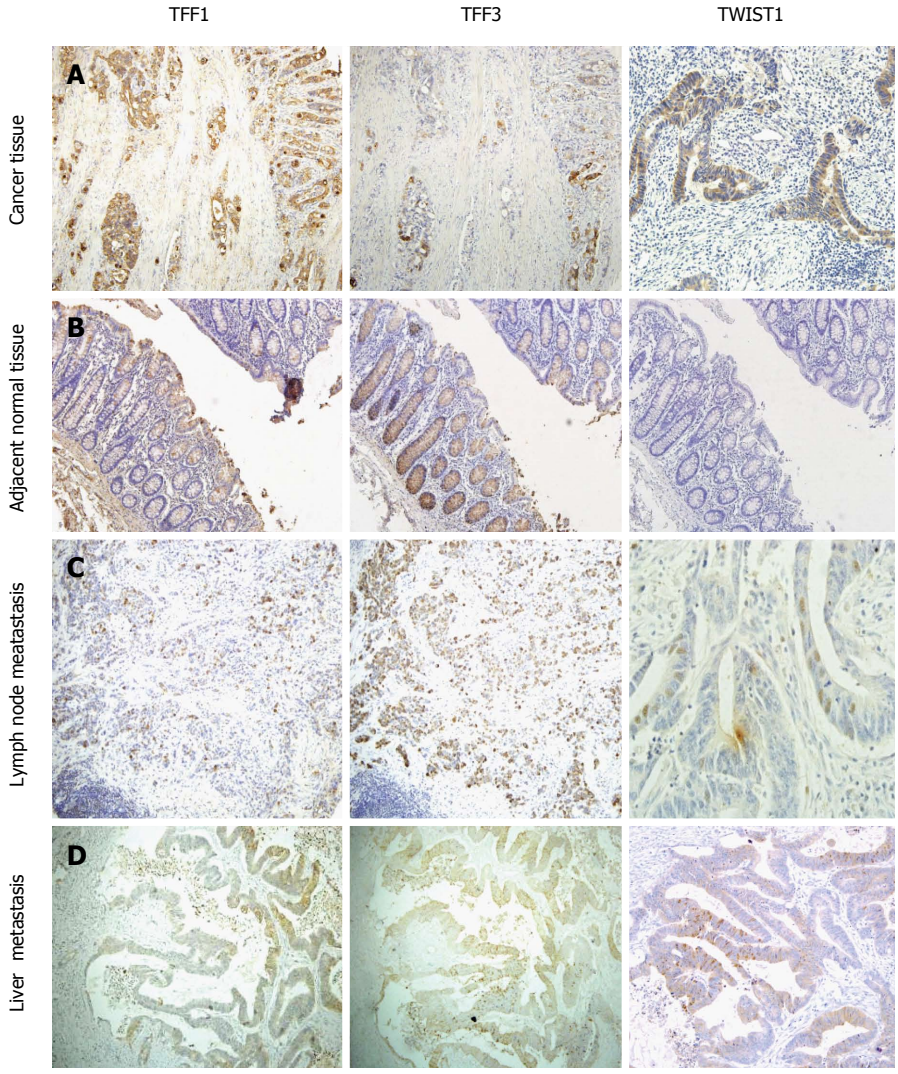


Figure 1 TFF1, TFF3 and TWIST1 expression trends in colorectal cancer (A), adjacent normal tissue (B), metastatic lymph node (C), and liver metastasis (D). Compared with normal mucosal tissue, colorectal cancer tissues expressed significantly higher TFF1 and TWIST1 and lower TFF3. Compared to primary colorectal cancer tissue, there were no statistical differences in TFF3 and TWIST1 expression in metastatic lymph node or liver metastatic tissue. But disparity in TFF1 expression between metastatic lymph node and primary colorectal cancer had statistical significance.

lymph node metastasis (P = 0.034, P = 0.000) and advanced stage (P = 0.031, P = 0.003). In contrast, TFF1 expression was correlated with differentiation (P = 0.029).

Relationship between TFF1, TFF3 and TWIST1 expression and patient's survival

The survival analysis indicated that the 3- and 5-year survival rates of the 75 patients were 71% (55 patients) and 54% (43 patients), respectively. The overall survival of the patients with higher TFF3 or TWIST1 expression levels was significantly less than that of the patients with lower TFF3 or TWIST1 expression levels (P = 0.042 for the TFF3 group; P = 0.003 for the TWIST1 group; Figure 2A and B). TFF1 expression demonstrated no correlation with patient survival (P = 0.952; Figure 2D). In contrast, higher expression of TFF3 and TWIST1 was correlated with an even worse overall survival (Figure 2C). Furthermore, a univariate analysis showed that the survival rate had a close relationship with age, infiltration depth, lymph node metastasis, distant metastasis, TFF3 expression, TWIST1 expression, and TNM stage (P < 0.05). A multivariate analysis demonstrated that only age, tumor stage, and TFF3 expression were correlated with survival (Table 5).

Expression of TFF1 and TFF3 proteins and mRNAs demonstrates the opposite tendency and is associated with EMT in vitro

Based on the immunohistochemistry results, we determined gene expression in colon cancer cell lines with different metastatic potentials. Our results showed that the expression levels of TFF3 and TWIST1 in cancer cell lines were higher than those in the normal human intestinal epithelial cell line (P < 0.05). Moreover, the expression intensities were inclined to rise both at the protein and mRNA levels with the increase in metastatic

Table 2 Comparison between low and high expression of TFF1, TFF3, and TWIST1 in different colorectal cancer tissues *n* (%)

Histology	*n*	TFF1			TFF3			TWIST1		
		Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
CRC	75	44 (38.6)	31 (64.6)	0.000	50 (60.2)	25 (31.6)	0.000	52 (40)	23 (71.9)	0.001
MNT	47	46 (40.4)	1 (2.1)	0.000	8 (9.6)	39 (49.4)	0.000	46 (35.4)	1 (3.1)	0.0001
MLN	30	19 (16.7)	11 (22.9)	0.659	20 (24.1)	10 (12.7)	0.818	25 (19.2)	5 (15.6)	0.142
Liver M	10	5 (4.4)	5 (10.4)	0.602	5 (6)	5 (6.3)	0.494	7 (5.4)	3 (9.4)	0.966

CRC: Colorectal cancer; MNT: Matched normal tissue; MLN: Metastatic lymph node; Liver M: Liver metastasis.

Table 3 Correlation among TFF1, TFF3 and TWIST1expression *n* (%)

Protein expression	TFF1			TFF3		
	Low	High	*P* value	Low	High	*P* value
TWIST1			0.297			0.623
Low	32 (72.7)	19 (61.3)		31 (66)	20 (71.4)	
High	12 (27.3)	12 (38.7)		16 (34)	8 (28.6)	
TFF3						
Low	22 (46.8)	25 (53.2)	0.007			
High	22 (78.6)	6 (21.4)				

Table 4 Correlation between TFF1, TFF3, and TWIST1 expression and clinicopathological Characteristics of colorectal cancers *n* (%)

Characteristic *n* = 75	TFF1			TFF3			TWIST1		
	Low	High	*P* value	Low	High	*P* value	Low	High	*P* value
Age (yr)			0.299			0.368			0.840
≤ 60	28 (63.6)	17 (54.8)		27 (57.4)	18 (64.3)		31 (60.8)	14 (58.3)	
> 60	16 (36.4)	14 (45.2)		20 (42.6)	10 (35.7)		20 (39.2)	10 (41.7)	
Gender			0.369			0.146			0.134
Male	30 (68.2)	18 (58.1)		33 (70.2)	15 (53.6)		30 (58.8)	18 (75.0)	
Female	14 (31.8)	13 (41.9)		14 (29.8)	13 (46.4)		21 (41.2)	6 (25.0)	
Histology			0.089			0.475			0.397
Adenocarcinoma	42 (95.5)	26 (83.9)		42 (89.4)	26 (92.9)		47 (92.2)	21 (87.5)	
Mucinous	2 (4.5)	5 (16.1)		5 (10.6)	2 (7.1)		4 (7.8)	3 (12.5)	
Differentiation			0.029			0.136			0.234
Well	10 (22.7)	12 (38.7)		15 (31.9)	7 (25.0)		18 (35.3)	4 (16.7)	
Moderate	16 (36.4)	15 (48.4)		22 (46.8)	9 (32.1)		20 (39.2)	11 (45.8)	
Poor	18 (40.9)	4 (12.9)		10 (21.3)	12 (42.9)		13 (25.5)	9 (37.5)	
Location			0.802			0.411			0.847
Colon	31 (70.5)	21 (67.7)		31 (66.0)	21 (75)		35 (68.6)	17 (70.8)	
Rectum	13 (29.5)	10 (32.3)		16 (34.0)	7 (25)		16 (31.4)	7 (30.7)	
T			0.545			0.651			0.246
1	2 (4.5)	0		1 (2.1)	1 (3.6)		1 (2.0)	1 (4.2)	
2	10 (22.7)	9 (29.0)		14 (29.8)	5 (17.9)		16 (31.4)	3 (12.5)	
3	13 (29.5)	7 (22.6)		11 (23.4)	9 (32.1)		11 (21.6)	9 (37.5)	
4	19 (43.2)	15 (48.4)		21 (44.7)	13 (46.4)		23 (45.1)	11 (45.8)	
N			0.143			0.034			0.000
No	26 (59.1)	13 (41.9)		20 (42.6)	19 (67.9)		34 (66.7)	5 (20.8)	
Yes	18 (40.9)	18 (58.1)		27 (57.4)	9 (32.1)		17 (33.3)	19 (79.2)	
M			0.484			0.267			0.288
No	41 (93.2)	28 (90.3)		42 (89.4)	27 (96.4)		48 (94.1)	21 (87.5)	
Yes	3 (6.8)	3 (9.7)		5 (10.6)	1 (3.6)		3 (5.9)	3 (12.5)	
Staging			0.917			0.031			0.003
Ⅰ	9 (20.5)	6 (19.4)		11 (23.4)	4 (14.3)		13 (25.5)	2 (8.3)	
Ⅱ	17 (38.6)	10 (32.3)		11 (23.4)	16 (57.1)		23 (45.1)	4 (16.7)	
Ⅲ	15 (34.1)	12 (38.7)		20 (42.6)	7 (25.0)		12 (23.5)	15 (62.5)	
Ⅳ	3 (6.8)	3 (9.7)		5 (10.6)	1 (3.6)		3 (5.9)	3 (12.5)	

CRC: Colorectal cancer; MNT: Matched normal tissue; MLN: Metastatic lymph node; Liver M: Liver metastasis.

Table 5 Significant predictive factors for cancer-specific survival in COX proportional-hazard analysis

Prognostic factor	Univariate HR (95%CI)	*P* value	Multivariate HR (95%CI)	*P* value
Age	0.404 (0.189-0.864)	0.019	0.262 (0.106-0.647)	0.004
Gender	1.137 (0.565-2.287)	0.719	1.065 (0.450-2.519)	0.887
Histopathological type	0.526 (0.126-2.193)	0.378	0.380 (0.080-1.809)	0.224
Tumor differentiation	0.939 (0.611-1.445)	0.775	0.701 (0.395-1.246)	0.226
T	1.556 (1.030-2.350)	0.036	0.979 (0.578-1.657)	0.937
N	2.961 (1.954-4.485)	0.000	1.668 (0.716-3.889)	0.236
M	12.519 (4.593-34.120)	0.000	2.728 (0.672-11.064)	0.160
Stage	4.507 (2.706-7.507)	0.000	2.718 (1.003-7.368)	0.039
TFF1	1.805 (0.929-3.509)	0.081	1.539 (0.661-3.579)	0.317
TFF3	0.382 (0.178-0.819)	0.013	0.224 (0.085-0.593)	0.003
TWIST1	2.819 (1.441-5.515)	0.002	1.904 (0.847-4.278)	0.119

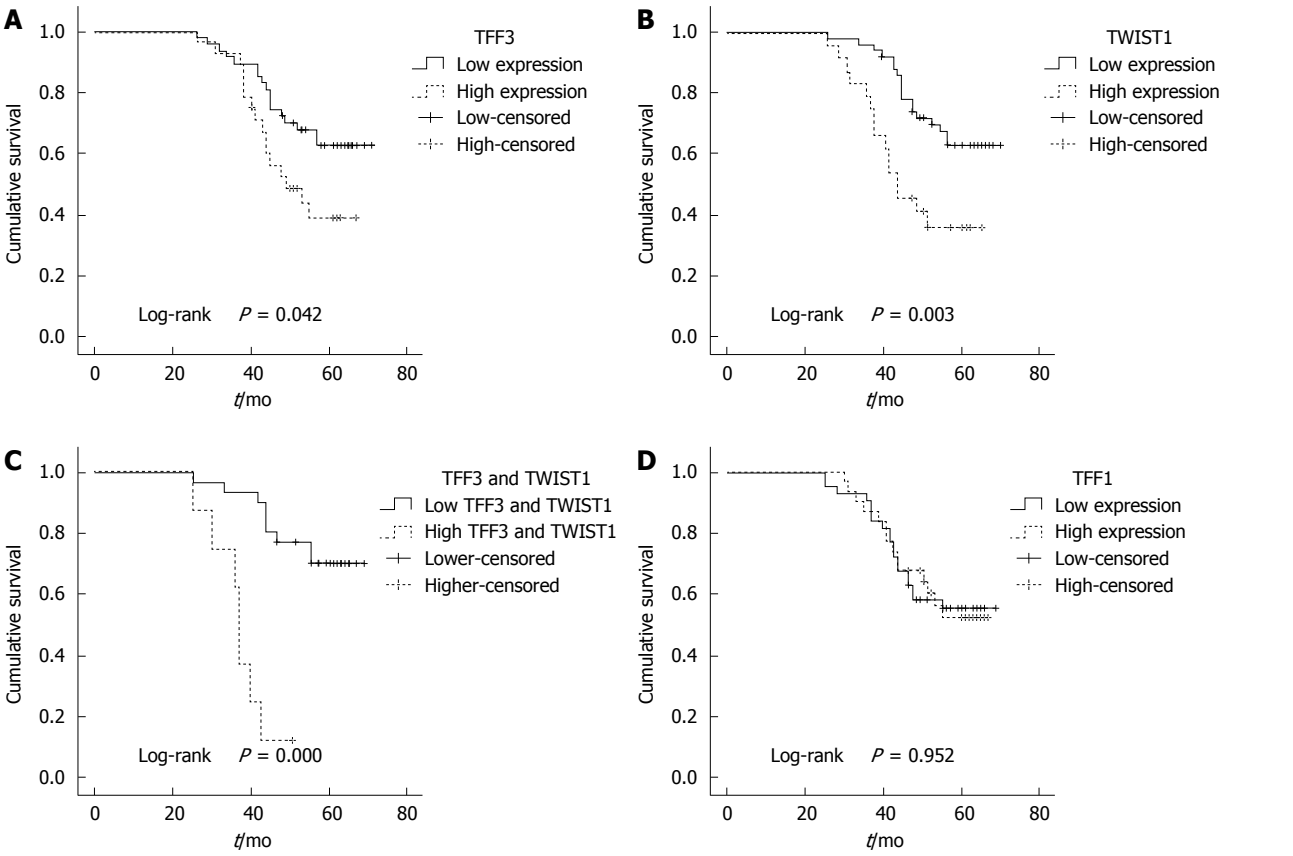


Figure 2 Correlation between protein expression in colorectal cancer and overall survival. The survival analysis indicated that the overall survival of the patients with higher TFF3 or TWIST1 expression was significantly less than that of the patients with lower TFF3 or TWIST1 expression (A and B); Higher expression of the TFF3 and TWIST1 correlated with even worse overall survival (C); TFF1 expression had no correlation with the survival (D).

potential. However, TFF1 expression demonstrated the opposite tendency (Figure 3).

Based on the above-mentioned outcome, we determined whether TFF1 and TFF3 expression was related to EMT. We investigated E-cadherin, vimentin, β-catenin and Snail, which are classic markers and critical transcription factors of EMT in different cell lines. It was observed that the expression levels of TFF1 protein and mRNA were decreased and that the expression levels of TWIST1 and TFF3 increased gradually with the increase in the metastatic potential of the cell lines. It was also observed that E-cadherin and β-catenin expression tended to decrease, while that of vimentin, TWIST1 and Snail tended to increase with the increase in metastatic potential (Figure 3).

DISCUSSION

Although TFFs display a number of beneficial effects in terms of cytoprotection and restitution, these proteins may lead to adverse outcomes when they are over-expressed in different tumor tissues. TFF1 protein was expressed in 89% of CRCs but not in normal mucosa[40], and in another study, the expression of TFF1 was found to be focally present in 60% of primary CRCs[7]. Our data demonstrated that the

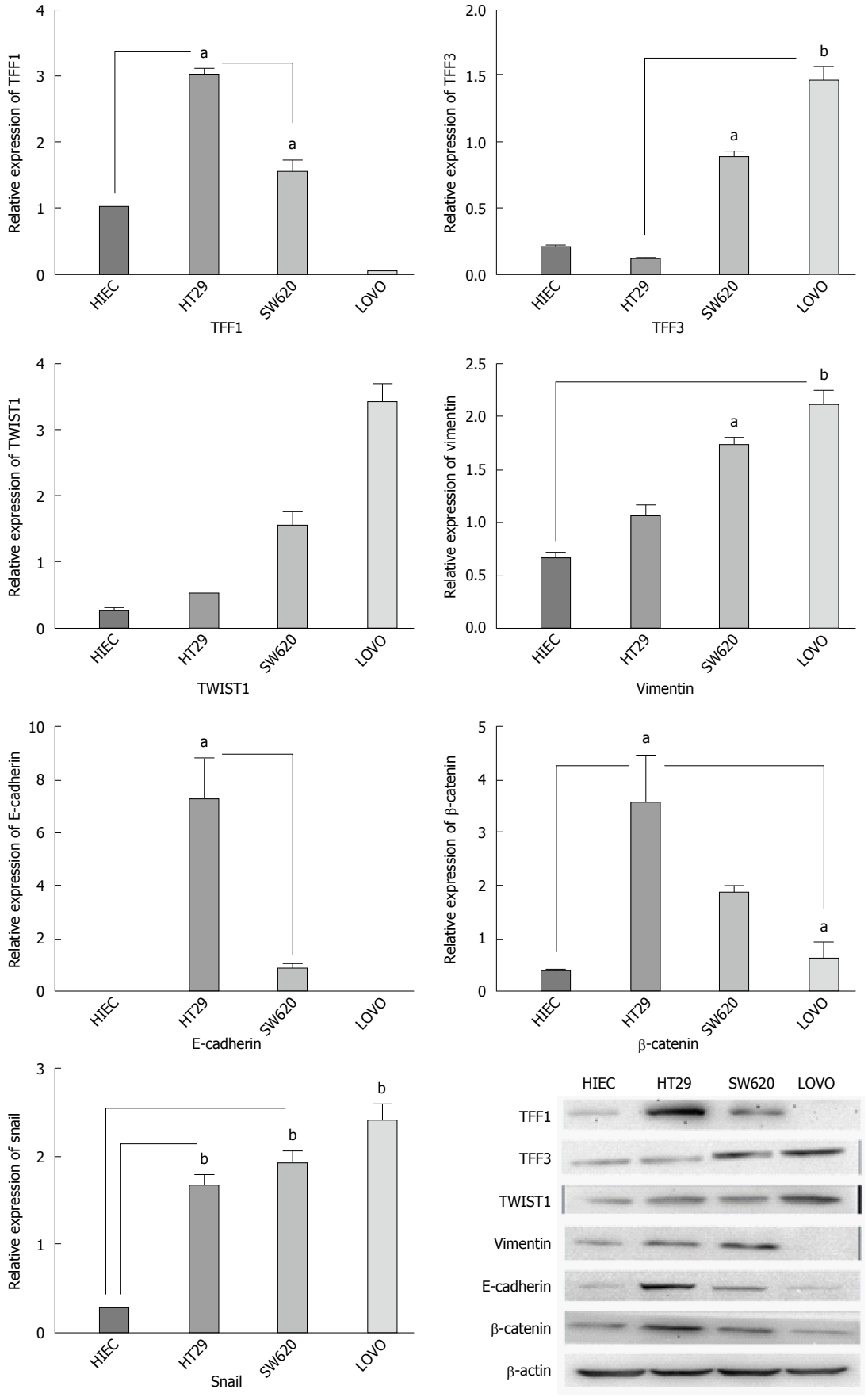


Figure 3 The mRNA and protein expression of TFF1, TFF3, TWIST1, E-cadherin, vimentin, and β-catenin in colon cancer cell lines with different invasion potentials. The expression of TFF3 and TWIST1 in cancer cell lines was higher than that in normal human intestinal epithelial cell line, and the expression intensity was inclined to rise with the increase in metastatic potential both at protein and mRNA levels. But TFF1 expression had the opposite tendency. The TFF1 protein and mRNA expression decreased and TWIST1 and TFF3 expression increased gradually with the increase in metastatic potential of cell lines. E-cadherin and β-catenin expression tended to decrease, while vimentin and TWIST1 expression was inclined to rise with the increase in metastatic potential. $^{a}P < 0.05$, $^{b}P < 0.01$.

TFF1 expression rate was 66.7%. The adjacent normal tissue also exhibited a positive expression rate of 27.3%, which is different to that mentioned above. TFF1 also tended to be increased in adjacent normal tissues, primary cancer tissues, and lymph node metastases successively, which may explain the oncogenic or pro-invasive features of TFF1. Reports on TFF3 and TWIST1 expression in CRC are rare, and the present data showed that their rates of positive expression were 78.7% and 54.7%, respectively, in cancer tissues, while adjacent normal tissues showed positive expression rates of 100% and 17%, respectively. TFF1 and TFF3 protein expression also showed a negative correlation in CRC, which suggests the possibility of mutual regulation among TFF family members. TFF1 protein expression is increased in gastric carcinoma with nodal metastases compared with carcinomas that lack such metastases[7,41]. Our study revealed no statistically significant association between TFF1 expression and clinicopathological features except for differentiation. Im *et al*[41] reported a much higher frequency of TFF1 expression in undifferentiated and diffuse types of gastric cancer compared with differentiated and intestinal types of gastric cancer. Contrary to this, TFF1 expression in CRC showed a higher frequency in well or moderately differentiated cases than in poorly differentiated cases. No correlation was observed between TFF1 expression and survival.

With regard to the clinical significance of TFF3 and TWIST1 expression, we found that higher TFF3 or TWIST1 expression was significantly correlated with lymph node metastasis and advanced stage. Additionally, we demonstrated that patients with higher expression of TFF3 or TWIST1 had a lower survival rate than those with lower expression of TFF3 or TWIST1. Therefore, it could be concluded that TFF3 and TWIST1 can be considered prognostic factors that may be linked to poor survival of patients with CRC.

TFF1 and TFF3 have been observed to be potent mitogens in both normal and cancer cells. The overexpression of TFF1 and TFF3 mRNAs has been found in all cases of ductal cancer *in situ*, lobular cancer *in situ*, invasive lobular cancer and in 21 of 24 invasive ductal cancers tested[42]. The migration and invasiveness of human gastric cancer cells are stimulated by TFF1 in a P13K-dependent manner[43]. TFF3 also induces a migratory and invasive phenotype in human colon cancer cells[22]. TWIST1 has been confirmed to contribute to metastasis through EMT regulation[44]. The inhibition of TWIST1 expression in highly metastatic mammary carcinoma cells specifically suppresses their ability to migrate from the mammary gland to the lung in a mouse model of breast cancer[45]. As for TFF3 and TWIST1 expression, although no significant differences were observed among primary cancers, metastatic lymph nodes and liver metastatic tissues, statistically significant differences were observed among colon cancer cell lines with different invasion potentials by qRT-PCR and Western blot assays.

TFF3, along with TFF1, has been used as a marker for the detection of disseminated breast cancer cells[46]. The decrease in TFF1 mRNA expression and the increase in TFF3 mRNA expression with increasing malignancy in cell lines may suggest opposite functions of these proteins in different types of colon cancer. The decrease in expression of epithelial markers (E-cadherin and β-catenin) and the increase in expression of mesenchymal markers (vimentin, TWIST1, and Snail) indicate that TFF3 may have some correlation with EMT. Further experiments including overexpression or RNA interference assays by transfection are needed to probe this potential correlation.

The expression levels of TFF3 and TWIST1 in CRC might be associated with patient survival after curative resection and are independent predictors of disease progression. In addition, simultaneous positive expression of TFF3 and TWIST1 induces a much worse survival rate. Therefore, it is assumed that TFF3 and TWIST1 expression plays a vital role in the development of CRC. Finally, TFF3 may be correlated to the invasiveness of CRC.

COMMENTS

Background

Despite that TFFs are primarily secreted by the epithelium of the gastrointestinal tract, their role in the progression of colorectal cancer (CRC) and their prognostic value have not been extensively expounded. TWIST1 is a key transcription factor that regulates epithelial-mesenchymal transition (EMT).

Research frontiers

Recent evidence indicates a pivotal role of TFFs in the oncogenic transformation, growth and metastasis of human solid tumors.TFF1 and TFF3 are found to co-express notably in the tumors of the human mammary gland and co-regulate each other in a positive feedback loop. The studies also show that TFF3 continuously up-regulates the expression of TWIST1 in HT29 cell line, which hints the involvement of TFFs in the process of EMT.

Innovations and breakthroughs

Reports on TFFs and TWIST1 expressions in CRC and correlations with metastasis and survival are quite rare. This research findings suggest that The expressions of TFF3 and TWIST1 are associated with CRC patients' survival after a curative resection and pivotal predictors of disease progression. TFF3 may be associated with the invasiveness of the CRC.

Applications

This research outcomes indicate that TFF3 and TWIST1 expression plays a vital role in the development of CRC and these two proteins might be important markers for disease progression and prognosis of CRC. The correlation of TFF3 with the EMT process may hint a potential target of CRC treatment.

Terminology

EMT is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal stem cells; these are multipotent stromal cells that can differentiate into a variety of cell types. EMT is essential for numerous developmental processes including mesoderm formation and neural tube formation. EMT has also been shown to occur in wound healing, in organ fibrosis, and in the initiation of

metastasis for cancer progression.

Peer-review

The studies reported in this manuscript are descriptive/correlative in nature and are well organized. Each discussion presented is intriguing and has some guiding significance for clinical practice and future research.

REFERENCES

1 **Cai SJ**, Peng JJ. Colorectal cancer epidemiology and prevention strategies [CSCO2014: China's Progress of Clinical Oncology]. People's Medical Publishing House, 2014: 294-301

2 **Li S**, Wang J, Lu Y, Fan D. Screening and early diagnosis of colorectal cancer in China: a 12 year retrospect (1994-2006). *J Cancer Res Clin Oncol* 2007; **133**: 679-686 [PMID: 17503079 DOI: 10.1007/s00432-007-0231-z]

3 **Howlader N**, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, Edwards BK. SEER Cancer Statistics Review, 1975-2008, National Cancer Institute. Bethesda, 2011

4 **Thim L**. A new family of growth factor-like peptides. 'Trefoil' disulphide loop structures as a common feature in breast cancer associated peptide (pS2), pancreatic spasmolytic polypeptide (PSP), and frog skin peptides (spasmolysins). *FEBS Lett* 1989; **250**: 85-90 [PMID: 2737304 DOI: 10.1016/0014-5793(89)80690-8]

5 **Sands BE**, Podolsky DK. The trefoil peptide family. *Annu Rev Physiol* 1996; **58**: 253-273 [PMID: 8815795 DOI: 10.1146/annurev.ph.58.030196.001345]

6 **Wright NA**, Poulsom R, Stamp G, Van Noorden S, Sarraf C, Elia G, Ahnen D, Jeffery R, Longcroft J, Pike C. Trefoil peptide gene expression in gastrointestinal epithelial cells in inflammatory bowel disease. *Gastroenterology* 1993; **104**: 12-20 [PMID: 8419234]

7 **Regalo G**, Wright NA, Machado JC. Trefoil factors: from ulceration to neoplasia. *Cell Mol Life Sci* 2005; **62**: 2910-2915 [PMID: 16374578 DOI: 10.1007/s00018-005-5478-4]

8 **Madsen J**, Nielsen O, Tornøe I, Thim L, Holmskov U. Tissue localization of human trefoil factors 1, 2, and 3. *J Histochem Cytochem* 2007; **55**: 505-513 [PMID: 17242463 DOI: 10.1369/jhc.6A7100.2007]

9 **Taupin D**, Podolsky DK. Trefoil factors: initiators of mucosal healing. *Nat Rev Mol Cell Biol* 2003; **4**: 721-732 [PMID: 14506475 DOI: 10.1038/nrm1203]

10 **Hernández C**, Santamatilde E, McCreath KJ, Cervera AM, Díez I, Ortiz-Masiá D, Martínez N, Calatayud S, Esplugues JV, Barrachina MD. Induction of trefoil factor (TFF)1, TFF2 and TFF3 by hypoxia is mediated by hypoxia inducible factor-1: implications for gastric mucosal healing. *Br J Pharmacol* 2009; **156**: 262-272 [PMID: 19076725 DOI: 10.1111/j.1476-5381.2008.00044.x]

11 **May FE**, Westley BR. Trefoil proteins: their role in normal and malignant cells. *J Pathol* 1997; **183**: 4-7 [PMID: 9370940 DOI: 10.1002/(SICI)1096-9896(199709)183]

12 **Poulsom R**, Hanby AM, Lalani EN, Hauser F, Hoffmann W, Stamp GW. Intestinal trefoil factor (TFF 3) and pS2 (TFF 1), but not spasmolytic polypeptide (TFF 2) mRNAs are co-expressed in normal, hyperplastic, and neoplastic human breast epithelium. *J Pathol* 1997; **183**: 30-38

13 **Henry JA**, Bennett MK, Piggott NH, Levett DL, May FE, Westley BR. Expression of the pNR-2/pS2 protein in diverse human epithelial tumours. *Br J Cancer* 1991; **64**: 677-682 [PMID: 1911216 DOI: 10.1038/bjc.1991.380]

14 **Chan VY**, Chan MW, Leung WK, Leung PS, Sung JJ, Chan FK. Intestinal trefoil factor promotes invasion in non-tumorigenic Rat-2 fibroblast cell. *Regul Pept* 2005; **127**: 87-94 [PMID: 15680474 DOI: 10.1016/j.regpep.2004.10.016]

15 **Yio X**, Zhang JY, Babyatsky M, Chen A, Lin J, Fan QX, Werther JL, Itzkowitz S. Trefoil factor family-3 is associated with aggressive behavior of colon cancer cells. *Clin Exp Metastasis* 2005; **22**: 157-165 [PMID: 16086236 DOI: 10.1007/s10585-005-6615-z]

16 **Dhar DK**, Wang TC, Tabara H, Tonomoto Y, Maruyama R, Tachibana M, Kubota H, Nagasue N. Expression of trefoil factor family members correlates with patient prognosis and neoangiogenesis. *Clin Cancer Res* 2005; **11**: 6472-6478 [PMID: 16166422 DOI: 10.1158/1078-0432.CCR-05-0671]

17 **Babyatsky M**, Lin J, Yio X, Chen A, Zhang JY, Zheng Y, Twyman C, Bao X, Schwartz M, Thung S, Lawrence Werther J, Itzkowitz S. Trefoil factor-3 expression in human colon cancer liver metastasis. *Clin Exp Metastasis* 2009; **26**: 143-151 [PMID: 18979216 DOI: 10.1007/s10585-008-9224-9]

18 **Efstathiou JA**, Noda M, Rowan A, Dixon C, Chinery R, Jawhari A, Hattori T, Wright NA, Bodmer WF, Pignatelli M. Intestinal trefoil factor controls the expression of the adenomatous polyposis coli-catenin and the E-cadherin-catenin complexes in human colon carcinoma cells. *Proc Natl Acad Sci USA* 1998; **95**: 3122-3127 [PMID: 9501226 DOI: 10.1073/pnas.95.6.3122]

19 **Taupin D**, Ooi K, Yeomans N, Giraud A. Conserved expression of intestinal trefoil factor in the human colonic adenoma-carcinoma sequence. *Lab Invest* 1996; **75**: 25-32 [PMID: 8683937]

20 **Tuna B**, Sökmen S, Sarioğlu S, Füzün M, Küpelioğlu A, Ellidokuz H. PS2 and HSP70 expression in rectal adenocarcinomas: an immunohistochemical investigation of 45 cases. *Appl Immunohistochem Mol Morphol* 2006; **14**: 31-36 [PMID: 16540727 DOI: 10.1097/01.pai.0000141544.28862.35]

21 **Williams R**, Elia G, Stamp GWH. Characterization of monoclonal antibodies raised to C-terminal peptides of pS2: a major trefoil peptide and motility factor expressed in adenocarcinomas and regions of mucosal injury. *Hum Pathol* 1996; **12**: 1259-1266 [DOI: 10.1016/S0046-8177(96)90334-2]

22 **Rivat C**, Rodrigues S, Bruyneel E, Piétu G, Robert A, Redeuilh G, Bracke M, Gespach C, Attoub S. Implication of STAT3 signaling in human colonic cancer cells during intestinal trefoil factor 3 (TFF3) -- and vascular endothelial growth factor-mediated cellular invasion and tumor growth. *Cancer Res* 2005; **65**: 195-202 [PMID: 15665295]

23 **Chan MW**, Chan VY, Leung WK, Chan KK, To KF, Sung JJ, Chan FK. Anti-sense trefoil factor family-3 (intestinal trefoil factor) inhibits cell growth and induces chemosensitivity to adriamycin in human gastric cancer cells. *Life Sci* 2005; **76**: 2581-2592 [PMID: 15769482 DOI: 10.1016/j.lfs.2004.11.014]

24 **Taupin DR**, Kinoshita K, Podolsky DK. Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. *Proc Natl Acad Sci USA* 2000; **97**: 799-804 [PMID: 10639160 DOI: 10.1073/pnas.97.2.799]

25 **Uchino H**, Kataoka H, Itoh H, Koono M. Expression of intestinal trefoil factor mRNA is downregulated during progression of colorectal carcinomas. *J Clin Pathol* 1997; **50**: 932-934 [PMID: 9462243 DOI: 10.1136/jcp.50.11.932]

26 **Taupin D**, Wu DC, Jeon WK, Devaney K, Wang TC, Podolsky DK. The trefoil gene family are coordinately expressed immediate-early genes: EGF receptor- and MAP kinase-dependent inter-regulation. *J Clin Invest* 1999; **103**: R31-R38 [PMID: 10225980 DOI: 10.1172/JCI3304]

27 **Vestergaard EM**, Borre M, Poulsen SS, Nexø E, Tørring N. Plasma levels of trefoil factors are increased in patients with advanced prostate cancer. *Clin Cancer Res* 2006; **12**: 807-812 [PMID: 16467092 DOI: 10.1158/1078-0432.CCR-05-1545]

28 **Kaise M**, Miwa J, Tashiro J, Ohmoto Y, Morimoto S, Kato M, Urashima M, Ikegami M, Tajiri H. The combination of serum trefoil factor 3 and pepsinogen testing is a valid non-endoscopic biomarker for predicting the presence of gastric cancer: a new marker for gastric cancer risk. *J Gastroenterol* 2011; **46**: 736-745 [PMID: 21455714 DOI: 10.1007/s00535-011-0396-8]

29 **Aikou S**, Ohmoto Y, Gunji T, Matsuhashi N, Ohtsu H, Miura H, Kubota K, Yamagata Y, Seto Y, Nakajima A, Goldenring JR, Kaminishi M, Nomura S. Tests for serum levels of trefoil factor family proteins can improve gastric cancer screening. *Gastroenterology* 2011; **141**: 837-845.e1-7 [PMID: 21699780 DOI: 10.1053/j.gastro.2011.05.040]

30 **Qu Y**, Yang Y, Ma D, Xiao W. Increased trefoil factor 3 levels in

the serum of patients with three major histological subtypes of lung cancer. *Oncol Rep* 2012; **27**: 1277-1283 [PMID: 22246423 DOI: 10.3892/or.2012.1627]

31 **Perry JK**, Kannan N, Grandison PM, Mitchell MD, Lobie PE. Are trefoil factors oncogenic? *Trends Endocrinol Metab* 2008; **19**: 74-81 [PMID: 18054496 DOI: 10.1016/j.tem.2007.10.003]

32 **Hebrok M**, Wertz K, Füchtbauer EM. M-twist is an inhibitor of muscle differentiation. *Dev Biol* 1994; **165**: 537-544 [PMID: 7958419 DOI: 10.1006/dbio.1994.1273]

33 **Chen ZF**, Behringer RR. twist is required in head mesenchyme for cranial neural tube morphogenesis. *Genes Dev* 1995; **9**: 686-699 [PMID: 7729687 DOI: 10.1101/gad.9.6.686]

34 **Ansieau S**, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, Fauvet F, Puisieux I, Doglioni C, Piccinin S, Maestro R, Voeltzel T, Selmi A, Valsesia-Wittmann S, Caron de Fromentel C, Puisieux A. Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell* 2008; **14**: 79-89 [PMID: 18598946 DOI: 10.1016/j.ccr.2008.06.005]

35 **Boumahdi S**, Driessens G, Lapouge G, Rorive S, Nassar D, Le Mercier M, Delatte B, Caauwe A, Lenglez S, Nkusi E, Brohée S, Salmon I, Dubois C, del Marmol V, Fuks F, Beck B, Blanpain C. SOX2 controls tumour initiation and cancer stem-cell functions in squamous-cell carcinoma. *Nature* 2014; **511**: 246-250 [PMID: 24909994 DOI: 10.1038/nature13305]

36 **Burns TF**, Dobromilskaya I, Murphy SC, Gajula RP, Thiyagarajan S, Chatley SN, Aziz K, Cho YJ, Tran PT, Rudin CM. Inhibition of TWIST1 leads to activation of oncogene-induced senescence in oncogene-driven non-small cell lung cancer. *Mol Cancer Res* 2013; **11**: 329-338 [PMID: 23364532 DOI: 10.1158/1541-7786.MCR-12-0456]

37 **Morel AP**, Hinkal GW, Thomas C, Fauvet F, Courtois-Cox S, Wierinckx A, Devouassoux-Shisheboran M, Treilleux I, Tissier A, Gras B, Pourchet J, Puisieux I, Browne GJ, Spicer DB, Lachuer J, Ansieau S, Puisieux A. EMT inducers catalyze malignant transformation of mammary epithelial cells and drive tumorigenesis towards claudin-low tumors in transgenic mice. *PLoS Genet* 2012; **8**: e1002723 [PMID: 22654675 DOI: 10.1371/journal.pgen.1002723]

38 **Tsai JH**, Donaher JL, Murphy DA, Chau S, Yang J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* 2012; **22**: 725-736 [PMID: 23201165 DOI: 10.1016/j.ccr.2012.09.022]

39 **Zhu YQ**, Tan XD. TFF3 modulates NF-{kappa}B and a novel negative regulatory molecule of NF-{kappa}B in intestinal epithelial cells via a mechanism distinct from TNF-{alpha}. *Am J Physiol Cell Physiol* 2005; **289**: C1085-C1093 [PMID: 16014704 DOI: 10.1152/ajpcell.00185.2005]

40 **Häckel C**, Falkenberg B, Günther T, Lippert H, Roessner A. The pS2 protein in colorectal carcinomas and metastases. *Pathol Res Pract* 1998; **194**: 171-176 [PMID: 9587935 DOI: 10.1016/S0344-0338(98)80018-6]

41 **Im S**, Yoo C, Jung JH, Choi HJ, Yoo J, Kang CS. Reduced expression of TFF1 and increased expression of TFF3 in gastric cancer: correlation with clinicopathological parameters and prognosis. *Int J Med Sci* 2013; **10**: 133-140 [PMID: 23329884 DOI: 10.7150/ijms.5500]

42 **Emami S**, Rodrigues S, Rodrigue CM, Le Floch N, Rivat C, Attoub S, Bruyneel E, Gespach C. Trefoil factor family (TFF) peptides and cancer progression. *Peptides* 2004; **25**: 885-898 [PMID: 15177885 DOI: 10.1016/j.peptides.2003.10.019]

43 **Yio X**, Diamond M, Zhang JY, Weinstein H, Wang LH, Werther L, Itzkowitz S. Trefoil factor family-1 mutations enhance gastric cancer cell invasion through distinct signaling pathways. *Gastroenterology* 2006; **130**: 1696-1706 [PMID: 16697734 DOI: 10.1053/j.gastro.2006.01.040]

44 **Vernon AE**, LaBonne C. Tumor metastasis: a new twist on epithelial-mesenchymal transitions. *Curr Biol* 2004; **14**: R719-R721 [PMID: 15341765 DOI: 10.1016/j.cub.2004.08.048]

45 **Yang J**, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; **117**: 927-939 [PMID: 15210113 DOI: 10.1016/j.cell.2004.06.006]

46 **Lacroix M**. Significance, detection and markers of disseminated breast cancer cells. *Endocr Relat Cancer* 2006; **13**: 1033-1067 [PMID: 17158753 DOI: 10.1677/ERC-06-0001]

P- Reviewer: Bhawal UK **S- Editor**: Qi Y **L- Editor**: Wang TQ **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.121

World J Gastroenterol 2017 January 7; 23(1): 121-126
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Estimation of quality of life in Cypriot patients with inflammatory bowel disease

Maria Tsoukka, Eleni Jelastopulu, Giagkos Lavranos, George Charalambous

Maria Tsoukka, Postgraduate Program Health Management, Frederick University, Nicosia, Cyprus University General Hospital of Larnaca, 7020 Larnaca, Cyprus

Eleni Jelastopulu, Department of Public Health, School of Medicine, University of Patras, 261 Patras, Greece

Giagkos Lavranos, Department of Health Sciences, School of Sciences, European University, 1516 Nicosia, Cyprus

George Charalambous, General Hospital of Athens "Hippocratio", 100 Athens, Greece

Author contributions: Tsoukka M was the primary researcher of the study as part of her PhD thesis and prepared the first draft of the manuscript; Jelastopulu E, Lavranos G and Charalambous G provided critical revisions and additions; both prior to submission and during the reviewer revision process.

Institutional review board statement: The study was reviewed and approved by the Research Committee of the Frederick University and ethical approval was given by the National Committee of Bioethics at the Ministry of Health.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Maria Tsoukka, PhD candidate, MSc Health Management, Postgraduate Program Health Management, Frederick University, Nicosia, Cyprus University General Hospital of Larnaca, Kalavardon No. 16, Dromolaxia, Larnaca 7020, Cyprus. mariate@windowslive.com
Telephone: +357-99538776
Fax: +357-2466017

Received: May 28, 2016
Peer-review started: May 30, 2016
First decision: September 20, 2016
Revised: October 20, 2016
Accepted: November 14, 2016
Article in press: November 16, 2016
Published online: January 7, 2017

Abstract

AIM

To investigate the health-related quality of life (HRQoL) of patients suffering with idiopathic inflammatory bowel disease (IBD).

METHODS

The Greek validated version of the Short Inflammatory Bowel Disease Questionnaire was used for evaluating the quality of life of IBD patients. The questionnaire was distributed to 100 consecutive patients suffering from IBD and presenting for a clinic appointment at the endoscopy unit of Larnaca General Hospital during the period from October to November 2012. The criteria for participating in this study were constituted by the documented diagnosis of either ulcerative colitis (UC) or Crohn's disease (CD) after endoscopy and histologic examination at least 6 months before the study, adult patients (18 years old or older), the capability of verbal communication and the patient's written consent for attending this study. The majority of the questionnaires were completed by a nurse practitioner who specializes

in IBD patient care.

RESULTS

Regarding the physical dimension in patients with UC, males scored significantly higher than females (4.2 *vs* 3.4, $P = 0.023$). Higher scores were also observed in UC patients younger than 35 or older than 50 years (4.0 and 4.2 *vs* 3.2, respectively, $P = 0.021$). The psychological dimension revealed similar results in patients with UC, with males, and older ages scoring higher (5.0 *vs* 3.0, $P = 0.01$ and 4.7 *vs* 2.7, $P < 0.5$, respectively), whereas regarding CD higher scores were observed in married compared to unmarried (3.83 *vs* 2.33, $P = 0.042$). No statistical differences in any parameters in the social dimension were observed. Regarding the treatment of, patients with CD, overall higher scores were observed when treated with biological factors compared to standard therapy in all dimensions but with statistical significant difference in the social dimension (5.00 *vs* 3.25, $P = 0.045$).

CONCLUSION

The study reveals a negative impact of IBD on HRQoL. Increased risks are age and gender in patients with UC and family status in patients with CD.

Key words: Crohn's disease; Health related quality of life; Quality of life; Short inflammatory bowel disease questionnaire; Ulcerative colitis

Core tip: The study reveals an overall negative impact of idiopathic inflammatory bowel diseases on health-related quality of life (HRQoL), mostly in patients with Crohn's disease (CD). Increased risks for decreased HRQoL are age and gender in patients with ulcerative colitis and family status in patients with CD. This is the first application of the validated Greek version of the short inflammatory bowel disease questionnaire in a patient population in Cyprus.

Tsoukka M, Jelastopulu E, Lavranos G, Charalambous G. Estimation of quality of life in Cypriot patients with inflammatory bowel disease. *World J Gastroenterol* 2017; 23(1): 121-126 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/121.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.121

INTRODUCTION

The idiopathic inflammatory bowel diseases (IBD) is a general term used to describe a group of chronic inflammations and relapsing disorders of the gastro enteric route, with Crohn's disease (CD) and ulcerative colitis (UC) being the most common ones. These diseases are characterized by an inadequate immune reaction that causes a characteristic inflammatory response[1]. The physical history of both diseases is characterized by relapses that can have a direct effect on the patient's lives[2] and can considerably influence their quality of life[3].

Activity indexes are used as a conventional way of evaluating the severity of IBD but they do not take into consideration other aspects that may affect the patient's life. As a result the indicators are proved less sensitive and come in contrast with the patient's perception of health[4,5].

This fact led researchers to the development of other tools to investigate the subjective sensation of health including the quality of life that is distinctively linked to medical care and health related experience[4].

The term health related quality of life (HRQoL) was developed in order to better understand and evaluate the consequences of the disease on quality of life. According to Drossman this is a general estimation of the patient's condition regarding their disease, how it influences their activities both in the psychological and social sectors and the way they are experiencing these activities[6].

The aim of this study was to assess the HRQoL in patients with IBD in Cyprus by using a validated disease-specific questionnaire and to investigate the factors that influence their quality of life.

MATERIALS AND METHODS

The validated Greek translation of the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) was used[7] for estimating the quality of life of IBD patients. It is a specific tool for measuring the quality of life of these patients[8] and was used after obtaining a licensing agreement from McMaster University, United States. The questionnaire is composed of 10 questions measuring psychological, social and physical status and is scored on a 7-point Likert scale from 1 (severe problem) to 7 (no problems at all). The absolute score ranges from 10-70, consistent with poor to optimum HRQoL. The SIBDQ has been tested for its validity and reliability revealing a Cronbach's factor of 0.78[7]. The Cronbach's alpha of the present sample was 0.89 and was judged as very satisfactory. The questionnaire was distributed to 100 consecutive patients suffering from IBD and presenting for an outpatient clinic appointment at the endoscopy/GI unit of Larnaca General Hospital during the period from October to November 2012. The criteria for participating in this study were a confirmed diagnosis of either UC or CD after endoscopy and histologic examination at least 6 mo before the study, adult patients (18 years old or older), the capability of fluent verbal communication in Greek and the patient's written consent for participating in this study. The completion of the questionnaires was overseen by a nurse practitioner who specializes in IBD patient care. The study was performed after approval by the Cyprus National Bioethics Committee, Cyprus Data Protection

Table 1 Demographic and disease characteristics of the study population *n* (%)

	Total *n* = 100	CD *n* = 40	UC *n* = 60	*P* value
Sex				0.198
Male	54 (53.5)	61.5%	48.3%	
Female	46 (46.5)	38.5%	51.7%	
Age (yr) (*n* = 97)		*n* = 38	*n* = 59	
18-35	39 (39.8)	16 (41.0)	23 (39.0)	0.979
35-50	28 (28.6)	11 (28.2)	17 (28.8)	
> 51	30 (31.6)	11 (30.8)	19 (32.2)	
Smoking	32 (33.0)	43.2%	26.7%	0.092
Education				0.673
Primary or less	17.3%	18.4%	16.7%	
Secondary	49.0%	42.1%	53.3%	
Tertiary or above	33.7%	39.5%	30.0%	
Employment status				0.349
Government officials	19.8%	22.9%	18.3%	
Private employee	44.8%	44.4%	45.0%	
Self-employed	4.2%	8.3%	1.7%	
Unemployed	31.3%	25.0%	35.0%	
Family status				0.822
Married	62.6%	59.0%	65.0%	
Unmarried	32.3%	35.9%	30.0%	
Divorced	5.1%	5.1%	5.0%	
Surgery	20.9%	36.1%	11.7%	0.004
Mean disease duration (yr)	12.63%	10.28%	14.07%	0.118
Treatment				0.019
Immunosuppressive treatment	65.0%	55.0%	71.7%	
Immunosuppressive treatment + corticosteroids	16.0%	12.5%	18.3%	
Treatment with biologic agents	19.0%	32.5%	10.0%	
Treatment with corticosteroids	22.0%	17.5%	25.0%	0.375%

CD: Crohn's disease; UC: Ulcerative colitis.

Authority and the Health Ministry Scientific Promotion Committee.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 for Windows (SPSS Inc. Chicago, IL, United States). Categorical data were expressed as numbers with percentages, and continuous data were expressed as medians with a range. *P* values less than 0.05 were considered to indicate statistical significance.

RESULTS

The demographic characteristics of the study population are shown in Table 1. One hundred patients with IBD were investigated, 40% with Crohn's disease and 60% with ulcerative colitis. In the CD group a predominance of men (61.5% *vs* 38.5%) was observed, whereas in the UC group the proportion of females was slightly higher (51.7% *vs* 48.3%).

Patients with CD scored overall lower on the SIBDQ (Table 2). Regarding the physical dimension in patients with ulcerative colitis, males scored significantly higher than females (4.2 *vs* 3.4, *P* = 0.023), whereas no gender difference was observed in patients with CD. Higher scores were also observed in ulcerative colitis patients younger than 35 or older than 50 years compared to the age group of 35-50 years old (4.0 and 4.2 *vs* 3.1, respectively, *P* = 0.021). Further details of the physical dimension are shown in Table 3.

Table 2 Short inflammatory bowel disease questionnaire score by dimension and type of disease (*n* = 100), mean (± SD) *n* (%)

	Crohn's disease	Ulcerative colitis
Physical dimension	18.53 (7.55)	19.52 (6.74)
Psychological dimension	10.68 (5.31)	11.88 (4.49)
Social dimension	7.92 (3.03)	8.63 (3.18)
Overall SIBDQ	33.79 (13.36)	36.30 (11.39)

SIBDQ: Short inflammatory bowel disease questionnaire.

Table 3 Physical Dimension scores of health-related quality of life in patients with Crohn's disease and ulcerative colitis and their association to demographic parameters

	Physical dimension	
	Crohn's disease, median	Ulcerative colitis, median
Gender		
Male	3.3	4.2
Female	3.5	3.4
Z	-0.015	-2.274
P value	0.988	0.023
Age		
< 35	3.1	4.0
35-50	3.8	3.2
> 50	3.8	4.2
χ^2	1.232	7.767
P value	0.540	0.021

The psychological dimension revealed similar results in patients with UC, with males, and older ages scoring higher (5.0 *vs* 3.0, *P* = 0.01 and 4.7 *vs* 2.7, *P* = 0.5, respectively), whereas regarding Crohn's disease higher scores were observed in married compared to unmarried (3.83 *vs* 2.33, *P* = 0.042) (Table 3). Further details of the psychological dimension are shown in Table 4.

No statistical differences in any parameters in both CD and UC were observed regarding the social dimension. Regarding the treatment of patients with CD, overall higher scores were observed when treated with biological factors compared to standard therapy in all dimensions but with statistical significant difference in the social dimension (5.00 *vs* 3.25, *P* = 0.045) (Table 5).

DISCUSSION

SIBDQ is a validated instrument measuring the subjective perception of health state and identifying problems in IBD patients. However, quality of life

Table 4 Psychological dimension scores of health-related quality of life in patients with Crohn's disease and ulcerative colitis and their association to demographic parameters

	Psychological dimension	
	Crohn's disease, median	Ulcerative colitis, median
Gender		
Male	3.17	5.00
Female	2.83	3.00
Z	-0.456	-3.474
P value	0.648	0.001
Age		
< 35	2.83	4.00
35-50	2.33	2.67
> 51	4.67	4.67
χ^2	1.011	5.979
P value	0.603	0.0503
Family status		
Married	3.83	4.00
Single	2.33	3.50
Z	6.351	-0.663
P value	0.042	0.507

HRQoL: Health-related quality of life.

Table 5 Scores of health-related quality of life in patients with Crohn's disease and ulcerative colitis in association to medication treatment

	Medication	Median	*Z*	*P* value
Physical dimension	Biological factors	5.00	-1.484	0.138
	Standard treatment	3.20		
Psychological dimension	Biological factors	4.67	-1.584	0.113
	Standard treatment	2.67		
Social dimension	Biological factors	5.00	-2.004	0.045
	Standard treatment	3.25		

HRQoL: Health-related quality of life.

measures have not been widely integrated into routine clinical practice till now, although they seem to be potentially useful in improving patients' health and delivering high quality health services that can come up to the patients' needs and expectations[9]. Furthermore, investigating the factors, which are associated to poor HRQoL, important information can be obtained on the way the disease influences all aspects of a patient's health and can also help health professionals in dealing with these problems more adequately[10].

In the present study the overall SIBDQ score as well as the single scores in all dimensions were lower in patients with CD, indicating thus the poorer quality of life in these patients. Furthermore, the scores were associated with demographic parameters such as gender and age in UC patients and to marital status in CD patients. In agreement with these results Mikocka-Walus *et al*[11] reported that 31 patients with CD had a worse physical HRQoL compared to 33 patients with UC.

Several studies have documented a relationship between gender and HRQoL, pointing out female patients with IBD having worse outcomes[12-17]. Possibly the lower scores are related to the higher sensibility of women compared to men towards gastrointestinal symptoms[18]. Besides surgical intervention Williet *et al*[19] mentioned that other factors, associated with depression and anxiety, affect IBD patients and here mainly women as the female gender is more susceptible to depression and anxiety. However, other researchers did not reveal any differences between gender regarding the HRQoL in both patients with UC and CD[20-22].

The effect of age on HRQoL in IBD was also reported in other studies, revealing patients in mid ages having lower scores[18]. Similarly, in a Greek study, IBD patients younger than 40 years old scored higher in HRQoL compared to higher age groups[23]. Several former studies reported also an association between age and HRQoL scores[24-26]. It is also worth mentioning that in the present study the differences in gender and age are observed mainly in patients with ulcerative colitis with effect to female UC patients revealing the similar low scores as females with CD.

In the physical dimension of quality of life of patients with IBD, women gathered lower scores, a fact that was mentioned by other researchers as well[13-18]. The results of the present study are related with the results of other studies that revealed lower score of women with gastrointestinal diseases compared to men[18]. Through a number of studies taken place among patients suffering by CD and UC no relation was emerged in between gender and HRQoL[21,22]. However, on the other hand many studies have reported that there is a relation between age and HRQoL[23-26], a fact that is already revealed through the present study. This correlation may result from the concerns and aspects of daily life, work and other factors at different stages of life with age being an important factor reflecting in measuring the quality of life in different age groups. Results of other studies come in full agreement with the present study revealing the fact that middle aged groups have lower scores of HRQoL[18-23].

The psychological dimension of this study highlights lower scores of HRQoL in women which stands in agreement with other studies that have reported higher levels of anxiety and depression among women than in male population[27], mentioning as well the deep concerns of women regarding their disease and especially the fact that they have to depend on others for help[28,29].

A number of potential interpretations and limitations for the results of the two dimensions of the SIBDQ can be provided. First, low scores of HRQoL in physical dimension may also reduce the scores of HRQoL in the psychological dimension or conversely and second there is a possibility of this interaction to be an additive effect of the two dimensions presenting

independently. The low score of HRQoL in unmarried patients may be attributed to the fact that family is a fundamental social institution that offers security, guidance and assistance to the members thus providing positive affects to their health and this is fundamental for the protection and promotion of their mental and physical health. Other researches mention the positive affection of the family to the progress of the illness[30,31].

The social dimension showed to be improved by taking biological agents. According to many researches positive reaction was observed to CD after the treatment with biological agents[32-34]. Kalafateli reports that the use of biological factors was found to have a negative impact on the HRQoL of all patients with IBD in relation to the systematic symptoms of the disease[35]. Since the introduction of biological agents in IBD treatment in Cyprus is relatively recent, it will be interesting to evaluate this dynamic in a subsequent follow up trial.

COMMENTS

Background

The idiopathic inflammatory bowel diseases (IBD) are nosological entities which are characterized by chronic relapsing of the immune system and inflammation of the gastrointestinal tract. They occur with remissions and relapses and their effect on the way of life of these patients is immediate, resulting in poorer quality of life (QoL) compared to the general population. However, in Cyprus the traditional way of assessing the gravity of the IBD does not include the overall impact of the disease on the health of the patient, nor the point of view of the patient regarding the disease and its impact mainly on the psychological and social sector. Identifying the factors associated with poor QoL, important information is offered on how the disease affects other aspects of the patient's health.

Research frontiers

In Cyprus the number of IBD patients in recent years has grown rapidly. However in Cyprus, the overall effect of the disease in relation to the psychological, physical and social well-being of the patient has never been researched. This research offers important information on how the disease affects all aspects of patient health; the result of this was for the research to be important help to the health care professionals in dealing with the problems of these patients in a more round and comprehensible perspective and thus contribute to make better their life through time.

Innovations and breakthroughs

It became apparent that the general health status of the patients with IBD is not only determined by clinical-laboratory assessment of the disease, but also by factors such as psychological situation, vocational, social and love life, cultural and other ideologies and primarily complications and side effects of the treatment. This study is the only one addressing Cypriot patients to measure the quality of life in relation to IBD. It became noticeable that the patients are affected regarding the physical, the psychological and the social dimension because of the disease. Women with ulcerative colitis (UC) seem to be more vulnerable than men in connection with the symptomatology and the psychological field. Patients with Crohn's disease (CD) that take biological agents were indicated to have better QoL in relation to their social activities and those who are married were designated to have better QoL compared to single people in the psychological field.

Applications

The results of this study could improve the treatment of patients with IBD, to reduce their symptoms and to help them depend less on health services. Promoting self-care of these patients, assistance in managing stress arising from diseases through alternative techniques, appointing clinical psychologist, creating specialized clinics with suitably trained individuals and social support programs especially for women at the ages 35-50, are possible ways to improve the HRQoL of IBD patients, in Cyprus.

Terminology

IBD means inflammatory bowel disease that are chronic inflammatory diseases of the intestine and are characterized by remissions and relapses. CD is a chronic inflammatory bowel disease that can infect any part of the digestive tract or the colon where as UC being also a chronic inflammatory bowel disease infects the colon. SIBDQ is a specific questionnaire used for measuring the quality of life of patients with IBD.

Peer-review

Available papers regarding measuring the quality of life of patients with IBD in Cyprus do not exist. The authors of this study investigated the QoL of patients with IBD related to physical, psychological, and social dimension. This study showed that aspects of life of these patients are affected more than those which are apparent in their laboratory tests. The results of the survey are interesting as they provide new information for Cypriot patients with IBD, enriching the scientific sector of Cyprus and open the way to new studies specializing even more in this sector aiming at improving the QoL of these patients.

REFERENCES

1 **Mikocka-Walus AA**, Turnbull DA, Moulding NT, Wilson IG, Andrews JM, Holtmann GJ. Controversies surrounding the comorbidity of depression and anxiety in inflammatory bowel disease patients: a literature review. *Inflamm Bowel Dis* 2007; **13**: 225-234 [PMID: 17206706 DOI: 10.1002/ibd.20062]

2 **Baumgart DC**, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; **369**: 1627-1640 [PMID: 17499605 DOI: 10.1016/S0140-6736(07)60750-8]

3 **Pontes RM**, Miszputen SJ, Ferreira-Filho OF, Miranda C, Ferraz MB. [Quality of life in patients with inflammatory bowel diseases: translation to Portuguese language and validation of the "Inflammatory Bowel Disease Questionnaire" (IBDQ)]. *Arq Gastroenterol* 2004; **41**: 137-143 [PMID: 15543390 DOI: 10.1590/S0004-28032004000200014]

4 **Verissimo R**. Quality of life in inflammatory bowel disease: psychometric evaluation of an IBDQ cross-culturally adapted version. *J Gastrointestin Liver Dis* 2008; **17**: 439-444 [PMID: 19104706]

5 **Garrett JW**, Drossman DA. Health status in inflammatory bowel disease. Biological and behavioral considerations. *Gastroenterology* 1990; **99**: 90-96 [PMID: 2188874]

6 **Moons Ph**. Quality of life in adults with congenital heart disease: Beyond the quantity of life. Doctoral Dissertation Katholike Universities Leuven. Faculty of Medicine School of Public Health, 2004

7 **Irvine EJ**, Zhou Q, Thompson AK. The Short Inflammatory Bowel Disease Questionnaire: a quality of life instrument for community physicians managing inflammatory bowel disease. CCRPT Investigators. Canadian Crohn's Relapse Prevention Trial. *Am J Gastroenterol* 1996; **91**: 1571-1578 [PMID: 8759664]

8 **Pallis AG**, Vllachonicolis IG, Mantzaris G, Leontides C, Yiannadakis E, Mouzas IA, Validation of the Greek Version of the Inflammatory Bowel Disease Questionnaire. *Arch Hell Med* 2001; **18**: 297-302

9 **Papanicolaou B**. Quality of Health Services: Principle, methods and applications. Athens: Papazisis Editions, 2007: 1-20

10 **Bowling A**. Measuring Disease. A review of quality of life measurement scales. Milton Kayenes: Open University, 1995

11 **Mikocka-Walus AA**, Turnbull DA, Andrews JM, Moulding NT, Wilson IG, Harley HNJ. Psychological problems in gastroenterology outpatients: a South Australian experience. Psychological co-morbidity in IBD, IBS and hepatitis C. Clinical Practice and Epidemiology in Mental Health, 2008 [DOI:

10.1186/1745-0179-4-15]
12 **Casellas F**, López-Vivancos J, Casado A, Malagelada JR. Factors affecting health related quality of life of patients with inflammatory bowel disease. *Qual Life Res* 2002; **11**: 775-781 [PMID: 12482161 DOI: 10.1023/A:1020841601110]
13 **Bernklev T**, Jahnsen J, Aadland E, Sauar J, Schulz T, Lygren I, Henriksen M, Stray N, Kjellevold O, Vatn M, Moum B. Health-related quality of life in patients with inflammatory bowel disease five years after the initial diagnosis. *Scand J Gastroenterol* 2004; **39**: 365-373 [PMID: 15125469 DOI: 10.1080/00365520310008386]
14 **Rubin GP**, Hungin AP, Chinn DJ, Dwarakanath D. Quality of life in patients with established inflammatory bowel disease: a UK general practice survey. *Aliment Pharmacol Ther* 2004; **19**: 529-535 [PMID: 14987321 DOI: 10.1111/j.1365-2036.2004.1873.x]
15 **Hjortswang H**, Järnerot G, Curman B, Sandberg-Gertzén H, Tysk C, Blomberg B, Almer S, Ström M. The influence of demographic and disease-related factors on health-related quality of life in patients with ulcerative colitis. *Eur J Gastroenterol Hepatol* 2003; **15**: 1011-1020 [PMID: 12923375 DOI: 10.1097/00042737-200309000-00012]
16 **Haapamaki J**. Health-Related Quality of Life, Symptoms and Comorbidity in Inflammatory Bowel Disease [Ph.D. dissertation], The Medical Faculty of the University of Helsinki 2011. Available from: URL: https://helda.helsinki.fi/bitstream/handle/10138/25298/healthre.pdf?sequence=1
17 **Sainsbury A**, Heatley RV. Review article: psychosocial factors in the quality of life of patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005; **21**: 499-508 [PMID: 15740531 DOI: 10.1111/j.1365-2036.2005.02380.x]
18 **Glise H**, Wiklund I. Health-related quality of life and gastrointestinal disease. *J Gastroenterol Hepatol* 2002; **17** Suppl: S72-S84 [PMID: 12000595]
19 **Williet N**, Sandborn WJ, Peyrin-Biroulet L. Patient-reported outcomes as primary end points in clinical trials of inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2014; **12**: 1246-1256.e6 [PMID: 24534550 DOI: 10.1016/j.cgh.2014.02.016]
20 **Zhou Y**, Ren W, Irvine EJ, Yang D. Assessing health-related quality of life in patients with inflammatory bowel disease in Zhejiang, China. *J Clin Nurs* 2010; **19**: 79-88 [PMID: 20500246 DOI: 10.1111/j.1365-2702.2009.03020.x]
21 **Nordin K**, Påhlman L, Larsson K, Sundberg-Hjelm M, Lööf L. Health-related quality of life and psychological distress in a population-based sample of Swedish patients with inflammatory bowel disease. *Scand J Gastroenterol* 2002; **37**: 450-457 [PMID: 11989837 DOI: 10.1080/003655202317316097]
22 **Yiakoumidakis C**, Kogoulis D, Elefsiniotis J, Pananoudaki H, Quality of Life in Inflammatory Bowel Disease. *Nursing* 2011; **50**: 95-103. Available from: URL: http://www.iatrotek.org/ioArt.asp?id=19414
23 **Pallis AG**, Vlachonikolis IG, Mouzas IA. Assessing health-related quality of life in patients with inflammatory bowel disease, in Crete, Greece. *BMC Gastroenterol* 2002; **2**: 1 [PMID: 11866863 DOI: 10.1186/1471-230X-2-1]
24 **Akerkar GA**, Peppercorn MA. Inflammatory bowel disease in the elderly. Practical treatment guidelines. *Drugs Aging* 1997; **10**: 199-208 [PMID: 9108893 DOI: 10.2165/00002512-199710030-00004]
25 **Gupta S**, Saverymuttu SH, Keshavarzian A, Hodgson HJ. Is the pattern of inflammatory bowel disease different in the elderly? *Age Ageing* 1985; **14**: 366-370 [PMID: 4072825 DOI: 10.1093/ageing/14.6.366]
26 **Cohen RD**. The quality of life in patients with Crohn's disease. *Aliment Pharmacol Ther* 2002; **16**: 1603-1609 [PMID: 12197839 DOI: 10.1046/j.1365-2036.2002.01323.x]
27 **Meder A**, Świątkowski M, Meder G, Koza J. Quality of life and coexisting anxiety-depressive disorders in patients with inflammatory bowel disease during relapse and a further 11 months observation. *Gastroenterologia Polska* 2010; **17**: 273-279
28 **Drossman DA**, Patrick DL, Mitchell CM, Zagami EA, Appelbaum MI. Health-related quality of life in inflammatory bowel disease. Functional status and patient worries and concerns. *Dig Dis Sci* 1989; **34**: 1379-1386 [PMID: 2766905]
29 **Blondel-Kucharski F**, Chircop C, Marquis P, Cortot A, Baron F, Gendre JP, Colombel JF. Health-related quality of life in Crohn's disease: a prospective longitudinal study in 231 patients. *Am J Gastroenterol* 2001; **96**: 2915-2920 [PMID: 11693326 DOI: 10.1111/j.1572-0241.2001.4681_b.x]
30 **Sajadinejad MS**, Asgari K, Molavi H, Kalantari M, Padibi P. Psychological Issues in Inflammatory Bowel Disease: An Overview Academic. In: Gionchetti P, editor. Review Article, 2012 [DOI: 10.1155/2012/106502]
31 **Viazis N**, Mantzaris G, Karmiris K, Polymeros D, Kouklakis G, Maris T, Karagiannis J, Karamanolis DG. Inflammatory bowel disease: Greek patients' perspective on quality of life, information on the disease, work productivity and family support. *Ann Gastroenterol* 2013; **26**: 52-58 [PMID: 24714294]
32 **Targan SR**, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997; **337**: 1029-1035 [PMID: 9321530 DOI: 10.1056/NEJM199710093371502]
33 **Baert FJ**, D'Haens GR, Peeters M, Hiele MI, Schaible TF, Shealy D, Geboes K, Rutgeerts PJ. Tumor necrosis factor alpha antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. *Gastroenterology* 1999; **116**: 22-28 [PMID: 9869598 DOI: 10.1016/S0016-5085(99)70224-6]
34 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549 [PMID: 12047962 DOI: 10.1016/S0140-6736(02)08512-4]
35 **Kalafateli M**, Triantos C, Theocharis G, Giannakopoulou D, Koutroumpakis E, Chronis A, Sapountzis A, Margaritis V, Thomopoulos K, Nikolopoulou V. Health-related quality of life in patients with inflammatory bowel disease: a single-center experience. *Ann Gastroenterol* 2013; **26**: 243-248 [PMID: 24714279]

P- Reviewer: Testa A **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.127

World J Gastroenterol 2017 January 7; 23(1): 127-134
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Health-related quality of life in gastroesophageal reflux patients with noncardiac chest pain: Emphasis on the role of psychological distress

Lei Zhang, Lei Tu, Jie Chen, Jun Song, Tao Bai, Xue-Lian Xiang, Rui-Yun Wang, Xiao-Hua Hou

Lei Zhang, Lei Tu, Jie Chen, Jun Song, Tao Bai, Xue-Lian Xiang, Xiao-Hua Hou, Division of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

Rui-Yun Wang, Division of Gerontology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, Hubei Province, China

Author contributions: Zhang L and Tu L contributed equally to this study; Hou XH designed the study; Tu L and Chen J contributed to data collection; Zhang L, Song J, Bai T and Xiang XL organized and analyzed the data; Zhang L and Wang RY drafted the paper.

Supported by the Key Clinical Construction Projects of Gastroenterology of the National Health and Family Planning Commission of China.

Institutional review board statement: This study was approved by the Local Ethical Committee for Clinical Studies in Humans of Huazhong University of Science and Technology, China [(2011) No, 025].

Informed consent statement: Patients provided informed verbal consent to participate in this study and complete the survey. All clinical data for questionnaires were obtained anonymously and innocuously.

Conflict-of-interest statement: All authors declare that they have no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Xiao-Hua Hou, MD, PhD, Division of Gastroenterology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Road, Wuhan 430022, Hubei Province, China. houxh@medmail.com
Telephone: +86-27-85726057
Fax: +86-27-85726057

Received: August 3, 2016
Peer-review started: August 5, 2016
First decision: September 5, 2016
Revised: September 18, 2016
Accepted: October 19, 2016
Article in press: October 19, 2016
Published online: January 7, 2017

Abstract

AIM

To investigate the effects of depression and anxiety on health-related quality of life (QoL) in gastroesophageal reflux disease (GERD) patients and those suffering from cardiac (CCP) and noncardiac (NCCP) chest pain in Wuhan, China.

METHODS

In this cross-sectional study, a total of 358 consecutive patients with GERD were enrolled in Wuhan, China, of which 176 subjects had complaints of chest pain. Those with chest pain underwent coronary angiography and were divided into a CCP group (52 cases) and NCCP group (124 cases). Validated GERD questionnaires were completed, and the 36-item Short-Form Health Survey and Hospital Anxiety/Depression Scale were used for evaluation of QoL and psychological symptoms, respectively.

RESULTS

There were similar ratios and levels of depression and anxiety in GERD with NCCP and CCP. However, the QoL was obviously lower in GERD with CCP than NCCP (48.34 ± 17.68 *vs* 60.21 ± 20.27, $P < 0.01$). In the GERD-NCCP group, rather than the GERD-CCP group, the physical and mental QoL were much poorer in subjects with depression and/or anxiety than those without anxiety or depression. Anxiety and depression had strong negative correlations with both physical and mental health in GERD-NCCP (all $P < 0.01$), but only a weak relationship with mental components of QoL in GERD-CCP.

CONCLUSION

High levels of anxiety and depression may be more related to the poorer QoL in GERD patients with NCCP than those with CCP. This highlights the importance of evaluation and management of psychological impact for improving QoL in GERD-NCCP patients.

Key words: Gastroesophageal reflux; Anxiety; Chest pain; Depression; Quality of life

Core tip: Comorbid anxiety and depression and reduced QoL are common problems in gastroesophageal reflux disease (GERD) and those suffering from cardiac (CCP) and noncardiac (NCCP) chest pain. In this study, the effects of depression and anxiety on QoL in Chinese GERD subjects with chest pain were assessed. These data demonstrated that high levels of anxiety and depression may have greater negative impact on poorer QoL in GERD patients with NCCP relative to those with CCP. Evaluation and management of the psychological impact could be of great benefit for improving QoL in GERD-NCCP patients.

Zhang L, Tu L, Chen J, Song J, Bai T, Xiang XL, Wang RY, Hou XH. Health-related quality of life in gastroesophageal reflux patients with noncardiac chest pain: Emphasis on the role of psychological distress. *World J Gastroenterol* 2017; 23(1): 127-134 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/127.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.127

INTRODUCTION

Gastro-esophageal reflux disease (GERD) is a common health problem consisting of typical symptoms such as acid regurgitation and heartburn at least once weekly, with a range of prevalence estimates of 2.5%-7.8% in East Asia, 8.8%-25.9% in Europe, and 18.1%-27.8% in North America[1]. GERD is frequently accompanied by chest pain[2], which is the most common atypical symptom of GERD[3]. In fact, gastroesophageal reflux is considered the primary mechanism responsible for chest pain without cardiac origin[4], commonly known as noncardiac chest pain (NCCP). As many as 50% of NCCP patients display abnormal esophageal acid exposure[5]. Typical GERD symptoms, such as heartburn and acid regurgitation, are independently associated with NCCP[6], and antacid therapy is usually effective for patients with NCCP[7,8]. Notably, a considerable portion (31%) of patients with cardiac chest pain (CCP) have comorbid reflux disease, according to GERD questionnaires[9,10]. It is speculated that the high prevalence of reflux symptoms may partly be due to the use of nitrates and Ca^{2+} antagonists in CCP patients[11,12].

Comorbid psychiatric disorders, such as anxiety and depression, are prevalent in patients with GERD, as well as GERD-related chest pain[13]. Approximately 60% of GERD patients reported worsening of the symptoms during stress[14]. Additionally, there were no significant correlations between the severity of GERD symptoms and the pathophysiological abnormalities detected by 24-h pH monitoring and esophageal manometry, further suggesting the influence of psychiatric factors in symptom perception[14]. It has already been documented that stress and psychological comorbidities may predispose individuals to be more vigilant for physiological sensations, which may result in enhanced response to a painful stimulus or a painful response to an innocuous stimulus and, in some instances, trigger or worsen chest pain of cardiac or esophageal origin[5,11,15].

NCCP patients have also been reported to experience a reduced quality of life (QoL) equal to that experienced by those with CCP[15,16]. Many patients who seek emergency services for chest pain are driven by a fear of myocardial infarction, even if they have been diagnosed as free of heart disease[11]. Psychiatric disorder and fear of pain were independently associated with mental and physical QoL, respectively[15]. However, the impact of depression and anxiety on QoL in GERD patients with NCCP and CCP is far from clear because research in this area is limited, especially in Chinese populations. Furthermore, in many cases, a greater emphasis is placed on the treatment of physical symptoms, and invisible psychological disorders in these subjects is often ignored.

In this observational study, we aimed to assess the differences in the roles of psychological distress on QoL in GERD patients with NCCP (predominantly of esophageal origin) and GERD patients with CCP (predominantly of cardiac origin). These data may provide useful indications for the management of GERD patients with chest pain, as an individualized biopsychosocial model has been proposed[17].

MATERIALS AND METHODS

Patients

In this cross-sectional study, a total of 358 con-

secutive patients with GERD from the Division of Gastroenterology, Union Hospital, Wuhan, China, were enrolled, of whom 176 had complaints of chest pain. Those with chest pain underwent coronary angiography, and accordingly divided into a CCP group (52 cases) and NCCP group (124 cases). NCCP was defined as patients without stenoses or with stenoses less than 30% in the epicardial coronary artery, and those with obvious stenosis were diagnosed as CCP[18]. All patients provided informed verbal consent and were invited to complete a standardized questionnaire as detailed below. This investigation was approved by the Local Ethical Committee for Clinical Studies in Human, Huazhong University of Science and Technology, China.

Questionnaire

Demographic characteristics: The self-reported questionnaire containing general characteristics, including age, sex, body mass index (BMI), education and occupation, as well as living habits, including smoking and alcohol, tea and coffee consumption, was used to collect baseline information.

Rose angina questionnaire: A translated Rose angina questionnaire[16], which had a specificity of 95%, sensitivity of 68%, and an intraclass correlation coefficient of 0.91, was used to estimate the duration, frequency, severity and characteristics of chest pain[16,19].

Gastroesophageal reflux symptom questionnaire: Clinical presentations and comorbid disorders were evaluated by a previously validated gastro-esophageal reflux symptom questionnaire[5]. On this questionnaire, esophageal and extraesophageal symptoms related to GERD were assessed. The frequency and severity of symptoms were graded on a 5-point Likert scale as previously described[11,16,20]. GERD was diagnosed on the basis of characteristic symptoms, such as heartburn and regurgitation, according to the Montreal standard[21]. A 7-item locally validated GERD questionnaire was used for the diagnosis of GERD, and a cut-off of 12 was recommended for a specificity of 84% and a sensitivity of 82%[18,22].

Hospital anxiety/depression scale: Depressive and anxious symptoms were assessed using a Chinese version of Hospital Anxiety/Depression Scale, which has robust psychometric properties and is brief and easy to administer[23]. The hospital anxiety/depression scale (HADS) consists of 14 items divided into two 21-point subscales for anxiety and depression, and a score of $\geqslant$ 8 was considered to be abnormal for either anxiety or depression[5,16].

36-item Short-Form Health Survey: The 36-item Short-Form Health Survey (SF-36) is an extensively used generic questionnaire for assessing health related QoL[24], which contained 8 dimensions [bodily pain (BP), physical function (PF), general health (GH), role-physical (RP), role-emotional (RE), mental health (MH), social functioning (SF) and vitality (VT)] and divides into two dimensions, the first four representing a physical component score (PCS) while the last four constituting a mental component score (MCS)[24]. It has good reliability and validity in the assessment of physical and mental QoL[25]. The score ranges from 0 to 100, and the higher score indicating a better health-related QoL[5,24].

Statistical analysis

Data entry was performed using EpiData 3.1, and SPSS 18.0 was used for statistical analysis. Comparisons of continuous variables were conducted by one-way analysis of variance or non-parametric Kruskal-Wallis tests, followed by the least significant difference test or Dunnett's T3 test for multiple comparisons, when required. Frequency variables were analyzed using chi-square tests. Two-tailed $P < 0.05$ was considered statistically significant. Spearman correlation analysis was used to identify correlations between psychological disorders and QoL in this study. Multiple regression analysis was further performed to investigate the independent factors impact on the QoL.

RESULTS

Demographics characteristics

Subjects with GERD-NCCP and GERD-CCP were significantly, but not substantially, older than those with GERD without chest pain (51.6 ± 11.4 and 61.13 ± 13.66 *vs* 46.2 ± 11.5, $P = 0.001$). Compared with GERD-NCCP patients, GERD-CCP were significantly older (61.13 ± 13.66 *vs* 51.59 ± 11.44, $P = 0.000$). However, there was no apparent difference in gender, BMI, and living habits, including smoking and alcohol intake, between these two groups (Table 1).

Chest pain and GERD scores in GERD patients with or without chest pain

GERD scores in patients with GERD, GERD-NCCP and GERD-CCP were similar (15.26 ± 3.79, 15.73 ± 3.54 and 15.00 ± 3.44, respectively, $P = 0.428$) (Table 2). Compared with GERD-NCCP patients, patients with GERD-CCP reported greater chest pain severity, with a significantly higher proportion having moderate to severe chest pain (78.9% *vs* 69.2%, $P = 0.038$). Chest pain was also more frequent in GERD-CCP patients than GERD-NCCP patients, with a higher proportion having chest pain attacks one or more times per week (42.3% *vs* 23.4%, $P = 0.046$) (Table 2).

Depression and anxiety in GERD patients with or without chest pain

There was a relatively higher proportion (43.5% and 46.2% *vs* 26.2%, $P = 0.022$ and 0.027, respectively)

Table 1 Patient characteristics

	GERD without CP (*n* = 182)	GERD with NCCP (*n* = 124)	GERD with CCP (*n* = 52)
Age (mean ± SD, yr)	46.16 ± 11.51	51.59 ± 11.44[b]	61.13 ± 13.66[b,d]
Sex (male/female)	104/78 (1.33:1)	78/46 (1.70:1)	36/16 (2.25:1)
BMI (mean ± SD, kg/m^2)	23.44 ± 4.23	22.97 ± 3.19	23.26 ± 3.57
Smoking, *n* (%)	39 (21.2)	37 (29.8)	14 (26.9)
Alcohol, *n* (%)	48 (26.4)	26 (21.0)	12 (23.1)

[b]*P* < 0.01 GERD with NCCP or CCP *vs* GERD without CP; [d]*P* < 0.01, GERD with CCP *vs* GERD with NCCP. BMI: Body mass index; CCP: Cardiac chest pain; CP: Chest pain; GERD: Gastroesophageal reflux disease; NCCP: Noncardiac chest pain.

Table 2 Gastroesophageal reflux disease scores and chest pain severity and frequency in gastroesophageal reflux disease patients with or without chest pain

	GERD without CP (*n* = 182)	GERD with NCCP (*n* = 124)	GERD with CCP (*n* = 52)	*P* value
GERD score (mean ± SD)	15.26 ± 3.79	15.73 ± 3.54	15.00 ± 3.44	0.428
Chest pain severity, *n* (%)	-	120	52	0.038
Mild		37 (30.8)	6 (11.5)	
Moderate		39 (32.5)	19 (36.5)	
Severe		37 (30.8)	22 (42.4)	
Incapacitating		7 (5.9)	5 (9.6)	
Chest pain frequency, *n* (%)	-	120	52	0.046
< once per month		43 (35.8)	14 (26.9)	
⩾ once per month		49 (40.8)	16 (30.8)	
⩾ once per week		28 (23.4)	22 (42.3)	

CCP: Cardiac chest pain; CP: Chest pain; GERD: Gastroesophageal reflux disease; NCCP: Noncardiac chest pain.

and level (6.86 ± 4.64 and 6.46 ± 4.09 *vs* 4.72 ± 4.13, *P* = 0.002 and 0.037, respectively) of anxiety in GERD patients with NCCP and CCP than those without chest pain; however, there was no significant difference in anxiety levels between the NCCP and CCP groups (6.86 ± 4.64 *vs* 6.46 ± 4.09, *P* = 0.584). (Table 3) For depression, both proportion and level were higher in GERD-NCCP patients than those with GERD and GERD-CCP, but the differences did not reach statistically significant (Table 3). These data suggested that patients with GERD-NCCP and GERD-CCP had equivalent levels of anxiety and depression.

QoL in GERD patients with or without chest pain

GERD patients with NCCP had lower BP, PF, RE and SF scores compared with those without chest pain; however, patients with GERD-CCP had poorer QoL in the broader aspects, including PF, BP, VT, RP, RE and SF. Particularly, GERD-CCP patients had lower PF, RP,

Table 3 Anxiety and depression in gastroesophageal reflux disease patients with or without chest pain

	GERD without CP (*n* = 182)	GERD with NCCP (*n* = 124)	GERD with CCP (*n* = 52)
HADS Depression, *n* (%)	72 (39.6)	61 (49.2)	19 (36.5)
(mean ± SD)	6.41 ± 4.76	7.00 ± 4.49	6.17 ± 4.05
HADS Anxiety, *n* (%)	48 (26.4)	54 (43.5)[a]	24 (46.2)[a]
(mean ± SD)	4.72 ± 4.13	6.86 ± 4.64[b]	6.35 ± 4.06[b]
HADS Depression and Anxiety, *n* (%)	45 (24.7%)	49 (39.5%)[a]	23 (44.3%)[a]

[a]*P* < 0.05, [b]*P* < 0.01, GERD with NCCP, with CCP *vs* GERD without CP. CCP: Cardiac chest pain; CP: Chest pain; GERD: Gastroesophageal reflux disease; NCCP: Noncardiac chest pain.

Table 4 Health-related quality of life in gastroesophageal reflux disease patients with or without chest pain

	GERD without CP (*n* = 182)	GERD with NCCP (*n* = 124)	GERD with CCP (*n* = 52)
Physical function	97.33 ± 5.33	84.55 ± 20.98[b]	55.87 ± 26.66[b,d]
Role-physical	54.92 ± 49.75	51.23 ± 46.67	25.48 ± 36.55[b,d]
Bodily pain	76.52 ± 19.71	60.83 ± 22.99[b]	54.46 ± 24.66[b]
General health	38.77 ± 17.69	39.92 ± 20.64	40.92 ± 21.88
Vitality	60.62 ± 24.75	61.33 ± 22.98	49.46 ± 22.66[a,d]
Social functioning	79.30 ± 26.06	67.25 ± 25.77[a]	53.41 ± 19.55[b,d]
Role-emotional	82.51 ± 37.81	53.82 ± 47.42[b]	41.89 ± 44.37[b]
Mental health	68.90 ± 24.46	62.77 ± 21.68	65.19 ± 19.73

All data are expressed as the mean ± SD. [b]*P* < 0.01, [a]*P* < 0.05 GERD with NCCP, with CCP *vs* GERD without CP; [d]*P* < 0.01 GERD with CCP *vs* GERD with NCCP. CCP: Cardiac chest pain; CP: Chest pain; GERD: Gastroesophageal reflux disease; NCCP: Noncardiac chest pain.

VT and SF scores than those with GERD-NCCP (all *P* < 0.01) (Table 4). In general, the levels of QoL were, in decreasing order, GERD, GERD-NCCP and then GERD-CCP. Compared with GERD-NCCP subjects, the GERD-CCP group had a significantly lower physical component (44.18 ± 19.11 *vs* 59.13 ± 20.16, *P* = 0.000), mental component (52.49 ± 19.92 *vs* 61.29 ± 23.14, *P* = 0.018) and total scores (48.34 ± 17.68 *vs* 60.21 ± 20.27, *P* = 0.000) (Figure 1).

QoL in patients with and without anxiety and depression

In the GERD and GERD-NCCP groups, the total SF-36 scores (Figure 2A), as well as PCS (Figure 2B) and MCS (Figure 2C), were significantly lower in subjects with depression, anxiety, and both depression and anxiety than those without depression or anxiety (all P< 0.05). However, this difference was not present in the GERD-CCP group (Figure 2).

Correlations between depression/anxiety and the QoL

Anxiety had a negative correlation with PCS, MCS and total SF-36 score in GERD (*r* = -0.64, -0.69 and -0.75, respectively; all *P* < 0.01) and GERD-NCCP (*r* = -0.49, -0.58 and -0.57, respectively; all *P* < 0.01) patients

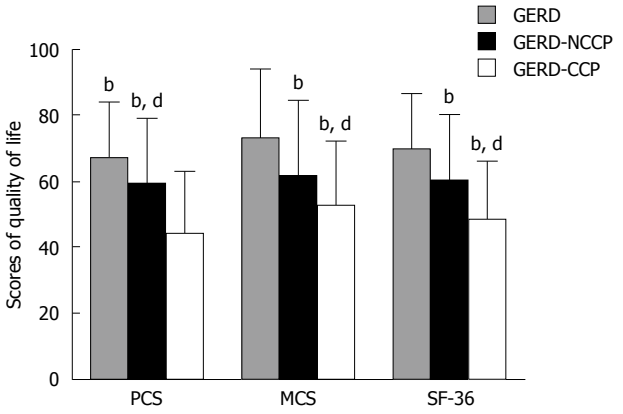


Figure 1 Health-related quality of life in gastroesophageal reflux disease patients with or without chest pain. The physical component score (PCS), mental component score (MCS) and total SF-36 score were highest in patients with gastroesophageal reflux disease (GERD), followed by GERD-NCCP and then GERD-CCP. [b]*P* < 0.01 GERD-NCCP or GERD-CCP *vs* GERD without CP, [d]*P* < 0.01 GERD-NCCP *vs* GERD-CCP.

Table 5 Influence of physical and psychological symptoms on quality of life in gastroesophageal reflux disease patients with chest pain: A multivariate analysis

	GERD with NCCP		GERD with CCP	
	β[1]	*P* value	β[1]	*P* value
Constant	-	0.000	-	0.000
Anxiety	-0.313	0.003	-0.169	0.048
Depression	-0.299	0.005	-0.077	0.218
Chest pain	-0.170	0.017	-0.422	0.001
GERD	-0.153	0.023	-0.236	0.043

[1]Standardized regression coefficient. CCP: Cardiac chest pain; GERD: Gastroesophageal reflux disease; NCCP: Noncardiac chest pain.

(Figure 3A). Similarly, depression was also negatively correlated with PCS, MCS and total QoL score in GERD (r = -0.61, -0.71 and -0.74, respectively; all *P* < 0.01) and GERD-NCCP (*r* = -0.57, -0.57 and -0.60, respectively; all *P* < 0.01) patients. (Figure 3B) However, anxiety had only a weak negative relation with MCS (*r* = -0.32, *P* < 0.05) in GERD-CCP patients, as did depression (*r* = -0.28, *P* < 0.05) (Figure 3).

Influence of physical and psychological symptoms on QoL in GERD patients with chest pain

The results of multiple analysis showed that anxiety, depression, GERD and chest pain were independent factors influencing the QoL of GERD patients with CCP and NCCP (the coefficient of determination reaches 0.675 and 0.682, respectively) (Table 5). In GERD patients with NCCP, the influence of anxiety (β = -0.313, *P* = 0.003) and depression (β = -0.299, *P* = 0.005) on the QoL were higher than chest pain (β = -0.170, *P* = 0.017) and GERD (β = -0.153, *P* = 0.023). On the contrary, the effects of chest pain (β = -0.422, *P* = 0.001) and GERD (β = -0.236, *P* = 0.043) on the QoL were dominant factors in GERD patients with CCP.

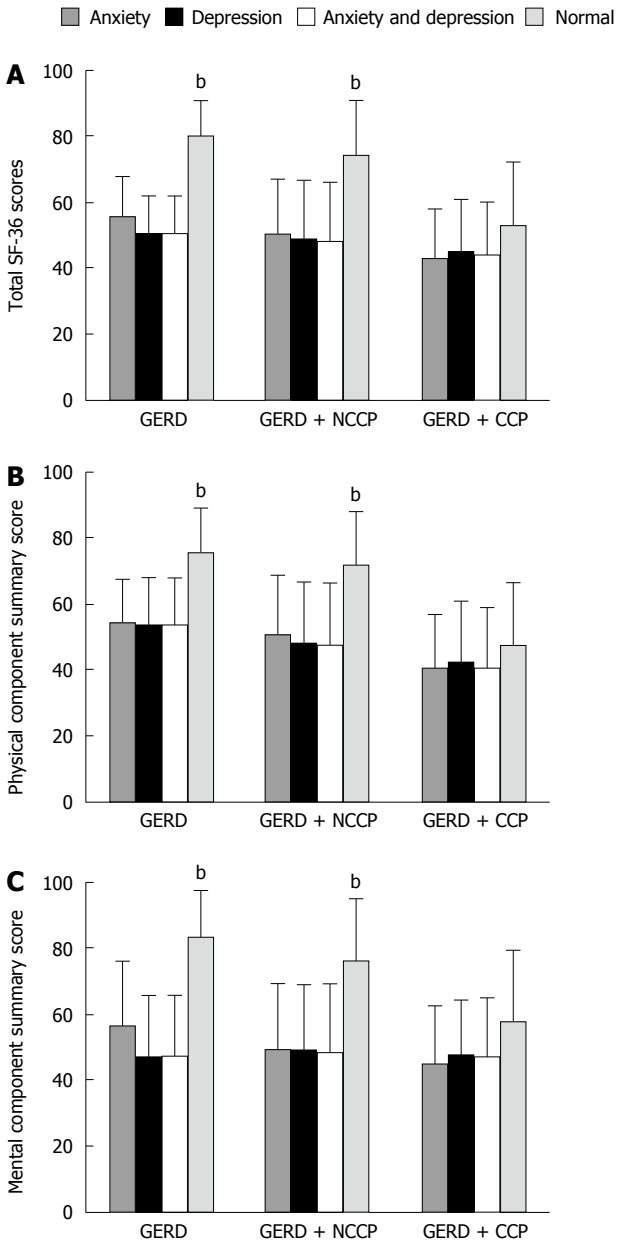


Figure 2 Health-related quality of life among gastroesophageal reflux disease patients with or without anxiety and depression. Total SF-36 scores (A), physical component (PCS) (B) and mental component (MCS) (C) scores were much lower in subjects with anxiety, depression, and both anxiety and depression than in those without anxiety and depression for both GERD and GERD-NCCP patients, while this difference was not present in GERD-CCPpatients. [b]*P* < 0.01, subjects without anxiety and depression *vs* subjects with anxiety, with depression, and with both anxiety and depression. CCP: Cardiac chest pain; GERD: Gastroesophageal reflux disease; NCCP: Noncardiac chest pain.

DISCUSSION

In this study, the influences of anxiety and depression on health-related QoL of GERD patients with CCP and NCCP were assessed. These data demonstrated that high levels of depression and anxiety, and impaired QoL were prevalent in GERD patients with CCP and NCCP. Importantly, anxiety and depression

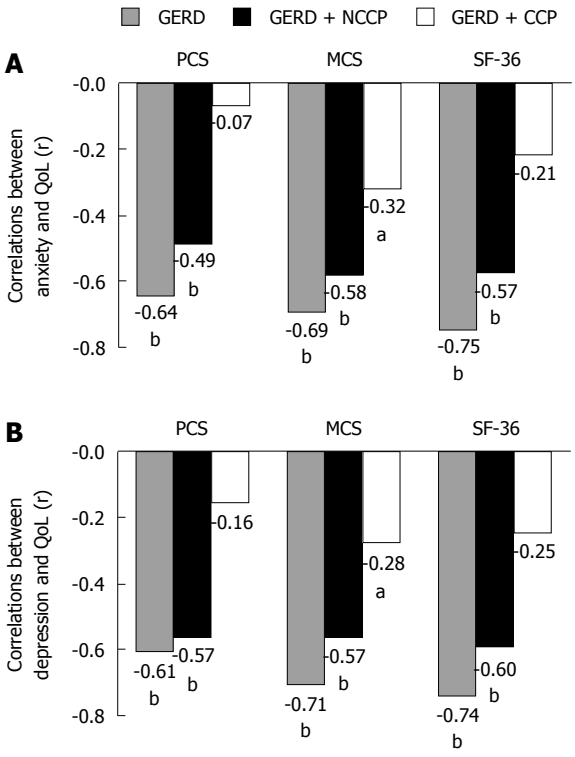


Figure 3 Correlations between psychological distress and quality of life in GERD patients with or without chest pain. Anxiety (A) and depression (B) were negatively correlated with physical component (PCS), mental component (MCS) and the total SF-36 scores in GERD and GERD-NCCP patients; however, there was only a weak negative correlation with MCS in GERD-CCP patients. The r represents the correlation coefficient. $^{a}P < 0.05$, $^{b}P < 0.01$ for the correlation coefficient.

may contribute differently to the QoL status in GERD patients with NCCP and with CCP.

Impairments of health-related QoL in patients with GERD, as well as those with NCCP and CCP, have been reported previously[18,26-28]. We demonstrated that GERD patients with chest pain had much poorer mental and physical QoL scores than those without chest pain. This difference may be partly because chest pain may be an alarm signal for fatal illness, which may contribute to high levels of psychological burden[3,5], and exacerbate the problem; however, it has been reported that the decreased QoL in patients with NCCP was equivalent to that in those suffering from CCP[29]. In this study, GERD-CCP patients displayed a much poorer QoL (both mental and physical) in comparison with GERD-NCCP ones. This suggests that the functional activity of GERD patients with CCP, which is usually accompanied by a more serious and potentially fatal chest pain, was more likely to be influenced by true heart trouble.

Physical symptoms, particularly chest pain, may also have a negative influence on mental status. There was a relatively higher proportion and level of psychiatric distress, and particularly anxiety symptoms, in GERD patients with NCCP or CCP relative to those without chest pain. In fact, depression and anxiety are two of the most common psychological symptoms related to GERD[26]. The effect of psychosocial factors on the pathogenesis of NCCP is also widely accepted[5,9,13]. These psychological and emotional factors may affect how patients perceive their symptoms[14]. This may partially explain why even slight physiologic stimuli can be interpreted as major symptoms by patients and significantly affect QoL, resulting in dissatisfaction with conventional treatment[14,30]. However, there was no difference in the levels of depression and anxiety between GERD-CCP and GERD-NCCP patients. Despite their lack of cardiac complications, NCCP patients had equivalent psychiatric morbidity, functional impairment, and medical utilization when compared to patients with CCP[31].

This study focused on the role of psychological distress on lower QoL in GERD patients with NCCP and CCP. This action may be different between two groups: (1) in the GERD-NCCP group, but not the GERD-CCP group, physical and mental QoL were much lower in subjects with depression and/or anxiety than those with normal mental status; (2) anxiety and depression exhibited strong negative correlations with QoL in GERD-NCCP patients, while both demonstrated a weak correlation only with the mental components of QoL in GERD-CCP patients; and (3) the independent influence of anxiety and depression on the QoL were stronger than chest pain in GERD-NCCP patients, while chest pain and gastroesophageal reflux were dominant factors in those with CCP. This further suggests that psychological distress plays a more important role in the determination of QoL in GERD patients with NCCP than those with CCP.

Physiological symptoms and psychological distress are two important factors with the potential to substantially affect QoL. As described above, there was a much poorer QoL in GERD-CCP than GERD-NCCP patients, but the levels of anxiety and depression between these two groups were analogous. This may be due to the fact that chest pain and associated symptoms of cardiac origin, rather than anxiety and depression, were stronger factors determining the QoL in CCP patients. On the contrary, relative to subjects with actual cardiac disorders, NCCP patients may experience more cardiac sensations, behavior restriction and illness vigilance[31-33]. In GERD-NCCP subjects, psychiatric distress, which is idiopathic or due to a long-term mental burden of disease, may play a greater important role in determining QoL. Thus, psychological and cognitive intervention may be of great benefit for QoL improvement in the condition of GERD with NCCP.

In conclusion, anxiety and depression, relative to physical illness, may play significant roles in determining the QoL of GERD patients with NCCP. However, in those with GERD-CCP, cardiac chest pain may play a more dominant role in QoL, even with high levels of comorbid depression and anxiety. Therefore, in addition to excluding the cardiac lesion and dealing with the organic illness, we should highlight the

importance of the identification and management of psychological impact in improving QoL in GERD-NCCP patients. Moreover, because patients in this study were predominately treated in a comprehensive medical center in Central China, conditions may be different to those visiting a primary physician. It should be more reasonable if a multi-center and widely covered survey could be conducted.

COMMENTS

Background

Noncardiac chest pain (NCCP) is the most common atypical symptom of gastroesophageal reflux disease (GERD). Notably, a considerable portion of patients with cardiac chest pain (CCP) also have comorbid reflux disease. Comorbid psychiatric disorders, such as anxiety and depression, and impaired quality of life (QoL) are prevalent in GERD patients, as well as GERD-related chest pain. However, the impact of psychological factors on QoL in GERD patients with NCCP and CCP is far from clear because research in this area is limited, especially in Chinese populations. In this observational study, we aimed to assess the differences in the roles of psychological distress on QoL in GERD patients with NCCP and those with CCP.

Research frontiers

The cause of impaired QoL in GERD and NCCP patients is complicated and multifactorial; besides the physiological dysfunction, psychological factors may not be ignored. Although proton pump inhibitors are now the dominant treatment for GERD and GERD with NCCP, it is not always effective. Interventions pointed at psychological disorders may be of great benefit on improving the QoL of these subjects. So, it is necessary to evaluate the roles of psychological factors on QoL.

Innovations and breakthroughs

This study demonstrated that high levels of depression/anxiety and impaired QoL were prevalent in GERD patients with CCP and NCCP. Importantly, anxiety and depression, relative to physical illness, may play a more significant role in determining the QoL in GERD patients with NCCP. However, in those with GERD-CCP, cardiac chest pain may play a more dominant role in QoL, even with high levels of comorbid anxiety and depression. It further confirmed the important role of psychological factors in QoL decreasing in NCCP patients.

Applications

In many cases, a greater emphasis is placed on the treatment of physical symptoms, and invisible psychological disorders in these subjects are often ignored. This study may help clinicians to pay more attention to the importance of identification and management of psychological impact in improving QoL in GERD-NCCP patients.

Terminology

NCCP: Recurrent episodes of angina-like retrosternal chest pain in patients without cardiac origin. CCP: Retrosternal angina precipitated by exertion and relieved by rest which due to ischaemic heart disease.

Peer-review

The authors report a study on 358 consecutive patients with gastroesophageal reflux with and without chest pain. The data suggest that depression and anxiety is the dominant factor for quality of life while presence or absence of cardiac disease has smaller effects.

REFERENCES

1 **El-Serag HB**, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2014; **63**: 871-880 [PMID: 23853213 DOI: 10.1136/gutjnl-2012-304269]

2 **Wong WM**, Fass R. Extraesophageal and atypical manifestations of GERD. *J Gastroenterol Hepatol* 2004; **19** Suppl 3: S33-S43 [PMID: 15324380 DOI: 10.1111/j.1440-1746.2004.03589.x]

3 **Heidelbaugh JJ**, Gill AS, Van Harrison R, Nostrant TT. Atypical presentations of gastroesophageal reflux disease. *Am Fam Physician* 2008; **78**: 483-488 [PMID: 18756656]

4 **Oranu AC**, Vaezi MF. Noncardiac chest pain: gastroesophageal reflux disease. *Med Clin North Am* 2010; **94**: 233-242 [PMID: 20380953 DOI: 10.1016/j.mcna.2010.01.001]

5 **Eslick GD**, Jones MP, Talley NJ. Non-cardiac chest pain: prevalence, risk factors, impact and consulting--a population-based study. *Aliment Pharmacol Ther* 2003; **17**: 1115-1124 [PMID: 12752348 DOI: 10.1046/j.0269-2813.2003.01557.x]

6 **Tan VP**, Wong BC, Wong WM, Leung WK, Tong D, Yuen MF, Fass R. Gastroesophageal Reflux Disease: Cross-Sectional Study Demonstrating Rising Prevalence in a Chinese Population. *J Clin Gastroenterol* 2016; **50**: e1-e7 [PMID: 25751371 DOI: 10.1097/MCG.0000000000000304]

7 **Karlaftis A**, Karamanolis G, Triantafyllou K, Polymeros D, Gaglia A, Triantafyllou M, Papanikolaou IS, Ladas SD. Clinical characteristics in patients with non-cardiac chest pain could favor gastroesophageal reflux disease diagnosis. *Ann Gastroenterol* 2013; **26**: 314-318 [PMID: 24714293]

8 **Coss-Adame E**, Erdogan A, Rao SS. Treatment of esophageal (noncardiac) chest pain: an expert review. *Clin Gastroenterol Hepatol* 2014; **12**: 1224-1245 [PMID: 23994670 DOI: 10.1016/j.cgh.2013.08.036]

9 **García-Campayo J**, Rosel F, Serrano P, Santed MA, Andrés E, Roca M, Serrano-Blanco A, León Latre M. Different psychological profiles in non-cardiac chest pain and coronary artery disease: a controlled study. *Rev Esp Cardiol* 2010; **63**: 357-361 [PMID: 20196998 DOI: 10.1016/S1885-5857(10)70070-0]

10 **Liu Y**, He S, Chen Y, Xu J, Tang C, Tang Y, Luo G. Acid reflux in patients with coronary artery disease and refractory chest pain. *Intern Med* 2013; **52**: 1165-1171 [PMID: 23728549 DOI: 10.2169/internalmedicine.52.0031]

11 **Wong WM**, Lai KC, Lau CP, Hu WH, Chen WH, Wong BC, Hui WM, Wong YH, Xia HH, Lam SK. Upper gastrointestinal evaluation of Chinese patients with non-cardiac chest pain. *Aliment Pharmacol Ther* 2002; **16**: 465-471 [PMID: 11876699 DOI: 10.1046/j.1365-2036.2002.01217.x]

12 **Hughes J**, Lockhart J, Joyce A. Do calcium antagonists contribute to gastro-oesophageal reflux disease and concomitant noncardiac chest pain? *Br J Clin Pharmacol* 2007; **64**: 83-89 [PMID: 17298478 DOI: 10.1111/j.1365-2125.2007.02851.x]

13 **Shapiro M**, Simantov R, Yair M, Leitman M, Blatt A, Scapa E, Broide E. Comparison of central and intraesophageal factors between gastroesophageal reflux disease (GERD) patients and those with GERD-related noncardiac chest pain. *Dis Esophagus* 2012; **25**: 702-708 [PMID: 22309285 DOI: 10.1111/j.1442-2050.2011.01317]

14 **Kamolz T**, Velanovich V. Psychological and emotional aspects of gastroesophageal reflux disease. *Dis Esophagus* 2002; **15**: 199-203 [PMID: 12444990 DOI: 10.1046/j.1442-2050.2002.00261.x]

15 **Eslick GD**, Talley NJ. Natural history and predictors of outcome for non-cardiac chest pain: a prospective 4-year cohort study. *Neurogastroenterol Motil* 2008; **20**: 989-997 [PMID: 18466221 DOI: 10.1111/j.1365-2982.2008.01133.x]

16 **Wong WM**, Lam KF, Cheng C, Hui WM, Xia HH, Lai KC, Hu WH, Huang JQ, Lam CL, Chan CK, Chan AO, Lam SK, Wong BC. Population based study of noncardiac chest pain in southern Chinese: prevalence, psychosocial factors and health care utilization. *World J Gastroenterol* 2004; **10**: 707-712 [PMID: 14991943 DOI: 10.3748/WJG.v10.i5.707]

17 **Chambers JB**, Marks EM, Russell V, Hunter MS. A multi-disciplinary, biopsychosocial treatment for non-cardiac chest pain. *Int J Clin Pract* 2015; **69**: 922-927 [PMID: 25363358 DOI: 10.1111/ijcp.12533]

18 **Cheung TK**, Hou X, Lam KF, Chen J, Wong WM, Cha H, Xia HH, Chan AO, Tong TS, Leung GY, Yuen MF, Wong BC. Quality of life and psychological impact in patients with noncardiac chest

pain. *J Clin Gastroenterol* 2009; **43**: 13-18 [PMID: 18698264 DOI: 10.1097/MCG.0b013e3181514725]
19 **Williams JF**, Sontag SJ, Schnell T, Leya J. Non-cardiac chest pain: the long-term natural history and comparison with gastroesophageal reflux disease. *Am J Gastroenterol* 2009; **104**: 2145-2152 [PMID: 19513020 DOI: 10.1038/ajg.2009.279]
20 **Mousavi S**, Tosi J, Eskandarian R, Zahmatkesh M. Role of clinical presentation in diagnosing reflux-related non-cardiac chest pain. *J Gastroenterol Hepatol* 2007; **22**: 218-221 [PMID: 17295874 DOI: 10.1111/j.1440-1746.2006.04416.x]
21 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943 [PMID: 16928254 DOI: 10.1111/j.1572-0241.2006.00630.x]
22 **Wong WM**, Lam KF, Lai KC, Hui WM, Hu WH, Lam CL, Wong NY, Xia HH, Huang JQ, Chan AO, Lam SK, Wong BC. A validated symptoms questionnaire (Chinese GERDQ) for the diagnosis of gastro-oesophageal reflux disease in the Chinese population. *Aliment Pharmacol Ther* 2003; **17**: 1407-1413 [PMID: 12786635 DOI: 10.1046/j.1365-2036.2003.01576.x]
23 **Yang Y**, Ding R, Hu D, Zhang F, Sheng L. Reliability and validity of a Chinese version of the HADS for screening depression and anxiety in psycho-cardiological outpatients. *Compr Psychiatry* 2014; **55**: 215-220 [PMID: 24199886 DOI: 10.1016/j.comppsych.2013.08.012]
24 **Hadlandsmyth K**, White KS, Krone RJ. Quality of life in patients with non-CAD chest pain: associations to fear of pain and psychiatric disorder severity. *J Clin Psychol Med Settings* 2013; **20**: 284-293 [PMID: 23338745 DOI: 10.1007/s10880-012-9347-7]
25 **Thumboo J**, Wu Y, Tai ES, Gandek B, Lee J, Ma S, Heng D, Wee HL. Reliability and validity of the English (Singapore) and Chinese (Singapore) versions of the Short-Form 36 version 2 in a multi-ethnic urban Asian population in Singapore. *Qual Life Res* 2013; **22**: 2501-2508 [PMID: 23475690 DOI: 10.1007/s11136-013-0381-1]
26 **Yang XJ**, Jiang HM, Hou XH, Song J. Anxiety and depression in patients with gastroesophageal reflux disease and their effect on quality of life. *World J Gastroenterol* 2015; **21**: 4302-4309 [PMID: 25892882 DOI: 10.3748/wjg.v21.i14.4302]
27 **Lee SW**, Lien HC, Lee TY, Yang SS, Yeh HJ, Chang CS. Heartburn and regurgitation have different impacts on life quality of patients with gastroesophageal reflux disease. *World J Gastroenterol* 2014; **20**: 12277-12282 [PMID: 25232262 DOI: 10.3748/wjg.v20.i34.12277]
28 **Ortiz-Garrido O**, Ortiz-Olvera NX, González-Martínez M, Morán-Villota S, Vargas-López G, Dehesa-Violante M, Ruiz-de León A. Clinical assessment and health-related quality of life in patients with non-cardiac chest pain. *Rev Gastroenterol Mex* 2015; **80**: 121-129 [PMID: 26148745 DOI: 10.1016/j.rgmx.2015.03.005]
29 **Remes-Troche JM**. The hypersensitive esophagus: pathophysiology, evaluation, and treatment options. *Curr Gastroenterol Rep* 2010; **12**: 417-426 [PMID: 20669058 DOI: 10.1007/s11894-010-0122-3]
30 **Nguyen TM**, Eslick GD. Systematic review: the treatment of noncardiac chest pain with antidepressants. *Aliment Pharmacol Ther* 2012; **35**: 493-500 [PMID: 22239853 DOI: 10.1111/j.1365-2036.2011.04978.x]
31 **Mourad G**, Jaarsma T, Hallert C, Strömberg A. Depressive symptoms and healthcare utilization in patients with noncardiac chest pain compared to patients with ischemic heart disease. *Heart Lung* 2012; **41**: 446-455 [PMID: 22652167 DOI: 10.1016/j.hrtlng.2012.04.002]
32 **White KS**, Craft JM, Gervino EV. Anxiety and hypervigilance to cardiopulmonary sensations in non-cardiac chest pain patients with and without psychiatric disorders. *Behav Res Ther* 2010; **48**: 394-401 [PMID: 20110083 DOI: 10.1016/j.brat.2010.01.001]
33 **Shelby RA**, Somers TJ, Keefe FJ, Silva SG, McKee DC, She L, Waters SJ, Varia I, Riordan YB, Knowles VM, Blazing M, Blumenthal JA, Johnson P. Pain catastrophizing in patients with noncardiac chest pain: relationships with pain, anxiety, and disability. *Psychosom Med* 2009; **71**: 861-868 [PMID: 19737857 DOI: 10.1097/PSY.0b013e3181b49584]

P- Reviewer: Moraes JP, Schweiger U **S- Editor**: Qi Y
L- Editor: O'Neill M **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.135

World J Gastroenterol 2017 January 7; 23(1): 135-140
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Prospective Study

Trefoil factor-3 is not a useful marker of mucosal healing in Crohn's disease treated with anti-TNF-α antibodies

Piotr Eder, Kamila Stawczyk-Eder, Katarzyna Korybalska, Natasza Czepulis, Joanna Luczak, Liliana Lykowska-Szuber, Iwona Krela-Kazmierczak, Krzysztof Linke, Janusz Witowski

Piotr Eder, Kamila Stawczyk-Eder, Liliana Lykowska-Szuber, Iwona Krela-Kazmierczak, Krzysztof Linke, Department of Gastroenterology, Human Nutrition and Internal Diseases, Poznan University of Medical Sciences, 60-355 Poznan, Poland

Katarzyna Korybalska, Natasza Czepulis, Joanna Luczak, Janusz Witowski, Department of Pathophysiology, Poznan University of Medical Sciences, 60-806 Poznan, Poland

Author contributions: Eder P and Witowski J designed the study, performed statistical analyses and wrote the manuscript; Korybalska K, Czepulis N and Luczak J performed the laboratory analyses; Stawczyk-Eder K, Lykowska-Szuber L, Krela-Kazmierczak I and Linke K managed the patients and helped to draft, review and edit the manuscript.

Supported by the Poznan University of Medical Sciences Grant, A helping hand (2014).

Institutional review board statement: The study was approved by the Bioethics Committee of the Poznan University of Medical Sciences (No. 409/2013).

Informed consent statement: Written informed consent was obtained from all the participants.

Conflict-of-interest statement: Eder P received lecture fees from Abbvie Poland and travel grants from Astellas and Abbvie Poland; Lykowska-Szuber L received travel grants from Alvogen and Abbvie Poland. Krela-Kazmierczak I received travel grants from Alvogen and Astellas. Linke K received travel grant from Abbvie Poland. Korybalska K, Stawczyk-Eder K, Czepulis N, Luczak J and Witowski J have nothing to disclose.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Piotr Eder, MD, PhD, Department of Gastroenterology, Human Nutrition and Internal Diseases, Poznan University of Medical Sciences, Heliodor Swiecicki Hospital, Przybyszewskiego Street 49, 60-355 Poznan, Poland. piotr.eder@op.pl
Telephone: +48-698050797
Fax: +48-618691686

Received: July 20, 2016
Peer-review started: July 22, 2016
First decision: October 20, 2016
Revised: November 2, 2016
Accepted: November 23, 2016
Article in press: November 28, 2016
Published online: January 7, 2017

Abstract

AIM

To evaluate whether repeated serum measurements of trefoil factor-3 (TFF-3) can reliably reflect mucosal healing (MH) in Crohn's disease (CD) patients treated with anti-tumor necrosis factor-α (anti-TNF-α) antibodies.

METHODS

Serum TFF-3 was measured before and after anti-TNF-α induction therapy in 30 CD patients. The results were related to clinical, biochemical and endoscopic parameters. MH was defined as a ⩾ 50% decrease in Simple Endoscopic Score for Crohn's disease (SES-CD).

RESULTS

SES-CD correlated significantly with CD clinical activity and several standard biochemical parameters (albumin,

leukocyte and platelet counts, C-reactive protein, erythrocyte sedimentation rate, fibrinogen). In contrast, SES-CD did not correlate with TFF-3 (P = 0.54). Moreover, TFF-3 levels did not change significantly after therapy irrespectively of whether the patients achieved MH or not. Likewise, TFF-3 did not correlate with changes in fecal calprotectin, which has been proposed as another biochemical marker of mucosal damage in CD.

CONCLUSION
Serum TFF-3 is not a convenient and reliable surrogate marker of MH during therapy with TNF-α antagonists in CD.

Key words: Adalimumab; Crohn's disease; Infliximab; Mucosal healing; Trefoil factors

Core tip: Mucosal healing (MH) is viewed as the holy grail of the efficacy of anti-tumor necrosis factor-α (anti-TNF-α) therapy for Crohn's disease (CD), however performance of repeated colonoscopies is questionable for economical and safety reasons. We aimed to assess, whether serum trefoil factor-3 (TFF-3), a parameter engaged in maintaining mucosal integrity, could be useful in the assessment of MH. We found no correlation between TFF-3 and CD endoscopic activity and fecal calprotectin. Changes in TFF-3 did not reflect the degree to which MH was achieved. Thus, TFF-3 does not seem to be a reliable surrogate marker for MH in CD patients undergoing anti-TNF-α therapy.

Eder P, Stawczyk-Eder K, Korybalska K, Czepulis N, Luczak J, Lykowska-Szuber L, Krela-Kazmierczak I, Linke K, Witowski J. Trefoil factor-3 is not a useful marker of mucosal healing in Crohn's disease treated with anti-TNF-α antibodies. *World J Gastroenterol* 2017; 23(1): 135-140 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/135.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.135

INTRODUCTION

A great deal of data shows that mucosal healing (MH) is the most sensitive marker of successful outcome of therapy with anti-tumor necrosis factor alpha (anti-TNF-α) antibodies for Crohn's disease (CD)[1]. These drugs have the greatest potential to induce MH when compared with other pharmacological agents[2]. It has been suggested that the occurrence of MH after anti-TNF-α therapy predicts successful long-term clinical remission and lower hospitalization and surgery rates[3]. There are, however, difficulties in rigorous and practical defining MH[4,5]. In this respect, repeated endoscopy seems to be the most appropriate method, however, its use may be limited by the associated invasiveness and costs[5]. Therefore, there is a need for new parameters that are simple and cheap to monitor reliably MH during anti-TNF-α therapy in CD.

Trefoil factors (TFFs) are a family of mucin-associated peptides secreted by goblet cells in the intestinal epithelium. They play an important role in maintaining mucosal barrier integrity[6]. It has recently been suggested that serum levels of TFF-3 can reflect MH in ulcerative colitis (UC)[7]. There are, however, no data on whether TFF-3 can play a similar role in CD.

The aim of the present study was to evaluate the usefulness of repeated serum TFF-3 measurements for the assessment of MH in CD patients treated with anti-TNF-α antibodies.

MATERIALS AND METHODS

Patients

Patients with diagnosed CD were prospectively enrolled to the analysis. The inclusion criteria were as follows: (1) failure of standard pharmacological treatment for CD according to current guidelines[8] in patients with Crohn's Disease Activity Index (CDAI) ⩾ 300 points or with active perianal lesions; and (2) full ileocolonoscopy with the Simple Endoscopic Score for Crohn's Disease (SES-CD) assessment[5].

For the induction therapy the patients received either infliximab (IFX; 5 mg/kg body weight intravenously at 0-2-6 wk) or adalimumab (ADA; 160 mg at week 0, 80 mg at week 2 and 40 mg every other week subcutaneously till week 12). Patients underwent clinical, biochemical, and endoscopic assessment at week 10 (IFX) or at week 14 (ADA). MH was defined as at least a 50% decrease in SES-CD (endoscopic criterion)[5]. Clinical response to therapy was defined as a decrease in CDAI by at least 100 points[9].

In addition, in some patients, fecal calprotectin (FC) was measured using PhiCal® Calprotectin ELISA (Immundiagnostik, Germany), according to the manufacturer's recommendations, as FC is believed to be the most useful surrogate marker of endoscopic activity in CD[10].

Serum concentrations of TFF-3 were measured using DuoSet® Immunoassay kit (R&D Systems, United States), as per manufacturer's instructions in parallel with clinical and endoscopic assessment before (week 0) and after the induction anti-TNF-α therapy (week 10 for IFX and week 14 for ADA). Patients who required any change in the concomitant treatment during the induction anti-TNF-α therapy were excluded from the analysis.

Statistical analysis

All continuous variables were checked for the normality of distribution. As the data did not display consistently normal distribution, they were analyzed using non-parametric statistics for paired (the Wilcoxon test) or unpaired (the Mann-Whitney U test) data, as ap-

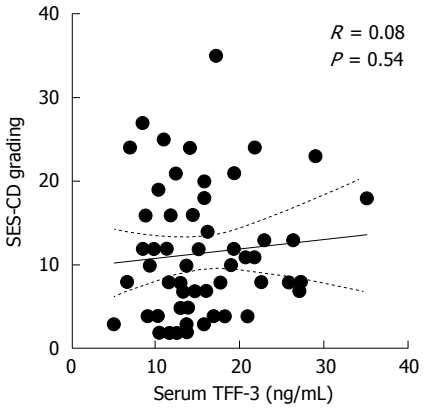


Figure 1 Correlation of serum trefoil factor-3 concentrations with Simple Endoscopic Score for Crohn's disease scores. Samples were collected from 29 patients before and after the induction therapy with anti-TNF-α agents (n = 58). TFF-3: Trefoil factor-3; SES-CD: Simple Endoscopic Score for Crohn's disease.

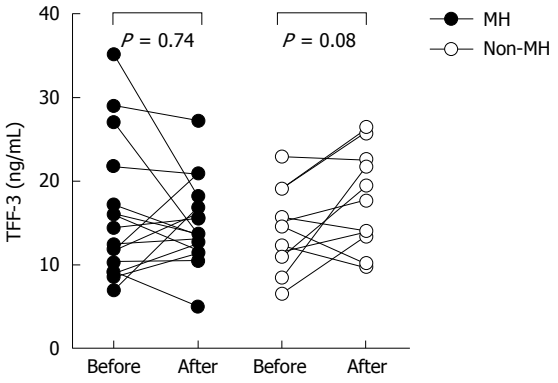


Figure 2 Individual changes in serum trefoil factor-3 during anti-TNF-α therapy in patients with or without successful mucosal healing as assessed by endoscopy. MH: Mucosal healing; TFF-3: Trefoil factor-3.

propriate. Categorized data were assessed with the Fisher's exact test. Correlations were assessed with the use of Spearman's rank correlation coefficient. A P value < 0.05 was considered significant. All data were analyzed using the GraphPad Prism 6.07 (GraphPad Software Inc., United States).

Ethical considerations

The study was approved by the Bioethics Committee of the Poznan University of Medical Sciences (No. 409/2013). Written informed consent was obtained from all the participants.

RESULTS

Patients characteristics

A total of 30 patients were enrolled, with one patient being excluded from the analysis owing to the incompleteness of biochemical data. Firstly we correlated SES-CD scores recorded before and after therapy with TFF-3 levels at the same time points (Figure 1). It turned out that absolute TFF-3 concentrations in serum did not correlate with the status of the mucosa as assessed by endoscopy. In sharp contrast, SES-CD correlated significantly with other parameters proposed as surrogate markers of severity of the disease (Table 1). In particular, SES-CD correlated well - in a positive and negative manner, respectively - with an index of clinical activity of the disease (CDAI) and albumin levels. Other significant correlations included leukocyte and platelet counts, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and fibrinogen. These observations indicated that the population of CD patients analyzed exhibited typical and expected responses to anti-TNF-α treatment[11].

Secondly in the next step, we analyzed changes in serum TFF-3 in patients with or without MH in response to therapy. To this end the patients were stratified according to the magnitude of decrease in SES-CD (with values ⩾ 50% and < 50% corresponding to successful and unsuccessful MH, respectively)[5].

Full clinical and demographic patient characteristics at baseline is presented in Table 2.

According to these criteria 18 out of 29 patients (62%) achieved successful MH. Baseline analysis revealed that patients with MH were younger and more often male (Table 2). Other parameters, including the indexes of clinical and endoscopic activity of the disease and several conventional biochemical markers did not differ between patients with and without MH. There was also no formal difference between the groups in TFF-3 levels both before and after the intervention (Figure 2). Comparison of TFF-3 levels before and after therapy separately for each group revealed no significant difference in patients with MH [(median and IQR): 13.50 (9.25-18.36) ng/mL *vs* 13.68 (12.33-17.26) ng/mL]. TFF-3 concentrations in patients with no MH tended to increase slightly over time [(median and IQR): 14.63 (10.98-19.02) *vs* 17.74 (13.34-22.53) ng/mL]. However, the effect was neither significant nor consistent (Figure 2). Likewise, there was no significant difference between the groups in TFF-3 changes expressed in either absolute or relative values. Moreover, the magnitude of changes in TFF-3 did not correlate with changes in CD clinical activity (not shown).

Fecal calprotectin

In 10 patients from the study group the endoscopic assessment of the gut was coupled with the measurement of FC before and after therapy. The levels of FC were found to correlate only weakly with SES-CD (R = 0.38) and did not correlate at all with serum TFF-3 (Figure 3). Moreover, changes in TFF-3 levels over the course of therapy were not reflected by corresponding changes in FC (not shown).

DISCUSSION

Anti-TNF-α therapy is used more and more frequently and at earlier stages of CD. There is however an

Table 1 Correlation of Crohn's disease endoscopic activity assessed by Simple Endoscopic Score for Crohn's disease with clinical and biochemical parameters recorded at the same time

	Simple endoscopic score for Crohn's disease *vs* biochemical parameters								
	CDAI	Albumin	WBC	PLT	CRP	Hb	Ferritin	Fibrinogen	ESR
R value	0.66	-0.62	0.3500	0.4400	0.57	-0.40	0.12	0.59	0.57
P value	< 0.0001	< 0.0001	0.0071	0.0005	< 0.0001	0.0018	0.41	< 0.0001	< 0.0001

Endoscopy was performed in 29 patients before and after the induction therapy with anti-TNF-α agents (n = 58). CDAI: Crohn's Disease Activity Index; WBC: White blood count; PLT: Platelets; CRP: C-reactive protein; Hb: Hemoglobin; ESR: Erythrocyte sedimentation rate; SES-CD: Simple Endoscopic Score for Crohn's Disease.

Table 2 Clinical, biochemical and demographic characteristics of Crohn's disease patients with or without successful mucosal healing in response to anti-TNF-α therapy *n* (%)

Feature	All (n = 29)	MH-group (n = 18)	Non-MH group (n = 11)	MH *vs* non-MH
Change in Simple Endoscopic Score for Crohn's disease over time (%)	-55 [-72-(-37)]	-70 [-81-(-56)]	-33 [-38-(-8)]	P < 0.0001
Age (yr)	27 (21-35)	22 (21-30)	35 (27-39)	P = 0.02
Men	21 (72)	15 (83)	5 (45)	P = 0.04
Disease duration (yr)	6 (3-11)	6 (5-10)	6 (3-12)	P = 0.77
Baseline Crohn's disease Activity Index (n)	319 (298-420)	310 (240-397)	348 (301-440)	P = 0.26
Baseline Simple Endoscopic Score for Crohn's disease (n)	15 (8-21)	16 (8-23)	12 (8-20)	P = 0.36
Baseline C-reactive protein (mg/L)	9.8 (2.8-31.2)	8.7 (2.3-18.2)	18.6 (3.7-34.5)	P = 0.15
Baseline hemoglobin (g/dL)	12.9 (10.1-14)	12 (9.9-13.5)	13.1 (10.2-14.8)	P = 0.60
Baseline albumin (mg/dL)	4.2 (3.6-4.4)	4.1 (3.5-4.4)	4.2 (3.7-4.4)	P = 0.84
Disease location				
L1 (ileal)	3/29 (10)	1/18 (5)	2/11 (18)	P = 0.53
L2 (colonic)	9/29 (31)	5/18 (28)	4/11 (36)	P = 0.69
L3 (ileocolonic)	17/29 (59)	12/18 (67)	5/11 (46)	P = 0.43
Disease behavior				
B1 (inflammatory)	24/29 (83)	14/18 (78)	10/11 (91)	P = 0.62
B2 (stricturing)	1/29 (3)	1/18 (5)	0/11 (0)	P = 1.00
B3 (penetrating)	4/29 (14)	3/18 (17)	1/11 (9)	P = 1.00
Medications				
Steroids	19/29 (65)	10/18 (55)	9/11 (82)	P = 0.23
Azathioprine	15/29 (52)	12/18 (67)	3/11 (27)	P = 0.06
Aminosalicylates	28/29 (96)	18/18 (100)	10/11 (91)	P = 0.37
Anti-TNF-α agent used: adalimumab/infliximab	17/12 (59/41)	11/7 (61/39)	6/5 (55/45)	P = 0.51

The data are presented as medians with interquartile ranges. MH: Mucosal healing.

ongoing debate on how to best monitor its efficacy and what should be its main therapeutic goal[12]. While MH is increasingly viewed as the best predictor of short- and long-term prognosis, it is not clear whether endoscopic assessment of MH could be replaced by any biochemical surrogate marker that is more convenient to apply in everyday clinical practice[4,5]. As a factor that plays an important role in maintaining the integrity of intestinal mucosa, TFF-3 could be considered as a potential candidate. TFF-3 is induced by mucosal injury and acts to promote epithelial repair[6]. Although, increased expression of TFF-3 in the inflamed mucosa was shown both in human IBD and in animal studies, it is not certain if this reflects IBD activity[7]. There are only limited data on TFF-3 in UC, and no data at all on its role in CD[6,7,13]. Srivastava *et al*[7] have recently demonstrated that median serum TFF-3 concentration in UC patients without MH was significantly higher than that in patients with MH and healthy controls. Our study was the first ever attempt to assess changes in TFF-3 in patients with CD treated with biological agents.

While our analysis confirmed that there is a need for new markers of CD activity (as significant improvement in several clinical and biochemical parameters occurred also in some patients without appreciable MH), it failed to demonstrate that TFF-3 could serve reliably as such a marker.

Firstly, TFF-3 concentrations did not correspond to the status of the mucosa as assessed by SES-CD. Secondly, the direction and the extent of intestinal changes in response to therapy did not correlate well with changes in serum TFF-3. Although the average levels of TFF-3 seemed to increase over time in patients with unsuccessful MH, such increases in TFF-3 did also occur in some patients with clear endoscopic improvement. Conversely, some patients with unsuccessful MH experienced a decrease in TFF-3. Thirdly, TFF-3 levels did not correlate with the levels of FC. To date, FC has been viewed as the most promising

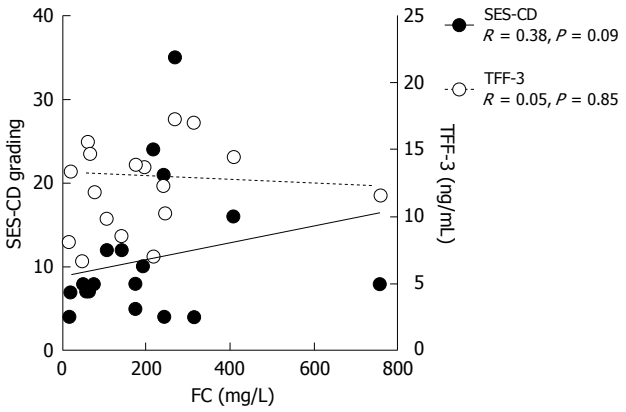


Figure 3 Correlation of fecal calprotectin with Simple Endoscopic Score for Crohn's disease and serum trefoil factor-3 in 10 patients assessed before and after the induction therapy with anti-TNF-α agents (*n* = 20). TFF-3: Trefoil factor-3; SES-CD: Simple Endoscopic Score for Crohn's Disease; FC: Fecal calprotectin.

new biomarker of the mucosal status, despite the fact that the disease location can significantly impact on how well FC correlates with MH[14-16]. Indeed, we have observed only weak-to-moderate correlation between FC with SES-CD in our group of patients. However, we could not detect any significant correlation between FC and TFF-3.

Our study has several limitations including a single centre design and a relatively small sample size. The latter was primarily related to a limited number of patients on whom repeated colonoscopic examination could be performed in a short period of time. Another issue was how to define MH, as different algorithms are in use. The criteria ultimately applied were strictly in accordance with the latest recommendations by the International Organization for the Study of Inflammatory Bowel Disease[5]. It is also a matter of debate when to assess MH in CD for the first time. There are different algorithms, however - taking into account that current guidelines define anti-TNF-α induction regimen as 7 doses of ADA or 3 doses of IFX - in the majority of trials MH was initially estimated between week 10-14 and at week 12-14 in case of IFX or ADA, respectively[8,11,17].

In conclusion, to the best of our knowledge this is the first ever study undertaken to assess the usefulness of serum TFF-3 for MH monitoring in CD patients treated with anti-TNF-α antibodies. While our observations should be validated in independent and larger cohorts of patients, they do not support the hypothesis that serum TFF-3 levels alone could serve as a convenient and reliable surrogate marker of MH during therapy with TNF-α antagonists in CD.

COMMENTS

Background

Mucosal healing (MH) is one of the most important goals of anti-TNF-α therapy for Crohn's disease (CD), because it predicts successful long-term clinical remission and lower hospitalization and surgery rates. It is crucial to find new non-invasive parameters that could reliably reflect MH, since repeated colonoscopies are problematic for both economical and safety reasons.

Research frontiers

MH is hot topic in the field of therapeutic monitoring in CD, especially during anti-TNF-α therapy, since TNF-α inhibitors have the highest potential to induce MH.

Innovations and breakthroughs

Trefoil factor-3 (TFF-3) is a mucin-associated peptide involved in the maintenance of intestinal mucosal integrity. It was hypothesized that TFF-3 can serve as a marker of MH in ulcerative colitis. Here, we have analyzed for the first time whether TFF-3 could be useful for monitoring MH in the course of anti-TNF-α therapy for CD.

Applications

The current study shows that serum TFF-3 is not a reliable surrogate marker of MH in CD patients treated with anti-TNF-α agents.

Terminology

Mucosal healing - reduction or disappearance of erosions and ulcerations in inflammatory bowel disease as a result of treatment. It predicts long-term clinical remission and fewer complications in the course of CD.

Peer-review

The authors present the results of a prospective study of the utility of measuring serum TFF-3 in patients with CD for predicting mucosal healing following induction therapy with anti-TNF agents. The results are clearly presented and will be of interest, if only because they offer a clear message that TFF-3 performs poorly as a biomarker of mucosal healing in CD.

REFERENCES

1 **Antunes O**, Filippi J, Hébuterne X, Peyrin-Biroulet L. Treatment algorithms in Crohn's - up, down or something else? *Best Pract Res Clin Gastroenterol* 2014; **28**: 473-483 [PMID: 24913386 DOI: 10.1016/j.bpg.2014.05.001]

2 **Neurath MF**, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. *Gut* 2012; **61**: 1619-1635 [PMID: 22842618 DOI: 10.1136/gutjnl-2012-302830]

3 **Pineton de Chambrun G**, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 15-29 [PMID: 19949430 DOI: 10.1038/nrgastro.2009.203]

4 **Rogler G**, Vavricka S, Schoepfer A, Lakatos PL. Mucosal healing and deep remission: what does it mean? *World J Gastroenterol* 2013; **19**: 7552-7560 [PMID: 24282345 DOI: 10.3748/wjg.v19.i43.7552]

5 **Vuitton L**, Marteau P, Sandborn WJ, Levesque BG, Feagan B, Vermeire S, Danese S, D'Haens G, Lowenberg M, Khanna R, Fiorino G, Travis S, Mary JY, Peyrin-Biroulet L. IOIBD technical review on endoscopic indices for Crohn's disease clinical trials. *Gut* 2016; **65**: 1447-1455 [PMID: 26353983 DOI: 10.1136/gutjnl-2015-309903]

6 **Aamann L**, Vestergaard EM, Gronbaek H. Trefoil factors in inflammatory bowel disease. *World J Gastroenterol* 2014; **20**: 3223-3230 [PMID: 24696606 DOI: 10.3748/wjg.v20.i12.3223]

7 **Srivastava S**, Kedia S, Kumar S, Pratap Mouli V, Dhingra R, Sachdev V, Tiwari V, Kurrey L, Pradhan R, Ahuja V. Serum human trefoil factor 3 is a biomarker for mucosal healing in ulcerative colitis patients with minimal disease activity. *J Crohns Colitis* 2015; **9**: 575-579 [PMID: 25964429 DOI: 10.1093/ecco-jcc/jjv075]

8 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based

Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]

9 **Levesque BG**, Sandborn WJ, Ruel J, Feagan BG, Sands BE, Colombel JF. Converging goals of treatment of inflammatory bowel disease from clinical trials and practice. *Gastroenterology* 2015; **148**: 37-51.e1 [PMID: 25127678 DOI: 10.1053/j.gastro.2014.08.003]

10 **Walsham NE**, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. *Clin Exp Gastroenterol* 2016; **9**: 21-29 [PMID: 26869808 DOI: 10.2147/CEG.S51902]

11 **Ben-Horin S**, Mao R, Chen M. Optimizing biologic treatment in IBD: objective measures, but when, how and how often? *BMC Gastroenterol* 2015; **15**: 178 [PMID: 26678147 DOI: 10.1186/s12876-015-0408-x]

12 **Danese S**, Vuitton L, Peyrin-Biroulet L. Biologic agents for IBD: practical insights. *Nat Rev Gastroenterol Hepatol* 2015; **12**: 537-545 [PMID: 26284562 DOI: 10.1038/nrgastro.2015.135]

13 **Grønbaek H**, Vestergaard EM, Hey H, Nielsen JN, Nexø E. Serum trefoil factors in patients with inflammatory bowel disease. *Digestion* 2006; **74**: 33-39 [PMID: 17068395 DOI: 10.1159/000096591]

14 **Stawczyk-Eder K**, Eder P, Lykowska-Szuber L, Krela-Kazmierczak I, Klimczak K, Szymczak A, Szachta P, Katulska K, Linke K. Is faecal calprotectin equally useful in all Crohn's disease locations? A prospective, comparative study. *Arch Med Sci* 2015; **11**: 353-361 [PMID: 25995752 DOI: 10.5114/aoms.2014.43672]

15 **Chang S**, Malter L, Hudesman D. Disease monitoring in inflammatory bowel disease. *World J Gastroenterol* 2015; **21**: 11246-11259 [PMID: 26523100 DOI: 10.3748/wjg.v21.i40.11246]

16 **Gecse KB**, Brandse JF, van Wilpe S, Löwenberg M, Ponsioen C, van den Brink G, D'Haens G. Impact of disease location on fecal calprotectin levels in Crohn's disease. *Scand J Gastroenterol* 2015; **50**: 841-847 [PMID: 25636819 DOI: 10.3109/00365521.2015.1008035]

17 **Dave M**, Loftus EV. Mucosal healing in inflammatory bowel disease-a true paradigm of success? *Gastroenterol Hepatol* (N Y) 2012; **8**: 29-38 [PMID: 22347830]

P- Reviewer: Can G, Doherty GA **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.141

World J Gastroenterol 2017 January 7; 23(1): 141-150
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Sitagliptin in patients with non-alcoholic steatohepatitis: A randomized, placebo-controlled trial

Tisha R Joy, Charles A McKenzie, Rommel G Tirona, Kelly Summers, Shannon Seney, Subrata Chakrabarti, Neel Malhotra, Melanie D Beaton

Tisha R Joy, Department of Medicine, Division of Endocrinology, St. Joseph's Hospital, Western University, London, Ontario N6A 4V2, Canada

Charles A McKenzie, Department of Medical Biophysics, Western University, London, Ontario N6A 4V2, Canada

Rommel G Tirona, Department of Physiology and Pharmacology, Western University, London, Ontario N6A 4V2, Canada

Kelly Summers, Shannon Seney, Department of Microbiology and Immunology, Western University, London, Ontario N6A 4V2, Canada

Subrata Chakrabarti, Department of Pathology and Laboratory Medicine, Western University, London, Ontario N6A 4V2, Canada

Neel Malhotra, Melanie D Beaton, Department of Medicine, Division of Gastroenterology, London Health Sciences Centre, Western University, London, Ontario N6A 5A5, Canada

Author contributions: Joy TR and Beaton MD contributed equally to this work and designed the research; Joy TR, Tirona RG, Summers K, Seney S, McKenzie CA and Beaton MD performed the research; Joy TR, McKenzie CA, Tirona RG, Summers K, Seney S, Malhotra N and Beaton MD contributed to the interpretation of the results; Joy TR and Beaton MD wrote the manuscript; and Joy TR, McKenzie CA, Tirona RG, Summers K, Seney S, Malhotra N and Beaton MD provided critical revisions of the manuscript.

Supported by the Physicians' Services Incorporated Foundation 10q2083 (Joy TR and Beaton MD); Academic Medical Organization of Southwestern Ontario, No. F10-002 (Beaton MD); as well as partly funded through academic research funds from the Program of Experimental Medicine (Joy TR); and Department of Medicine Academic Funds (Joy TR) from Western University, London, Ontario, Canada.

Institutional review board statement: This study was reviewed and approved by the Western University Research Ethics Board (REB#17389), London, Ontario, Canada.

Clinical trial registration statement: This study was registered at www.Clinicaltrials.gov. The registration identification number is NCT01260246.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: Joy TR has received speakers' honoraria from Merck, Novo Nordisk, Eli Lilly, Boehringer Ingelheim, Janssen, Astra Zeneca; served as an advisory board member or consultant to Amgen, Sanofi, Novo Nordisk, Merck, and participated in clinical trials with Amgen and Astra Zeneca. Beaton MD has served as an advisory board member or consultant for Abbvie, Allergan and Takeda, and participated in clinical trials with Gilead and Intercept. The remaining authors have no conflict of interest to report.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Correspondence to: Dr. Melanie D Beaton, Associate Professor, Department of Medicine, Division of Gastroenterology, London Health Sciences Centre, Western University, 339 Windermere Road, London, Ontario N6A 5A5, Canada. melanie.beaton@lhsc.on.ca
Telephone: +1-519-6633344
Fax: +1-519-6633220

Received: September 29, 2016
Peer-review started: September 30, 2016
First decision: December 2, 2016

Revised: December 9, 2016
Accepted: December 21, 2016
Article in press: December 21, 2016
Published online: January 7, 2017

Abstract

AIM

To evaluate the effect of sitagliptin *vs* placebo on histologic and non-histologic parameters of non-alcoholic steatohepatitis (NASH).

METHODS

Twelve patients with biopsy-proven NASH were randomized to sitagliptin (100 mg daily) (n = 6) or placebo (n = 6) for 24 wk. The primary outcome was improvement in liver fibrosis after 24 wk. Secondary outcomes included evaluation of changes in NAFLD activity score (NAS), individual components of NAS (hepatocyte ballooning, lobular inflammation, and steatosis), glycemic control and insulin resistance [including measurements of glycated hemoglobin (HbA1C) and adipocytokines], lipid profile including free fatty acids, adipose distribution measured using magnetic resonance imaging (MRI), and thrombosis markers (platelet aggregation and plasminogen activator inhibitor 1 levels). We also sought to determine the correlation between changes in hepatic fat fraction (%) [as measured using the Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation (IDEAL) MRI technique] and changes in hepatic steatosis on liver biopsy.

RESULTS

Sitagliptin was not significantly better than placebo at reducing liver fibrosis score as measured on liver biopsy (mean difference between sitagliptin and placebo arms, 0.40, P = 0.82). There were no significant improvements evident with the use of sitagliptin *vs* placebo for the secondary histologic outcomes of NAS total score as well as for the individual components of NAS. Compared to baseline, those patients who received sitagliptin demonstrated improved HbA1C (6.7% ± 0.4% *vs* 7.9% ± 1.0%, P = 0.02), and trended towards improved adiponectin levels (4.7 ± 3.5 μg/mL *vs* 3.9 ± 2.7 μg/mL, P = 0.06) and triglyceride levels (1.26 ± 0.43 mmol/L *vs* 2.80 ± 1.64 mmol/L, P = 0.08). However, when compared with placebo, sitagliptin did not cause a statistically significant improvement in HbA1C (mean difference, -0.7%, P = 0.19) nor triglyceride levels (mean difference -1.10 mmol/L, P = 0.19) but did trend towards improved adiponectin levels only (mean difference, 0.60 μg/mL, P = 0.095). No significant changes in anthropometrics, liver enzymes, other adipocytokines, lipid profile, thrombosis parameters, or adipose distribution were demonstrated. The MRI IDEAL procedure correlated well with steatosis scores obtained on liver biopsy in both groups at baseline and post-treatment, and the Spearman correlation coefficients ranged from r = 0.819 (baseline) to r = 0.878 (post-treatment), P = 0.002.

CONCLUSION

Sitagliptin does not improve fibrosis score or NAS after 24 wk of therapy. The MRI IDEAL technique may be useful for non-invasive measurement of hepatic steatosis.

Key words: Sitagliptin; Randomized controlled trial; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Fibrosis; Magnetic resonance imaging; Hepatic steatosis; Insulin resistance; Platelet aggregation

Core tip: Presently, there is no approved medical therapy for non-alcoholic steatohepatitis (NASH). In this randomized placebo-controlled trial, the effect of sitagliptin on liver fibrosis in patients with NASH after 24 wk was evaluated. There was no significant improvement with the use of sitagliptin on liver fibrosis, total non-alcoholic fatty liver disease activity score or its individual components. Similarly, there were no significant improvements in liver enzymes, adipocytokines, lipid profile, thrombosis parameters, or adipose distribution. There was a strong correlation between hepatic fat % measured using the MRI IDEAL technique and hepatic steatosis on liver biopsy.

Joy TR, McKenzie CA, Tirona RG, Summers K, Seney S, Chakrabarti S, Malhotra N, Beaton MD. Sitagliptin in patients with non-alcoholic steatohepatitis: A randomized, placebo-controlled trial. *World J Gastroenterol* 2017; 23(1): 141-150 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/141.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.141

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the United States, affecting approximately 95 million adults[1-3]. The spectrum of disease ranges from simple steatosis to steatohepatitis with or without fibrosis (NASH)[4]. The pathogenesis of NASH has been associated with not only insulin resistance, metabolic syndrome, and diabetes but also oxidative stress and lipotoxicity[3]. Approximately 50%-70% of patients with type 2 diabetes (DM2) have hepatic steatosis[5,6]. More importantly, those with DM2 and/or insulin resistance are at a greater risk and have a greater likelihood for progression of NASH[7]. Although lifestyle modification is the mainstay of treatment, achievement and/or maintenance of dietary goals and weight loss is often difficult[3,8].

Currently, there is no approved pharmacologic agent for the management of NASH. Given the

importance of insulin resistance, several anti-diabetic agents have been investigated in NASH but have yielded variable outcomes[9-14]. Sitagliptin is an oral antidiabetic agent that inhibits dipeptidyl peptidase IV (DPP-IV), a naturally occurring enzyme that degrades the incretins - glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 and GIP are hormones secreted from the gastrointestinal system in response to food intake and cause increased insulin secretion and suppressed glucagon secretion, resulting in improved serum glucose levels[15]. There are two available incretin-based anti-diabetic classes - GLP-1 analogues and DPP-IV inhibitors, both of which are being actively investigated for NASH.

DPP-IV activity correlates with hepatic steatosis and NASH grading as well as with markers of liver damage such as gamma glutamyl transferase (GGT) and alanine aminotransferase (ALT) levels[16,17]. In rodents, reduced DPP-IV levels are associated with reduced lipogenesis and decreased hepatic steatosis[18,19]. Thus, DPP-IV itself may be important to the pathogenesis of NASH. Use of the DPP-IV inhibitor, sitagliptin, improves lipid metabolism, and attenuates the progression of hepatic fibrosis in mice with NASH, as well as decreases platelet aggregation *in vitro*[20,21]. Thus, sitagliptin may be an attractive therapeutic option for NASH, especially given its low risk of hypoglycemia, weight-neutrality, and demonstrated safety profile in individuals with moderate hepatic insufficiency[22,23].

In uncontrolled human studies, sitagliptin improved serum ALT, aspartate aminotransferase (AST), and GGT levels in patients with DM2 and NASH[24] as well as significantly decreased hepatocyte ballooning and NASH scores[25]. Recently, a randomized placebo-controlled trial[26] examining 24 wk of sitagliptin therapy in patients with non-alcoholic fatty liver disease demonstrated no significant improvement in liver fat as measured using magnetic resonance imaging (MRI). However, liver biopsies were not performed in that trial.

Thus, the aim of this randomized placebo-controlled trial was to evaluate the efficacy of sitagliptin *vs* placebo in patients with DM2 and biopsy-proven NASH in reducing liver fibrosis histologically using paired liver biopsies. Additionally, we evaluated the effects of sitagliptin on NAFLD activity score (NAS), mediators of insulin resistance (adipocytokines and adipose distribution), lipid profile and thrombosis parameters. We also aimed to examine the use of MRI-derived hepatic fat fraction as a surrogate for histologic assessment of hepatic steatosis.

MATERIALS AND METHODS

Study design and patients

We conducted an investigator-initiated, randomized, double-blinded, allocation-concealed, placebo-controlled clinical trial of 24 wk' duration of sitagliptin 100 mg daily versus placebo in patients with DM2 and biopsy-confirmed NASH. Participants were recruited from the Endocrinology and Gastroenterology outpatient clinics at St. Joseph's Hospital and London Health Sciences Centre, respectively, at Western University. The trial was registered at www.clinicaltrials.gov (registration number: NCT01260246). The clinical trial was approved by the Western University Research Ethics Board (REB No. 17389), and all patients provided written informed consent.

Inclusion criteria included age 18 years or older, established diagnosis of type 2 diabetes on lifestyle management alone or with approved treatment (see exclusions below) with a hemoglobin A1C (HbA1C) of 7.1%-8.9%, and a diagnosis of NASH based on the American Association for the Study of Liver Disease criteria[3] including histological evidence of NASH on the basis of pre-randomization liver biopsy. Patients were excluded for substantial alcohol consumption (> 20 g/d for women or > 30 g/d for men); prior exposure to DPP-IV inhibitor, GLP-1 analogue, or thiazolidinedione; Child's class B or C cirrhosis; any contraindication for MRI; any contraindication for liver biopsy; current or prior use of medications that can induce steatohepatitis; participation in another clinical trial; prior history of pancreatitis; pregnancy, breastfeeding, or intention to become pregnant.

Randomization and masking

The St. Joseph's Hospital clinical trial pharmacy team randomized patients into either sitagliptin or placebo groups 1:1, stratified by gender, using computer-generated numbers. Blinding and allocation concealment was maintained by use of identical-looking bottles and capsules, in which sitagliptin or placebo were compounded by the hospital pharmacy. Physicians and all other study personnel were also blinded to drug allocation. Unblinding of treatment allocation was done only after all study procedures were completed in all study patients.

Study visits and procedures

After screening, patients underwent a baseline assessment (Visit 1) including medical history; physical examination; documentation of anthropometric measures [weight, waist-to-hip ratio, and body mass index (BMI)]; blood work for glycemic control (HbA1C), markers of insulin resistance [homeostasis model of assessment for insulin resistance (HOMA-IR)] (which used fasting glucose and insulin levels) and adipocytokines [adiponectin, adipsin, leptin, resistin, visfatin, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), free fatty acids (FFA), lipid profile [total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), liver parameters [AST, ALT, alkaline phosphatase (Alk Phos), GGT] markers of thrombosis [platelet aggregation and plasminogen activator

inhibitor 1 (PAI-1)]; and MRI assessment of adipose distribution[% fat in the liver, visceral adipose tissue (VAT), subcutaneous abdominal adipose tissue (SAAT), and subcutaneous peripheral adipose tissue (SPAT) of the left thigh].

After randomization, patients returned for study visits at weeks 12 (Visit 2) and 24 (Visit 3). At visit 2, adverse effects were noted, and compliance was assessed using pill count. Anthropometric measures and blood work for HbA1C, fasting glucose and insulin, FFA, lipid profile, and liver parameters were measured. Visit 3 study procedures included documentation of adverse effects and compliance as well as all study procedures as visit 1, including a repeat liver biopsy.

Histologic evaluation: Ultrasonography-guided percutaneous liver biopsies were obtained from all subjects prior to initiation of therapy and at completion of the study. All biopsy specimens were placed in formalin solution for fixation and embedded in paraffin blocks. An independent liver histopathologist (SC) who was blinded to study treatment allocation and clinical or laboratory information assessed baseline and end-of-treatment liver biopsies. The grade and stage of liver disease severity was assessed according to the scoring system proposed by the NASH Clinical Research Network[27]. This was recorded as the sum of the scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2). Fibrosis (0-4) was scored separately. For the purposes of analysis, fibrosis stages 1a, 1b, and 1c were considered as stage 1. A diagnosis of NASH required the presence of steatohepatitis (NAS $\geqslant$ 3) with a hepatocyte ballooning score of $\geqslant$ 1 and fibrosis score of $\geqslant$ 1.

MRI: MRI examinations were performed using 3 Tesla Discovery MR750 MRI (General Electric). Quantification of adiposity in the hepatic, VAT, SAAT, and thigh SPAT depots used the Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation (IDEAL) procedure[28], a computer-based quantification method separating fat and water signals in MRI images. Excellent correlation (r^2 = 0.99, slope = 1.00 ± 0.01) for hepatic fat quantification using IDEAL *vs* the gold standard method, magnetic resonance spectroscopy (MRS) has been previously established[29-32]. Regions of interest (ROIs) were placed in vessel free regions of the lower right lobe of the liver to obtain water-only and fat-only images. Then, hepatic fat-fraction (HFF) was obtained [HFF (%) = fat/(water + fat) × 100] and mean fat fraction calculated[32].

Left thigh SPAT (%) was quantified using ImageJ version 1.34 n image analysis software, specifically utilizing the Connected Threshold Grower and Voxel Counter tools. Percent adipose tissue was calculated by dividing the total voxels determined for fat intensity signals connected to the subcutaneous adipose seed point by the total voxels for the slice. Similarly, single slices (1 cm) at the L4 region were obtained to quantify VAT and SAAT using these techniques, as previously published by our group[33,34].

Biochemical parameters: Blood work for fasting glucose, insulin, HbA1C, FFA, AST, ALT, Alk Phos, GGT, and lipid profile were collected and analyzed per standard hospital procedures within our hospital core laboratory. The analytes (adiponectin, adipsin, resistin, leptin, visfatin IL-6, TNF-α, PAI-1) were measured from venous blood samples collected in BD™ P800 EDTA tubes pre-coated with general protease inhibitors (to allow for accurate measurement). Samples were centrifuged and stored at -80 ℃ until thawing for grouped analysis at the completion of the study. They were analyzed using the Human Diabetes Bio-Plex Panel and a Bio-Plex™ 200 readout System (Bio-Rad Laboratories, CA, United States), which utilizes Luminex® xMAP™ multiplexed immunoassay technology (Luminex Corp., TX, United States). Levels of analytes were automatically calculated from standard curves using Bio-Plex Manager software (v.4.1.1, Bio-Rad). VerifyNow-P2Y12 (Accumetrics, CA, United States) is a rapid platelet-function cartridge-based assay that was used to directly measure platelet aggregation[35]. The data are expressed as platelet reaction units (PRU) (ref. range 194-418).

Outcomes

The primary outcome was improvement in liver fibrosis on histology from baseline to end of treatment. Secondary histological outcomes included changes in overall NAS and individual components of NAS (steatosis, hepatocyte ballooning, and lobular inflammation)[27]. Other secondary outcome measures included changes from baseline to 24 wk in serum liver enzyme concentrations, fasting lipid and FFA concentrations, measures of insulin resistance (adipocytokines and HOMA-IR), glycemic control (HbA1C), thrombosis (platelet aggregation and PAI-1 levels), and adipose distribution.

Statistical analysis

Statistical review of the study was completed by a biomedical statistician (LS). The sample size was calculated based on a mean difference in fibrosis score[27] between pre and post conditions of -0.55, or a decrease of slightly more than one half score on 4 point scale (SD of difference = 0.68). Using the methods of Cohen[36], a sample size of 16 patients would provide 80% power to detect at least this difference with alpha (2-tailed) = 5%. We anticipated a 25% drop out rate, and thus our recruitment target was increased to 20 individuals.

All evaluable patients who underwent an end-of-treatment biopsy at week 24 were included in the modified intent-to-treat analysis. All data are expressed as mean ± SD (standard deviation) for

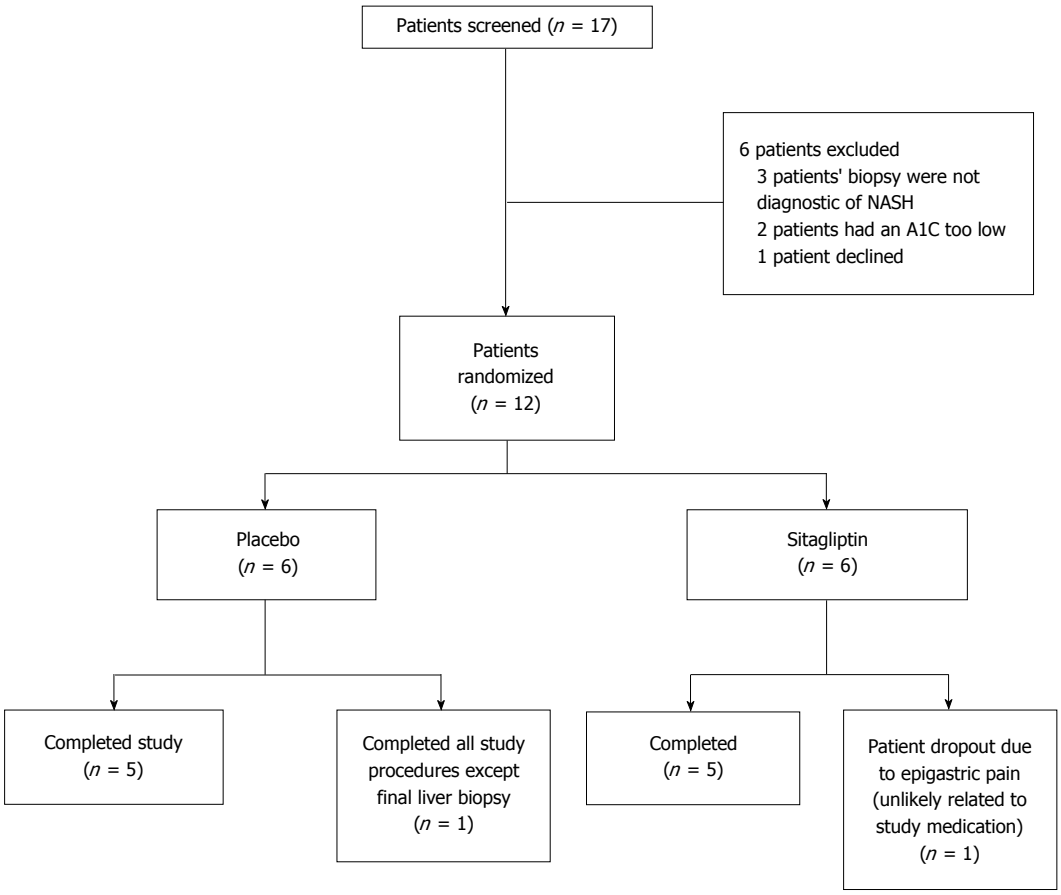


Figure 1 Study subjects' flow diagram. HbA1C: Glycated hemoglobin; NASH: Non-alcoholic steatohepatitis.

normally distributed data and median (interquartile range) for non-normally distributed data. Comparisons between sitagliptin and placebo were made using unpaired *t*-tests or Wilcoxon two sample tests for continuous variables and χ^2 tests or, where expected counts were less than five, Fisher's exact test for categorical variables. The association between liver steatosis and MRI assessment for hepatic fat was evaluated using Spearman rank correlations. A two-tailed *P* value $\leqslant$ 0.05 was considered statistically significant. Statistical analysis was performed using SAS 9.4 (Cary NC, United States).

RESULTS

Between September 2011 and August 2014, we randomly assigned 12 patients with histologically confirmed NASH to receive sitagliptin (*n* = 6) or placebo (*n* = 6) (Figure 1). One patient from the sitagliptin arm withdrew from the study due to epigastric pain (see below), and one patient from the placebo arm completed all end of study procedures except the liver biopsy. In the participants who completed the trial, compliance was above 95% in both arms. Participants were predominantly female (66% *vs* 50%, sitagliptin *vs* placebo, *P* = 1.00), with similar durations of diabetes and NASH, and similar fibrosis score and NAS (Table 1).

Sitagliptin was not significantly better than placebo at reducing liver fibrosis score as measured on liver biopsy (mean difference between sitagliptin and placebo arms, 0.40, *P* = 0.82) (Table 2). Individual patient data on changes in liver fibrosis stratified by treatment group are shown in Figure 2. There were no significant improvements evident with the use of sitagliptin *vs* placebo for the secondary histologic outcomes of NAS total score as well as for the individual components of NAS (Table 2).

Compared to baseline, those patients who received sitagliptin demonstrated improved glycemic control (HbA1C) (6.7% ± 0.4% *vs* 7.9% ± 1.0%, *P* = 0.02), and trended towards improved adiponectin levels (4.7 ± 3.5 μg/mL *vs* 3.9 ± 2.7 μg/mL, *P* = 0.06) and triglyceride levels (1.26 ± 0.43 mmol/L *vs* 2.80 ± 1.64 mmol/L, *P* = 0.08) (Table 3). However, when compared with placebo, sitagliptin did not cause a statistically significant improvement in HbA1C (mean difference, -0.7%, *P* = 0.19) nor triglyceride levels (mean difference -1.10 mmol/L, *P* = 0.19) but did trend towards improved adiponectin levels (mean difference, 0.60 μg/mL, *P* = 0.095). No significant changes in anthropometrics, liver enzymes, adipocytokines, lipid parameters, thrombosis, or adipose distribution were demonstrated (Table 3).

Table 1 Baseline characteristics of subjects

	Placebo (n = 6)	Sitagliptin (n = 6)
Demographics		
Age (yr)	54.7 ± 9.8	56.7 ± 9.9
Gender - male, n (%)	2 (33)	3 (50)
White, n (%	6 (100	5 (83)
Weight (kg)	105.8 ± 23.5	100.4 ± 28.7
BMI (kg/m^2)	37.4 ± 4.7	35.9 ± 6.6
Diabetes duration (yr)[1]	6.0 (5.0, 6.0)	6.5 (5.0, 23.0)
NASH Duration (yr)[1]	4.0 (1.0, 4.0)	1.3 (0.1, 2.0)
Biochemical profile		
HbA1C (%)	8.2 ± 0.9	7.9 ± 1.0
HOMA-IR	3.5 ± 2.2	3.4 ± 1.2
Fasting glucose (mmol/L)	11.3 ± 4.9	8.1 ± 3.1
Fasting insulin (pmol/L)	165 ± 134	168 ± 63
AST (IU/L)	39 ± 19	44 ± 22
ALT (IU/L)	46 ± 36	72 ± 50
Alkaline phosphatase (IU/L)	85 ± 34	93 ± 41
GGT (IU/L)	100 ± 98	164 ± 182
Triglycerides (mmol/L)	2.33 ± 2.00	2.80 ± 1.64
HDL-C (mmol/L)	1.10 ± 0.34	1.12 ± 0.46
LDL-C (mmol/L)	1.61 ± 0.71	1.42 ± 0.97
Free fatty acids (μmol/L)	645 ± 195	559 ± 158
Platelet aggregation (PRU)	292 ± 37	236 ± 28
Histologic profile		
Fibrosis	2.2 ± 0.8	2.2 ± 1.0
NAS	4.2 ± 1.5	3.8 ± 0.8
Steatosis	1.8 ± 0.8	1.8 ± 0.8
Lobular Inflammation	1.2 ± 0.4	1.0 ± 0
Ballooning	1.2 ± 0.4	1.0 ± 0
MRI		
Hepatic fat (%)	21.9 ± 10.6	19.0 ± 9.7
SAAT (%)	40.5 ± 7.1	38.0 ± 22.0
VAT (%)	30.5 ± 3.4	26.8 ± 11.6
Left thigh fat (%)	37.8 ± 9.9	39.5 ± 15.8

[1]Data are presented as median (Q1, Q3). Data are presented as mean ± SD, unless otherwise specified. ALT: Alanine aminotransferase; AST: Aspartate transferase; BMI: Body mass index; HbA1C: Hemoglobin A1C; HOMA-IR: Homeostasis model of assessment for insulin resistance; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; NAS: Non-alcoholic fatty liver disease activity score; NASH: Non-alcoholic steatohepatitis; MRI: Magnetic resonance imaging; PRU: Platelet reaction units; SAAT: Subcutaneous abdominal adipose tissue; VAT: Visceral adipose tissue.

The MRI IDEAL technique correlated well with steatosis scores obtained on liver biopsy in both groups at baseline and post-treatment, and the Spearman correlation coefficients ranged from r = 0.819 (baseline) to r = 0.878 (post-treatment), P = 0.002.

Treatment with sitagliptin was well tolerated. A total of 3 patients had adverse events. Two weeks after randomization, one patient developed a serious adverse event of a right subdural hemorrhage, manifest as left leg weakness and numbness, most likely caused by treatment with his blood thinner (warfarin) for atrial fibrillation. He elected to continue in the trial with blinded medication and was deemed to have had an event unrelated to study medication. A second patient had a non-serious adverse event of low back pain and right foot pain, diagnosed as lumbar spondylolysis, which resolved within 48 h of oral analgesic use. The adverse event was deemed unrelated to study medication and did not result in discontinuation of study medication as well. The third patient developed epigastric pain approximately 1 month after starting sitagliptin. She was hospitalized for her symptoms; pancreatitis was ruled out. The final discharge diagnosis was possible gastritis. She had a history of epigastric pain occurring 1 to 3 times per year for the 4 years prior to her study entry. She withdrew from the trial at the time of her hospitalization, and had another bout of epigastric discomfort 1 mo following her hospitalization. Although she was classified as having a serious adverse event, it was deemed by the investigators that it was unlikely related to the study medication.

DISCUSSION

In this randomized double-blinded placebo-controlled trial, sitagliptin did not significantly improve liver fibrosis or any parameter of NAS after 24 wk of therapy. Those receiving sitagliptin trended towards having improved adiponectin levels, but no other improvements in parameters of insulin resistance, adipose distribution, thrombosis, liver enzymes, or lipid profile. This trial however did show a strong correlation between hepatic steatosis on liver biopsy and hepatic fat fraction measured using MRI with the IDEAL technique, thus providing further data regarding the validity of this non-invasive technique in assessing and monitoring changes in hepatic steatosis in patients with NASH.

This study has a number of strengths. This is the first randomized, placebo-controlled trial to report the effect of a DPP-IV inhibitor on liver histology in patients with NASH. We utilized a single, expert histopathologist who remained blinded to study treatment as well as laboratory data throughout the study. Thus, he was able to provide unbiased determinations of histologic features. Second, all patients included in this trial had histologically-proven NASH, were extensively phenotyped and well-matched for baseline features. Third, to the best of our knowledge this is the first trial to document the validity of the IDEAL technique for MRI in relation to paired liver biopsies assessing histologic changes in hepatic steatosis. And finally, the trial was conducted by experienced investigators from multidisciplinary backgrounds which allowed for us to examine the effect of sitagliptin on a number of important outcomes related to the pathogenesis of NASH (insulin resistance, lipid parameters) in a rigidly executed placebo-controlled trial.

In rodent models, sitagliptin use has resulted in improved features of NASH[20,37,38]. In humans, randomized controlled trials examining the effect of sitagliptin on liver histology has not previously been examined. Several studies have instead examined changes in liver enzymes as a marker of NASH but

Table 2 Changes in primary and secondary histologic outcomes after 6 mo of treatment with sitagliptin *vs* placebo

	Placebo (n = 6)			Sitagliptin (n = 6)			Difference	
	Baseline	Post-treatment[1]	*P* value	Baseline	Post-treatment[1]	*P* value	(95%CI)	*P* value
Primary outcome								
Fibrosis	2.2 ± 0.8	2.0 ± 1.0	0.85	2.2 ± 1.0	2.4 ± 1.1	0.85	0.4 (-0.98, 1.78)	0.82
Secondary outcomes								
NAS	4.2 ± 1.5	3.8 ± 1.9	0.50	3.8 ± 0.8	3.4 ± 1.5	0.85	0.2 (-1.62, 2.02)	1.00
Steatosis	1.8 ± 0.8	1.6 ± 0.9	0.62	1.8 ± 0.8	1.4 ± 0.9	0.55	0 (-1.08, 1.08)	0.91
Hepatocyte ballooning	1.2 ± 0.4	1.4 ± 0.9	0.89	1.0 ± 0	0.8 ± 0.4	0.36	-0.40 (-1.05, 0.25)	0.23
Lobular inflammation	1.2 ± 0.4	0.8 ± 0.4	0.22	1.0 ± 0	1.2 ± 0.4	0.36	0.60 (-0.13, 1.33)	0.12

[1]Data only available for 5 subjects. Data are presented as mean ± SD. NAS: Non-alcoholic fatty liver disease activity score.

Table 3 Changes in anthropometric, biochemical, and MRI parameters after 6 mo of treatment with sitagliptin *vs* placebo

	Placebo (n = 6)		Sitagliptin (n = 6)		Difference
	Baseline	Post-treatment	Baseline	Post-treatment[1]	(95%CI)
Anthropometric and biochemical parameters					
Weight (kg)	105.8 ± 23.5	104.7 ± 23.7	100.4 ± 28.7	101.1 ± 32.4	0.8 (-6.3, 8.0)
BMI (kg/m^2)	37.4 ± 4.7	36.8 ± 4.6	35.9 ± 6.6	35.8 ± 8.0	0.6 (-2.8, 4.0)
HbA1C (%)	8.2 ± 0.9	8.0 ± 1.7	7.9 ± 1.0	6.7 ± 0.4[a]	-0.7 (-1.7, 0.4)
HOMA-IR	3.5 ± 2.2	2.73 ± 1.631	3.4 ± 1.2	3.5 ± 2.9	-0.36 (-3.3,2.6)
Triglycerides (mmol/L)	2.33 ± 2.00	2.29 ± 1.40	2.80 ± 1.64	1.26 ± 0.43[b]	-1.10 (-2.88, 0.66)
HDL-C (mmol/L)	1.10 ± 0.34	1.11 ± 0.29	1.12 ± 0.46	1.12 ± 0.40	-0.05 (-0.32, 0.22)
LDL-C (mmol/L)	1.61 ± 0.71	1.42 ± 0.34	1.42 ± 0.97	1.54 ± 0.51	0.30 (-0.17, 0.77)
Free fatty acids (μmol/L)	645 ± 195	914 ± 411	559 ± 158	488 ± 270	-143 (-788, 503)
Platelet aggregation (PRU)	292 ± 37	266 ± 63	236 ± 28	251 ± 74	-11 (-92, 70)
AST (IU/L)	39 ± 19	42 ± 23	44 ± 22	35 ± 9	-5 (-36, 26)
ALT (IU/L)	46 ± 36	48 ± 28	72 ± 50	51 ± 15	-5 (-51, 41)
Alk phos (IU/L)	85 ± 34	97 ± 43	93 ± 41	76 ± 28	-14 (-36, 7)
GGT (IU/L)	100 ± 98	153 ± 176	164 ± 182	90 ± 76	-62 (-164, 39)
Adiponectin (μg/mL)	2.01 ± 1.30	2.09 ± 1.14	3.93 ± 2.65	4.70 ± 3.46[b]	0.60 (-0.13, 1.32)[c]
Adipsin (μg/mL)	0.62 ± 0.26	0.61 ± 0.26	0.66 ± 0.14	0.62 ± 0.07	-0.07 (-0.26, 0.13)
Visfatin (ng/mL)	3.21 ± 3.64	3.94 ± 3.59	1.82 ± 2.33	1.70 ± 1.08	-0.58 (-3.26, 2.09)
Leptin (ng/mL)	14.62 ± 12.52	17.64 ± 16.20	9.61 ± 3.29	8.87 ± 5.54	-3.5 (-8.6, 1.6)
Resistin (ng/mL)	3.66 ± 1.01	4.31 ± 1.66	7.19 ± 8.66	4.55 ± 1.73	-4.05 (-11.33, 2.34)
TNF-α (pg/mL)	3.69 ± 1.22	4.95 ± 4.79	2.85 ± 0.66	5.14 ± 3.20	0.95 (-4.70, 6.61)
IL-6 (pg/mL)	5.89 ± 1.37	6.19 ± 2.37	7.15 ± 6.22	6.40 ± 3.41	-1.44 (-7.76, 4.87)
MRI parameters					
Hepatic fat (%)	21.9 ± 10.6	19.1 ± 9.6	19.0 ± 9.7	16.1 ± 12.9	2.0 (-7.3, 11.2)
SAAT (%)	40.5 ± 7.1	39.8 ± 7.7	38.0 ± 22.0	34.1 ± 20.1	0.7 (-2.3, 3.7)
VAT (%)	30.5 ± 3.4	30.3 ± 4.4	26.8 ± 11.6	27.6 ± 13.4	0 (-6.4, 6.4)

[1]One subject was excluded from the post-treatment, placebo group homeostasis model of assessment for insulin resistance (HOMA-IR) calculation due to blood glucose level above the acceptable steady-state glucose value for calculation of HOMA-IR. [a]$P < 0.05$ *vs* baseline; [b]$P < 0.10$ *vs* baseline; [c]$P < 0.10$ *vs* control.

effects have not been consistent[24,26,39,40] Recently, a randomized double-blinded placebo-controlled trial of 50 patients by Cui *et al*[26] demonstrated no improvement in hepatic steatosis using the MRI-based biomarker of proton density-fat fraction (MRI-PDFF), following 24 wk of sitagliptin therapy. Our results further extend these findings by demonstrating no improvement in the histologic features of NASH with sitagliptin in patients with histologically-proven NASH. These results are in contrast with those of Yilmaz *et al*[25], where sitagliptin demonstrated improved NAS and hepatocyte ballooning with a trend towards improved hepatic steatosis, after 1 year of therapy in 15 patients. However, the latter trial was open-label without a comparator arm.

Our data are similar to a larger randomized controlled trial examining the effects of another incretin-based antidiabetic agent, the GLP-1 analogue liraglutide. Armstrong *et al*[14] randomized 52 patients to liraglutide or placebo for 48 wk to determine the effects on liver histology, liver enzymes, FFA, lipid parameters. Their primary endpoint was improvement in liver histology (defined as disappearance of hepatocyte ballooning without worsening of fibrosis) and their secondary histologic endpoints were changes in NAS and fibrosis score. Although they demonstrated improvement in the primary endpoint, they did not demonstrate any improvements in NAS, fibrosis score, HOMA-IR, FFA, or liver enzymes (apart from GGT). Thus, improvement in liver histology or liver enzymes may be difficult to achieve with incretin-based agents, although it is possible that a longer treatment period

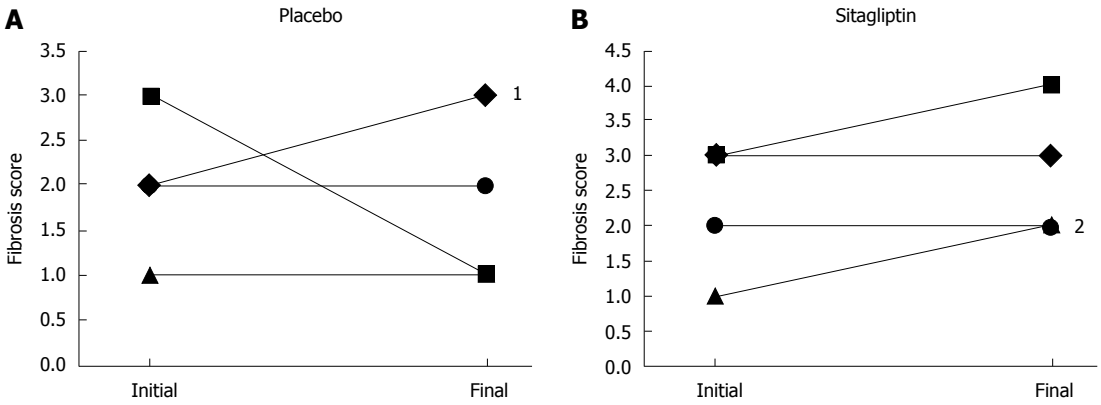


Figure 2 Changes in fibrosis scores in paired liver biopsies (*n* = 5 per arm) before and after 24 wk of treatment with placebo (A) or sitagliptin (B). [1]In the placebo arm, 2 patients' fibrosis scores increased from 2 to 3; [2]In the sitagliptin arm, 2 patients' fibrosis scores increased from 1 to 2.

beyond 48 wk may be required to document an effect.

While the HbA1C mean difference between sitagliptin *vs* placebo of -0.7% did not reach statistical significance, the result is consistent with the expected drop in HbA1C following the addition of sitagliptin to patients with DM2 having a mean baseline HbA1C of 8.0%[41]. This demonstrates that sitagliptin did indeed improve glycemic control to the expected degree from published data. Furthermore, this effect was accompanied by a trend towards improved adiponectin levels, an adipocytokine associated with insulin sensitivity, suggesting that improvement in insulin resistance is still possible with sitagliptin even in patients with NASH. Since high levels of VAT and low levels of SPAT correlate with insulin resistance, we examined adipose distribution as one of our secondary outcomes. Our results demonstrating no improvement in VAT or SPAT with sitagliptin are in contrast to those by Lima-Martínez *et al*[42], who demonstrated decreases in VAT of 12% after 24 wk of sitagliptin therapy. However, the latter trial was open-label without a comparator arm, and utilized a less precise technique (bioimpedance analyzer) compared to MRI to document VAT.

The hepatic fat % did not improve significantly with the use of sitagliptin *vs* placebo, as evident also in the trial by Cui *et al*[26]. However, the MRI IDEAL technique did have high correlation with hepatic steatosis on histology. This technique, while having been previously validated with MRS[29-32], is certainly much more feasible than MRS. This trial therefore supports that hepatic fat fraction measurements through the IDEAL technique may be an accurate non-invasive method to longitudinally monitor hepatic steatosis changes in patients with NASH.

Our study has some important limitations. Firstly, our sample size was small. This may have affected our ability to assess for changes in the outcomes measured as well as the validity of MRI IDEAL technique. For the primary histologic outcome, the mean potentially detectable difference in fibrosis score with our attained sample size was 0.8. However, given that our negative findings were supported by the trials by Cui *et al*[26] and Armstrong *et al*[14], it is unlikely that a true histologic improvement with 24 wk of sitagliptin therapy has been missed. Certainly, a larger sample size and longer duration of therapy would be helpful to address this limitation. Secondly, this study enrolled patients with milder NASH compared to other studies. Our baseline NAS in the sitagliptin arm was 3.8 ± 0.8, slightly lower than the mean scores in the studies by Armstrong *et al*[14] and the observational study by Yilmaz *et al*[25], where the mean baseline scores were 4.9 ± 0.9 and 5.6 ± 1.6, respectively. Thus, whether sitagliptin would have impacted histologic and non-histologic outcomes differently in patients with more advanced histologic NASH severity remains uncertain. Although our results lend support to the use of MRI as a non-invasive technique in patients with NASH, additional multicenter trials are required to assess the utility of the IDEAL technique for measuring longitudinal changes in hepatic steatosis. Regardless, the current data allow our centre to use the IDEAL technique for future research in NASH patients to monitor hepatic steatosis. With the emergence of additional novel MR technologies, such as MR Elastography to assess fibrosis, it is likely that in coming years non-invasive means of assessing disease severity and response to therapy may supplant liver biopsy in this condition.

Our trial therefore demonstrates that sitagliptin 100 mg daily for 24 wk, compared to placebo, does not improve histologic features of NASH significantly. Sitagliptin was well-tolerated in patients with NASH and demonstrated the expected improved in glycemic control, as measured through HbA1C. Importantly, we did demonstrate the feasibility and validity of the MRI IDEAL technique for non-invasive measurement of hepatic steatosis longitudinally. Future studies of longer duration, larger sample size and in patients with worse severity of NASH may demonstrate different results and should be considered.

ACKNOWLEDGMENTS

We would like to sincerely thank Leanne Vanderhaeghe and Christopher Reynaert of the Pharmacy Department at St. Joseph's Hospital for their help with the conduct of this trial, particularly with regards to generation of randomization schemes, purchase and compounding of active and placebo capsules, and dispensing of study medications. Similarly, the invaluable efforts of our research assistant, Patricia Rosas-Arellanos, as well as participation of the patients are greatly appreciated.

COMMENTS

Background

This study was undertaken to evaluate the efficacy of an established and effective antidiabetic medication, sitagliptin, on features of nonalcoholic fatty liver disease (NAFLD). As NAFLD and type 2 diabetes share many pathophysiologic mechanisms, including insulin resistance, it was theorized that Sitagliptin may also improve liver disease in NAFLD.

Research frontiers

There are presently no approved medical therapies for NAFLD. As such the identification of potential drug therapy for this condition is very important; given it is a very common disorder with the potential to cause significant liver injury.

Innovations and breakthroughs

In this study sitagliptin did not improve liver fibrosis in a small group of NAFLD patients treated for 24 wk. There was a trend toward improvement in adiponectin level, an important hormone in the regulation of glucose regulation and fatty acid oxidation.

Applications

Sitagliptin was safe and well tolerated in this population with known liver disease. It is possible that further studies, of larger size and longer duration, may be needed to accurately assess whether a treatment effect may be evident.

Terminology

NAFLD the presence of hepatic steatosis in the absence of other causes for secondary fat accumulation; Non-alcoholic steatohepatitis - the presence of hepatic steatosis in addition to inflammation and hepatocyte injury with or without fibrosis.

Peer-review

As the authors pointed out sitagliptin did not significantly improve liver fibrosis or any parameter of NAFLD activity score after 24 wk of therapy, and this result is in agreement with previous literature. The major concern, however, from the clinical applicability's point of view is the size of the sample, which was too small and could have limited the ability to assess for changes in the outcomes measured and broaden the information in relation to identify appropriate surrogates for histologic assessment of hepatic steatosis.

REFERENCES

1 **Federico A**, Zulli C, de Sio I, Del Prete A, Dallio M, Masarone M, Loguercio C. Focus on emerging drugs for the treatment of patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 16841-16857 [PMID: 25492998 DOI: 10.3748/wjg.v20.i45.16841]

2 **Loomba R**, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 686-690 [PMID: 24042449 DOI: 10.1038/nrgastro.2013.171]

3 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

4 **Kunde SS**, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. *Hepatology* 2005; **42**: 650-656 [PMID: 16037946 DOI: 10.1002/hep.20818]

5 **Creutzfeldt W**, Frerichs H, Sickinger K. Liver diseases and diabetes mellitus. *Prog Liver Dis* 1970; **3**: 371-407 [PMID: 4908707]

6 **Leite NC**, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver Int* 2009; **29**: 113-119 [PMID: 18384521 DOI: 10.1111/j.1478-3231.2008.01718.x]

7 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

8 **Musso G**, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 2010; **52**: 79-104 [PMID: 20578268 DOI: 10.1002/hep.23623]

9 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

10 **Loomba R**, Lutchman G, Kleiner DE, Ricks M, Feld JJ, Borg BB, Modi A, Nagabhyru P, Sumner AE, Liang TJ, Hoofnagle JH. Clinical trial: pilot study of metformin for the treatment of non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2009; **29**: 172-182 [PMID: 18945255 DOI: 10.1111/j.1365-2036.2008.03869.x]

11 **Ratziu V**, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, Podevin P, Lacorte JM, Bernhardt C, Bruckert E, Grimaldi A, Poynard T. Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. *Gastroenterology* 2008; **135**: 100-110 [PMID: 18503774 DOI: 10.1053/j.gastro.2008.03.078]

12 **Ratziu V**, Charlotte F, Bernhardt C, Giral P, Halbron M, Lenaour G, Hartmann-Heurtier A, Bruckert E, Poynard T. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. *Hepatology* 2010; **51**: 445-453 [PMID: 19877169 DOI: 10.1002/hep.23270]

13 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

14 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]

15 **Campbell JE**, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab* 2013; **17**: 819-837 [PMID: 23684623 DOI: 10.1016/j.cmet.2013.04.008]

16 **Balaban YH**, Korkusuz P, Simsek H, Gokcan H, Gedikoglu G, Pinar A, Hascelik G, Asan E, Hamaloglu E, Tatar G. Dipeptidyl peptidase IV (DDP IV) in NASH patients. *Ann Hepatol* 2007; **6**: 242-250 [PMID: 18007554]

17 **Firneisz G**, Varga T, Lengyel G, Fehér J, Ghyczy D, Wichmann

B, Selmeci L, Tulassay Z, Rácz K, Somogyi A. Serum dipeptidyl peptidase-4 activity in insulin resistant patients with non-alcoholic fatty liver disease: a novel liver disease biomarker. *PLoS One* 2010; **5**: e12226 [PMID: 20805868 DOI: 10.1371/journal.pone.0012226]

18 **Ben-Shlomo S**, Zvibel I, Shnell M, Shlomai A, Chepurko E, Halpern Z, Barzilai N, Oren R, Fishman S. Glucagon-like peptide-1 reduces hepatic lipogenesis via activation of AMP-activated protein kinase. *J Hepatol* 2011; **54**: 1214-1223 [PMID: 21145820 DOI: 10.1016/j.jhep.2010.09.032]

19 **Conarello SL**, Li Z, Ronan J, Roy RS, Zhu L, Jiang G, Liu F, Woods J, Zycband E, Moller DE, Thornberry NA, Zhang BB. Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc Natl Acad Sci USA* 2003; **100**: 6825-6830 [PMID: 12748388 DOI: 10.1073/pnas.0631828100]

20 **Onoyama T**, Koda M, Okamoto T, Kishina M, Matono T, Sugihara T, Murawaki Y. Therapeutic effects of the dipeptidyl peptidase-IV inhibitor, sitagliptin, on non-alcoholic steatohepatitis in FLS-ob/ob male mice. *Mol Med Rep* 2015; **12**: 6895-6902 [PMID: 26397061 DOI: 10.3892/mmr.2015.4329]

21 **Gupta AK**, Verma AK, Kailashiya J, Singh SK, Kumar N. Sitagliptin: anti-platelet effect in diabetes and healthy volunteers. *Platelets* 2012; **23**: 565-570 [PMID: 22950787 DOI: 10.3109/09537104.2012.721907]

22 **Williams-Herman D**, Round E, Swern AS, Musser B, Davies MJ, Stein PP, Kaufman KD, Amatruda JM. Safety and tolerability of sitagliptin in patients with type 2 diabetes: a pooled analysis. *BMC Endocr Disord* 2008; **8**: 14 [PMID: 18954434 DOI: 10.1186/1472-6823-8-14]

23 **Migoya EM**, Stevens CH, Bergman AJ, Luo WL, Lasseter KC, Dilzer SC, Davies MJ, Wagner JA, Herman GA. Effect of moderate hepatic insufficiency on the pharmacokinetics of sitagliptin. *Can J Clin Pharmacol* 2009; **16**: e165-e170 [PMID: 19221403]

24 **Iwasaki T**, Yoneda M, Inamori M, Shirakawa J, Higurashi T, Maeda S, Terauchi Y, Nakajima A. Sitagliptin as a novel treatment agent for non-alcoholic Fatty liver disease patients with type 2 diabetes mellitus. *Hepatogastroenterology* 2011; **58**: 2103-2105 [PMID: 22024083 DOI: 10.5754/hge11263]

25 **Yilmaz Y**, Yonal O, Deyneli O, Celikel CA, Kalayci C, Duman DG. Effects of sitagliptin in diabetic patients with nonalcoholic steatohepatitis. *Acta Gastroenterol Belg* 2012; **75**: 240-244 [PMID: 22870790]

26 **Cui J**, Philo L, Nguyen P, Hofflich H, Hernandez C, Bettencourt R, Richards L, Salotti J, Bhatt A, Hooker J, Haufe W, Hooker C, Brenner DA, Sirlin CB, Loomba R. Sitagliptin vs. placebo for non-alcoholic fatty liver disease: A randomized controlled trial. *J Hepatol* 2016; **65**: 369-376 [PMID: 27151177 DOI: 10.1016/j.jhep.2016.04.021]

27 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

28 **Reeder SB**, Pineda AR, Wen Z, Shimakawa A, Yu H, Brittain JH, Gold GE, Beaulieu CH, Pelc NJ. Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL): application with fast spin-echo imaging. *Magn Reson Med* 2005; **54**: 636-644 [PMID: 16092103 DOI: 10.1002/mrm.20624]

29 **Reeder SB**, Robson PM, Yu H, Shimakawa A, Hines CD, McKenzie CA, Brittain JH. Quantification of hepatic steatosis with MRI: the effects of accurate fat spectral modeling. *J Magn Reson Imaging* 2009; **29**: 1332-1339 [PMID: 19472390 DOI: 10.1002/jmri.21751]

30 **Hines CD**, Yu H, Shimakawa A, McKenzie CA, Brittain JH, Reeder SB. T1 independent, T2* corrected MRI with accurate spectral modeling for quantification of fat: validation in a fat-water-SPIO phantom. *J Magn Reson Imaging* 2009; **30**: 1215-1222 [PMID: 19856457 DOI: 10.1002/jmri.21957]

31 **Hines CD**, Yu H, Shimakawa A, McKenzie CA, Warner TF, Brittain JH, Reeder SB. Quantification of hepatic steatosis with 3-T MR imaging: validation in ob/ob mice. *Radiology* 2010; **254**: 119-128 [PMID: 20032146 DOI: 10.1148/radiol.09090131]

32 **Meisamy S**, Hines CD, Hamilton G, Sirlin CB, McKenzie CA, Yu H, Brittain JH, Reeder SB. Quantification of hepatic steatosis with T1-independent, T2-corrected MR imaging with spectral modeling of fat: blinded comparison with MR spectroscopy. *Radiology* 2011; **258**: 767-775 [PMID: 21248233 DOI: 10.1148/radiol.10100708]

33 **Al-Attar SA**, Pollex RL, Robinson JF, Miskie BA, Walcarius R, Little CH, Rutt BK, Hegele RA. Quantitative and qualitative differences in subcutaneous adipose tissue stores across lipodystrophy types shown by magnetic resonance imaging. *BMC Med Imaging* 2007; **7**: 3 [PMID: 17352814 DOI: 10.1186/1471-2342-7-3]

34 **Alabousi A**, Al-Attar S, Joy TR, Hegele RA, McKenzie CA. Evaluation of adipose tissue volume quantification with IDEAL fat-water separation. *J Magn Reson Imaging* 2011; **34**: 474-479 [PMID: 21780238 DOI: 10.1002/jmri.22603]

35 **Malinin A**, Pokov A, Spergling M, Defranco A, Schwartz K, Schwartz D, Mahmud E, Atar D, Serebruany V. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: the VERIfy Thrombosis risk ASsessment (VERITAS) study. *Thromb Res* 2007; **119**: 277-284 [PMID: 16563469 DOI: 10.1016/j.thromres.2006.01.019]

36 **Cohen J**. Statistical Power Analysis for the Behavioural Sciences. New Jersey: Lawrence Erlbaum Associates Inc., 1988

37 **Akaslan SB**, Degertekin CK, Yilmaz G, Cakir N, Arslan M, Toruner FB. Effects of sitagliptin on nonalcoholic fatty liver disease in diet-induced obese rats. *Metab Syndr Relat Disord* 2013; **11**: 243-250 [PMID: 23544853 DOI: 10.1089/met.2012.0128]

38 **Maiztegui B**, Borelli MI, Madrid VG, Del Zotto H, Raschia MA, Francini F, Massa ML, Flores LE, Rebolledo OR, Gagliardino JJ. Sitagliptin prevents the development of metabolic and hormonal disturbances, increased β-cell apoptosis and liver steatosis induced by a fructose-rich diet in normal rats. *Clin Sci* (Lond) 2011; **120**: 73-80 [PMID: 20795946 DOI: 10.1042/CS20100372]

39 **Fukuhara T**, Hyogo H, Ochi H, Fujino H, Kan H, Naeshiro N, Honda Y, Miyaki D, Kawaoka T, Tsuge M, Hiramatsu A, Imamura M, Kawakami Y, Aikata H, Chayama K. Efficacy and safety of sitagliptin for the treatment of nonalcoholic fatty liver disease with type 2 diabetes mellitus. *Hepatogastroenterology* 2014; **61**: 323-328 [PMID: 24901133]

40 **Arase Y**, Kawamura Y, Seko Y, Kobayashi M, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Sezaki H, Saito S, Hosaka T, Ikeda K, Kumada H, Ohmoto-Sekine Y, Hsieh SD, Amakawa K, Ogawa K, Matsumoto N, Iwao A, Tsuji H, Hara S, Mori Y, Okubo M, Sone H, Kobayashi T. Efficacy and safety in sitagliptin therapy for diabetes complicated by non-alcoholic fatty liver disease. *Hepatol Res* 2013; **43**: 1163-1168 [PMID: 23489256 DOI: 10.1111/hepr.12077]

41 **Charbonnel B**, Karasik A, Liu J, Wu M, Meininger G. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled with metformin alone. *Diabetes Care* 2006; **29**: 2638-2643 [PMID: 17130197 DOI: 10.2337/dc06-0706]

42 **Lima-Martínez MM**, Paoli M, Rodney M, Balladares N, Contreras M, D'Marco L, Iacobellis G. Effect of sitagliptin on epicardial fat thickness in subjects with type 2 diabetes and obesity: a pilot study. *Endocrine* 2016; **51**: 448-455 [PMID: 26233684 DOI: 10.1007/s12020-015-0710-y]

P- Reviewer: Lee HC, Ramos-Gomez M, Thompson JR
S- Editor: Yu J **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.151

World J Gastroenterol 2017 January 7; 23(1): 151-166
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

SYSTEMATIC REVIEWS

Prevalence of hepatitis C virus infection among hemodialysis patients in the Middle-East: A systematic review and meta-analysis

Soheil Ashkani-Esfahani, Seyed Moayed Alavian, Mohammad Salehi-Marzijarani

Soheil Ashkani-Esfahani, Seyed Moayed Alavian, Mohammad Salehi-Marzijarani, Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran 14155-3651, Iran

Soheil Ashkani-Esfahani, Student Research Committee, Shiraz University of Medical Sciences, Shiraz 71348-45794, Iran

Mohammad Salehi-Marzijarani, Department of Biostatistics, Shiraz University of Medical Sciences, Shiraz 71348-45794, Iran

Author contributions: Alavian SM designed research; Ashkani-Esfahani S performed research and gathered data; Salehi-Marzijarani M analyzed data and designed charts and tables; Ashkani-Esfahani S and Alavian SM wrote and edited the paper.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Seyed Moayed Alavian, MD, Professor of Gastroenterology, Baqiyatallah Research Center for Gastroenterology and Liver Diseases, Baqiyatallah University of Medical Sciences, Tehran 14155-3651, Iran. soashkani@gmail.com
Telephone: +98-218126407
Fax: +98-2181264070

Received: September 28, 2016
Peer-review started: September 29, 2016
First decision: October 20, 2016
Revised: November 3, 2016
Accepted: December 16, 2016
Article in press: December 19, 2016
Published online: January 7, 2017

Abstract

AIM

To determine hepatitis C virus (HCV) infection prevalence in each country of the Middle-East and the overall prevalence of the region.

METHODS

In this systematic review, we gathered all documents related to HCV infection prevalence among hemodialysis patients in 17 middle-east countries from April 2006 to March 2016. We selected only cross-sectional studies that had proper sampling and measurement methods as well as a valid statistical analysis.

RESULTS

After screening of 7311 documents, 56 studies were selected reporting the prevalence of HCV infection among hemodialysis patients from 10 countries of the region. Seven countries including United Arab Emirates, Afghanistan, Qatar, Bahrain, Kuwait, Oman, Israel, and Cyprus did not have any relevant document; thus, their latest reports were just mentioned. We performed the meta-analysis and determined the prevalence rates for each country as well as the whole region. The overall HCV infection prevalence among hemodialysis patients in the region was reported to be 25.3%; Egypt and Syria had the highest reported rates while Iran and Lebanon had the lowest. Further investigations are still needed to provide more reliable databases, find main risk factors, and to improve diagnosis and treatment plans, particularly in higher prevalent countries.

CONCLUSION
Controlling the prevalence and improving the management methods of HCV infection among hemodialysis patients are of a great concern in the Middle-East region.

Key words: Hepatitis C; Hemodialysis; Prevalence; Middle-East; Meta-Analysis; Review

Core tip: This paper is a systematic review and meta-analysis of the reports published from April 2006 to March 2016 on the prevalence of hepatitis C infection among 17 countries of the Middle-East region including: Iran, Turkey, Iraq, Saudi Arabia, Syria, Yemen, Palestine, United Arab Emirates, Jordan, Lebanon, Oman, Egypt, Cyprus, Qatar, Afghanistan, Bahrain, Israel, and Kuwait.

Ashkani-Esfahani S, Alavian SM, Salehi-Marzijarani M. Prevalence of hepatitis C virus infection among hemodialysis patients in the Middle-East: A systematic review and meta-analysis. *World J Gastroenterol* 2017; 23(1): 151-166 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/151.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.151

INTRODUCTION

Hepatitis C virus (HCV) infection is a major concern for the public health worldwide in both developing and developed countries[1] . Transmission of HCV infection is mainly by exposure to infected devices and tools despite rigid hygienic control, infected blood or blood products, hemodialysis, intravenous (IV) drug abuse, and organ transplantation[2]. The estimation of national prevalence and ways of transmission of HCV should be completed in order to allow the national authorities to prioritize preventive measures and have the best and most appropriate use of available resources. Epidemiological surveys on the roles of potential risk factors, such as injections for medications, vaccinations, medical procedures, tattooing, and injections outside of medical settings, have shown a wide geographical variation with major implications for the populations and potential management, prevention, and control plans[3,4]. Prospective investigations have revealed that about 80% of the acute hepatitis C cases progress to chronic infection; about 10%-20% of these cases will develop chronic liver disease complications, like liver cirrhosis, within two to three decades of onset, and about 1%-5% will end up with liver cancer[4,5].

Among the risk factors of HCV infection which had been evaluated through many studies, the following can be mentioned in brief: Sex (male > female), education (more than 12 years > less than 12 years), ethnicity (whites and Hispanics < others), number of sexual partners, age of starting intercourse, intravenous drug use, addiction, vaccination history, blood transfusion, occupation and employment situation, history of hemodialysis, and organ transplantation, *etc*[6].

Screening for HCV mainly focuses on testing those who have an individual risk factor for exposure, who have evidence of liver disease, and who belong to specific demographic groups that have a high-prevalence of infection[6,7]. Without screening, many infected individuals will be identified late which may lead to longer hospitalization and death[6,7]. In recent guidelines, the CDC (Center for disease control and prevention) recommended that testing for HCV should be performed routinely in patients at increased risk of infection; specifically for those who have ever had illegal drug injections, received blood or organs before July 1992, received clotting factors produced before 1987, were ever on chronic hemodialysis, any evidence of liver diseases, and those infected with HIV[6,7]. The initial screening test for HCV infection is an HCV antibody test. Various antibody tests are available, including laboratory based enzyme-linked Immunosorbent assay (ELISA), HCV RNA by reverse transcription Polymerase chain reaction (RT-PCR), and tests performed on samples that the patient may collect at home[8]. A reactive or indeterminate/equivocal antibody test should be followed by HCV RNA testing to determine occult infections[8,9]. Immunocompromised patients, patients on hemodialysis, transplant recipients, and advanced HIV infected ones might have higher false negative rates of antibody testing than immune-competent patients[8-10].

HCV infection is common and associated with significant morbidity and mortality among dialysis patients and is more common in dialysis patients than in healthy populations. Dialysis Outcomes and Practice Patterns Study, which provides reliable data regarding the prevalence of HCV infection among dialysis patients, is a prospective, observational survey among adult hemodialysis patients who are randomly selected from 308 representative dialysis facilities in many countries such as Japan, France, Germany, Spain, Italy, the United Kingdom, and the United States. In the 2004 report, the overall prevalence was 13.5% (compared to global prevalence in the general population of approximately 3%)[11]. The reported prevalence of anti-HCV antibodies among hemodialysis patients in different countries were from 5.5% to 14% in the United States[12], 13.5% to 31% in Italy[11,13-15], 10% to 42% in France[16], 75% in Moldavia[17], 3.8% in Germany, 14.8% in Japan, 22.9% in Spain, and 2.6% in the United Kingdom[11]. Nonetheless, the relatively high incidence of anti-HCV antibodies in hemodialysis units is a concern for today's health policy makers and care providers. A number of risk factors have

been identified for HCV infection among hemodialysis patients; the number of blood transfusions[18], duration of the hemodialysis treatment[18], and also nosocomial transmissions due to poor infection-control measures are among the most important ones[19,20].

No comprehensive report was presented, particularly during the last decade, in order to give a sight about the prevalence of HCV infection among hemodialysis patients of the Middle-East countries. We found that evaluation and estimation of the prevalence in these countries and performing comparisons among them may help researchers and health policy makers create or modify research projects, preventive programs and management plans for the hemodialysis patients in the region.

In the present study, we have systematically reviewed papers and reports related to HCV infection prevalence among hemodialysis patients in 17 countries in the Middle-East region.

MATERIALS AND METHODS

We studied the prevalence of HCV infection related to hemodialysis in the Middle-East countries and the changes in the trends during the past decade through a comprehensive systematic review of literature followed by integrating the data and analysis of the outcome.

Study question

The populations of interest in this survey was hemodialysis receiving patients among the general population of the Middle-East countries and the interested outcome was presence of positive HCV-antibody in their blood samples based on ELISA test even if other laboratory evaluations are not identified clearly, from April 2006 to March 2016. We intended to find the prevalence of HCV infection related to hemodialysis and the possible alterations in each country regarding this prevalence during the last decade.

Search strategy

For searching in each one of the databases, we used the following terms "Hepatitis C", "HCV," and "Hemodialysis" altogether with the name of each country in the Middle-East region including[21]: Afghanistan, Bahrain, Cyprus, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, Turkey, United Arab Emirates (UAE), and Yemen, as key words for titles and/or abstracts in a MeSH word search. Cross-sectional studies were selected and screened for further evaluations. The searching process was performed in the first week of June 2016.

Electronic databases and Gray literature

We searched 10 international electronic databases of biomedical sciences including Medline (Pubmed), Proquest, Embase, Scopus, Google scholar, CINAHL, CABI, Index Medicus for Eastern Mediterranean Region (IMEMR), Cochrane library, and EMROMedex. Also, three Iranian national databases of medical sciences papers were evaluated including Iranmedex, Scientific Information Database (SID), IranDoc, and Magiran for the papers which were not added to the international electronic databases. Since the language of many countries of the region is Arabic, the keywords were also searched in Arabic to find any data in this regard.

The Gray literature evaluation included 82 international and regional congresses and seminars that were held in the study time period around the world and specifically in the region, and we selected and hand searched the abstract books that were obtainable as well as possible by two independent reviewers. We also searched national reports from CDC centers of the countries, those which were accessible, and the websites of the university, thesis, and reports which were related to the subject during the study time period. Moreover, forward and backward citations of the searched items were performed.

Critical appraisal and selection of studies

Documents were catalogued using Endnote X4. Two independent reviewers who were trained in this field reviewed all citations thoroughly for eligibility criteria to be included in the analysis. The inclusion criteria were all descriptive and/or analytical cross-sectional surveys which had specified temporal and geographic characteristics of the study, sufficient populations, correct and proper sampling methods with identical and valid measurement tools for all study subjects, and proper analytical methods considering the sampling design and the demographic data. A previously obtained method which was a revision of the criteria developed by Sharifi *et al*[22] was occupied for this purpose.

Data extraction and analysis

The extracted data were first author, year of the study, location, sample population, sampling method and sample size, HCV detection method, age, male to female ratio, and HCV point prevalence in the subjects. Cochrane Q-test was used with a significance level of less than 0.1 for statistical heterogeneity of the results. I^2, presented a range of 0% (no heterogeneity) to 100% (significant heterogeneity), was employed to assessing level of heterogeneity; values of 25%, 50% and 75% were considered as representing low, medium, and high heterogeneity, respectively[23]. Wherever Cochrane Q-test and I^2 confirmed the studies heterogeneity, random effect meta-analysis based on DerSimonian and Laird method was used to combine the outcomes; otherwise, fixed effect meta-analysis was used. Statistical analyses were carried out with "metan" command in Stata, version 11.0 (Stata

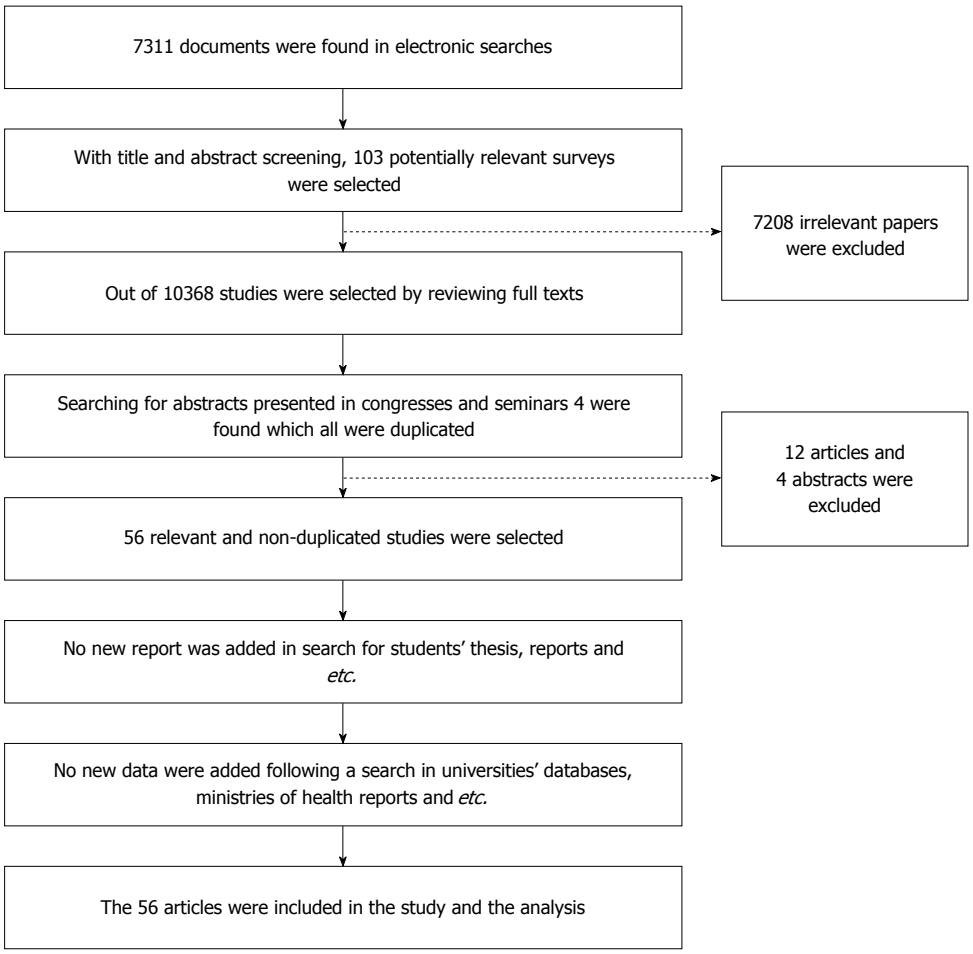


Figure 1 Follow diagram of systematic review and searches for hepatitis C virus infection prevalence among hemodialysis patients in the Middle-East countries.

Corp, College Station, TX, United States)[24].

RESULTS

In our primary search in databases with the aforementioned search strategy 7311 documents were found. After assessment of the documents according to their titles and also their abstracts, 7208 unrelated documents were omitted. We found 103 relevant citations out of all searched documents among which 68 studies were not overlapping (duplicate studies found by many search routes); of these 68 papers 56 were included in this investigation according to their publication year which are mentioned in the result part; studies which were not cross-sectional, had inappropriate methods of sampling, or inappropriate data analysis and invalid data reporting were excluded after full-text screening. In some countries such as Afghanistan, Cyprus, Qatar, Kuwait, UAE, Oman, and Bahrain, no related document was found that was published after; thus, no study could be included in the meta-analysis for these countries[4,25-45]. In gray literature evaluations 4 studies were found of which all had overlapping with the published articles. The detailed search process is exhibited in Figure 1.

The research method in all included studies was cross-sectional conducted within the hemodialysis patients from April 2006 to March 2016. Since the investigations in this field were limited, we did not set any exclusion criteria for the population of the study, hence, the sample size varied from 31 to 22070 patients in different centers in the Middle-East countries. The age of the studies subjects was between 8.3± 2.4 and 80.9 ± 4.9 years old. In the majority of the surveys (49 of 56), 50.6 to 69.9 percent of the subjects were males, while in the other 7 documents 50 to 58.1 percent of the participants were females. ELISA methods were the most dominant methods used for HCV antibody detection; some studies had also used other more specific methods like RT-PCR beside ELISA which had led to lower rates of false negative results. The reported prevalences in this study are based on ELISA test results. All papers that used ELISA for HCV detection had implemented second or third generations of the test.

The overall prevalence of HCV infection among hemodialysis patients in the Middle-East countries was 25.3% (95%CI: 20.2%-30.5%). As it is shown in Table

Table 1 The prevalence of Hepatitis C infection in Middle-East countries. Summary of studies by countries[1], age and hemodialysis duration are presented

Subgroup	Number of studies	Prevalence (95%CI)	Between studies		Between subgroups	
			I^2	$P_{Heterogeneity}$	Q	$P_{Heterogeneity}$
Country						
Iran	21	12% (10%-15%)	90.0%	0.001	30052.52	0.001
Iraq	9	20% (12%-28%)	97.3%	0.001		
Egypt	7	50% (46%-55%)	94.7%	0.001		
Jordan	4	35% (17%-54%)	97.4%	0.001		
Yemen	4	42% (28%-56%)	93.4%	0.001		
Turkey	4	23% (18%-28%)	66.3%	0.031		
Palestine	3	18% (5%-30%)	94.8%	0.001		
Lebanon	2	9% (1%-17%)	98.4%	0.001		
Syria	1	54% (50%-59%)	-	-		
Saudi	1	19% (13%-25%)	-	-		
Age(yr)						
< 40	4	24% (11%-38%)	95.4%	0.001	334.82	0.001
40-50	16	35% (26%-45%)	97.8%	0.001		
⩾ 50	26	20% (10%-29%)	99.7%	0.001		
Hemodialysis duration (yr)						
< 3	13	22% (15%-29%)	96.4%	0.001	3085.57	0.001
3-5	8	22% (4%-40%)	99.7%	0.001		
⩾ 5	9	30% (16%-45%)	99.0%	0.001		

[1]United Arab Emirates, Afghanistan, Qatar, Bahrain, Kuwait, Oman, Israel, and Cyprus, were not added due to insufficient data. The prevalences are reported as ratio (95%CI).

1, the reported percentages were heterogeneous and showed statistical significance (test for heterogeneity: Q = 30052.5, df = 9, P < 0.001).

Iran

An overall anti-HCV antibody prevalence of less than 1% was reported in the general population of Iran[46-48]. The most dominant genotype of HCV in Iran was subtype 1a (44.9%) followed by 3a (39.6%), and 1b (11.3%) among the general population[49]. The reported prevalences of hepatitis C infection among hemodialysis patient in different areas are as follows: In 2006, 38% (19/50) and 4.9% (10/204) in Urmia city and Markazi province, respectively[50,51]; in 2007, 10.3% (9/89) in Ghazvin[52], 20.4% (66/324) in Tabriz[53], and 8.5% (11/130) in Tehran[54]; In 2008 6.5% (44/674) in Eastern-Azerbaijan[55], 24.7% in Golestan[56], 6.8% in Markazi[57], 21% (39/186) in Sari and Ghaemshahr[58], and another 12.3% in Sari[59]; In 2009, 18.4% (30/163), 7.9% (34/214), and 5.3% (6/112), in Gilan, Khuzestan, and Tehran, respectively[60-62]; in 2010, 20.1% (67/334) in Amol, Tonekabon, Rasht, and Ramsar[63]; in 2011, 11.9% in Gilan[64], and 31.5% (64/203) in Kerman province[65]; in 2012, 6% (9/160) and 7% (16/228) in Yazd and Kerman provinces, respectively[66,67]; in 2013, 7.2% (13/181) in Shiraz[68]; in 2014, 8.1% (37/455) in Tabriz[69], and 5.2% (26/499) in Isfahan[69]; in 2015, 5.9% (11/185) in Alborz Province[70].

As it is exhibited in Figure 2, the overall prevalence of HCV infection among hemodialysis patients in Iran according to the last decade's publications was estimated as 12% (95%CI: 9%-15%).

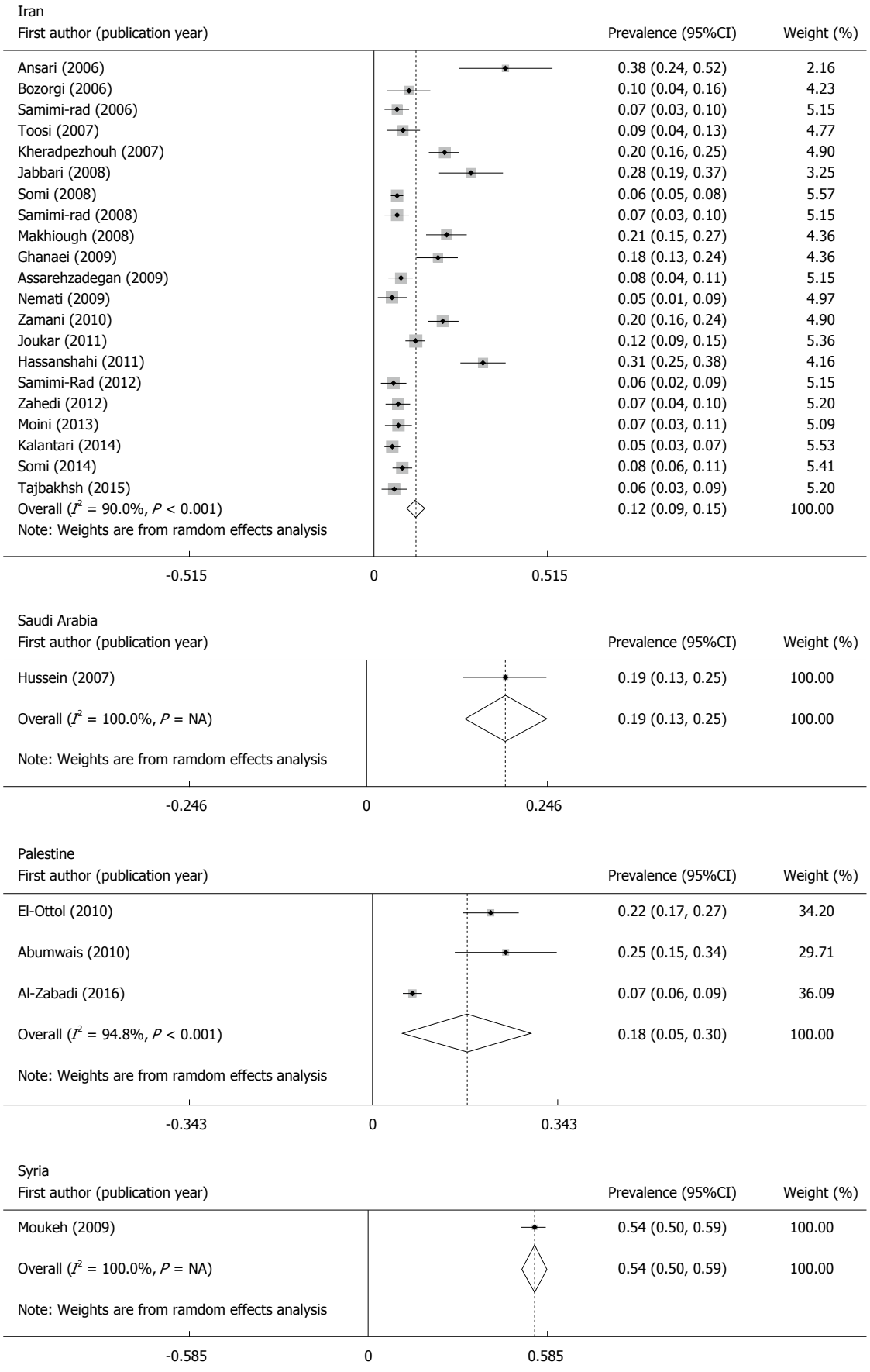
Turkey

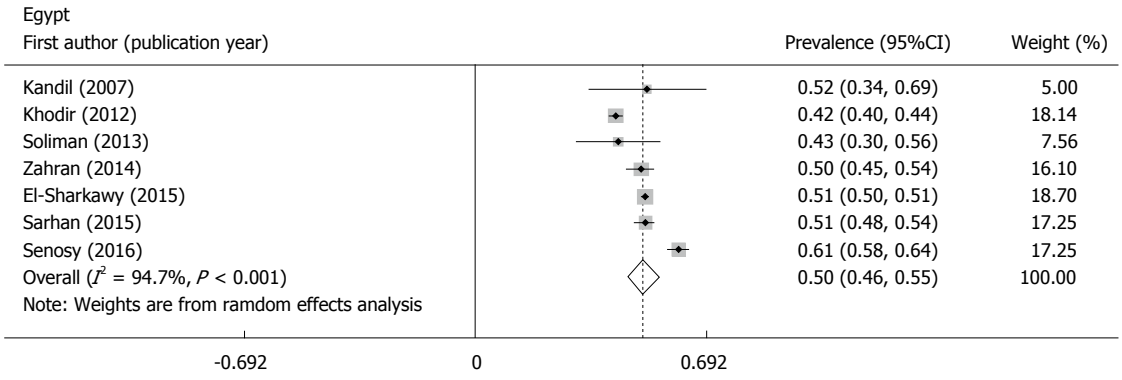
The prevalence of HCV infection among the general population of turkey was reported as 1.6%[71]. Genotype 1b was found to be the most common genotype of HCV (67.7%) followed by genotype 1 (7.7%), 4 (7.3%) and genotype 3 (6.7%) among the general population[72,73]. Investigations on the prevalence of HCV infection among hemodialysis patients are limited in this country and are as follows: in 2004, 19% (19/83) in Izmir[74], in 2006, 26% (245/934)[75], and 28.7% (54/188)[76], in the whole country, and 35.6% (26/73) in Ankara[77]; in 2009, 19% (83/437) in the Ege region[78]; in 2014, 19.9% (40/201) in Antalya[79]. As it is demonstrated in Figure 2, the prevalence of HCV infection among hemodialysis patients in Turkey according to the publications of the last 10 years was estimated as 23% (95%CI: 13%-28%).

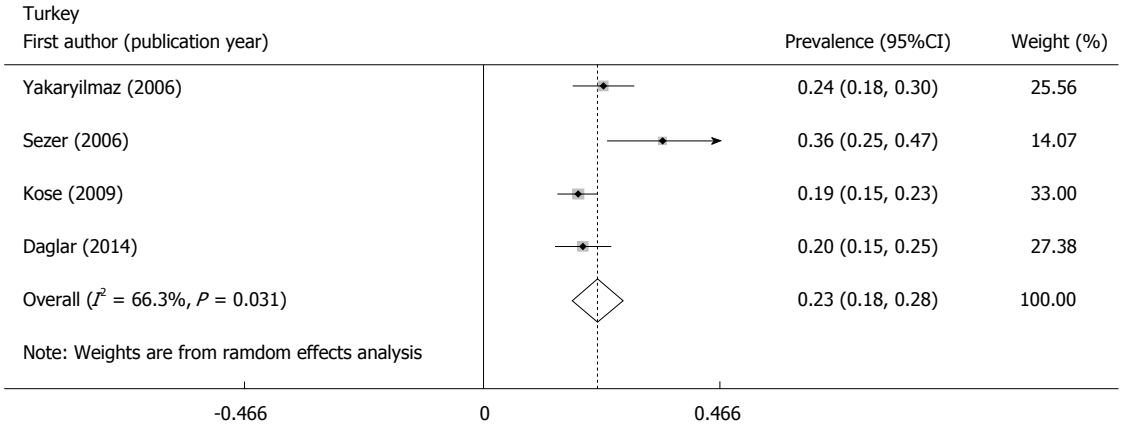
Saudi Arabia

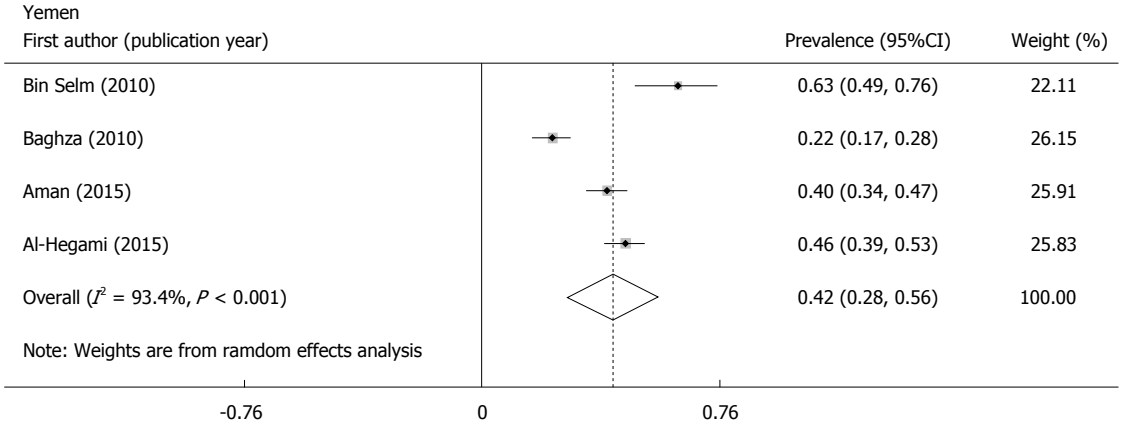
A report compiled by the WHO mentions 437,292 official reports of HCV infections among the general population of Saudi Arabia, revealing an estimated prevalence of nearly 1.8%[80]. The most prevalent genotype of the virus in Saudi Arabia was genotype 4 followed by 1a and 1b, whereas genotypes 2a/2b, 3, 5, and 6 were rarely detected among the general population[42,81,82].In one study in 2007, a prevalence of 18.9% (34/180 patients) was reported[83].

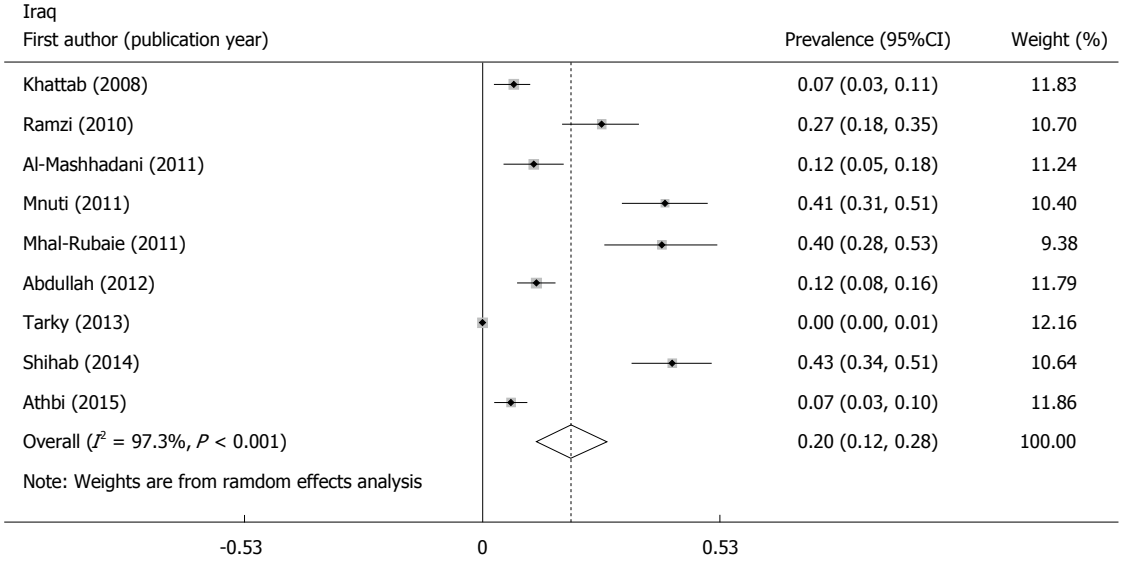
As shown in Figure 2, the overall prevalence in the


Iran
First author (publication year)
Prevalence (95%CI)
Weight (%)
Ansari (2006) 0.38 (0.24, 0.52) 2.16
Bozorgi (2006) 0.10 (0.04, 0.16) 4.23
Samimi-rad (2006) 0.07 (0.03, 0.10) 5.15
Toosi (2007) 0.09 (0.04, 0.13) 4.77
Kheradpezhouh (2007) 0.20 (0.16, 0.25) 4.90
Jabbari (2008) 0.28 (0.19, 0.37) 3.25
Somi (2008) 0.06 (0.05, 0.08) 5.57
Samimi-rad (2008) 0.07 (0.03, 0.10) 5.15
Makhiough (2008) 0.21 (0.15, 0.27) 4.36
Ghanaei (2009) 0.18 (0.13, 0.24) 4.36
Assarehzadegan (2009) 0.08 (0.04, 0.11) 5.15
Nemati (2009) 0.05 (0.01, 0.09) 4.97
Zamani (2010) 0.20 (0.16, 0.24) 4.90
Joukar (2011) 0.12 (0.09, 0.15) 5.36
Hassanshahi (2011) 0.31 (0.25, 0.38) 4.16
Samimi-Rad (2012) 0.06 (0.02, 0.09) 5.15
Zahedi (2012) 0.07 (0.04, 0.10) 5.20
Moini (2013) 0.07 (0.03, 0.11) 5.09
Kalantari (2014) 0.05 (0.03, 0.07) 5.53
Somi (2014) 0.08 (0.06, 0.11) 5.41
Tajbakhsh (2015) 0.06 (0.03, 0.09) 5.20
Overall (I^2 = 90.0%, P < 0.001) 0.12 (0.09, 0.15) 100.00
Note: Weights are from ramdom effects analysis
-0.515
0
0.515
Saudi Arabia
First author (publication year)
Prevalence (95%CI)
Weight (%)
Hussein (2007) 0.19 (0.13, 0.25) 100.00
Overall (I^2 = 100.0%, P = NA) 0.19 (0.13, 0.25) 100.00
Note: Weights are from ramdom effects analysis
-0.246
0
0.246
Palestine
First author (publication year)
Prevalence (95%CI)
Weight (%)
El-Ottol (2010) 0.22 (0.17, 0.27) 34.20
Abumwais (2010) 0.25 (0.15, 0.34) 29.71
Al-Zabadi (2016) 0.07 (0.06, 0.09) 36.09
Overall (I^2 = 94.8%, P < 0.001) 0.18 (0.05, 0.30) 100.00
Note: Weights are from ramdom effects analysis
-0.343
0
0.343
Syria
First author (publication year)
Prevalence (95%CI)
Weight (%)
Moukeh (2009) 0.54 (0.50, 0.59) 100.00
Overall (I^2 = 100.0%, P = NA) 0.54 (0.50, 0.59) 100.00
Note: Weights are from ramdom effects analysis
-0.585
0
0.585


Egypt
First author (publication year)
Prevalence (95%CI)
Weight (%)
Kandil (2007) 0.52 (0.34, 0.69) 5.00
Khodir (2012) 0.42 (0.40, 0.44) 18.14
Soliman (2013) 0.43 (0.30, 0.56) 7.56
Zahran (2014) 0.50 (0.45, 0.54) 16.10
El-Sharkawy (2015) 0.51 (0.50, 0.51) 18.70
Sarhan (2015) 0.51 (0.48, 0.54) 17.25
Senosy (2016) 0.61 (0.58, 0.64) 17.25
Overall (I^2 = 94.7%, P < 0.001) 0.50 (0.46, 0.55) 100.00
Note: Weights are from ramdom effects analysis
-0.692
0
0.692


Turkey
First author (publication year)
Prevalence (95%CI)
Weight (%)
Yakaryilmaz (2006) 0.24 (0.18, 0.30) 25.56
Sezer (2006) 0.36 (0.25, 0.47) 14.07
Kose (2009) 0.19 (0.15, 0.23) 33.00
Daglar (2014) 0.20 (0.15, 0.25) 27.38
Overall (I^2 = 66.3%, P = 0.031) 0.23 (0.18, 0.28) 100.00
Note: Weights are from ramdom effects analysis
-0.466
0
0.466


Yemen
First author (publication year)
Prevalence (95%CI)
Weight (%)
Bin Selm (2010) 0.63 (0.49, 0.76) 22.11
Baghza (2010) 0.22 (0.17, 0.28) 26.15
Aman (2015) 0.40 (0.34, 0.47) 25.91
Al-Hegami (2015) 0.46 (0.39, 0.53) 25.83
Overall (I^2 = 93.4%, P < 0.001) 0.42 (0.28, 0.56) 100.00
Note: Weights are from ramdom effects analysis
-0.76
0
0.76


Iraq
First author (publication year)
Prevalence (95%CI)
Weight (%)
Khattab (2008) 0.07 (0.03, 0.11) 11.83
Ramzi (2010) 0.27 (0.18, 0.35) 10.70
Al-Mashhadani (2011) 0.12 (0.05, 0.18) 11.24
Mnuti (2011) 0.41 (0.31, 0.51) 10.40
Mhal-Rubaie (2011) 0.40 (0.28, 0.53) 9.38
Abdullah (2012) 0.12 (0.08, 0.16) 11.79
Tarky (2013) 0.00 (0.00, 0.01) 12.16
Shihab (2014) 0.43 (0.34, 0.51) 10.64
Athbi (2015) 0.07 (0.03, 0.10) 11.86
Overall (I^2 = 97.3%, P < 0.001) 0.20 (0.12, 0.28) 100.00
Note: Weights are from ramdom effects analysis
-0.53
0
0.53

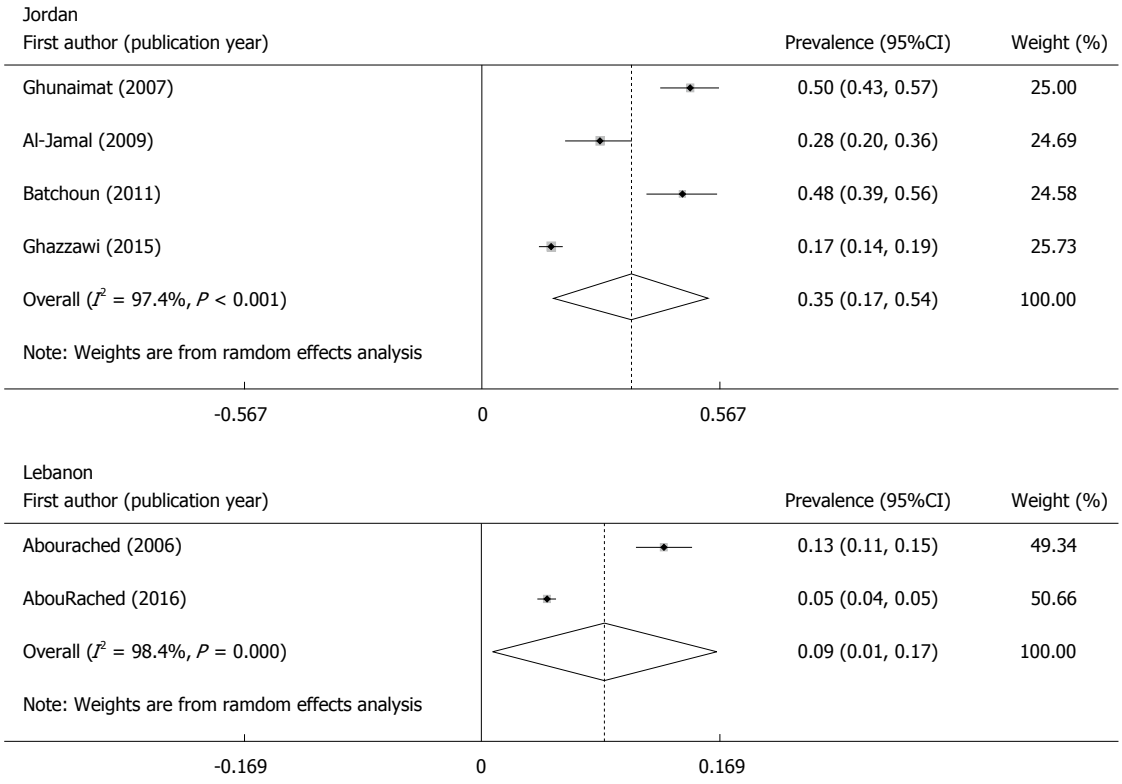


Figure 2 Prevalence of hepatitis C virus infection among hemodialysis patients in the Middle-East region during the last decade according to the results of enzyme-linked Immunosorbent assay. The reported prevalence of each country separated by First author name and sorted by publication year. The overall prevalence and 95%CI is also reported for each country. Prevalence data are reported as ratio (95%CI). United Arab Emirates, Afghanistan, Qatar, Bahrain, Kuwait, Oman, Israel, and Cyprus, were not added due to insufficient data.

last decade's only one publication was estimated as 19% (95%CI: 13%-25%).

Kuwait

The latest reports of WHO showed a prevalence of 1.8% of HCV infection in general population of Kuwait[26]. The most common genotypes of HCV in Kuwait was genotype 4 with a rate of 43% (95%CI: 33%-52%) followed by genotype 1 with a rate of 28% (95% CI: 21%-34%)[29]. There was no published study during the last decade regarding the prevalence of HCV infection in Kuwait.

UAE

Regarding the latest reports, the prevalence of HCV infection in UAE was about 1% in general population[31]. In 1995, a prevalence of 24% (64/262) of HCV infection among hemodialysis patients was reported[32]. The predominant genotypes were 4, 3, and 1[33]. No study was found reporting the prevalence of HCV infection among hemodialysis patients during the last decade in UAE.

Qatar

According to the literature, HCV prevalence in the general population of Qatar was 0.5% among Qataris people, and 1% (95%CI: 0.43%-1.82%) overall. Genotype 4 is the most common HCV genotype reported in Qatar which accounts for almost 100% of all infections[36,38]. No report was found for Qatar on the prevalence of HCV infection during the last decade.

Yemen

HCV prevalence in the general population was estimated to be 1.8%[84]. In 2010, the anti-HCV antibody prevalence rate among the hemodialysis patients was reported as 62.7%[85]; In 2014, 22.5% (45/200)[86]; In 2015, it was reported as 40.2% (88/219; 95%CI: 33.6%-46.73%)[87], and 46% (98/213)[88]. The most prevalent genotype of HCV was 4 with a prevalence of 63.7% followed by genotype 1a + 1b with a rate of 26.9% among the general population[89].

As it is presented in Figure 2, the overall prevalence of HCV infection among hemodialysis patients in Yemen according to the publications of the last decade was estimated as 42% (95%CI: 28%-56%).

Iraq

A meta-analysis in 2015 estimated the prevalence of HCV infection to be 0.2% among the general population of Iraq (range: 0%-7.2%; 95%CI: 0.1%-0.3%)[90]; HCV genotype 4 is the most common genotype[38]. Regarding the prevalence of hepatitis C among hemodialysis patients during the past decade, the reported rates ranged from 0 to 42.6%[91-98]: In 2007, 11.7% (10/87)[94]; in 2008, 7.1% (12/169)[98];

in 2009, 40.3% (23/57)[99]; in 2010, 26.7% (27/101)[96]; in 2012, 12.3% (29/236)[92]; in 2014, 42.6% (52/122)[97]; in 2015, 6.6% (11/165)[100].

As Figure 2 shows, the overall prevalence of HCV infection among hemodialysis patients in Iraq according to the last decade's publications was estimated as 20% (95%CI: 12%-28%).

Afghanistan

HCV prevalence in the population at large in Afghanistan appears to be around 1%; however, there are no reliable data on the prevalence of HCV infection and hepatitis C among hemodialysis patients as a high risk group[39]. Regarding the common genotypes in this country, only one study reported HCV genotype data among 71 HCV positive from Mazar-i-Sharif, Herat, and Jalalabad between 2006 and 2008[40]. In this report, about two-thirds of participants were infected with genotype 3a while the rest of them were infected with genotype 1[40].

Palestine

The prevalence of HCV infection in Palestine was reported as 0.2% rate (range: 0%-9.0%; 95%CI: 0.2%-0.3%). The most dominant genotype was found to be genotype 4 followed by 1 and 3a among the general population[101,102]. Studies on the prevalence of hepatitis C among hemodialysis patients were limited in this country. The reported prevalence rates were as follows: 24.7% (19/77) and 22% (54/246) in 2010[102,103]; 7.4% (64/868) in 2016[104]. As it is demonstrated in Figure 2, the overall prevalence of HCV infection among hemodialysis patients in Palestine according to the last decade's publications was calculated as 18% (95%CI: 5%-30%).

Oman

HCV infection prevalence among the general population in Oman, nationals and expatriates, was reported to be below 1%, ranging from 0.4% to 0.9% in blood donors[30]. To our knowledge, there are no reports on the prevalence of HCV among hemodialysis patients in the past decade in this country.

Bahrain

Regarding the prevalence of HCV infection in Bahrain's general population, two recent studies were found which reported a low prevalence of 0.3%[42,43]. The predominant genotype among Bahraini patients was type 1 (36.7%), followed by genotypes 3 and 4 (15.6% each)[44]. We searched all of the databases and no more recent studies were found on the prevalence of HCV among hemodialysis patients to estimate the possible changes in its rate and the trend of the disease.

Syria

The prevalence of HCV infection among the general population of Syria was estimated as 0.4% (range: 0.3%-0.9%; 95%CI: 0.4%-0.5%)[90]. The dominant genotype in this country were genotype 4 and then genotype 1 among the general population[90]. Regarding the prevalence of HCV among hemodialysis patients in 1995 a prevalence of 75% (90/120) and 48.9% (68/139) in 1996 were reported which were not included in our meta-analysis[105,106]. The latest and the only found survey in 2006 revealed a rate of 54.4% (299/550)[107].

The prevalence of HCV infection among hemodialysis patients in Syria regarding the last decade's publications was estimated as 54% (95%CI: 50%-59%; Figure 2).

Jordan

According to the recent reports, the prevalence of HCV infection in Jordan's general population was estimated to be ranging from 0.3% to 2.1%[4,90]. The most dominant genotypes of HCV was genotypes 4 and 1 according to their research among the general population[108]. Regarding the prevalence of hepatitis C among hemodialysis patients, in 1994 and 2001, 24.5% and 34.6% were reported, respectively[108,109]. During the last decade the rates of 49.8% (104/209)[110], 28% (34/120)[111], 47.7% (64/134)[112], and 16.5% (117/712)[113], in 2007, 2009, 2011, and 2015, respectively. The overall prevalence of HCV infection among hemodialysis patients in Jordan considering the last decade's publications was measured as 35% (95%CI: 17%-54%; Figure 2).

Lebanon

The prevalence of HCV in Lebanon is reported to be 0.2% according to a recent report[114]. HCV genotype 4 is reported to be the most dominant genotype among the general population as well as among hemodialysis patients[30,115]. The prevalences of HCV infection among hemodialysis patients were 13% (134/1030) in 2007[116] and 4.7% (177/3769) in 2016[117]. Prior to that, in 1995 a prevalence of 27% (range: 10%-39%) was reported which could not be included in our study[118]. As it is exhibited in Figure 2, the overall prevalence of HCV infection among hemodialysis patients in Lebanon according to the last ten years' publications was estimated as 9% (95%CI: 1%-17%).

Egypt

Egypt is one of the countries which are heavily affected by HCV having a prevalence of 14.7%; HCV genotype 4 is by far the most common genotype in Egypt[30]. During the past two decades literature reported prevalences ranging from 48.2% to 87.5% for HCV infection among hemodialysis patients which were not taken into account in our analysis[119-125]. The reports of the prevalence of hepatitis C infection among hemodialysis patients during the last decade are as follows: In 2007, 51.6% (16/31)[126]; in 2012,

43% (36/83)[127], and 42.2% (992/2351)[128]; in 2014, 49.6% (255/514)[129]; in 2015, 51% (503/987)[130], and 50.7% (11189/22070)[131]; in 2016, 60.9% (591/971)[132].

Considering the chart in Figure 2, the overall prevalence of HCV infection among hemodialysis patients in Egypt was calculated as 50% (95%CI: 46%-55%).

Israel

The latest reports on the prevalence of HCV infection among general Israeli population reveal a rate of 1.96%[133]. HCV genotype 1 (70%) and 3 (20%) were the most inspected ones among the individuals[133]. No recent study was found for Israel regarding the HCV infection among hemodialysis patients.

Cyprus

The general prevalence of HCV infection in Cyprus is reported as 0.5% and the most dominant genotype in Cyprus was reported to be genotype 1[4,45]. To our knowledge, there is no publication on the prevalence of HCV infection among hemodialysis patients in this country.

DISCUSSION

In hemodialysis centers, hepatitis C virus infection remains a major concern. Blood transfusion as well as nosocomial infection continue to play important roles in the transmission of HCV[134]. An overall prevalence of 25.3% of HCV infection was reported among hemodialysis patients in the Middle-East region according to the present study. Regarding the result of the present investigation, the prevalence was higher in ages 40 to 50 years old; this might be due to higher rate of renal diseases in older ages. Also, among patients who were under hemodialysis for more than 5 years the prevalence of hepatitis C was higher than those who were treated for a shorter time period. Other than some countries such as Afghanistan, Israel, Cyprus, Qatar, Kuwait, UAE, Oman, and Bahrain, which had no recent data on the prevalence of HCV infection among hemodialysis patients, among the other countries a rate of 9% in Lebanon to 54% in Syria were observed. Despite insufficient data on Syria, according to the only presented paper from this country, it had a higher prevalence even than Egypt, a country with the highest reported HCV infection among the Middle-East countries[90]. Lebanon (9%), Iran (12%), Saudi Arabia (19%), Iraq (20%), Turkey (23%), and Palestine (18%), depicted lower prevalences although the number of studies seems to be not sufficient in Lebanon, Saudi Arabia, and Palestine. More investigations are expected and suggested to give out more accurate conclusion in these countries. Moreover, providing updated surveys in UAE, Oman, Afghanistan, Israel, Cyprus, Qatar, Kuwait, and Bahrain, are greatly recommended.

In Saudi Arabia before 2000, prevalence reports ranged from 15% to 90% among different hemodialysis centers[81]. Afterwards, studies showed a range of 14.7% to 43.9%[42,82]. Many of these studies suggested that the duration of the dialysis session was more related to the chance of infection than the repeated blood transfusions[42,82]. Despite the further increase in dialysis services, the prevalence did not have a significant change during the recent years in Saudi Arabia which may be due to better implementation of infection-control policies and also the screening methods in certain hemodialysis units[81]. However, the studies on the epidemiology of HCV infection related to hemodialysis are noticeably insufficient. Our estimated prevalence in Iran is also lower than the previously reported 13.57% in 2010 by Alavian *et al*[135]. In their systematic review that was performed from April 2001 to March 2008 and even before, 12 studies were evaluated, of a total sample size of 5280, a prevalence of 12.91 (95%CI: 10.25-15.56) was reported; however, most of the studies are conducted in a limited number of provinces which applies some limitations to the outcome. In Iraq, Adherence to infection precautions, screening of transfusions, the use of separate machines for the infected patients, and using erythropoietin instead of blood transfusions may be the cause of the overall lower prevalence rates[92,94,96-100].

In Yemen, sensitivity analyses suggest that there may be an underestimation for HCV prevalence since measured HCV prevalence in this country increased from 1.9% in baseline analysis to 2.8% and 2.4% in the two sensitivity analyses, respectively[84]; HCV prevalence among hemodialysis patients increased from 40.0% in 1999 to 62.7% in 2007[85,136], and 42% in our estimation. Insufficient data on the prevalence of HCV infection, particularly among the patients on maintenance hemodialysis is a barrier for determining the alterations in the trend and risk factors of transmission; thus, present literature show an increase in the overall prevalence in Yemen. Moreover, in Syria, the higher rates of infection, ranged from 48.9% to 75%[105,106], seems to be continued during the past two decades as well as our present evaluation (54.4%). This might be due less than optimal screening of blood and blood products and poor sterilization of equipment in these patients[105-107]. Studies in Jordan showed a decline in the rates of HCV infection among hemodialysis patients (from 49.8% to 16.5%) though the overall rate was high (35%)[108-112]; standardized infection control protocols including the use of disposable gloves, kits, needles, dialyzers, and single use vials as well as disinfection of surfaces and dialysis machines between hemodialysis sessions with appropriate solutions were the reasons for the decline in the prevalence rates[113].

In Kuwait, Qatar, and UAE, non-nationals comprise more than three-fourths of the population[35].

Documents depict a high prevalence of HCV infection before 2006 among hemodialysis patients in Kuwait, ranging between 27% and 71%[27,28,30]. Obviously, studies on the prevalence of HCV infection and the possible changes in its trend are not well investigated in Kuwait country during the last decade, even the reports of the health ministry revealed no data in this case. In UAE, it seems that the medical care providers still do not take HCV infection as a major concern, especially among patients undergoing dialysis treatment, and studies are lacking and the changes in the trend are not measurable. In Qatar, HCV prevalence was as high as 44.6% in hemodialysis patients according to a recent systematic review[36]. Qadi *et al*[42] reported the prevalence of HCV infection among the Bahraini hemodialysis patients, a rate of 7.4% among 81 patients recruited from tertiary health centers of the country in 2004. The prevalence of HCV infection among Israeli hemodialysis patients in 1997 and 2001 reports showed rates of 12.3%[137] and 18%[138]. The latest reports published for Oman in 1992 and 1993 by Al-Dhahry *et al*[41] revealed a 26.5% prevalence for HCV infection in these patients. Although the prevalence of HCV infection is not high in these countries considering the latest reports, it is assumed that conducting new epidemiological surveys is needed for a better estimation.

Latest reports depicted the prevalence of HCV infection in the United States hemodialysis centers to be in a range of 8% to 16.8%[12,139], which was about 5 times greater than the prevalence within the country's general population (1.6%)[140]. The time spent on dialysis therapy has been suggested as an independent risk factor for the infection[141]. In Europe, a prevalence of 11.5% was reported in 2003, while Japan's HCV infection prevalence among hemodialysis patients was 13.4%[142]. In some other reports from European developed countries such as Belgium, Germany, Spain, France, Sweden, Poland, Hungary, United kingdom, and Italy, prevalence rates of 6.8%, 6%, 12%, 30%, 9%, 44%, 15%, 3%, and 16% were reported, respectively[143]. As it is demonstrated, in contrast with the developed countries, some Middle-East countries such as Iran, Iraq, Turkey, Lebanon, Palestine, and Saudi Arabia may have had better hygienic condition; however, the lower rates of infection can be due to lower number of patients and the sample population and also number of dialysis units in the country, for example in areas like Iraq and Palestine, specifically Gaza strip[102-104].

The mechanisms responsible for HCV infection transmission in hemodialysis services in the Middle-East countries has not been recognized properly yet. However, some investigations have reported that cross infection through hemodialysis machines may be in charge for the transmission which necessitates more attention on sterilization and control of infection in dialysis units[144]. Diagnosis and treatment of all hemodialysis patients who are infected with the virus, education of nurses and all health care providers involved with these cases, and organizing prevention programs regarding the natural characteristics of each country and its population are suggested as prevention programs which can be initiated in Middle-East countries for better evaluation and reduction of HCV infection[144,145]. Nevertheless, successful control of the infection needs further investigations to assess the effectiveness of different preventive and diagnostic policies. Preventive programs varies in different regions and various societies. Several studies are focused on isolating hemodialysis patients while some others attempted to use specified equipment and services for these patients and disinfection of the devices and the environment[135,146,147].

As a limitation of this study, the limited number of studies in some countries can be noticed. The low sample populations in a number of studies in some centers can be mentioned as another limiting factor for which the outcome could not be generalized. However, since the prevalence of HCV infection and also the number of the studies in this field were not significant, we could not omit these surveys.

Overall, in this paper we reported the prevalence of HCV infection among the countries of the Middle-East region considering the documents published during the past decade. Health policy makers and health care system should focus on the possible risk factors of each country individually in order to plan for effectively reduce the transmission rates and improve treatment methods for the infected ones. Also, experiences of the countries which were succeeded in reducing the incidence rate and the infection prevalence might be helpful if being shared.

ACKNOWLEDGMENTS

Authors wanted to thank Dr. Sahar Hosseini for helping in gathering the data, and Dr. Sedigheh Ebrahimi for her guides in study design and reviewing the paper. Authors also give their special thanks are also expressed to the staff at Tehran Hepatitis center and Shiraz medical student research committee for their help and support.

COMMENTS

Background

Hemodialysis is a major risk factor for hepatitis C virus (HCV) infection among the patients suffering from renal disease. Knowing the prevalence of this infection may help the health policy makers and care providers plan for better screening, management, and treatment of the infection.

Research frontiers

In this systematic review and meta-analysis the authors determine the prevalence of hepatitis C infection among 17 countries of the Middle-East region according to the related documents published during the last decade.

Innovations and breakthroughs

This research provides data for the health care system to have a sight on the

rates of the infection, complete and update their information, and plan for the future. Countries which lack data in this field should do their efforts toward providing them and those with high rates of infection should plan for better management of the case.

Applications

The results of the present study can be applied in health policy making and programming for better management of the dialysis centers as well as the infected individuals who may need for dialysis treatment in the future. Knowing the infection rate, the possible ways of transmission and complications of the infection are beneficial for future's planning.

Peer-review

This is a very interesting review article on the prevalence of hepatitis C among hemodialysis patients among the Middle-East countries. The author is investigating the infection state of HCV of the Middle-East region in the precise. Description of an Middle-Eastern medical state and the discussion about a route of infection think a requirement.

REFERENCES

1 **Simmonds P**, Holmes EC, Cha TA, Chan SW, McOmish F, Irvine B, Beall E, Yap PL, Kolberg J, Urdea MS. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; **74** (Pt 11): 2391-2399 [PMID: 8245854 DOI: 10.1099/0022-1317-74-11-2391]

2 **Alavian SM**, Hajarizadeh B, Ahmadzad-Asl M, Kabir A, Bagheri-Lankarani K. Hepatitis B Virus infection in Iran: A systematic review. *Hepat Mon* 2008; **8**: 281-294

3 **Smith DB**, Mellor J, Jarvis LM, Davidson F, Kolberg J, Urdea M, Yap PL, Simmonds P. Variation of the hepatitis C virus 5' non-coding region: implications for secondary structure, virus detection and typing. The International HCV Collaborative Study Group. *J Gen Virol* 1995; **76** (Pt 7): 1749-1761 [PMID: 9049380]

4 **Lavanchy D**. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831]

5 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969]

6 **Chou R**, Clark EC, Helfand M. Screening for hepatitis C virus infection: a review of the evidence for the US Preventive Services Task Force. *Ann Int Med* 2004; **140**: 465-479 [PMID: 15023713]

7 **Alter MJ**, Kuhnert WL, Finelli L. Control CfD, Prevention. Guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. Massachusetts Medical Society, 2003

8 **Centers for Disease Control and Prevention (CDC)**. Testing for HCV infection: an update of guidance for clinicians and laboratorians. *MMWR Morb Mortal Wkly Rep* 2013; **62**: 362-365 [PMID: 23657112]

9 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875]

10 **Pereira BJ**, Levey AS. Hepatitis C virus infection in dialysis and renal transplantation. *Kidney Int* 1997; **51**: 981-999 [PMID: 9083262]

11 **Fissell RB**, Bragg-Gresham JL, Woods JD, Jadoul M, Gillespie B, Hedderwick SA, Rayner HC, Greenwood RN, Akiba T, Young EW. Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents: the DOPPS. *Kidney Int* 2004; **65**: 2335-2342 [PMID: 15149347]

12 **Finelli L**, Miller JT, Tokars JI, Alter MJ, Arduino MJ. National surveillance of dialysis-associated diseases in the United States, 2002. *Semin Dial* 2005; **18**: 52-61 [PMID: 15663766]

13 **Fabrizi F**, Lunghi G, Raffaele L, Guarnori I, Bacchini G, Corti M, Pagano A, Erba G, Locatelli F. Serologic survey for control of hepatitis C in haemodialysis patients: third-generation assays and analysis of costs. *Nephrol Dial Transplant* 1997; **12**: 298-303 [PMID: 9132649]

14 **Biamino E**, Caligaris F, Ferrero S, Montalcini G, Bongiorno P, Scuvera I. [Prevalence of anti-HCV antibody positivity and seroconversion incidence in hemodialysis patients]. *Minerva Urol Nefrol* 1999; **51**: 53-55 [PMID: 10429410]

15 **Di Napoli A**, Pezzotti P, Di Lallo D, Petrosillo N, Trivelloni C, Di Giulio S. Epidemiology of hepatitis C virus among long-term dialysis patients: a 9-year study in an Italian region. *Am J Kidney Dis* 2006; **48**: 629-637 [PMID: 16997059]

16 **Couroucé AM**, Bouchardeau F, Chauveau P, Le Marrec N, Girault A, Zins B, Naret C, Poignet JL. Hepatitis C virus (HCV) infection in haemodialysed patients: HCV-RNA and anti-HCV antibodies (third-generation assays). *Nephrol Dial Transplant* 1995; **10**: 234-239 [PMID: 7538650]

17 **Covic A**, Iancu L, Apetrei C, Scripcaru D, Volovat C, Mititiuc I, Covic M. Hepatitis virus infection in haemodialysis patients from Moldavia. *Nephrol Dial Transplant* 1999; **14**: 40-45 [PMID: 10052474]

18 **Natov SN**, Lau JY, Bouthot BA, Murthy BV, Ruthazer R, Schmid CH, Levey AS, Pereira BJ. Serologic and virologic profiles of hepatitis C infection in renal transplant candidates. New England Organ Bank Hepatitis C Study Group. *Am J Kidney Dis* 1998; **31**: 920-927 [PMID: 9631834]

19 **Sypsa V**, Psichogiou M, Katsoulidou A, Skoutelis G, Moutafis S, Hadjiconstantinou V, Kakavas J, Kalapothaki V, Boletis J, Hatzakis A. Incidence and patterns of hepatitis C virus seroconversion in a cohort of hemodialysis patients. *Am J Kidney Dis* 2005; **45**: 334-343 [PMID: 15685512]

20 **Centers for Disease Control and Prevention (CDC)**. Hepatitis C virus transmission at an outpatient hemodialysis unit--New York, 2001-2008. *MMWR Morb Mortal Wkly Rep* 2009; **58**: 189-194 [PMID: 19265779]

21 **Wikipedia contributors**. List of Middle East countries by population. Wikipedia, The Free Encyclopedia 2016. [acccessed 2016 July 15]. Available from: URL: https://en.wikipedia.org/w/index.php?title=List_of_Middle_East_countries_by_population&oldid=729873384

22 **Sharifi V**, Mesgarpour B, Basirnia A, Amin-Esmaeeli M, Farhoudian A, Amini H, Mohammadi MR, Rahimi-Movaghar A, Salesian N, Yousefi-Nooraie R. Quality of Studies on the Prevalence of Psychiatric Disorders in Iran. *Iran J Psychiat Clin Psychol* 2012; **18**: 138-149

23 **Ades AE**, Lu G, Higgins JP. The interpretation of random-effects meta-analysis in decision models. *Med Decis Making* 2005; **25**: 646-654 [PMID: 16282215]

24 **Harris R**, Bradburn M, Deeks J, Harbord R, Altman D, Sterne J. Metan: fixed-and random-effects meta-analysis. *Stata J* 2008; **8**: 3

25 **Ameen R**, Sanad N, Al-Shemmari S, Siddique I, Chowdhury RI, Al-Hamdan S, Al-Bashir A. Prevalence of viral markers among first-time Arab blood donors in Kuwait. *Transfusion* 2005; **45**: 1973-1980 [PMID: 16371052]

26 **World Health Organization**. Country Cooperation Strategy for WHO and Kuwait 2012–2016. Available from: URL: http://apps.who.int/iris/bitstream/10665/113231/1/CCS_Kuwait_2014_EN_15234.pdf

27 **El-Reshaid K**, Kapoor M, Sugathan T, Al-Mufti S, Al-Hilali N. Hepatitis C virus infection in patients on maintenance dialysis in kuwait: epidemiological profile and efficacy of prophylaxis. *Saudi J Kidney Dis Transpl* 1995; **6**: 144-150 [PMID: 18583854]

28 **Wreghitt TG**. Blood-borne virus infections in dialysis units--a review. *Rev Med Virol* 1999; **9**: 101-109 [PMID: 10386337]

29 **Sadeghi F**, Salehi-Vaziri M, Almasi-Hashiani A, Gholami-Fesharaki M, Pakzad R, Alavian SM. Prevalence of Hepatitis C Virus Genotypes Among Patients in Countries of the Eastern Mediterranean Regional Office of WHO (EMRO): A Systematic Review and Meta-Analysis. *Hepat Mon* 2016; **16**: e35558 [PMID: 27274353]

30 **Mohamoud YA**, Riome S, Abu-Raddad LJ. Epidemiology of hepatitis C virus in the Arabian Gulf countries: Systematic review and meta-analysis of prevalence. *Int J Infect Dis* 2016; **46**: 116-125 [PMID: 26996460 DOI: 10.1016/j.ijid.2016.03.012]

31 **Aghemo A**, Dore GJ, Hatzakis A, Wedemeyer H, Razavi H. Estimating HCV disease burden - volume 3 (editorial). *J Viral*

Hepat 2015; **22** Suppl 4: 1-3 [PMID: 26513444]
32 **El Shahat YI**, Varma S, Bari MZ, Shah Nawaz M, Abdulrahman S, Pingle A. Hepatitis C virus infection among dialysis patients in United arab emirates. *Saudi J Kidney Dis Transpl* 1995; **6**: 157-162 [PMID: 18583857]
33 **Alfaresi MS**. Prevalence of hepatitis C virus (HCV) genotypes among positive UAE patients. *Mol Biol Rep* 2011; **38**: 2719-2722 [PMID: 21104026]
34 **Abro AH**, Al-Dabal L, Younis NJ. Distribution of hepatitis C virus genotypes in Dubai, United Arab Emirates. *J Pak Med Assoc* 2010; **60**: 987-990 [PMID: 21381547]
35 **Fent T.** Department of Economic and Social Affairs, Population Division, United Nations Expert Group Meeting on Social and Economic Implications of Changing Population Age Structures. *Eur J Population/Revue européenne de Démographie* 2008; **24**: 451-452 [DOI: 10.1007/s10680-008-9165-7]
36 **Mohamoud Y**. The epidemiology of hepatitis C virus in Qatar: A systematic review and meta-analysis. Qatar Foundation Annual Research Conference, 2013
37 **Bener A**, Al-Kaabi S, Derbala M, Al-Marri A, Rikabi A. The epidemiology of viral hepatitis in Qatar. *Saudi J Kidney Dis Transpl* 2009; **20**: 300-306 [PMID: 19237827]
38 **Messina JP**, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**: 77-87 [PMID: 25069599 DOI: 10.1002/hep.27259]
39 **Chemaitelly H**, Mahmud S, Rahmani AM, Abu-Raddad LJ. The epidemiology of hepatitis C virus in Afghanistan: systematic review and meta-analysis. *Int J Infect Dis* 2015; **40**: 54-63 [PMID: 26417880]
40 **Sanders-Buell E**, Rutvisuttinunt W, Todd CS, Nasir A, Bradfield A, Lei E, Poltavee K, Savadsuk H, Kim JH, Scott PT, de Souza M, Tovanabutra S. Hepatitis C genotype distribution and homology among geographically disparate injecting drug users in Afghanistan. *J Med Virol* 2013; **85**: 1170-1179 [PMID: 23918535]
41 **Al-Dhahry SH**, Aghanashinikar PN, al-Hasani MK, Buhl MR, Daar AS. Prevalence of antibodies to hepatitis C virus among Omani patients with renal disease. *Infection* 1993; **21**: 164-167 [PMID: 7690010]
42 **Qadi AA**, Tamim H, Ameen G, Bu-Ali A, Al-Arrayed S, Fawaz NA, Almawi WY. Hepatitis B and hepatitis C virus prevalence among dialysis patients in Bahrain and Saudi Arabia: a survey by serologic and molecular methods. *Am J Infect Control* 2004; **32**: 493-495 [PMID: 15573057]
43 **Almawi WY**, Qadi AA, Tamim H, Ameen G, Bu-Ali A, Arrayid S, Abou Jaoude MM. Seroprevalence of hepatitis C virus and hepatitis B virus among dialysis patients in Bahrain and Saudi Arabia. *Transplant Proc* 2004; **36**: 1824-1826 [PMID: 15350487]
44 **Janahi EM**, Al-Mannai M, Singh H, Jahromi MM. Distribution of Hepatitis C Virus Genotypes in Bahrain. *Hepat Mon* 2015; **15**: e30300 [PMID: 26977163]
45 **Demetriou VL**, van de Vijver DA, Kostrikis LG. Molecular epidemiology of hepatitis C infection in Cyprus: evidence of polyphyletic infection. *J Med Virol* 2009; **81**: 238-248 [PMID: 19107977]
46 **Alavian SM**, Adibi P, Zali MR. Hepatitis C virus in Iran: Epidemiology of an emerging infection. *Arch Iranian Med* 2005; **8**: 84-90
47 **Liakina V**, Hamid S, Tanaka J, Olafsson S, Sharara AI, Alavian SM, Gheorghe L, El Hassan ES, Abaalkhail F, Abbas Z, Abdou A, Abourached A, Al Braiki F, Al Hosani F, Al Jaberi K, Al Khatry M, Al Mulla MA, Al Quraishi H, Al Rifai A, Al Serkal Y, Alam A, Alashgar HI, Alawadhi S, Al-Dabal L, Aldins P, Alfaleh FZ, Alghamdi AS, Al-Hakeem R, Aljumah AA, Almessabi A, Alqutub AN, Alswat KA, Altraif I, Alzaabi M, Andrea N, Assiri AM, Babatin MA, Baqir A, Barakat MT, Bergmann OM, Bizri AR, Blach S, Chaudhry A, Choi MS, Diab T, Djauzi S, El Khoury S, Estes C, Fakhry S, Farooqi JI, Fridjonsdottir H, Gani RA, Ghafoor Khan A, Goldis A, Gottfredsson M, Gregorcic S, Hajarizadeh B, Han KH, Hasan I, Hashim A, Horvath G, Hunyady B, Husni R, Jafri W, Jeruma A, Jonasson JG, Karlsdottir B, Kim DY, Kim YS, Koutoubi Z, Lesmana LA, Lim YS, Löve A, Maimets M, Makara M, Malekzadeh R, Matičič M, Memon MS, Merat S, Mokhbat JE, Mourad FH, Muljono DH, Nawaz A, Nugrahini N, Priohutomo S, Qureshi H, Rassam P, Razavi H, Razavi-Shearer D, Razavi-Shearer K, Rozentale B, Sadik M, Saeed K, Salamat A, Salupere R, Sanai FM, Sanityoso Sulaiman A, Sayegh RA, Schmelzer JD, Sibley A, Siddiq M, Siddiqui AM, Sigmundsdottir G, Sigurdardottir B, Speiciene D, Sulaiman A, Sultan MA, Taha M, Tarifi H, Tayyab G, Tolmane I, Ud Din M, Umar M, Valantinas J, Videčnik-Zorman J, Yaghi C, Yunihastuti E, Yusuf MA, Zuberi BF, Gunter J. Historical epidemiology of hepatitis C virus (HCV) in select countries - volume 3. *J Viral Hepat* 2015; **22** Suppl 4: 4-20 [PMID: 26513445]
48 **Taherkhani R**, Farshadpour F. Epidemiology of hepatitis C virus in Iran. *World J Gastroenterol* 2015; **21**: 10790-10810 [PMID: 26478671 DOI: 10.3748/wjg.v21.i38.10790]
49 **Jahanbakhsh Sefidi F**, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Bokharaei-Salim F. Distribution of hepatitis C virus genotypes in Iranian chronic infected patients. *Hepat Mon* 2013; **13**: e7991 [PMID: 23550108]
50 **MohammadHassan KA**, Mir-Davood O. Evaluation of Diagnostic Value of Elisa Method (EIA) and PCR in Diagnosis of Hepatitis C Virus In Hemodialysis Patients. *Hepat Mon* 2006; **2006**: 19-23
51 **Samimirad K**, Shahbaz B, Noroozi M, Mahmoodi M, Fayaz-Vaseghi M. Prevalence of Hepatitis C virus antibody and related risk factors among hemodialysis patients in Markazi province (2004). *Arak Med Univ J* 2006; **9**: 1-12
52 **Bozorghi SH**, Ramezany H, Vahid T, Mostajeri A, Kareghar Fard H, Rezayi M, Ashayeri N, Alaviyan SM. Assessment of prevalence and risk factors of hepatitis C virus infection in haemodialysis patients in Ghazvi. *Sci J Iranian Blood Trans Organ* 2006; **2**: 331-337
53 **Kheradpezhouh M**, Taremi M, Gachkar L, Aghabozorgi S, Khoshbaten M. Presence and significance of transfusion-transmitted virus infection in Iranian patients on maintenance hemodialysis. *J Microbiol Immunol Infect* 2007; **40**: 106-111 [PMID: 17446957]
54 **Toosi MN**, Larti F, Rasteh M, Foroutan H, Salarieh N, Lessan-Pezeshki M, Abdollahi A, Seifi S, Razeghi E, Rahbar M. Risk factors and seroprevalence of hepatitis B and C infections among hemodialysis patients in Tehran. *Iran J Pathol* 2007; **2**: 181-186
55 **Somi MH**, Keivani H, Ardalan MR, Farhang S, Pouri AA. Hepatitis C virus genotypes in patients with end-stage renal disease in East Azerbaijan, Iran. *Saudi J Kidney Dis Transpl* 2008; **19**: 461-465 [PMID: 18445914]
56 **Jabbari A**, Besharat S, Khodabakshi B. Hepatitis C in hemodialysis centers of Golestan province, northeast of Iran (2005). *Hepat Mon* 2008; **8**: 61-65
57 **Samimi-Rad K**, Hosseini M. Hepatitis C virus infection and hcv genotypes of hemodialysis patients. *Iran J Pub Heal* 2008; **37**: 146-152
58 **Makhlough A**, Jamshidi M, Mahdavi MR. Hepatitis C prevalence studied by polymerase chain reaction and serological methods in haemodialysis patients in Mazandaran, Iran. *Singapore Med J* 2008; **49**: 921-923 [PMID: 19037560]
59 **Taziki O**, Espahbodi F. Prevalence of hepatitis C virus infection in hemodialysis patients. *Saudi J Kidney Dis Transpl* 2008; **19**: 475-478 [PMID: 18445917]
60 **Mansour-Ghanaei F**, Sadeghi A, Mashhour MY, Joukar F, Besharati S, Roshan ZA, Khosh-Sorur M. Prevalence of hepatitis B and C infection in hemodialysis patients of Rasht (Center of Guilan Province, Northern Part of Iran). *Hepat Mon* 2009; **9**: 45-49
61 **Assarehzadegan MA**, Shakerinejad G, Noroozkohnejad R, Amini A, Rahim Rezaee SA. Prevalence of hepatitis C and B infection and HC V genotypes among hemodialysis patients in Khuzestan province, southwest Iran. *Saudi J Kidney Dis Transpl* 2009; **20**: 681-684 [PMID: 19587521]
62 **Nemati E**, Alavian SM, Taheri S, Moradi M, Pourfarziani V, Einollahi B. Hepatitis C virus infection among patients on hemodialysis: a report from a single center in Iran. *Saudi J Kidney*

Dis Transpl 2009; **20**: 147-153 [PMID: 19112238]
63 **Zamani F**, Ameli M, Razmjou S, Shakeri R, Amiri A, Darvish R. Incidence of hepatitis C infection in patients on hemodialysis: a multicenter study of northern part of Iran. *Saudi J Kidney Dis Transpl* 2010; **21**: 1169-1171 [PMID: 21060201]
64 **Joukar F**, Besharati S, Mirpour H, Mansour-Ghanaei F. Hepatitis C and hepatitis B seroprevalence and associated risk factors in hemodialysis patients in Guilan province, north of Iran: HCV and HBV seroprevalence in hemodialysis patients. *Hepat Mon* 2011; **11**: 178-181 [PMID: 22087139]
65 **Hassanshahi G**, Arababadi MK, Assar S, Hakimi H, Karimabad MN, Abedinzadeh M, Rafatpanah H, Derakhshan R. Post-transfusion-transmitted hepatitis C virus infection: a study on thalassemia and hemodialysis patients in southeastern Iran. *Arch Virol* 2011; **156**: 1111-1115 [PMID: 21340738]
66 **Samimi-Rad K**, Hosseini M, Mobeini G, Asgari F, Alavian SM, Tahaei ME, Salari H. Hepatitis C virus infection among multi-transfused patients and personnel in haemodialysis units in central Islamic Republic of Iran. *East Mediterr Health J* 2012; **18**: 227-235 [PMID: 22574475]
67 **Zahedi MJ**, Darvish Moghaddam S, Alavian SM, Dalili M. Seroprevalence of Hepatitis Viruses B, C, D and HIV Infection Among Hemodialysis Patients in Kerman Province, South-East Iran. *Hepat Mon* 2012; **12**: 339-343 [PMID: 22783346 DOI: 10.5812/hepatmon.5969]
68 **Moini M**, Ziyaeyan M, Aghaei S, Sagheb MM, Taghavi SA, Moeini M, Jamalidoust M, Hamidpour L. Hepatitis C virus (HCV) Infection Rate among Seronegative Hemodialysis Patients Screened by Two Methods; HCV Core Antigen and Polymerase Chain Reaction. *Hepat Mon* 2013; **13**: e9147 [PMID: 24032048]
69 **Somi MH**, Etemadi J, Ghojazadeh M, Farhang S, Faramarzi M, Foroutan S, Soleimanpour M. Risk factors of HCV seroconversion in hemodialysis patients in tabriz, iran. *Hepat Mon* 2014; **14**: e17417 [PMID: 24976839]
70 **Tajbakhsh R**. Prevalence of hepatitis C and B virus infections among hemodialysis patients in Karaj, Iran. *Saudi J Kidney Dis Transpl* 2015; **26**: 792-796 [PMID: 26178560]
71 **Çeldir M**, Kara I, Coşkuner S, Keskin B, Küçüker M, Orer H, Ergönül O. Hepatitis C prevalence in Turkey: estimation through meta-analysis. *Eur J Pub Heal* 2014; **24**: cku163.032 [DOI: 10.1093/eurpub/cku163.032]
72 **Altindis M**, Dal T, Akyar I, Karatuna O, Gokahmetoglu S, Ulger ST, Kulah C, Uzun B, Şener AG, Ozdemir M. Six-year distribution pattern of hepatitis C virus in Turkey: a multicentre study. *Biotechnol Biotec Eq* 2016; **30**: 335-340 [DOI: 10.1080/13102818.2015.1093430]
73 **Karaca C**, Cakaloğlu Y, Demir K, Ozdil S, Kaymakoğlu S, Badur S, Okten A. Risk factors for the transmission of hepatitis C virus infection in the Turkish population. *Dig Dis Sci* 2006; **51**: 365-369 [PMID: 16534682]
74 **Olut AI**, Ozsakarya F, Dilek M. Seroprevalence of hepatitis C virus infection and evaluation of serum aminotransferase levels among haemodialysis patients in Izmir, Turkey. *J Int Med Res* 2005; **33**: 641-646 [PMID: 16372581]
75 **Selcuk H**, Kanbay M, Korkmaz M, Gur G, Akcay A, Arslan H, Ozdemir N, Yilmaz U, Boyacioglu S. Distribution of HCV genotypes in patients with end-stage renal disease according to type of dialysis treatment. *Dig Dis Sci* 2006; **51**: 1420-1425 [PMID: 16868830]
76 **Yakaryilmaz F**, Gurbuz OA, Guliter S, Mert A, Songur Y, Karakan T, Keles H. Prevalence of occult hepatitis B and hepatitis C virus infections in Turkish hemodialysis patients. *Ren Fail* 2006; **28**: 729-735 [PMID: 17162434]
77 **Sezer S**, Tutal E, Aldemir D, Türkoglu S, Demirel OU, Afsar B, Ozdemir FN, Haberal M. Hepatitis C infection in hemodialysis patients: Protective against oxidative stress? *Transplant Proc* 2006; **38**: 406-410 [PMID: 16549132]
78 **Kose S**, Gurkan A, Akman F, Kelesoglu M, Uner U. Treatment of hepatitis C in hemodialysis patients using pegylated interferon alpha-2a in Turkey. *J Gastroenterol* 2009; **44**: 353-358 [PMID: 19277451]
79 **Dağlar D**, Ergani A, Demirbakan H, Ozhak Baysan B, Ongüt G, Koçak H, Oğünç D, Akbaş H, Yıldırım B, Colak D. [Investigation of hepatitis B and hepatitis C virus infections by serological and molecular methods in hemodialysis patients]. *Mikrobiyol Bul* 2014; **48**: 143-150 [PMID: 24506725]
80 **World Health Organization**. The growing threats of hepatitis B and C in the Eastern Mediterranean region: a call for action. 2009. Available from: URL: http://apps.who.int/iris/bitstream/10665/122800/3/EM_RC56_r5_fr.pdf
81 **Karkar A**. Hepatitis C in dialysis units: the Saudi experience. *Hemodial Int* 2007; **11**: 354-367 [PMID: 17576302]
82 **Saxena AK**, Panhotra BR, Naguib M, Aboras MN, Sundaram DS, Venkateshappa CK, Khan WU. Prevalence of hepatitis C antibodies among hemodialysis patients in Al-hasa region of saudi arabia. *Saudi J Kidney Dis Transpl* 2001; **12**: 562-565 [PMID: 18209404]
83 **Hussein MM**, Mooij JM, Hegazy MS, Bamaga MS. The impact of polymerase chain reaction assays for the detection of hepatitis C virus infection in a hemodialysis unit. *Saudi J Kidney Dis Transpl* 2007; **18**: 107-113 [PMID: 17237902]
84 **Chaabna K**, Kouyoumjian SP, Abu-Raddad LJ. Hepatitis C Virus Epidemiology in Djibouti, Somalia, Sudan, and Yemen: Systematic Review and Meta-Analysis. *PLoS One* 2016; **11**: e0149966 [PMID: 26900839 DOI: 10.1371/journal.pone.0149966].]
85 **Selm SB**. Prevalence of hepatitis C virus infection among hemodialysis patients in a single center in Yemen. *Saudi J Kidney Dis Transpl* 2010; **21**: 1165-1168 [PMID: 21060200]
86 **Baghza NM**. The Prevalence of Hepatitis C Virus among Hemodialysis Patients in Yemen. *J Purity Utility React Environ* 2014; **3**: 62-66
87 **Aman K**, Al-Dubai SA, Aman R, Hawash A, Alshagga M, Kassim S. Prevalence and associated factors of hepatitis C virus infection among renal disease patients on maintenance hemodialysis in three health centers in Aden, Yemen: a cross sectional study. *Saudi J Kidney Dis Transpl* 2015; **26**: 380-385 [PMID: 25758898]
88 **Al-Hegami MA**, Al-Mamari A, Al-Kadasse AS, Al-Gasha'a FA, Al-Hag S, Al-Hegami AA. Prevalence and Risk Factors of Hepatitis B and Hepatitis C Virus Infections among Patients with Chronic Renal Failure in Zabeed City, Yemen Republic. *Open J Med Microbiol* 2015; **5**: 136 [DOI: 10.4236/ojmm.2015.53017]
89 **Al-Shamahy HA**, Abdu SSA. Genotyping of Hepatitis C Virus (HCV) in infected patients from Yemen. *Eur J Basic Med Sci* 2013; **3**: 78-82
90 **Banister RF**. [Leadership skill]. *Xianggang Hu Li Za Zhi* 1974; **16**: 40-44 [PMID: 4546629]
91 **Abdullah BA**, Khaled MD, Maarouf MN. Detection of hepatitis C virus (HCV) by ELISA, RIBA and Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) technique among kidney dialysis patients in Nineveh governorate/Iraq. *Sci J Thi-Qar* 2012; **3**: 55-67
92 **Abdullah AM**, Hardan A, Latif II. Genotyping of hepatitis C virus isolates from Iraqi hemodialysis patients by reverse transcription-PCR and one step nested RT-PCR. *Diyala J Med* 2012; **3**: 9-18
93 **Al-Dulaimi SBK**, Al-Ubadi AE, Al-Ubadi AE, Al-Bayatti EN, Al-Saday SDK. Toxoplasma gondii, HCV, and HBV seroprevalence in Haemodialysis patients with chronic renal failure in Al-Kindy Hospital Baghdad, Iraqi. *Al-Mustansiriyah J Sci* 2012; **23**: 33-38
94 **Al-Mashhadani JI**. Hepatitis C virus infection among haemodialysis patients in Al-Anbar governorate. *Iraqi J Com Med* 2007; **20**: 20-23
95 **Mnuti JK**, Al-Abbudi FA. Hepatitis C virus infection assessment among chronic hemodialysis patients in Al-Kadhmiya Teaching Hospital. *Iraqi Postgrad Med J* 2011; **10**: 460-464
96 **Ramzi ZS**, Abdulla AA, Al-Hadithi T, Al-Tawil N. Prevalence and risk factors for hepatitis C virus infection in hemodialysis patients in Sulaimani. *Zanco J Med Sci* 2010; **14**: 44-50
97 **Shihab SS**, Al-Hmudi HA, Al-Edani HS, Mahdi KH. Viral hepatitis infections in Basrah haemodialysis unit: serological diagnosis and viral loading. *Eur J Exp Biol* 2014; **4**: 106-112
98 **Khattab OS**. Prevalence and risk factors for hepatitis C virus

infection in hemodialysis patients in an Iraqi renal transplant center. *Saudi J Kidney Dis Transpl* 2008; **19**: 110-115 [PMID: 18087139]
99 **Al-Rubaie HMH**, Malik AS. Seroconversion Rate of Hepatitis C Virus Infection among Haemodialysis Patients in AL-Kadhimiya Teaching Hospital (Dialysis Unit). *Iraqi J Med Sci* 2011; **9**: 343-349
100 **Jasim NA**, Athbi HA. Prevalence and Risk Factors for Hepatitis Cand B Viruses Infection among Hemodialysis Patients in Holy Karbala, Iraq. Holy Karbala, Iraq, 2015
101 **Ayesh BM**, Zourob SS, Abu-Jadallah SY, Shemer-Avni Y. Most common genotypes and risk factors for HCV in Gaza strip: a cross sectional study. *Virol J* 2009; **6**: 105 [PMID: 19607718 DOI: 10.1186/1743-422X-6-105]
102 **El-kader Y El-Ottol A**, Elmanama AA, Ayesh BM. Prevalence and risk factors of hepatitis B and C viruses among haemodialysis patients in Gaza strip, Palestine. *Virol J* 2010; **7**: 210 [PMID: 20809985 DOI: 10.1186/1743-422X-7-210]
103 **Abumwais JQ**, Idris OF. Prevalence of hepatitis C, hepatitis B, and HIV infection among hemodialysis patients in Jenin District (Palestine). *Iran J Virol* 2010; **4**: 38-44
104 **Al Zabadi H**, Rahal H, Fuqaha R. Hepatitis B and C prevalence among hemodialysis patients in the West Bank hospitals, Palestine. *BMC Infect Dis* 2016; **16**: 41 [PMID: 26830673 DOI: 10.1186/s12879-016-1359-8]
105 **Abdulkarim AS**, Zein NN, Germer JJ, Kolbert CP, Kabbani L, Krajnik KL, Hola A, Agha MN, Tourogman M, Persing DH. Hepatitis C virus genotypes and hepatitis G virus in hemodialysis patients from Syria: identification of two novel hepatitis C virus subtypes. *Am J Trop Med Hyg* 1998; **59**: 571-576 [PMID: 9790432]
106 **Othman B**, Monem F. Prevalence of antibodies to hepatitis C virus among hemodialysis patients in Damascus, Syria. *Infection* 2001; **29**: 262-265 [PMID: 11688903]
107 **Moukeh G**, Yacoub R, Fahdi F, Rastam S, Albitar S. Epidemiology of hemodialysis patients in Aleppo city. *Saudi J Kidney Dis Transpl* 2009; **20**: 140-146 [PMID: 19112237]
108 **Bdour S**. Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping. *J Med Microbiol* 2002; **51**: 700-704 [PMID: 12171303]
109 **Said RA**, Hamzeh YY, Mehyar NS, Rababah MS. Hepatitis C virus infection in hemodialysis patients in jordan. *Saudi J Kidney Dis Transpl* 1995; **6**: 140-143 [PMID: 18583853]
110 **Ghunaimat M**, Al-Mrayat Z, Abbadi R, Akash N. Point prevalence of hepatitis C antibodies among hemodialysis patients at king Hussein Medical Center. *J Royal Med Serv* 2007; **14**: 63-67
111 **Al-Jamal M**, Al-Qudah A, Al-Shishi KF, Al-Sarayreh A, Al-Quraan L. Hepatitis C virus (HCV) infection in hemodialysis patients in the south of Jordan. *Saudi J Kidney Dis Transpl* 2009; **20**: 488-492 [PMID: 19414962]
112 **Batchoun RG**, Al-Najdawi MA, Al-Taamary S. Anti-ENA antibody profile in hepatitis C patients undergoing hemodialysis. *Saudi J Kidney Dis Transpl* 2011; **22**: 682-688 [PMID: 21743211]
113 **Ghazzawi I**, Yassin M, Alshebly H, Sheyyab S, Alqudah B, SN NA. Prevalence of Hepatitis B and C Viruses in Hemodialysis Patients at JRMS. *JRMS* 2015; **22**: 69-75
114 **Abou Rached A**, Abou Kheir S, Saba J, Ammar W. Epidemiology of hepatitis B and hepatitis C in Lebanon. *Arab J Gastroenterol* 2016; **17**: 29-33 [PMID: 27032492]
115 **Matar GM**, Sharara HM, Abdelnour GE, Abdelnoor AM. Genotyping of hepatitis C virus isolates from Lebanese hemodialysis patients by reverse transcription-PCR and restriction fragment length polymorphism analysis of 5' noncoding region. *J Clin Microbiol* 1996; **34**: 2623-2624 [PMID: 8880537]
116 **Abourached A**, Saniour P, Bou Jaoude J. prevalence des hepatities B et C chez les donneurs de sang, les hémodialysés et les toxicomanes au Liban. J Boujaoude, JFPD, GCB, 2007
117 **Abou Rached A**, El Khoury L, El Imad T, Geara AS, Jreijiry J, Ammar W. Incidence and prevalence of hepatitis B and hepatitis C viruses in hemodialysis patients in Lebanon. *World J Nephrol* 2016; **5**: 101-107 [PMID: 26788469 DOI: 10.5527/wjn.v5.i1.101]
118 **Naman RE**, Mansour I, Klayme S, Khalil G. [Hepatitis C virus in hemodialysis patients and blood donors in Lebanon]. *J Med Liban* 1996; **44**: 4-9 [PMID: 8965318]
119 **el-Ahmady O**, Halim AB, Mansour O, Salman T. Incidence of hepatitis C virus in Egyptians. *J Hepatol* 1994; **21**: 687 [PMID: 7814820]
120 **Khattab MA**, Eslam M, Sharwae MA, Hamdy L. Seroprevalence of hepatitis C and B among blood donors in Egypt: Minya Governorate, 2000-2008. *Am J Infect Control* 2010; **38**: 640-641 [PMID: 20400204]
121 **Abdel-Wahab MF**, Zakaria S, Kamel M, Abdel-Khaliq MK, Mabrouk MA, Salama H, Esmat G, Thomas DL, Strickland GT. High seroprevalence of hepatitis C infection among risk groups in Egypt. *Am J Trop Med Hyg* 1994; **51**: 563-567 [PMID: 7527186]
122 **Gohar SA**, Khalil RY, Elaish NM, Khedr EM, Ahmed MS. Prevalence of antibodies to hepatitis C virus in hemodialysis patients and renal transplant recipients. *J Egypt Public Health Assoc* 1995; **70**: 465-484 [PMID: 17214170]
123 **Hassan AA**, Khalil R. Hepatitis C in dialysis patients in egypt: relationship to dialysis duration, blood transfusion, and liver disease. *Saudi J Kidney Dis Transpl* 2000; **11**: 72-73 [PMID: 18209303]
124 **Zekri AR**, Sedkey L, el-Din HM, Abdel-Aziz AO, Viazov S. The pattern of transmission transfusion virus infection in Egyptian patients. *Int J Infect Dis* 2002; **6**: 329-331 [PMID: 12725217]
125 **Gad A**, Tanaka E, Orii K, Rokuhara A, Nooman Z, El-Hamid Serwah A, El-Sherif A, El-Essawy M, Yoshizawa K, Kiyosawa K. Clinical significance of T.T. virus infection in maintenance hemodialysis patients of an endemic area for hepatitis C infection. *Hepatol Res* 2002; **22**: 13-19 [PMID: 11804829]
126 **Kandil ME**. Hepatitis C and B Viruses Among Some High Risk Groups of Egyptian Children. *J Med Sci* 2007; **7**: 1259-1267
127 **Soliman AR**, Momtaz Abd Elaziz M, El Lawindi MI. Evaluation of an isolation program of hepatitis C virus infected hemodialysis patients in some hemodialysis centers in egypt. *ISRN Nephrol* 2013; **2013**: 395467 [PMID: 24967226 DOI: 10.5402/2013/395467]
128 **Khodir SA**, Alghateb M, Okasha KM, Shalaby Sel-S. Prevalence of HCV infections among hemodialysis patients in Al Gharbiyah Governorate, Egypt. *Arab J Nephrol Transplant* 2012; **5**: 145-147 [PMID: 22967252]
129 **Zahran AM**. Prevalence of seroconversion of hepatitis C virus among hemodialysis patients in Menoufia Governorate, Egypt. *Arab J Nephrol Transplant* 2014; **7**: 133-135 [PMID: 25366511]
130 **Sarhan II**, Kamel CR. Prevalence of hepatitis C virus seroconversion among hemodialysis patients in Egypt. *Egypt Liver J* 2015; **5**: 34-39 [DOI: 10.1097/01.elx.0000463167.48017.41]
131 **ElSharkawy M**, Sarhan I, Afifi A, Aboseif k, Mady G, Eltayeb M, Soliman Y, ElShenawy H, ElSayed H, Elsharawy A. Fp722 current status of HCV infection in hemodialysis patients in Egypt. *Nephrol Dial Transpl* 2015; **30**: iii317-iii318 [DOI: 10.1093/ndt/gfv183.40]
132 **Senosy SA**, El Shabrawy EM. Hepatitis C virus in patients on regular hemodialysis in Beni-Suef Governorate, Egypt. *J Egypt Pub Heal Assoc* 2016; **91**: 86-89 [DOI: 10.1097/01.EPX.0000484091.57255.c0]
133 **Zuckerman E**. Liver Unit, Carmel Medical Center, Haifa, Israel. Sources for the epidemiology of hepatitis C in Israel. Conversation with: Razavi HA. USA: Center for Disease Analysis, Kromite, Louisville, CO, 2010
134 **Su Y**, Norris JL, Zang C, Peng Z, Wang N. Incidence of hepatitis C virus infection in patients on hemodialysis: a systematic review and meta-analysis. *Hemodial Int* 2013; **17**: 532-541 [PMID: 23072424]
135 **Alavian SM**, Kabir A, Ahmadi AB, Lankarani KB, Shahbabaie MA, Ahmadzad-Asl M. Hepatitis C infection in hemodialysis patients in Iran: a systematic review. *Hemodial Int* 2010; **14**: 253-262 [PMID: 20491973]
136 **Chaabna K**, Abu Raddad L. The Epidemiology Of Hepatitis C Virus In Yemen: A Systematic Review And Meta-analysis. Qatar Foundation Annual Research Conference, 2014
137 **Weinstein T**, Zevin D, Ori Y, Korzets A, Chagnac A, Herman M, Tur-Kaspa R, Gafter U. Hepatitis C infection in renal transplant

recipients in Israel. *Transplant Proc* 1997; **29**: 2696-2698 [PMID: 9290794]
138 **Weinstein T**, Tur-Kaspa R, Chagnac A, Korzets A, Ori Y, Zevin D, Herman M, Gafter U. Hepatitis C infection in dialysis patients in Israel. *Isr Med Assoc J* 2001; **3**: 174-177 [PMID: 11303373]
139 **Bergman S**, Accortt N, Turner A, Glaze J. Hepatitis C infection is acquired pre-ESRD. *Am J Kidney Dis* 2005; **45**: 684-689 [PMID: 15806471]
140 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714 [PMID: 16702586]
141 **Patel PR**, Thompson ND, Kallen AJ, Arduino MJ. Epidemiology, surveillance, and prevention of hepatitis C virus infections in hemodialysis patients. *Am J Kidney Dis* 2010; **56**: 371-378 [PMID: 20570422]
142 **Goodkin DA**, Bragg-Gresham JL, Koenig KG, Wolfe RA, Akiba T, Andreucci VE, Saito A, Rayner HC, Kurokawa K, Port FK, Held PJ, Young EW. Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *J Am Soc Nephrol* 2003; **14**: 3270-3277 [PMID: 14638926]
143 **Jadoul M**, Poignet J-L, Geddes C, Locatelli F, Medin C, Krajewska M, Barril G, Scheuermann E, Sonkodi S, Goubau P. The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. *Nephrol Dial Transpl* 2004; **19**: 904-909 [DOI: 10.1093/ndt/gfh012]
144 **Alavian SM**. A shield against a monster: Hepatitis C in hemodialysis patients. *World J Gastroenterol* 2009; **15**: 641-646 [PMID: 19222088 DOI: 10.3748/wjg.15.641]
145 **Alavian SM**. We Need a New National Approach to Control Hepatitis C: It is Becoming too Late. *Hepat Mon* 2008; **8**: 165-169
146 **Barril G**, Traver JA. Decrease in the hepatitis C virus (HCV) prevalence in hemodialysis patients in Spain: effect of time, initiating HCV prevalence studies and adoption of isolation measures. *Antiviral Res* 2003; **60**: 129-134 [PMID: 14638409]
147 **Alter MJ**, Lyerla RL, Tokars JI, Miller ER, Arduino MJ. Recommendations for preventing transmission of infections among chronic hemodialysis patients. Morbidity and Mortality Weekly Report: Recommendations and Reports, 2001: 1-43

P- Reviewer: Ahmed Said ZN, Alexopoulou A, Chemaitelly H, Farshadpour F, Larrubia JR, Nakajima H, Vilibic-Cavlek T
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.167

World J Gastroenterol 2017 January 7; 23(1): 167-172
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Ileo-ileal intussusception caused by lymphangioma of the small bowel treated by single-incision laparoscopic-assisted ileal resection

Atsushi Kohga, Akihiro Kawabe, Yuto Hasegawa, Kiyoshige Yajima, Takuya Okumura, Kimihiro Yamashita, Jun Isogaki, Kenji Suzuki, Akira Komiyama

Atsushi Kohga, Akihiro Kawabe, Yuto Hasegawa, Kiyoshige Yajima, Takuya Okumura, Kimihiro Yamashita, Jun Isogaki, Kenji Suzuki, Division of Surgery, Fujinomiya City General Hospital, Fujinomiya, Shizuoka 4180076, Japan

Akira Komiyama, Division of Pathology, Fujinomiya City General Hospital, Fujinomiya, Shizuoka 4180076, Japan

Author contributions: All authors contributed to the acquisition of data and the writing and revision of this manuscript.

Institutional review board statement: This study was approved by the Institutional Review Board of Fujinomiya City General Hospital.

Informed consent statement: This case report is provided for academic communication only, not for other purposes. In this case report, the images do not disclose the patient's personal information. Consent was acquired from the patient for publication of this case report.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Manuscript source: Unsolicited manuscript

Correspondence to: Atsushi Kohga, MD, Division of Surgery, Fujinomiya City General Hospital, 3-1, Nishiki-cho, Fujinomiya, Shizuoka 4180076, Japan. akohga11@yahoo.co.jp
Telephone: +81-54-4273151
Fax: +81-54-4237232

Received: July 27, 2016
Peer-review started: July 30, 2016
First decision: September 28, 2016
Revised: October 7, 2016
Accepted: October 30, 2016
Article in press: October 31, 2016
Published online: January 7, 2017

Abstract

Intraabdominal lymphangiomas are uncommon; additionally, those affecting the gastrointestinal tract are rare and account for less than 1% of cases. Intussusception caused by a cystic lymphangioma of the small bowel is extremely rare. The patient was a 20-year-old woman who visited our emergency room with a complaint of abdominal pain. A computed tomography image revealed ileo-ileal intussusception with a leading hypovascular mass measuring 1 cm in a diameter. Single-incision laparoscopic-assisted ileal resection was performed. The surgical specimen consisted of a soft polycystic mass. Macroscopically, a pedunculated polyp with a convolutional pattern was found. Microscopically, the inner surfaces of the cysts were covered with a single layer of endothelial cells. On immunohistochemical examination, the endothelial cells were partially positive for D2-40 and CD34. Smooth muscle cells were also found around the cysts. The lesion was diagnosed as a cystic lymphangioma. Dozens of cases of small bowel lymphangiomas have previously been reported. Of these, cases with intussusception were very rare. This is the first case of small bowel intussusception due to lymphangioma treated by single-incision laparoscopic-assisted surgery.

Key words: Intussusception; Single-incision laparoscopic-

assisted surgery; Lymphangioma

Core tip: We observed an extremely rare case of small bowel intussusception caused by cystic lymphangioma. Dozens of lymphangiomas of the small bowel have previously been reported. Of these, few cases with intussusception have been reported. In the present case, single-incision laparoscopic-assisted surgery was useful for treating the telescoped lesion. To our knowledge, no cases of small bowel intussusception due to lymphangioma have been treated by laparoscopic surgery.

Kohga A, Kawabe A, Hasegawa Y, Yajima K, Okumura T, Yamashita K, Isogaki J, Suzuki K, Komiyama A. Ileo-ileal intussusception caused by lymphangioma of the small bowel treated by single-incision laparoscopic-assisted ileal resection. *World J Gastroenterol* 2017; 23(1): 167-172 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/167.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.167

INTRODUCTION

Compared to the rate observed in children, intussusception in adults is uncommon. Ninety-five percent of cases of intussusception occur in children[1,2]. Small bowel neoplasms such as metastatic tumours, adenocarcinoma, gastrointestinal tumours, lymphoma, carcinoid tumours and other tumours including benign lesions may induce intussusception in adults[3,4]. Lymphangioma is a congenital lymphatic system malformation[5], and a small proportion of lymphangiomas occur in the gastrointestinal tract[6]. Intussusception caused by lymphangioma of the small bowel is extremely rare[7]. Here, we report the case of a young woman with intussusception caused by lymphangioma of the small bowel who was treated by single-incision laparoscopic-assisted ileal resection.

CASE REPORT

The patient was a 20-year-old woman who visited our emergency room due to a complaint of abdominal pain. A physical examination revealed mild tenderness in the upper right abdomen without peritoneal irritation signs. The patient had no past history of comorbid medical or surgical illness. Laboratory data showed slight leukocytosis (WBC 108 × $10^2/\mu L$) with a moderately elevated C-reactive protein level (CRP, 4.19 mg/dL).

Ultrasonography of the upper right abdomen indicated no significant findings. A computed tomography (CT) image revealed ileo-ileal intussusception in the lower abdomen with a leading hypovascular mass measuring 1 cm in a diameter (Figure 1). The preoperative diagnosis was intussusception of the small bowel due to inverted Meckel's diverticulum or a benign tumour.

Single-incision laparoscopic-assisted ileal resection was immediately performed. A single incision measuring approximately 2.5 cm long was performed at the umbilicus, and two 5-mm trocars were placed in the incision using a disposable protractor, one for camera port and the other for the forceps. An ileo-ileal intussusception was found via laparoscopic inspection. The involved segment of the small bowel was removed through the incision using forceps and hands (Figure 2). The intussusception was released by applying the Hutchinson manoeuvre. We then observed that the soft mass was the leading point of the intussusception (Figure 3). Ileal resection was performed, and the mass was resected.

The surgical specimen consisted of a soft polycystic mass. Macroscopically, a pedunculated polyp with a convolutional pattern was found. A polycystic appearance was noted on the cut surface. We performed additional resection of the ileum on both the oral and anal side due to an insufficient margin. The cut sections of the specimen revealed multiple cystic lesions located mainly in the mucosal to submucosal layer (Figure 4). Microscopically, the inner surfaces of the cysts were covered with a single layer of endothelial cells. No blood cells were found in the cysts (Figure 5). On immunohistochemical examination, the endothelial cells were partially positive for D2-40 and CD34. Smooth muscle cells were also found around the cysts (Figure 6). The lesion was diagnosed as cystic lymphangioma. The postoperative course was uneventful, and the patient was discharged on the 7th postoperative day without postoperative complications.

DISCUSSION

In the present report, we present an extremely rare case of small bowel intussusception caused by cystic lymphangioma of the ileum. Cystic lymphangioma was first described by Radenbacker[8]. Lymphangioma is a congenital disease and occurs more frequently in the head, neck and axilla; however, intraabdominal lymphangiomas are uncommon, and those that affect the gastrointestinal tract are rare and account for less than 1% of cases[5,9,10].

Generally, lymphangiomas primarily occur in children. However, reported cases of small bowel lymphangiomas exhibit a wide age range. Morris-Stiff *et al*[11] reported that a Japanese/Taiwanese predisposition to small bowel lymphangiomas may exist. According to the Japanese literature, Kurokawa reviewed 40 patients with small bowel lymphangiomas and suggested a slight male predominance[12].

Pathologically, lymphangiomas are divided into three

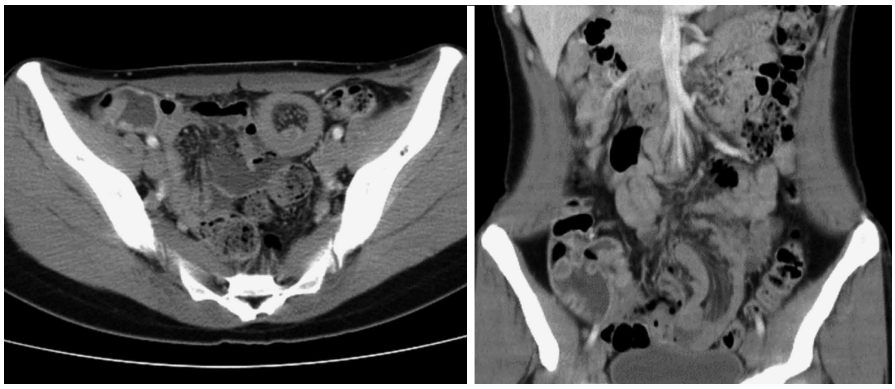


Figure 1 Computed tomography of abdomen: Computed tomography revealed an ileo-ileal intussusception. The leading point revealed hypovascular mass measuring 1 cm in a diameter.

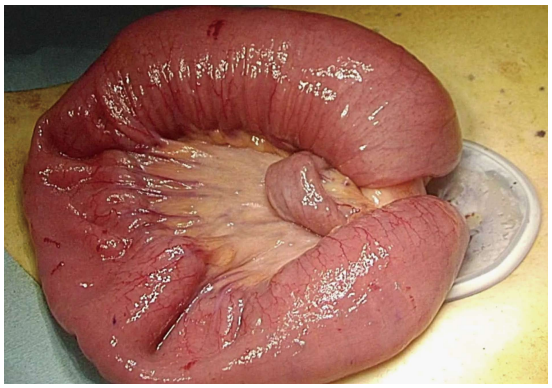


Figure 2 The lesion was externalized using forceps and hands through the umbilical incision.

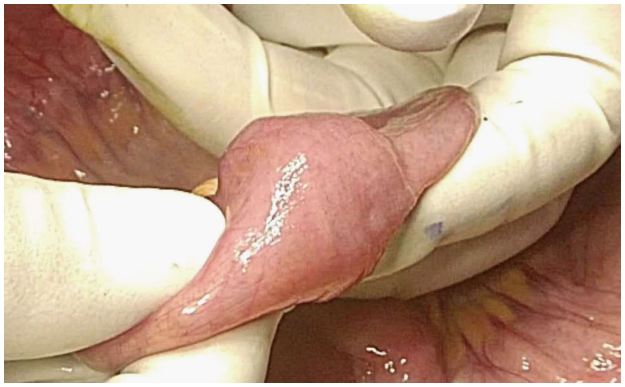


Figure 3 Leading point of intussusception was palpable and soft mass was confirmed.

groups: simple capillary lymphangioma, cavernous lymphangioma, and cystic lymphangioma[7,13,14]. Of these, the present case was diagnosed as a cystic lymphangioma consisting of a large macroscopic lymphatic space with investitures of collagen and smooth muscle[15].

Small bowel lymphangiomas usually present no symptoms but can sometimes cause melena, abdominal pain, intussusception, ileus and protein-losing gastroenteropathy[5,16]. Although CT images are useful for the diagnosis of intussusception, radiologic studies are not a conclusive test for diagnosis[1,7].

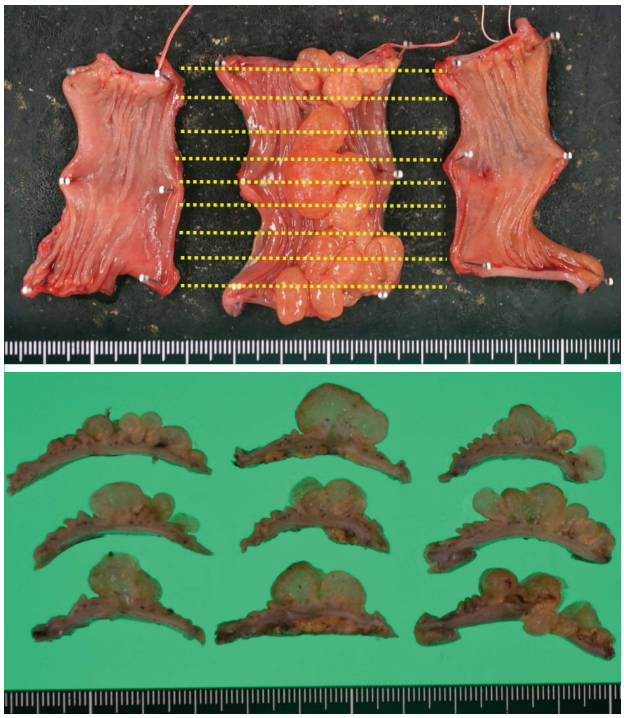


Figure 4 Microscopic findings of the resected specimen. Polycystic mass with convolutional pattern was found. Cutting lines are shown by lines.

In the present case, the presence of a mild tenderness in the upper right abdomen interfered with the diagnosis of ileo-ileal intussusception by ultrasonography.

Dozens of lymphangiomas of the small bowel have previously been reported[17-20]. Of these, a few cases have been reported to result in intussusception[7,21]. In the Japanese literature, Kurokawa reported 7 cases of small bowel lymphangioma with intussusception[12].

Surgical resection is the standard treatment. Relapses may occur if vesicles or part of the tumour remain unresectable[5,22-24]. Therefore, it seems plau-

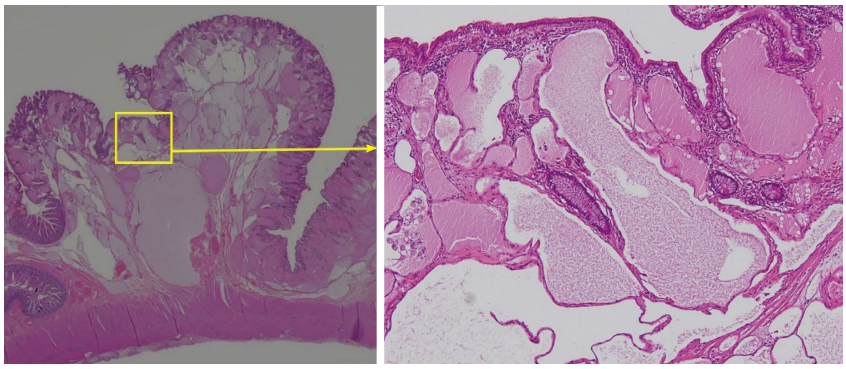


Figure 5 Microscopic findings: Cysts were lined by a flat epithelial endothelium. No blood cells were found.

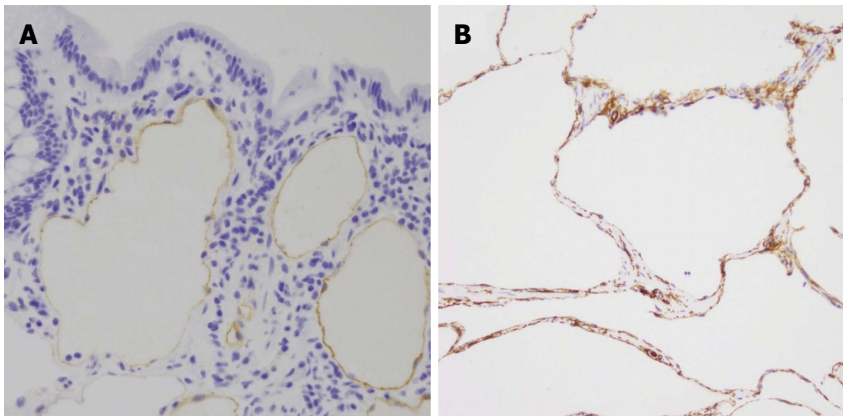


Figure 6 Immunohistochemical examination: The endothelial cells were partially positive for D2-40 (A) and CD34 (B).

sible that additional resection should be performed if the surgical margin is not sufficient.

Generally, single-incision laparoscopic-assisted surgery (SILS) is considered a less invasive and more aesthetic method than conventional multi-port laparoscopic surgery[25]. However, SILS currently has limitations for its indication[26,27]. With regard to small intestine resection, Nickerson *et al*[28] suggested that SILS is effective and feasible for resection of small bowel tumours. Additionally, some authors have reported cases of small bowel intussusception treated by SILS[25,29]. In the present case, we removed the telescoped segment through the umbilical incision; then, reduction was performed under direct vision. We consider SILS a feasible procedure for small bowel intussusception only if the intussusception is thought to be caused by a benign lesion and the telescoped segment is sufficiently short to pass the umbilical incision. To our knowledge, no cases of small bowel intussusception due to lymphangioma have been treated by laparoscopic surgery or SILS.

Recently, several cases of small bowel lymphangioma causing intestinal bleeding have been treated by endoscopic polypectomy using double-balloon enteroscopy[16,30]. Endoscopic polypectomy might be a useful method to treat small bowel lymphangioma without intussusception.

In conclusion, we reported a rare case of small bowel intussusception caused by a cystic lymphangioma. SILS was useful for the small bowel intussusception caused by a benign lesion in the present case.

COMMENTS

Case characteristics

A 20-year-old woman presented with an abdominal pain. Mild tenderness in upper right abdomen without peritoneal irritation signs was found.

Clinical diagnosis

Intussusception of small bowel.

Differential diagnosis

Intussusception of small bowel due to inverted Meckel's diverticulum or a benign tumor.

Laboratory diagnosis

Laboratory data showed slight leukocytosis (WBC, 108 × 10^2/μL) with moderately elevated C-reavtive protein (CRP, 4.19 mg/dL), suggesting presence of inflammation.

Imaging diagnosis

Ileo-ileal intussusception in the lower abdomen with the leading point of hypovascular mass measuring 1 cm in a diameter.

Pathological diagnosis
Cystic lymphangioma of ileum.

Treatment
Surgical resection.

Related reports
Previously, dozens of lymphangioma of the small bowel had been reported. Of these, a few cases have been reported to set up intussusception.

Term explanation
Lymphangiomas is congenital disease and occur more frequently in the head, neck and axilla, however, intraabdominal lymphangiomas are uncommon. Pathologically, Lymphangiomas are divided into three groups: simple capillary lymphangioma, cavernous lymphangioma, and cystic lymphangioma.

Experiences and lessons
The authors experienced a rare case of small bowel intussusception caused by cystic lymphangioma. single-incision laparoscopic-assisted surgery was useful for the small bowel intussusception caused by benign lesion as the present case.

Peer-review
The authors offered an interesting case with well treatment. Indeed, it is rare. The authors described a patient got ileo-ileal intussusception caused by lymphangioma of small bowel, and treated by single-incision lapascopic-assisted ileal resection.

REFERENCES

1 **Begos DG**, Sandor A, Modlin IM. The diagnosis and management of adult intussusception. *Am J Surg* 1997; **173**: 88-94 [PMID: 9074370 DOI: 10.1016/S0002-9610(96)00419-9]
2 **Marinis A**, Yiallourou A, Samanides L, Dafnios N, Anastasopoulos G, Vassiliou I, Theodosopoulos T. Intussusception of the bowel in adults: a review. *World J Gastroenterol* 2009; **15**: 407-411 [PMID: 19152443 DOI: 10.3748/wjg.15.407]
3 **Hodin RA**, Matthews JB. Small intestine. In: Norton JA, Barie PS, Bollinger RR, Chang AE, Lowry SF, Mulfihill SJ, editors. Surgery: Basic Science and Clinical Evidence. 2nd ed. New York: Springer, 2008: 963-990
4 **Coco C**, Rizzo G, Manno A, Mattana C, Verbo A. Surgical treatment of small bowel neoplasms. *Eur Rev Med Pharmacol Sci* 2010; **14**: 327-333 [PMID: 20496543]
5 **Uncu H**, Erdem E, Kuterdem E. Lymphangiomas of the ileum: a report of two cases and a review of the literature. *Surg Today* 1997; **27**: 542-545 [PMID: 9306548 DOI: 10.1007/BF02385808]
6 **Good CA**. Tumors of the small intestine. *Am J Roentgenol Radium Ther Nucl Med* 1963; **89**: 685-705 [PMID: 13949051]
7 **Limaiem F**, Khalfallah T, Marsaoui L, Bouraoui S, Lahmar A, Mzabi S. Jejunal lymphangioma: an unusual cause of intussusception in an adult patient. *Pathologica* 2015; **107**: 19-21 [PMID: 26591627]
8 **Daniel S**, Lazarevic B, Attia A. Lymphangioma of the mesentery of the jejunum: report of a case and a brief review of the literature. *Am J Gastroenterol* 1983; **78**: 726-729 [PMID: 6637962]
9 **Huguet KL**, Metzger PP, Menke DM. Colorectal lymphangioma. *Am Surg* 2007; **73**: 414-416 [PMID: 17439042]
10 **Wan YL**, Lee TY, Hung CF, Ng KK. Ultrasound and CT findings of a cecal lymphangioma presenting with intussusception. *Eur J Radiol* 1998; **27**: 77-79 [PMID: 9587772 DOI: 10.1016/S0720-048X(97)00061-2]
11 **Morris-Stiff G**, Falk GA, El-Hayek K, Vargo J, Bronner M, Vogt DP. Jejunal cavernous lymphangioma. *BMJ Case Rep* 2011; **2011**: [PMID: 22696733 DOI: 10.1136/bcr.03.2011.4022]
12 **Kurokawa T**, Kanai M, Kaneko Y, Takahashi, Motohara T. A case of Lymphangioma of the Jejunum Presenting with Intussusception. *Jpn J Gastroenterol Surg* 2011; **44**: 1597-1603 [DOI: 10.5833/jjgs.44.1597]
13 **Wegener G**. Ueber. lymphangiome. *Arch Klin Chir* 1877; **20**: 641-707
14 **Chen CW**, Hsu SD, Lin CH, Cheng MF, Yu JC. Cystic lymphangioma of the jejunal mesentery in an adult: a case report. *World J Gastroenterol* 2005; **11**: 5084-5086 [PMID: 16124074 DOI: 10.3748/wjg.v11.i32.5084]
15 **Rieker RJ**, Quentmeier A, Weiss C, Kretzschmar U, Amann K, Mechtersheimer G, Bläker H, Herwart OF. Cystic lymphangioma of the small-bowel mesentery: case report and a review of the literature. *Pathol Oncol Res* 2000; **6**: 146-148 [PMID: 10936792 DOI: 10.1007/BF03032366]
16 **Kida A**, Matsuda K, Hirai S, Shimatani A, Horita Y, Hiramatsu K, Matsuda M, Ogino H, Ishizawa S, Noda Y. A pedunculated polyp-shaped small-bowel lymphangioma causing gastrointestinal bleeding and treated by double-balloon enteroscopy. *World J Gastroenterol* 2012; **18**: 4798-4800 [PMID: 23002353 DOI: 10.3748/wjg.v18.i34.4798]
17 **Childs WA**, Rosenstiel HC. Lymphangioma of the small intestine. *Surgery* 1955; **37**: 650-652 [PMID: 14373279]
18 **Barquist ES**, Apple SK, Jensen DM, Ashley SW. Jejunal lymphangioma. An unusual cause of chronic gastrointestinal bleeding. *Dig Dis Sci* 1997; **42**: 1179-1183 [PMID: 9201081 DOI: 10.1023/A:1018889620827]
19 **Kunimura T**, Inagaki T, Hayashi R, Katou H, Iwaku K, Morohoshi T. A case of multiple colonic lymphangiomas causing intussusception. *J Gastroenterol* 2005; **40**: 316-318 [PMID: 15830294 DOI: 10.1007/s00535-004-1544-1]
20 **Bucciero F**, Marsico M, Galli A, Tarocchi M. Small bowel lymphangioma: A rare case of intestinal bleeding. *Dig Liver Dis* 2015; **47**: 815 [PMID: 25936693 DOI: 10.1016/j.dld.2015.03.025]
21 **Oshita M**, Okazaki T, Yamataka A, Kusafuka J, Lane GJ, Matsumoto T, Miyano T. Jejuno-jejunal intussusception secondary to submucosal lymphangioma in a child. *Pediatr Surg Int* 2005; **21**: 1001-1003 [PMID: 16235059 DOI: 10.1007/s00383-005-1529-z]
22 **Raszkowski HJ**, Rehbock DJ, Cooper FG. Mesenteric and retroperitoneal lymphangioma. *Am J Surg* 1959; **97**: 363-367 [PMID: 13627371 DOI: 10.1016/0002-9610(59)90321-6]
23 **Chen CF**, Chuang CH, Lu CY, Hu C, Kuo TL, Hsieh JS. Adult intussusception secondary to lymphangioma of the cecum: a case report. *Kaohsiung J Med Sci* 2009; **25**: 347-352 [PMID: 19561001 DOI: 10.1016/S1607-551X(09)70527-3]
24 **Fernandes BF**, Moraes ÉN, de Oliveira FR, Benevides GN, Felipe-Silva A, Ferreira CR, de Alcântara PS, Tokeshi F, Martinês JA, Ferronato ÂE. Subserous lymphangioma of the sigmoid colon: an uncommon cause of acute abdomen in pediatric patients. *Autops Case Rep* 2015; **5**: 65-70 [PMID: 26894047 DOI: 10.4322/acr.2015.026]
25 **Yu H**, Wu S, Zhu R, Yu X. Single-incision laparoscopic-assisted ileal resection for adult intussusception. *J Minim Access Surg* 2016; **12**: 182-185 [PMID: 27073316 DOI: 10.4103/0972-9941.169986]
26 **Kim SG**, Moon JI, Choi IS, Lee SE, Sung NS, Chun KW, Lee HY, Yoon DS, Choi WJ. Risk factors for conversion to conventional laparoscopic cholecystectomy in single incision laparoscopic cholecystectomy. *Ann Surg Treat Res* 2016; **90**: 303-308 [PMID: 27274505 DOI: 10.4174/astr.2016.90.6.303]
27 **Trastulli S**, Cirocchi R, Desiderio J, Guarino S, Santoro A, Parisi A, Noya G, Boselli C. Systematic review and meta-analysis of randomized clinical trials comparing single-incision versus conventional laparoscopic cholecystectomy. *Br J Surg* 2013; **100**: 191-208 [PMID: 23161281 DOI: 10.1002/bjs.8937]
28 **Nickerson TP**, Aho JM, Bingener J. Single-incision laparoscopic resection of small bowel tumours: Making it easier for patient and surgeon. *J Minim Access Surg* 2016; **12**: 235-239 [PMID: 27279394 DOI: 10.4103/0972-9941.158958]
29 **Pitiakoudis M**, Romanidis K, Giatromanolaki A, Courcoutsakis N, Nagorni EA, Foutzitzi S, Tsaroucha A, Zezos P, Kouklakis G. Single-incision assisted laparoscopic surgery (SILS) in the treatment of an intussusception induced by a solitary

hamartomatous polyp: a case report and review of the literature. *J Med Case Rep* 2015; **9**: 125 [PMID: 26031291 DOI: 10.1186/s13256-015-0606-8]

30 **Li F**, Osuoha C, Leighton JA, Harrison ME. Double-balloon enteroscopy in the diagnosis and treatment of hemorrhage from small-bowel lymphangioma: a case report. *Gastrointest Endosc* 2009; **70**: 189-190 [PMID: 19152891 DOI: 10.1016/j.gie.2008.09.036]

P- Reviewer: Lakatos PL, Lee HC **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Wang CH

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.173

World J Gastroenterol 2017 January 7; 23(1): 173-177
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Synchronous quintuple primary gastrointestinal tract malignancies: Case report

Soo-Hong Kim, Byung-Soo Park, Hyun Sung Kim, Jae Hun Kim

Soo-Hong Kim, Byung-Soo Park, Hyun Sung Kim, Department of Surgery, Pusan National University Yangsan Hospital, Yangsan, Gyungsangnam-do 50612, South Korea

Jae Hun Kim, Department of Surgery, Pusan National University Hospital, Busan 602-739, South Korea

Author contributions: Kim SH and Park BS designed the report; Kim HS and Kim JH collected the patient's clinical information; Park BS analyzed the patient's data; Kim SH wrote the paper.

Institutional review board statement: The case report was exempt from institutional review board of the Pusan National University Yangsan Hospital.

Informed consent statement: The patient provided informed written consent prior to the treatment.

Conflict-of-interest statement: All the authors have no conflicts of interests to declare.

Manuscript source: Unsolicited manuscript

Correspondence to: Byung-Soo Park, MD, Department of Surgery, Pusan National University Yangsan Hospital, 20 Geumo-ro, Mulgeum-eup, Yangsan, Gyungsangnam-do 50612, South Korea. colonsurgeon@naver.com
Telephone: +82-55-3602124
Fax: +82-55-3602154

Received: September 9, 2016
Peer-review started: September 12, 2016
First decision: September 28, 2016
Revised: October 5, 2016
Accepted: October 30, 2016

Article in press: October 31, 2016
Published online: January 7, 2017

Abstract

Multiple primary malignancy is defined as two or more malignancies detected in an individual person. In particular, synchronous quintuple primary malignancy is extremely rare. A 52-year-old male with anal pain and intermittent blood-tinged stool was diagnosed with malignancies in the stomach, jejunum, ascending colon, transverse colon and rectum. He underwent a subtotal gastrectomy, segmental resection of the jejunum and total protocolectomy with end ileostomy. The postoperative pathologic findings were moderate differentiated gastric adenocarcinoma (pT1bN0M0, pStage ⅠA), combined adenocarcinoma and neuroendocrine carcinoma of the jejunum (pT3N0M0, pStage ⅡA), three mucinous adenocarcinoma of the ascending colon (pT3N0M0, pStage ⅡA), transverse colon (pT1N0M0, pStage Ⅰ) and rectum (pT3N1aM0, pStage ⅢB). The tumors did not lack MLH-1 and MSH-2 expression, as the markers (bat26, D5S346, bat25, D2S123) suggest MSI-H presence. Adjuvant chemoradiotherapy was started according to regimen, FOLFOX 4 for advanced rectal cancer. Six years post-operation, the patient is currently attending regular follow-ups without recurrence or metastasis.

Key words: Small bowel neoplasm; Stomach neoplasm; Synchronous quintuple primary cancer; Colon neoplasm

Core tip: We have experienced a case of synchronous quintuple primary gastrointestinal tract malignancies. Reports on synchronous quintuple primary malignancies are extremely rare. Hence, we report on the case, which developed in the stomach, jejunum, ascending

colon, transverse colon and rectum with literature review.

Kim SH, Park BS, Kim HS, Kim JH. Synchronous quintuple primary gastrointestinal tract malignancies: Case report. *World J Gastroenterol* 2017; 23(1): 173-177 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/173.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.173

INTRODUCTION

The occurrence of multiple primary malignancy, which is defined as two or more malignancies detected in an individual person, is becoming more frequent[1,2]. When multiple primary malignancies are diagnosed in multiple organs, they are classified into synchronous or metachronous subcategories according to the time of detection[3]. While there are a number of reports on cases of triple or quadruple primary malignancies, and metachronous quintuple primary malignancies, reports on synchronous quintuple primary malignancies are extremely rare. Here, we report on a case of synchronous quintuple primary gastrointestinal tract malignancy, which developed in the stomach, jejunum, ascending colon, transverse colon and rectum with literature review.

CASE REPORT

A 52-year-old male legal office worker was referred to our center as a result of anal pain and intermittent blood-tinged stool. Prior to his referral, the patient had quit smoking and consumption alcohol for an approximate 2 year period. However, the patient had a history of 30 years of smoking one pack a day and social drinking. His sister had been diagnosed with colon cancer at the age of 50 and underwent an operation. The other family members have no distinct medical history associated with malignancies. On a digital rectal examination, a rectal mass near the anus was found. Colonoscopy revealed three masses in the ascending colon, transverse colon and the rectum. Each mass was identified with mucinous adenocarcinoma, respectively. Esophagogastroduodenoscopy revealed an early gastric cancer (EGC) type Ⅱc lesion at the antrum, posterior wall of the stomach with atrophic gastritis (Figure 1). A computed tomography (CT) scan for staging found another mass with lymphadenopathy at the jejunum and showed no significant lymph node enlargement around the stomach, colon and rectum (Figure 2). A positron emission tomography (PET)/CT showed abnormal increases in fluorodeoxy glucose (FDG) uptake in the ascending colon, rectum and jejunum but no definite abnormal FDG uptake along the gastric wall and transverse colon was noted. Diffuse increased FDG uptake was found at both thyroid glands, which allowed for the diagnosis of thyroiditis (Figure 3). The patient underwent a subtotal gastrectomy, segmental resection of the jejunum and a total proctocolectomy with the end ileostomy simultaneously during one operation.

The pathologic results following the gastrectomy determined the gastric tumor to be EGC type Ⅱc, tubular adenocarcinoma, moderate differentiated, intestinal type by the Lauren classification system, with a depth of invasion into the submucosa (T1b) and no lymph node metastasis in 19 lymph nodes (pT1bN0M0, pStage Ⅰ A). The pathologic results at the jejunum revealed a 7.0 cm × 4.5 cm sized, combined adenocarcinoma and neuroendocrine carcinoma, with a depth of invasion into the subserosa (T3) and no lymph node metastasis in 8 lymph nodes (pT3N0M0, pStage Ⅱ A). The specimen gained from the total proctocolectomy had three adenocarcinomas at the ascending colon, 5.5 cm × 4.5 cm sized, mucinous adenocarcinoma, with modified Astler-Coller's stage C2, with a depth of invasion into the subserosa. At the transverse colon, mucinous adenocarcinoma arising from high grade tubulovillous adenoma was presented with a depth of involvement up until the muscularis mucosa without penetration. Results from analysis of the rectum showed 6.5 cm × 3.8 cm sized, mucinous adenocarcinoma with modified Astler-Coller's stage C2, invasion to perirectal fat tissue was identified. A total of 67 lymph nodes were dissected by proctocolectomy, with 1 perirectal lymph node showing metastasis. The stages of the ascending colon, transverse colon and rectal cancer were pStage Ⅱ A (pT3N0M0), pStage Ⅰ (pT1N0M0) and pStage Ⅲ B (pT3N1aM0), respectively. The tumors of the colon and rectum were evaluated with MLH-1 and MSH-2 expression for Lynch syndrome, both gene expressions were present and functioning. The gastric and colorectal tumors were evaluated with microsatellite instability (MSI). The results showed MSI-high (MSI-H) for MSI markers (bat26, D5S346, bat25, D2S123) and microsatellite stable (MSS) for the other marker (D17S250). Consequently, postoperative adjuvant chemoradiotherapy was started according to the regimen, FOLFOX 4 (Oxaliplatin, 5-fluorouracil (5-FU) and leucovorin) for rectal cancer. Six years post operation, the patient is currently attending regular follow-ups and is without recurrence or metastasis, reporting a normal bill of health.

DISCUSSION

According to the Warren and Gates criteria, multiple primary malignancies are defined if the following 4 conditions are satisfied: (1) each tumor is malignant; (2) each tumor has its own pathological features; (3) tumors occur in different parts of the organs, and are not continuous with each other; and (4) each tumor has its own metastatic pathway and the diagnosis of metastatic or recurrent tumors can be excluded[4,5]. In the case of this study, though three malignancies

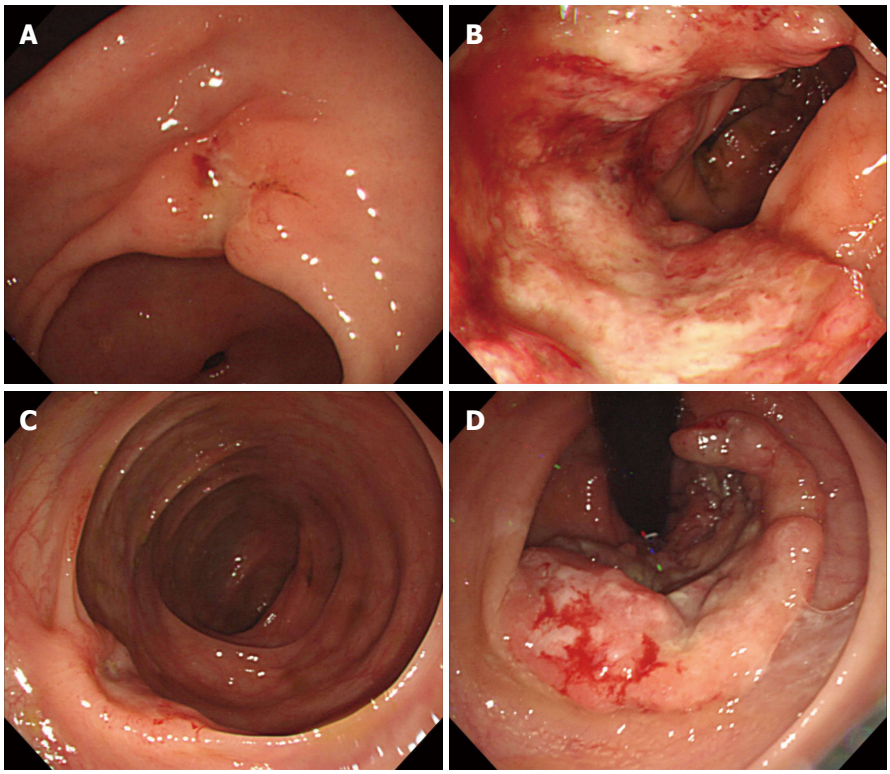


Figure 1 Endoscopic findings. A: Early gastric cancer type Ⅱc lesion at antrum, posterior wall of stomach; B: Ulcerative mass at proximal ascending colon, diagnosed with adenocarcinoma; C: Concave mass at transverse colon, diagnosed with adenocarcinoma; D: Ulcerative rectal mass near anus, diagnosed with adenocarcinoma pathologically.

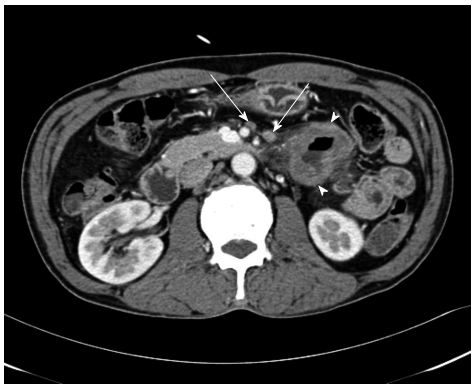


Figure 2 Computed tomography finding. Focal irregular wall thickening at proximal jejunum with lymphadenopathy, suggesting adenocarcinoma.

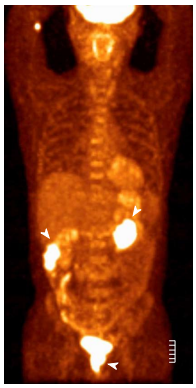


Figure 3 Positron emission tomography/computed tomography findings. Revealed abnormal increased fluorodeoxy glucose (FDG) uptakes in ascending colon, rectum and jejunum but no definite abnormal FDG uptake along the gastric wall and transverse colon was noted. Diffuse increased FDG uptake was found at both thyroid glands, which allowed for the diagnosis of thyroiditis.

gathered at the colon and rectum, each of these malignancies were determined to be primary cancers. They were distinguished as primary malignancies which originated from independent polyps and masses with distinct margins. Synchronous malignancies are defined as more than two primary cancers occurring within a 6 mo period after diagnosis of the first tumor, post the 6 mo period patients with further diagnosed malignancies can be referred to as having metachronous cancers[6]. According to these definitions, the patient in this particular case was thus diagnosed with synchronous quintuple primary cancer.

The occurrences of multiple primary malignancies have increased in recent years. Many factors can be attributed to this increase, including an increasing proportion of elderly patients in the general population, regular medical check-ups and increased number of cancer survivors[2,7]. Reported incidences of multiple primary malignancies are approximately 1%-10%. Metachronous multiple primary malignancies are more common than synchronous malignancies with a ratio 2.7:1. Double primary tumors are most common and triple, quadruple tumors are relatively rare. This is exemplified by the lack of publications on the tumors

Table 1 Published cases of synchronous quintuple primary malignancies in English literature

Year	Country	Age/sex	Location	Pathology
1995	Germany	67/M	Descending colon	Adenocarcinoma
			Kidney	Adenocarcinoma
			Prostate	Adenocarcinoma
			Bladder	Transitional cell carcinoma
			Bladder	malignant fibrous histocytoma
2012	Japan	46/F	Right ovary	Clear cell adenocarcinoma
			Endometrium	Endometrioid adenocarcinoma
			Ascending colon	Adenocarcinoma
			Rectum	Adenocarcinoma
			Left lung	Papillary adenocarcinoma
2015	United States	57/F	Right popliteal fossa	Malignant melanoma
			Left breast	Invasive lobular carcinoma
			Left axillary lymph node	Diffuse large B cell lymphoma
			Left axillary lymph node	Nodular lymphocyte predominant Hodgkin lymphoma
			Left tibial soft tissue	Giant cell tumor

F: Female; M: Male.

with no more than 20 published cases of quintuple (or more numbers) primary malignancies and less than 5 cases of synchronous quintuple (or more) cases being presented, this figure includes current cases in English literature[3,8] (Table 1).

The patient was suspected for Lynch syndrome, however, the tumors all possessed *MLH1*, *MSH2* gene expression. Therefore, the patient was unlikely to have Lynch syndrome. On the other hand, the tumors had MSI-H for MSI markers (bat26, D5S346, bat25, D2S123) and MSS for the other marker (D17S250). MSI is believed to be a factor in carcinogenesis[9]. Hence, diagnostics suggested MSI may be responsible for carcinogenesis in the patient.

To decide treatment option for multiple primary malignancy patient, the stages of each synchronous malignancy is the most important factor[2]. In the current case, with the exception of rectal cancer, there was no evidence of lymph node metastasis and had stages with relatively more favorable outcomes than stage ⅢB rectal cancer. Therefore, the adjuvant therapy was focused on the rectal cancer and favorable results were achieved.

In conclusion, surgeons should consider the possibility of multiple primary malignancies before surgery for intestinal tract malignancies. It is essential to perform full preoperative evaluations including esophagogastroduodenoscopy, colonoscopy, CT scan, PET/CT, and other imaging modalities, if needed. In addition, the stage of each malignancy is the most important factor to determine treatment options for multiple primary synchronous malignancy patients.

COMMENTS

Case characteristics

A 52-year-old male was diagnosed with synchronous quintuple primary malignancies in the stomach, jejunum, ascending colon, transverse colon and rectum.

Clinical diagnosis

The patient was diagnosed with malignancies in the stomach, jejunum, ascending colon, transverse colon and rectum.

Imaging diagnosis

Esophagogastroduodenoscopy revealed an early gastric cancer (EGC) at the antrum. Colonoscopy revealed three masses in the ascending colon, transverse colon and the rectum. A computed tomography (CT) scan found a mass at the jejunum and showed no significant lymph node enlargement around the stomach, colon and rectum. A positron emission tomography (PET)/CT showed abnormal increases in fluorodeoxy glucose (FDG) uptake in the ascending colon, rectum and jejunum but no definite abnormal FDG uptake along the gastric wall and transverse colon was noted.

Pathological diagnosis

The pathologic results determined the gastric tumor to be tubular adenocarcinoma. The mass at the jejunum revealed combined adenocarcinoma and neuroendocrine carcinoma. The specimen gained from the total proctocolectomy had three adenocarcinomas.

Treatment

The patient underwent a subtotal gastrectomy, segmental resection of the jejunum and a total proctocolectomy with the end ileostomy simultaneously during one operation.

Related reports

Three case reports of synchronous quintuple malignancy were published in English literature.

Experiences and lessons

Surgeons should consider the possibility of multiple primary malignancies before surgery for intestinal tract malignancies. The stage of each malignancy is the most important factor to determine treatment options for multiple primary synchronous malignancy patients.

Peer-review

It's a well written, well-illustrated, prolonged follow-up of the patient case report.

REFERENCES

1 **Maruyama T**, Nakasone T, Maruyama N, Matayoshi A, Arasaki A. Synchronous quadruple multiple primary cancers of the tongue, bilateral breasts, and kidney in a female patient with a disease-free

survival time of more than 5 years: a case report. *World J Surg Oncol* 2015; **13**: 263 [PMID: 26310238 DOI: 10.1186/s12957-015-0684-5]
2 **Oh SJ**, Bae DS, Suh BJ. Synchronous triple primary cancers occurring in the stomach, kidney, and thyroid. *Ann Surg Treat Res* 2015; **88**: 345-348 [PMID: 26029681 DOI: 10.4174/astr.2015.88.6.345]
3 **Komiyama S**, Nishio E, Ichikawa R, Miyamura H, Kawamura K, Komiyama M, Nishio Y, Udagawa Y. Asymptomatic synchronous quintuple primary cancers. *Gynecol Obstet Invest* 2012; **74**: 324-328 [PMID: 22776788 DOI: 10.1159/000339135]
4 **Warren S**, Gates O. Multiple primary malignant tumors: survey of the literature and a statistical study. *Am J Cancer* 1932; **16**: 1358-1414
5 **Xu LL**, Gu KS. Clinical retrospective analysis of cases with multiple primary malignant neoplasms. *Genet Mol Res* 2014; **13**: 9271-9284 [PMID: 24682981 DOI: 10.4238/2014.March.12.19]
6 **Moertel CG**, Dockerty MB, Baggenstoss AH. Multiple primary malignant neoplasms. I. Introduction and presentation of data. *Cancer* 1961; **14**: 221-230 [PMID: 13771652 DOI: 10.1002/1097-0142(196103/04)14:2<221::AID-CNCR2820140202>3.0.CO;2-6]
7 **Cercato MC**, Colella E, Ferraresi V, Diodoro MG, Tonachella R. Report of two cases of quintuple primary malignancies and review of the literature. *Anticancer Res* 2008; **28**: 2953-2958 [PMID: 19031939]
8 **Testori A**, Cioffi U, De Simone M, Bini F, Vaghi A, Lemos AA, Ciulla MM, Alloisio M. Multiple primary synchronous malignant tumors. *BMC Res Notes* 2015; **8**: 730 [PMID: 26613933 DOI: 10.1186/s13104-015-1724-5]
9 **Li B**, Liu HY, Guo SH, Sun P, Gong FM, Jia BQ. Detection of microsatellite instability in gastric cancer and dysplasia tissues. *Int J Clin Exp Med* 2015; **8**: 21442-21447 [PMID: 26885089]

P- Reviewer: Biondi A, Ennaifer R **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Liu WX

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.178

World J Gastroenterol 2017 January 7; 23(1): 178-184
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Cutting balloon treatment of anastomotic biliary stenosis after liver transplantation: Report of two cases

Fan Ding, Hui Tang, Chi Xu, Zai-Bo Jiang, Shu-Hong Yi, Hua Li, Nan Jiang, Wen-Jie Chen, Qing Yang, Yang Yang, Gui-Hua Chen

Fan Ding, Hui Tang, Chi Xu, Shu-Hong Yi, Hua Li, Nan Jiang, Qing Yang, Yang Yang, Gui-Hua Chen, Department of Hepatic Surgery and Liver Transplantation Center of The Third Affiliated Hospital, Organ Transplantation Institute, Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China

Fan Ding, Hui Tang, Chi Xu, Shu-Hong Yi, Hua Li, Nan Jiang, Qing Yang, Yang Yang, Gui-Hua Chen, Organ Transplantation Research Center of Guangdong Province, Guangzhou 510630, Guangdong Province, China

Zai-Bo Jiang, Department of Vascular and Interventional Radiology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China

Wen-Jie Chen, Yang Yang, Gui-Hua Chen, Guangdong Key Laboratory of Liver Disease Research, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China

Author contributions: Ding F, Xu C, Yang Y and Chen GH designed the report; Jiang ZB performed the cutting balloon treatment; Jiang N, Yang Q, Yang Y and Chen GH performed the orthotopic liver transplantation; Ding F, Tang H and Chen WJ collected the patients' clinical data; Ding F, Tang H, Xu C, Yi SH and Li H analyzed the data and wrote the paper.

Supported by Key Scientific and Technological Projects of Guangdong Province, No. 2014B020228003, No. 2014B030301041 and No. 2015B020226004; the Natural Science Foundation of Guangdong Province, No. 2015A030312013; and the Science and Technology Planning Project of Guangzhou, No. 201400000001-3 and No. 158100076.

Institutional review board statement: This case report was exempt from Institutional Review Board review [(2016)2-114] at The Third Affiliated Hospital of Sun Yat-Sen University in Guangzhou, China.

Informed consent statement: The patients involved in this report gave their written informed consent for the use and disclosure of their health information.

Conflict-of-interest statement: The authors declare that there is no conflict of interest related to this report.

Manuscript source: Unsolicited manuscript

Correspondence to: Chi Xu, MD, Deputy Director, Department of Hepatic Surgery and Liver Transplantation Center of The Third Affiliated Hospital, Organ Transplantation Institute, Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China. chixuzoe@163.com
Telephone: +86-20-85252177
Fax: +86-20-85252276

Received: August 8, 2016
Peer-review started: August 9, 2016
First decision: September 6, 2016
Revised: September 21, 2016
Accepted: October 19, 2016
Article in press: October 19, 2016
Published online: January 7, 2017

Abstract

Biliary stenosis is a common complication after liver transplantation, and has an incidence rate ranging from 4.7% to 12.5% based on our previous study. Three types of biliary stenosis (anastomotic stenosis, non-anastomotic peripheral stenosis and non-anastomotic central hilar stenosis) have been identified. We report the outcome of two patients with anastomotic stricture after liver transplantation who underwent successful

cutting balloon treatment. Case 1 was a 40-year-old male transplanted due to subacute fulminant hepatitis C. Case 2 was a 57-year-old male transplanted due to hepatitis B virus-related end-stage cirrhosis associated with hepatocellular carcinoma. Both patients had similar clinical scenarios: refractory anastomotic stenosis after orthotopic liver transplantation and failure of balloon dilation of the common bile duct to alleviate biliary stricture.

Key words: Liver transplantation; Cutting balloon; Anastomotic; Biliary stenosis; Cholangiography; Balloon dilation

Core tip: Biliary stenosis is the relatively common complication after liver transplantation. Our case report represents one of few documenting evidence of the cutting balloon treatment as a safe and effective procedure in refractory anastomotic stenosis after orthotopic liver transplantation. The cutting balloon treatment could be an alternative therapy to the endoscopic application or the surgical application.

Ding F, Tang H, Xu C, Jiang ZB, Yi SH, Li H, Jiang N, Chen WJ, Yang Q, Yang Y, Chen GH. Cutting balloon treatment of anastomotic biliary stenosis after liver transplantation: Report of two cases. *World J Gastroenterol* 2017; 23(1): 178-184 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/178.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.178

INTRODUCTION

Cutting balloon is an angioplasty device, which appropriately combines microsurgical incision with mechanical dilation. The system was invented by Barath *et al*[1] and was initially used in percutaneous coronary interventions. Compared with traditional balloon dilation technology, cutting balloon can effectively incise the vascular wall with concentrated and low-dilated pressure.

Cutting balloon technology plays an important role in complex coronary artery lesions[2], but is rarely reported in the field of biliary stenosis after orthotopic liver transplantation[3,4]. This report summarizes the application of cutting balloon treatment in two cases with anastomotic biliary stenosis after liver transplantation.

CASE REPORT

Case 1 was a 40-year-old male with hepatitis C virus (HCV)-related end-stage cirrhosis associated with portal hypertension. The patient, who weighed 71.5 kg, had undergone splenectomy 5 years previously and had no clinical history of other systemic diseases. Laboratory examinations revealed high levels of hepatobiliary enzymes, coagulation factors and quantitative HCV RNA: aspartate aminotransferase (AST) was 123.0 U/L, alanine aminotransferase (ALT) was 64 U/L, albumin (ALB) was 34.1 g/L, total bilirubin (TBILI) was 91.62 μmol/L, direct bilirubin (DBILI) was 36.87 μmol/L, prothrombin time (PT) was 15.1 s, the international normalized ratio of prothrombin time (PT-INR) was 1.25, and HCV RNA was 1.21×10^6 IU/mL. Case 2 was a 57-year-old male with hepatitis B virus (HBV)-related end-stage cirrhosis associated with hepatocellular carcinoma (HCC). The patient, who weighed 69.0 kg, was diagnosed with type Ⅱ diabetes 12 years previously and had not undergone abdominal surgery. Blood examination results were as follows: AST 41.0 U/L, ALT 33 U/L, ALB 39.6 g/L, TBILI 73.7 μmol/L, DBILI 49 μmol/L, PT 19.0 s, PT-INR 1.59, and HBV DNA 270 IU/mL. The clinical characteristics of these two patients are described in Table 1.

Case 1

Due to the failure of medical therapy, Case 1 underwent orthotopic liver transplantation (OLT) on June 6, 2012 (the liver graft warm ischemia time was 6 min and cold ischemia time was 7 h). Biliary anastomoses were performed by continuous anastomosis with absorbable suture (6-0 PDS suture). Postoperative pathology revealed nodular cirrhosis associated with cholestasis in hepatocytes and capillaries (Figure 1). Immunosuppressive therapy consisting of cyclosporine and mycophenolate mofetil was administered. Five months later, the patient was readmitted due to xanthochromia and pruritus. Re-examination of hepatic function showed the following results: AST 76 U/L, ALT 52 U/L, TBILI 90.8 μmol/L, DBILI 66.7 μmol/L, γ-glutamyl transpeptidase (GGT) 179.0 μmol/L and alkaline phosphatase (ALP) 315 μmol/L. Magnetic resonance cholangiopancreatography (MRCP) revealed post-OLT anastomotic stenosis of the choledochal duct, intra-hepatic bile duct dilation, and biliary sludge in the common hepatic duct and bilateral hepatic ducts; the patient was diagnosed with transplantation-related ischemic injury involving the biliary tract (Figure 2). On November 16, 2012, percutaneous transhepatic cholangial drainage (PTCD) was performed (Figure 3). Minor complications occurred during and after surgery, all of which were resolved following appropriate treatment and nursing. On January 15, 2013, re-examination by cholangiography showed that the anastomotic stenosis was reduced by nearly 20% (Figure 4); thus, we decided to remove the biliary drainage. One year later, the patient was referred to the Clinical Center again because of xanthochromia and pruritus. Clinical laboratory examination results were as follows: AST 129.0 U/L, ALT 42 U/L, TBILI 47.2 μmol/L, DBILI 35 μmol/L, GGT 755 μmol/L, and

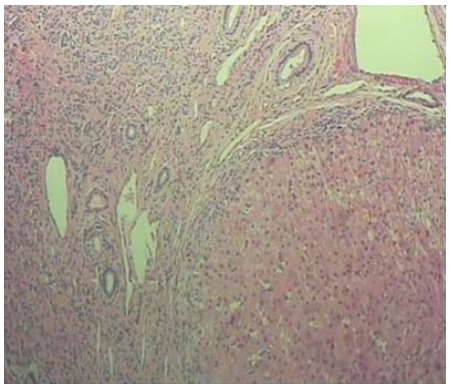


Figure 1 Postoperative pathology. Nodular cirrhosis associated with hepatocyte and capillary bile cholestasis.

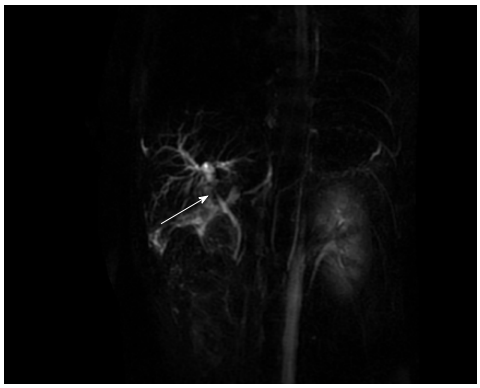


Figure 2 Magnetic resonance cholangiopancreatography findings. Post-orthotopic liver transplantation anastomotic stenosis of the choledochal duct, intra-hepatic bile duct dilation, and biliary sludge in the common hepatic duct and bilateral hepatic ducts; patient diagnosed with transplantation-related ischemic injury involving the biliary tract.

Table 1 Patient characteristics

Case No.	Age, yr	Sex	Diagnosis	Child-Pugh scores	MELD scores
Case 1	40	Male	Subacute fulminant hepatitis C	9	15
Case 2	57	Male	Hepatitis B virus-related end-stage cirrhosis associated with HCC	6	17

HCC: Hepatocellular carcinoma.

ALP 4895 μmol/L. On November 22, 2013, based on the clinical history and out-patient examinations, we performed cutting balloon treatment (Figure 5). The key surgical procedures were: the patient was placed in the left position and his abdominal skin was sterilized. The guidewire was then successfully placed in the correct position and the surgeon implanted the cutting balloon into the stenosis site and inflated the balloon (diameter 6 mm, length 4 cm; inflated pressure 6 atm, dilatation time 3 min). The surgeon subsequently consolidated the cutting site with conventional balloon

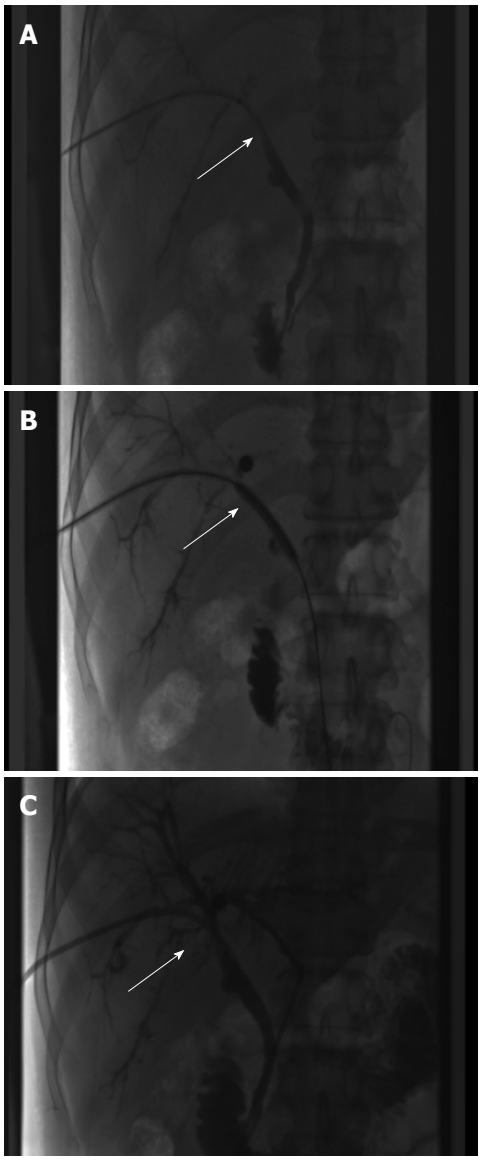


Figure 3 Percutaneous transhepatic cholangial drainage combined with balloon dilation. A: Anastomotic stenosis of the choledochal duct (straight arrow); B: The inflated balloon (diameter 8 mm, length 4 cm) has a waist at the narrowest part of the stenosis (straight arrow); C: Resolution of the stenosis after balloon dilation (straight arrow).

dilatation (diameter 8 mm, length 4 cm). The operation was successful. Complications included abdominal pain, nausea and emesis, which were minor and tolerable. On January 7, 2014, cholangiography indicated that the anastomotic stenosis was resolved (Figure 6). Liver function gradually recovered to physiological level within the 3-year follow-up period.

Case 2

Having met the standard of the "Milan criteria", OLT was performed in Case 2 on September 14, 2014 (the liver graft warm ischemia time was 0 min and cold ischemia time was 6 h). Biliary anastomosis was performed by continuous anastomosis with absorbable suture (7-0 PDS suture). Postoperative pathology indicated moderately differentiated HCC and nodular cirrhosis

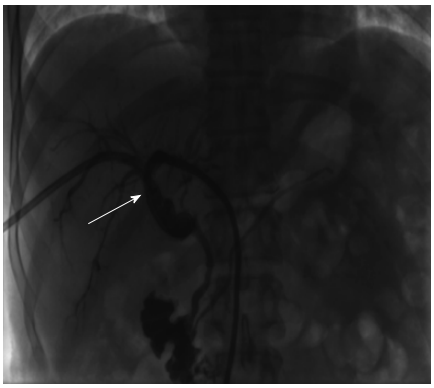


Figure 4 Cholangiography findings. The anastomotic stenosis was reduced by about 20 % (straight arrow).

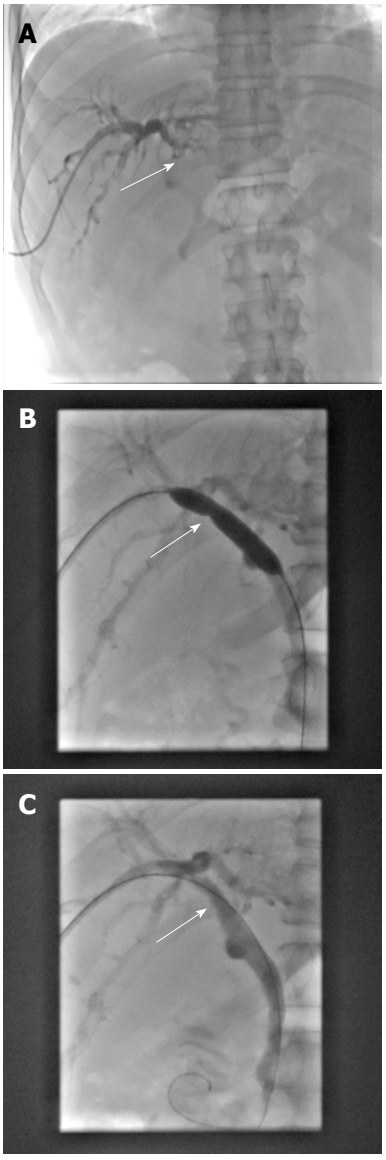


Figure 5 Cutting balloon therapy. A: Cholangiography showed the development of anastomotic stenosis (straight arrow); B: The inflated cutting balloon (diameter 6 mm, length 4 cm) has a waist at the narrowest part of the stenosis (straight arrow); C: Resolution of the stenosis after balloon dilation (straight arrow).

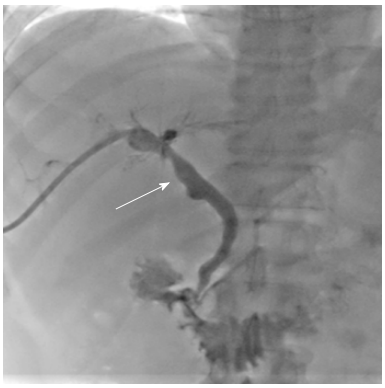


Figure 6 Cholangiography findings. The anastomotic stenosis was resolved (straight arrow).

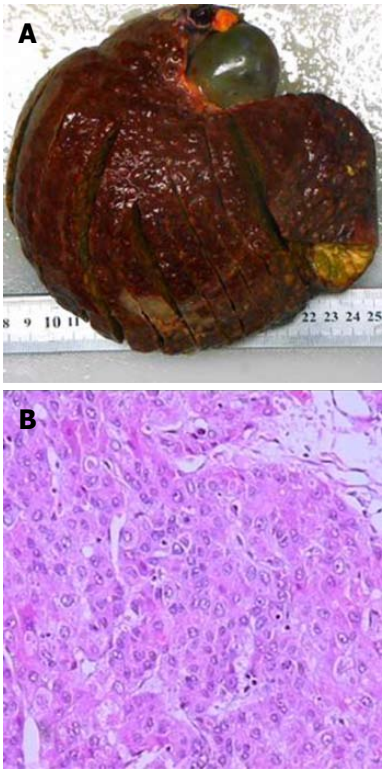


Figure 7 Postoperative pathology. A: Moderately differentiated hepatocellular carcinoma; B: Peripheral hepatic tissues revealed nodular cirrhosis pathologic changes.

pathological changes in peripheral hepatic tissues (Figure 7). Immunosuppressive therapy consisting of tacrolimus and mycophenolate mofetil was administered. Fifteen days after surgery, the patient developed cutaneous or sclera icterus, and emergency examination results were: AST 33 U/L, ALT 41 U/L, TBILI 74.50 μmol/L, DBILI 47.1 μmol/L, GGT 470.0 μmol/L, ALP 537 μmol/L; MRCP revealed severe anastomotic stenosis of the choledochal duct, and severe choledochectasia involving the intrahepatic bile ducts and left-right hepatic bile ducts above the anastomotic stomas. The patient was diagnosed with biliary anastomotic stenosis (Figure

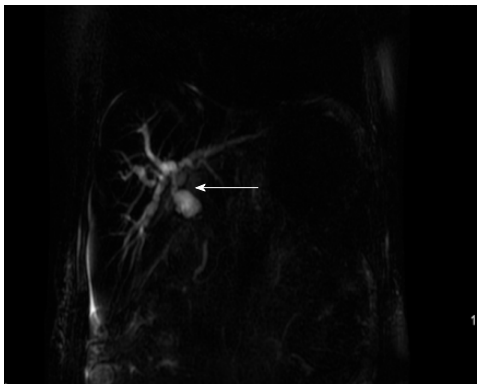


Figure 8 Magnetic resonance cholangiopancreatography findings. Severe anastomotic stenosis of the choledochal duct, severe choledochectasia involving the intrahepatic bile ducts and left-right hepatic bile ducts above the anastomotic stomas; Patient diagnosed with biliary anastomotic stenosis.

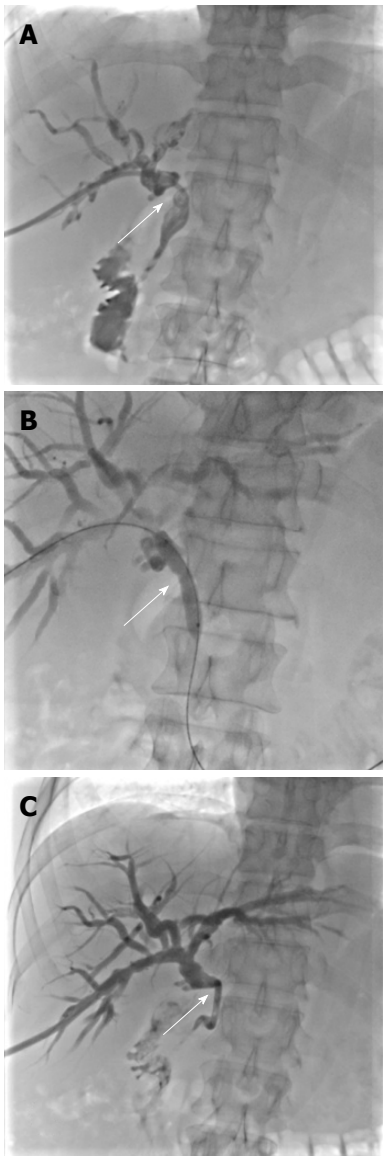


Figure 9 Percutaneous transhepatic cholangial drainage combined with balloon dilation. A: Severe anastomotic stenosis of the choledochal duct (straight arrow); B: The inflated balloon (diameter 8 mm, length 4 cm) has a waist at the narrowest part of the stenosis (straight arrow); C: Resolution of the stenosis after balloon dilation (straight arrow).

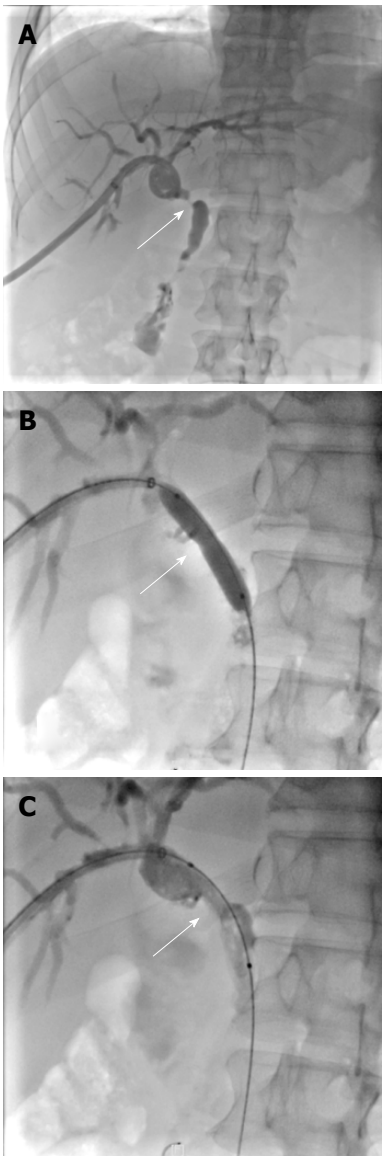


Figure 10 Cutting balloon therapy. A: Cholangiography showed that the anastomotic stenosis had resolved; B: The inflated cutting balloon (diameter 5 mm, length 2 cm) has a waist at the narrowest part of the stenosis (straight arrow); C: Resolution of the stenosis after balloon dilation (straight arrow).

8). On October 4, 2014, PTCD was performed without severe complications (Figure 9). Five months later, cholangiography revealed the presence of anastomotic stenosis, hence cutting balloon treatment was carried out (Figure 10). The surgical procedures were as follows. The patient was placed in the left position and his abdominal skin was sterilized. The guidewire was successfully placed in the correct position. The surgeon implanted the cutting balloon into the stenosis site and inflated the balloon (diameter 5 mm, length 2 cm; inflated pressure 6 atm, dilatation time 3 min). The surgeon subsequently consolidated the cutting site with conventional balloon dilatation (diameter 8 mm, length 4 cm). The surgery was successful. The patient had transient hemorrhage on the first night after surgery. Emergency blood examinations showed no change. Under the standardized management of

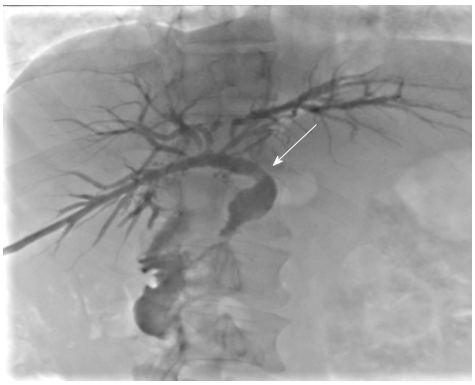


Figure 11 Cholangiography findings. The anastomotic stenosis was reduced by about 30% (straight arrow).

styptic measures, the prognosis was favorable. On March 10, 2015, cholangiography revealed that the anastomotic stenosis was reduced by 30% (Figure 11), and therefore biliary drainage was immediately removed. The clinical indicators gradually recovered and were maintained within the physiological range during the 10-mo follow-up period.

DISCUSSION

Postoperative anastomotic biliary stenosis can occur after surgery in the bile ducts of transplanted or non-transplanted liver. The majority of postoperative anastomotic stenosis encountered by the organ transplantation team are most often seen in liver transplant recipients. Three types of biliary stenosis (anastomotic, peripheral, and central) have been reported[5,6]. The causes of biliary stenosis are shown in Table 2. In addition to ischemia and fibrosis, immunological processes and ABO blood type incompatibility are suspected to contribute to biliary stenosis after liver transplantation[7-9].

Over the past two decades, with the development of technology and endoscopic treatment, the surgical management of biliary stenosis has undergone a rapid decline. Endoscopic treatment has the obvious advantage of high efficiency and a low incidence of procedure-related complications[10]. ERCP was first reported in 1968, and has been used for endoscopic visualization of the ampulla of Vater and minimally invasive cannulation of the pancreatic duct or biliary duct[11]. In 1974, Kawai *et al*[12] reported their clinical experience of endoscopic electrosurgical sphincterotomy of the ampulla of Vater to remove gallstones in the common bile duct. This new application in the field of surgical endoscopy was soon accepted as a safe, direct technique for evaluating biliary and pancreatic disease. ERCP has evolved from a diagnostic tool to an almost exclusively therapeutic technique[13]. While ERCP combined with balloon dilation or stent placement is generally effective for biliary stenosis after liver transplantation, uncertainties regarding the optimal therapy remain and can be seen in the variable outcomes described in previous reports of endoscopic treatment[14-17].

Table 2 Etiologies of biliary stenosis

Procedure-related factors	Non-procedure-related factors
Biliary anastomosis	Chronic pancreatitis
Cholecystectomy	Inflammation and infections
Ischemic injury	Primary sclerosing cholangitis
Choledocholithiasis	Radiation therapy
Post-endoscopic biliary sphincterotomy	Autoimmune cholangiopathy
Trauma	Sphincter of Oddi dysfunction

The cutting balloon system, which incorporates three or four radially-directed microsurgical blades on the surface of the balloon, is an alternative device that has been used in calcified or rigid lesions[18]. Compared with conventional angioplasty, by creating endovascular micro-incisions during dilatation, the cutting balloon reduces vascular tone, yielding a greater luminal diameter and lower incidence of residual stenosis, which is conducive for lower inflation pressure and a reduced incidence of postoperative complications[19]. This device is particularly suitable for biliary stenosis, which is characterized by a high concentration of elastic and muscle fibers that can generate substantial recoil following balloon inflation[20,21]. We have used cutting balloon treatment in patients who have a high risk of refractory anastomotic stenosis and this treatment has yielded satisfactory results, with no severe postoperative complications, such as bile leakage or catheter-related complications.

In conclusion, cutting balloon treatment for biliary anastomotic stenosis after liver transplantation may be an alternative therapy to endoscopic or surgical treatment, and avoids unnecessary routine stents, directly incising stenosis scars, and has a favorable long-term prognosis.

COMMENTS

Case characteristics

Two patients were diagnosed with biliary stricture after liver transplantation. Both patients were treated immediately by percutaneous transhepatic cholangial drainage combined with balloon dilatation. However, cholangiography revealed postoperative restenosis (Case 1 approximately 2 mo later, Case 2 approximately 5 mo later). Both patients underwent cutting balloon treatment with a good prognosis.

Clinical diagnosis

Case 1: Initial diagnosis was subacute fulminant hepatitis C complicated by post-orthotopic liver transplantation (OLT) anastomotic stenosis of the choledochal duct. Case 2: Initial diagnosis was hepatitis B virus-related end-stage cirrhosis associated with hepatocellular carcinoma, complicated by severe anastomotic stenosis of the choledochal duct.

Differential diagnosis

Biliary infection; hepatic insufficiency; ischemic cholangitis.

Laboratory diagnosis

Hyperbilirubinemia.

Imaging diagnosis
Case 1: Magnetic resonance cholangiopancreatography (MRCP) showed post-OLT anastomotic stenosis of the choledochal duct (Figure 2). Case 2: MRCP revealed severe anastomotic stenosis of the choledochal duct (Figure 8).

Pathological diagnosis
Case 1: Nodular cirrhosis associated with hepatocyte and capillary bile cholestasis (Figure 1). Case 2: Moderately differentiated hepatocellular carcinoma and peripheral hepatic tissues revealed nodular cirrhosis pathologic changes (Figure 7).

Treatment
Cutting balloon treatment with the aim of resolving anastomotic biliary stenosis.

Related reports
The safety and efficacy of cutting balloon treatment in vascular surgery has been widely reported. Hence, this technology has gradually been used to treat biliary or ureteral stenosis. In the few reports on post-OLT biliary stenosis, although the efficacy requires further clinical evidence, this technology shows huge potential according to the findings in the two cases reported.

Term explanation
Cutting balloon treatment and refractory anastomotic stenosis after OLT.

Experiences and lessons
Cutting balloon treatment may be an alternative therapy to endoscopic or surgical treatment.

Peer-review
The report provides clinical support for the safety and efficacy of cutting balloon treatment used in post-OLT refractory anastomotic stenosis.

REFERENCES

1 **Barath P**, Fishbein MC, Vari S, Forrester JS. Cutting balloon: a novel approach to percutaneous angioplasty. *Am J Cardiol* 1991; **68**: 1249-1252 [PMID: 1842213]
2 **Baber U**, Kini AS, Sharma SK. Stenting of complex lesions: an overview. *Nat Rev Cardiol* 2010; **7**: 485-496 [PMID: 20725106 DOI: 10.1038/nrcardio.2010.116]
3 **Saad WE**, Davies MG, Saad NE, Waldman DL, Sahler LG, Lee DE, Kitanosono T, Sasson T, Patel NC. Transhepatic dilation of anastomotic biliary strictures in liver transplant recipients with use of a combined cutting and conventional balloon protocol: technical safety and efficacy. *J Vasc Interv Radiol* 2006; **17**: 837-843 [PMID: 16687750 DOI: 10.1097/01.rvi.0000209343.80105.b4]
4 **Fonio P**, Calandri M, Faletti R, Righi D, Cerrina A, Brunati A, Salizzoni M, Gandini G. The role of interventional radiology in the treatment of biliary strictures after paediatric liver transplantation. *Radiol Med* 2015; **120**: 289-295 [PMID: 25030968 DOI: 10.1007/s11547-014-0432-x]
5 **Zajko AB**, Campbell WL, Bron KM, Schade RR, Koneru B, Van Thiel DH. Diagnostic and interventional radiology in liver transplantation. *Gastroenterol Clin North Am* 1988; **17**: 105-143 [PMID: 3292423]
6 **Ward EM**, Kiely MJ, Maus TP, Wiesner RH, Krom RA. Hilar biliary strictures after liver transplantation: cholangiography and percutaneous treatment. *Radiology* 1990; **177**: 259-263 [PMID: 2399328 DOI: 10.1148/radiology.177.1.2399328]
7 **Friedrich K**, Smit M, Brune M, Giese T, Rupp C, Wannhoff A, Kloeters P, Leopold Y, Denk GU, Weiss KH, Stremmel W, Sauer P, Hohenester S, Schirmacher P, Schemmer P, Gotthardt DN. CD14 is associated with biliary stricture formation. *Hepatology* 2016; **64**: 843-852 [PMID: 26970220 DOI: 10.1002/hep.28543]
8 **Weeder PD**, van Rijn R, Porte RJ. Machine perfusion in liver transplantation as a tool to prevent non-anastomotic biliary strictures: Rationale, current evidence and future directions. *J Hepatol* 2015; **63**: 265-275 [PMID: 25770660 DOI: 10.1016/j.jhep.2015.03.008]
9 **Song GW**, Lee SG, Hwang S, Kim KH, Ahn CS, Moon DB, Ha TY, Jung DH, Park GC, Kang SH, Jung BH, Yoon YI, Kim N. Biliary stricture is the only concern in ABO-incompatible adult living donor liver transplantation in the rituximab era. *J Hepatol* 2014; **61**: 575-582 [PMID: 24801413 DOI: 10.1016/j.jhep.2014.04.039]
10 **Chang JH**, Lee I, Choi MG, Han SW. Current diagnosis and treatment of benign biliary strictures after living donor liver transplantation. *World J Gastroenterol* 2016; **22**: 1593-1606 [PMID: 26819525 DOI: 10.3748/wjg.v22.i4.1593]
11 **William S McCune**, Paul E Shorb, Herbert Moscovitz. Endoscopic cannulation of the ampulla of Vater: a preliminary report. By William S. McCune, Paul E. Shorb, and Herbert Moscovitz, 1968. *Gastrointest Endosc* 1988; **34**: 278-280 [PMID: 3292347]
12 **Kawai K**, Akasaka Y, Murakami K, Tada M, Koli Y. Endoscopic sphincterotomy of the ampulla of Vater. *Gastrointest Endosc* 1974; **20**: 148-151 [PMID: 4825160]
13 **Classen M**, Demling L. [Endoscopic sphincterotomy of the papilla of vater and extraction of stones from the choledochal duct (author's transl)]. *Dtsch Med Wochenschr* 1974; **99**: 496-497 [PMID: 4835515 DOI: 10.1055/s-0028-1107790]
14 **Adler DG**, Baron TH, Davila RE, Egan J, Hirota WK, Leighton JA, Qureshi W, Rajan E, Zuckerman MJ, Fanelli R, Wheeler-Harbaugh J, Faigel DO. ASGE guideline: the role of ERCP in diseases of the biliary tract and the pancreas. *Gastrointest Endosc* 2005; **62**: 1-8 [PMID: 15990812 DOI: 10.1016/j.gie.2005.04.015]
15 **Gürakar A**, Wright H, Camci C, Jaboour N. The application of SpyScope® technology in evaluation of pre and post liver transplant biliary problems. *Turk J Gastroenterol* 2010; **21**: 428-432 [PMID: 21331998]
16 **Dawson SL**, Mueller PR. Nonoperative management of biliary obstruction. *Annu Rev Med* 1985; **36**: 1-11 [PMID: 3888048 DOI: 10.1146/annurev.me.36.020185.000245]
17 **Cotton PB**. Critical appraisal of therapeutic endoscopy in biliary tract diseases. *Annu Rev Med* 1990; **41**: 211-222 [PMID: 2184724 DOI: 10.1146/annurev.me.41.020190.001235]
18 **Lee MS**, Singh V, Nero TJ, Wilentz JR. Cutting balloon angioplasty. *J Invasive Cardiol* 2002; **14**: 552-556 [PMID: 12205358]
19 **Okura H**, Hayase M, Shimodozono S, Kobayashi T, Sano K, Matsushita T, Kondo T, Kijima M, Nishikawa H, Kurogane H, Aizawa T, Hosokawa H, Suzuki T, Yamaguchi T, Bonneau HN, Yock PG, Fitzgerald PJ. Mechanisms of acute lumen gain following cutting balloon angioplasty in calcified and noncalcified lesions: an intravascular ultrasound study. *Catheter Cardiovasc Interv* 2002; **57**: 429-436 [PMID: 12455075 DOI: 10.1002/ccd.10344]
20 **Kakani NK**, Puckett M, Cooper M, Watkinson A. Percutaneous transhepatic use of a cutting balloon in the treatment of a benign common bile duct stricture. *Cardiovasc Intervent Radiol* 2006; **29**: 462-464 [PMID: 16447007 DOI: 10.1007/s00270-004-0279-y]
21 **Atar E**, Bachar GN, Eitan M, Graif F, Neyman H, Belenky A. Peripheral cutting balloon in the management of resistant benign ureteral and biliary strictures: long-term results. *Diagn Interv Radiol* 2007; **13**: 39-41 [PMID: 17354194]

P- Reviewer: Hosny K **S- Editor**: Yu J
L- Editor: Filipodia **E- Editor**: Liu WX

World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v23.i1.185

World J Gastroenterol 2017 January 7; 23(1): 185-190
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Spontaneous rupture of hepatic epithelioid hemangioendothelioma: A case report

Jun-Wu Yang, Yong Li, Kai Xie, Wei Dong, Xian-Tong Cao, Wei-Dong Xiao

Jun-Wu Yang, Yong Li, Wei Dong, Xian-Tong Cao, Wei-Dong Xiao, Department of General Surgery, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

Kai Xie, Department of Radiology, The First Affiliated Hospital of Nanchang University, Nanchang 330006, Jiangxi Province, China

Author contributions: Yang JW wrote the report; Li Y, Dong W, Cao XT and Xiao WD performed the surgery; Xie K helped to collect the relevant data.

Supported by The First Affiliated Hospital of Nanchang University, Jiangxi Province, China.

Institutional review board statement: The study was reviewed by the Institutional Review Board of the First Affiliated Hospital of Nanchang University, Nanchang, China.

Informed consent statement: The patient in this study provided informed written consent prior to the manuscript preparation.

Conflict-of-interest statement: The authors declared that there is no conflict of interest related to this report.

Manuscript source: Unsolicited manuscript

Correspondence to: Yong Li, MD, Professor, Department of General Surgery, The First Affiliated Hospital of Nanchang University, No. 17, Yongwai Zhengjie, Nanchang 330006, Jiangxi Province, China. yfyly@163.com
Telephone: +86-791-88694131
Fax: +86-791-88694131

Received: July 21, 2016
Peer-review started: July 25, 2016
First decision: September 20, 2016
Revised: September 27, 2016
Accepted: October 27, 2016
Article in press: October 27, 2016
Published online: January 7, 2017

Abstract

Hepatic epithelioid hemangioendothelioma (HEH) is a rare tumor of vascular endothelial origin. Spontaneous rupture of HEH is a life-threatening complication and is extremely rare. HEH has variable malignant potential, and the clinical diagnosis remains challenging. Here we report a case of HEH with spontaneous rupture. A 44-year-old man presented with constant cutting pains over the right upper abdomen after eating. He had hemoptysis 11 d previously. Diagnostic abdominal puncture demonstrated active bleeding. Chest and abdominal computer tomography scan showed multiple ground-glass nodules over the lungs, multiple low-density intrahepatic nodules and massive hemorrhage. Transcatheter arterial embolization and exploratory laparotomy were performed and subsequent immunohistochemical examination confirmed a diagnosis of HEH.

Key words: Hepatic epithelioid hemangioendothelioma; Spontaneous rupture

Core tip: Hepatic epithelioid hemangioendothelioma (HEH) is a rare tumor of vascular endothelial origin. Spontaneous rupture of HEH is a life-threatening complication and very few cases have been reported. We report a case of HEH with spontaneous rupture. Transcatheter arterial embolization and exploratory

laparotomy were performed, and subsequent immunohistochemical examination confirmed a diagnosis of HEH. The patient died 6 mo after surgery. We also reviewed the literature and described the diagnosis and treatment of this disease.

Yang JW, Li Y, Xie K, Dong W, Cao XT, Xiao WD. Spontaneous rupture of hepatic epithelioid hemangioendothelioma: A case report. *World J Gastroenterol* 2017; 23(1): 185-190 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i1/185.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i1.185

INTRODUCTION

Hepatic epithelioid hemangioendothelioma (HEH) is a rare malignant tumor of vascular endothelial origin. Although it can occur at any age, the mean age was 41.7 years[1]. Spontaneous rupture of HEH is extremely rare and seldom reported. The exact cause of the tumor rupture is still unknown. Therefore, there is a lack of experience in the treatment of ruptured HEH. Here, we report a case of spontaneous rupture of HEH, which was treated with transcatheter arterial embolization (TAE) and exploratory laparotomy. The patient finally died at postoperative 6 mo.

CASE REPORT

A 44-year-old man was admitted to the outpatient department of our hospital because of a sudden hemoptysis on February 9, 2015. Chest and abdominal computer tomography (CT) scan showed multiple low-density intrahepatic nodules, and lesions growing adjacent to the hepatic capsule with a clear margin. The maximum lesion was located in the right lobe with a 75-mm diameter. Contrast-enhanced CT showed heterogeneous enhancement and stronger enhancement at the portal venous phase. In addition, there were multiple ground-glass nodules over the lungs (Figure 1). Based on the imaging findings, we considered a possibility of HEH and lung metastasis. The patient went home without any treatment. On February 20, 2015, he was sent to the emergency department of our hospital with the complaint of constant cutting-like pains over the right upper abdomen after eating, which radiated from the back to the right shoulder. On physical examination, his right upper abdomen was tender and slightly spastic, but without rebound tenderness, liver and spleen were not palpable, and there was pain with fist percussion over the liver. Laboratory examinations on admission revealed: white blood cell count of 12.01 $\times$ 10^9/L (normal range 4-10 $\times$ 10^9/L), hemoglobin (HB) 59 g/L (normal range 110-150 g/L), alanine aminotransferase 125 U/L (normal range 5-35 U/L), aspartate transaminase 189 U/L (normal range 5-40 U/L), prothrombin time 14.7 s (normal range 9.8-12.1 s), prothrombin-time ratio 1.26 (normal range 0.85-1.15), prothrombin-time activity 64% (normal range 70%-130%), international normalized ratio 1.26 (normal range 0.85-1.15), and plasma protamine paracoagulation test was positive. Serum tumor markers, carbohydrate antigen 12-5 (CA12-5) was 190.2 U/mL (normal range 0-35 U/mL), and α-fetoprotein (AFP), carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) were all within normal ranges. Hepatitis B surface antigen was negative. Diagnostic abdominal puncture demonstrated active bleeding suspicious of tumor rupture. TAE was performed for the purpose of hemostasis. On March 5, 2015, the patient felt chest stufly, tachypnea and stomachache suddenly, the blood pressure was 91/55 mmHg, heart rate was 86 times/min, and HB was 61 g/L, and CT scan revealed massive hematocoelia suspicious of tumor rupture with massive hemorrhage. After consultation with surgeons, he underwent hepatobiliary surgery and an exploratory laparotomy was performed. Hematocoelia of about 5000 mL and multiple nodular tumors within the liver were found during operation. A tumor originated from the S8 of the liver adhering to the diaphragm, and active bleeding occurred from two peritumoral veins. Besides, a neoplasm (2.0 cm $\times$ 3.0 cm) was seen over the adhesive diaphragm. Finally, segmental resection of S8 tumors was performed, suturing was made to stop bleeding and diaphragm neoplasm was resected. The patient received 600mL fresh frozen plasma and 20 units of cryoprecipitate during the operation, and the hepatic portal was blocked for 20 min. Pathological diagnosis of the tumor over S8 was HEH based on the microscopy which showed that carcinoma cells with almost consistent size appeared nest-like or papillary around the blood vessels, and the nuclei were round or ovoid (Figure 2). The major part of the diaphragm neoplasm was blood clot, with a few blood vessels and striated muscle tissue. Immunohistochemistry showed that CD31 (+++) (Figure 3A), CD34 (+++) (Figure 3B), FVIII (Figure 3C), and Ki-67 (40%+) (Figure 3D) were positive in tumor cells. The patient died in 6 mo after surgery during the regular follow-up.

DISCUSSION

HEH is a rare low-grade malignant tumor with an incidence of < 1 per 1000000, and it was first reported by Ishak in 1984[2]. The tumor occurs in adults of all ages, averaging approximately 41.7 years, and the female: male ratio was 3:2[1]. . The etiology of HEH is unclear, possibly related to oral contraceptives and alcohol intake, and viral hepatitis[3]. The initial clinical presentation is non-specific (mostly right upper abdominal pain, hepatomegaly and weight loss), and some patients were discovered incidentally with no symptoms. When patients present with a sudden onset of abdominal pain, especially with hemorrhagic shock, we should be aware of tumor rupture. Few patients

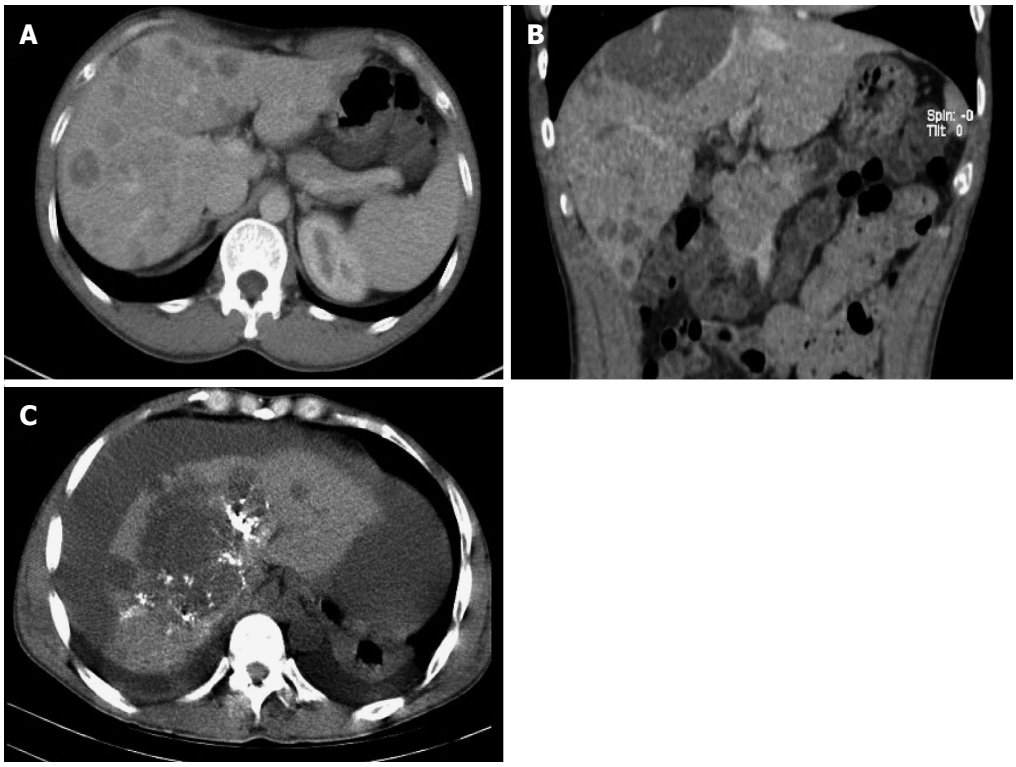


Figure 1 Computer tomography. A: Venous phase image revealed multiple low-density intrahepatic nodules in both lobes, and part of nodules showed inhomogeneous enhancement; B: The maximum lesion was located in the right lobe under the hepatic capsule; C: Computer tomography scan after transcatheter arterial embolization revealed massive hematocoelia.

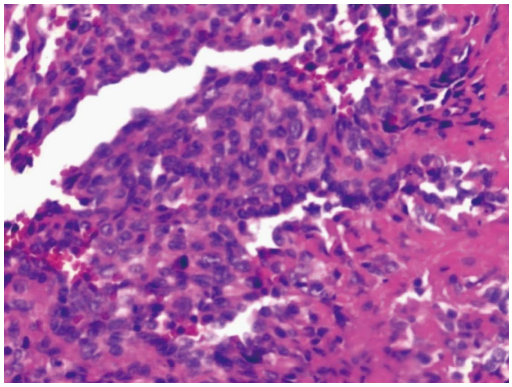


Figure 2 Histopathologic findings of hepatic epithelioid hemangioendothelioma. Carcinoma cells appeared nest-like or papillary around the blood vessels, the nuclei were round or ovoid (hematoxylin-eosin staining; magnification × 200).

had jaundice and finally died of hepatic failure[4].

HEH has characteristic imaging findings. The tumors tend to have multiple nodules, distributing along the liver periphery and under the hepatic capsule[5]. In addition, these nodules may coalesce over time[5]. HEH was shown as hypoechoic lesions in ultrasound examination[3]. CT scans revealed multiple low-density intrahepatic nodules with some lesions in sight of calcification[3]. MRI showed inhomogenously hypointense lesions on T1WI, while inhomogenously hyperintense on T2WI[6]. Contrast-enhanced scans show peripheral ring-like enhancement at hepatic arterial phase and stronger enhancement at portal venous phase[7]. A "halo" and "capsular retraction" sign, as well as "lollipop" sign are characteristic findings of HEH in CT and MRI[6,8]. Angiographic examination can demonstrate the relationship between the tumor and blood vessels. In addition, abdominal tapping revealing blood in the peritoneal cavity is helpful in the diagnosis of tumor rupture. Imaging examination can help identify extrahepatic metastases, the common sites were lungs, peritoneum, lymph nodes and bones[1]. All these examinations can provide a clue to the preoperative diagnosis of HEH.

The confirmed diagnosis of HEH is based on pathological and immunohistochemical results. HEH usually had a pale or white cut surface, with necrosis and sclerosis in the central part[3]. Generally, the central and portal veins were invaded by the tumor[9]. Histologically, the tumor was composed of sheets of dendritic and epithelioid cells. Microscopically, tumor cells appeared spindle, irregular and epithelial, and accidental red cells were visible and accompanied by mucoid or fibrous stroma[10,11]. Intracytoplasmic lumina is the characteristic finding in pathological examination. Immunohistochemically, at least one endothelial marker (CD34, FVIII, CD31) was positive in HEH, while cytokeratin and alpha-fetoprotein immunocytochemistry was negative[3,12]. According to the statistics, positive rate of CD34 was 93.3%, FVIII was 90.0% and CD31 was 75.0%[3,12]. In the present case report, CD34, FVIII and CD31 were all positive.

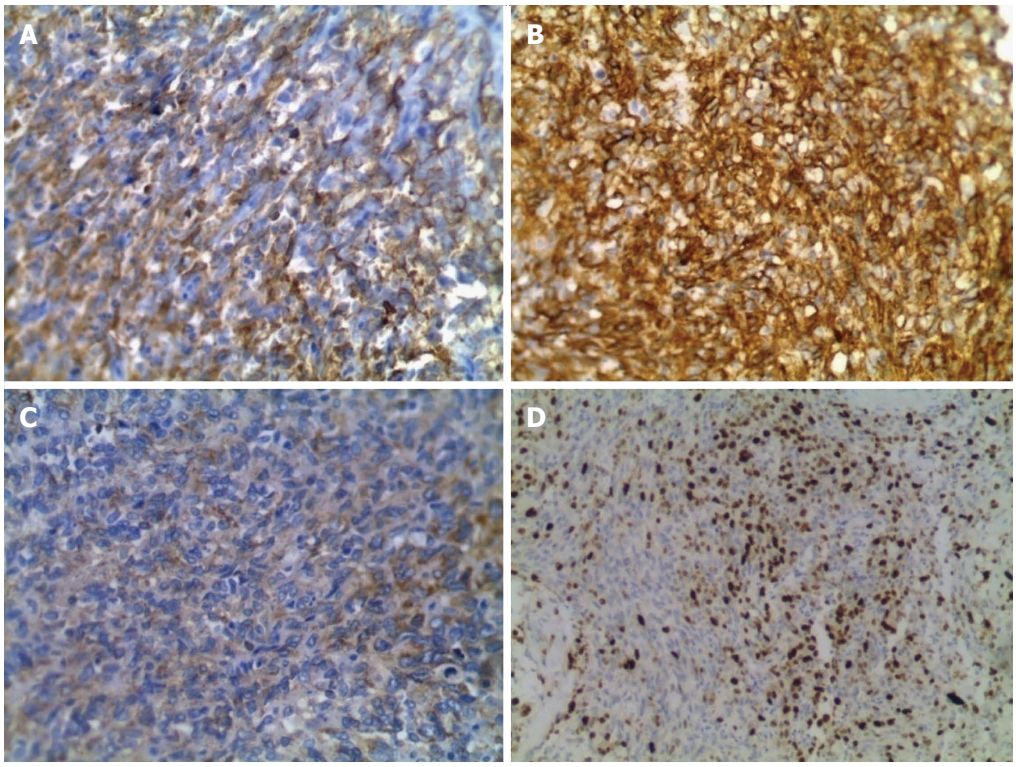


Figure 3 Immunohistochemical examination of hepatic epithelioid hemangioendothelioma. A: Specimen stained positive for CD31 (magnification × 200); B: Specimen stained positive for CD34 (magnification × 200); C: Specimen stained positive for FVIII (magnification × 200); D: Ki-67 proliferation index is less than 40% (magnification × 100).

Treatment modalities mainly depend on the patient's individual situation. Hepatic resection is a good choice for located lesions and liver transplantation for multiple nodules. In the reported series, liver transplant resulted in an excellent prognosis in patients with HEH and it can be used for patients with extrahepatic metastasis as well[9,13]. Marino reported a projected 5-year survival rate of 76% after liver transplantation in five patients with metastatic involvement[9]. Some patients die from their HEH recurrence after liver transplantation, so clinical follow-up treatment is necessary. The common post-operative complications were perihepatic abscess, portal vein thrombosis and post-operative bleeding, which may result in a second operation[14]. In addition, TAE, chemotherapy, radiotherapy, hormone therapy and thermoablation are the treatment of choice for patients with metastasis. Mascarenhas reported a patient with a metastasis to the lungs who was successfully treated with oral thalidomide therapy[15]. The present patient was treated with TAE and exploratory laparotomy because of the rupturing of the tumor and abdominal hemorrhea.

Dietze *et al*[16] reported that 6 of 12 patients with HEH had liver failure, suggesting that it may be related to extensive involvement of both lobes. Although its growth may lead to hepatic failure, extrahepatic metastases and even death, its prognosis is considered better than that of other hepatic malignancies[11]. Tumor size, number of nodules and number of hepatic segments can influence prognosis. The survival rate for patients with a tumor > 10 cm in diameter was significantly lower than for patients with a tumor < 5 cm[14]. Prognosis of HEH was closely related to the choice of treatment. The 1-year and 5-year survival rates were 96% and 54.5% after liver transplantation, 73.3% and 30% after chemotherapy or radiotherapy, 100% and 75% after hepatic resection and were 39.3% and 4.5% without treatment[11]. Dong *et al*[17] investigated the implications of the pathological characteristics for the prognosis of HEH, and found that patients without cellular atypia and nuclear fission and with Ki-67 < 10% had a relatively good prognosis. Patients with spontaneous rupture of hepatic malignancies often have a poor prognosis, possibly due to the intraperitoneal metastasis after tumor rupture. HEH with spontaneous rupture is extremely rare. Our patient in the present report died in 6 months after surgery. The cause of death is unknown.

Spontaneous rupture of HEH is extremely rare. The exact mechanism is unclear. Generally, rupture of hepatic malignant tumors is often related to rapid expansion and central necrosis, tumor venous invasion, abdominal trauma and pressure of the diaphragm[18]. Spontaneous rupture of HEH in this report was evoked by eating, possibly due to the large size of the tumors and peripheral location. A typical symptom is the persistent pain over right upper abdomen with shock[19]. Diagnostic puncture is very important to confirm the bleeding caused by rupture of hepatic malignant tumors. The priority treatment

for the rupture of HEH is hemostasis and saving lives. Other treatments, such as local liver resection or liver transplantation, should be chosen accordingly.

We report a patient with spontaneous rupture of HEH along with clinical diagnosis, treatment and prognosis of the disease. Due to limited clinical experience with HEH, especially with spontaneous rupture, further studies are needed for the clinical diagnosis and management of HEH.

COMMENTS

Case characteristics

A 44-year-old man had a sudden onset of chest stufly, tachypnea and stomachache, and he had had hemoptysis and constant cutting-like pains over the right upper abdomen.

Clinical diagnosis

Diagnostic abdominal puncture demonstrated active bleeding suspicious of tumor rupture.

Differential diagnosis

Differential diagnosis included cholangiocarcinoma, angiosarcoma, hepatocellular carcinoma, sclerosing hemangioma and metastatic carcinoma.

Laboratory diagnosis

White blood cell count 12.01 × 10^9/L, hemoglobin 59 g/L, alanine aminotransferase 125 U/L, aspartate transaminase 189 U/L, prothrombin time 14.7 s, prothrombin time ratio 1.26, prothrombin time activity 64%, international normalized ratio 1.26. Plasma protamine paracoagulation test was positive. Serum tumor markers: CA12-5 was 190.2 U/mL, and AFP, CEA and CA19-9 were all within normal ranges.

Imaging diagnosis

Chest and abdominal computer tomography scan showed multiple low-density intrahepatic nodules, the largest nodule being 75 mm in diameter in the S8 of the liver adhering to the diaphragm. In addition, there were multiple ground-glass nodules over the lungs.

Pathological diagnosis

Pathological diagnosis of the tumor in the S8 of the liver was hepatic epithelioid hemangioendothelioma (HEH), and carcinoma cells were positive for CD31 (+++), CD34 (+++), FVIII, and Ki-67 (40%+).

Treatment

Segmental resection of S8 tumors, suture for stopping bleeding and diaphragm neoplasm resection were performed for the purpose of hemostasis.

Related reports

There have been very few reports about spontaneous rupture of HEH. The exact etiology of HEH is unknown and the mean age of the patients was 41.7 years. The exact cause of the tumor rupture is still unclear.

Term explanation

HEH is a rare low-grade malignant tumor with an incidence of < 1 per 1000000.

Experiences and lessons

The authors are lacking of experience in the clinical diagnosis and treatment of ruptured HEH. However, saving life is always the priority treatment for the ruptured tumor.

Peer-review

It is a very interesting case, well written and detailed.

REFERENCES

1 **Mehrabi A**, Kashfi A, Fonouni H, Schemmer P, Schmied BM, Hallscheidt P, Schirmacher P, Weitz J, Friess H, Buchler MW, Schmidt J. Primary malignant hepatic epithelioid hemangioendothelioma: a comprehensive review of the literature with emphasis on the surgical therapy. *Cancer* 2006; **107**: 2108-2121 [PMID: 17019735 DOI: 10.1002/cncr.22225]

2 **Ishak KG**, Sesterhenn IA, Goodman ZD, Rabin L, Stromeyer FW. Epithelioid hemangioendothelioma of the liver: a clinicopathologic and follow-up study of 32 cases. *Hum Pathol* 1984; **15**: 839-852 [PMID: 6088383 DOI: 10.1016/S0046-8177(84)80145-8]

3 **Makhlouf HR**, Ishak KG, Goodman ZD. Epithelioid hemangioendothelioma of the liver. *Cancer* 1999; **85**: 562-582 [DOI: 10.1002/(SICI)1097-0142(19990201)85:3<562::AID-CNCR7>3.0.CO;2-T]

4 **Earnest F**, Johnson CD. Case 96: Hepatic epithelioid hemangioendothelioma. *Radiology* 2006; **240**: 295-298 [PMID: 16793987 DOI: 10.1148/radiol.2401032099]

5 **Azzam RI**, Alshak NS, Pham HP. AIRP best cases in radiologic-pathologic correlation: Hepatic epithelioid hemangioendothelioma. *Radiographics* 2012; **32**: 789-794 [PMID: 22582359 DOI: 10.1148/rg.323115010]

6 **Gan LU**, Chang R, Jin H, Yang LI. Typical CT and MRI signs of hepatic epithelioid hemangioendothelioma. *Oncol Lett* 2016; **11**: 1699-1706 [PMID: 26998064]

7 **Zhou L**, Cui MY, Xiong J, Dong Z, Luo Y, Xiao H, Xu L, Huang K, Li ZP, Feng ST. Spectrum of appearances on CT and MRI of hepatic epithelioid hemangioendothelioma. *BMC Gastroenterol* 2015; **15**: 69 [PMID: 26088585 DOI: 10.1186/s12876-015-0299-x]

8 **Alomari AI**. The lollipop sign: a new cross-sectional sign of hepatic epithelioid hemangioendothelioma. *Eur J Radiol* 2006; **59**: 460-464 [PMID: 16644166 DOI: 10.1016/j.ejrad.2006.03.022]

9 **Marino IR**, Todo S, Tzakis AG, Klintmalm G, Kelleher M, Iwatsuki S, Starzl TE, Esquivel CO. Treatment of hepatic epithelioid hemangioendothelioma with liver transplantation. *Cancer* 1988; **62**: 2079-2084 [PMID: 3052779]

10 **Ahmad N**, Adams DM, Wang J, Prakash R, Karim NA. Hepatic epithelioid hemangioendothelioma in a patient with hemochromatosis. *J Natl Compr Canc Netw* 2014; **12**: 1203-1207 [PMID: 25190690]

11 **Lyburn ID**, Torreggiani WC, Harris AC, Zwirewich CV, Buckley AR, Davis JE, Chung SW, Scudamore CH, Ho SG. Hepatic epithelioid hemangioendothelioma: sonographic, CT, and MR imaging appearances. *AJR Am J Roentgenol* 2003; **180**: 1359-1364 [PMID: 12704052]

12 **Mistry AM**, Gorden DL, Busler JF, Coogan AC, Kelly BS. Diagnostic and therapeutic challenges in hepatic epithelioid hemangioendothelioma. *J Gastrointest Cancer* 2012; **43**: 521-525 [PMID: 22544493 DOI: 10.1007/s12029-012-9389-y]

13 **Lerut JP**, Orlando G, Adam R, Schiavo M, Klempnauer J, Mirza D, Boleslawski E, Burroughs A, Sellés CF, Jaeck D, Pfitzmann R, Salizzoni M, Söderdahl G, Steininger R, Wettergren A, Mazzaferro V, Le Treut YP, Karam V. The place of liver transplantation in the treatment of hepatic epitheloid hemangioendothelioma: report of the European liver transplant registry. *Ann Surg* 2007; **246**: 949-957; discussion 957 [PMID: 18043096 DOI: 10.1097/SLA]

14 **Grotz TE**, Nagorney D, Donohue J, Que F, Kendrick M, Farnell M, Harmsen S, Mulligan D, Nguyen J, Rosen C, Reid-Lombardo KM. Hepatic epithelioid haemangioendothelioma: is transplantation the only treatment option? *HPB* (Oxford) 2010; **12**: 546-553 [PMID: 20887322 DOI: 10.1111/j.1477-2574.2010.00213.x]

15 **Mascarenhas RC**, Sanghvi AN, Friedlander L, Geyer SJ, Beasley HS, Van Thiel DH. Thalidomide inhibits the growth and progression of hepatic epithelioid hemangioendothelioma. *Oncology* 2004; **67**: 471-475 [PMID: 15714004 DOI: 10.1159/000082932]

16 **Dietze O**, Davies SE, Williams R, Portmann B. Malignant epithelioid haemangioendothelioma of the liver: a clinicopathological and histochemical study of 12 cases. *Histopathology* 1989; **15**: 225-237 [PMID: 2807182 DOI: 10.1111/j.1365-2559.1989.tb03073.x]

17 **Dong K**, Wang XX, Feng JL, Liu H, Zu KJ, Chang J, Lv FD. Pathological characteristics of liver biopsies in eight patients with hepatic epithelioid hemangioendothelioma. *Int J Clin Exp Pathol* 2015; **8**: 11015-11023 [PMID: 26617819]
18 **Hung MC**, Wu HS, Lee YT, Hsu CH, Chou DA, Huang MH. Intraperitoneal metastasis of hepatocellular carcinoma after spontaneous rupture: a case report. *World J Gastroenterol* 2008; **14**: 3927-3931 [PMID: 18609723 DOI: 10.3748/wjg.14.3927]
19 **Lau WY**, Dewar GA, Li AK. Spontaneous rupture of hepatic epithelioid haemangio-endothelioma. *Aust N Z J Surg* 1989; **59**: 972-974 [PMID: 2597104 DOI: 10.1111/j.1445-2197.1989.tb07643.x]

P- Reviewer: Mayor-Ibarguren A **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

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

ISSN 1007-9327

9 771007 932045