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

World Journal of *Gastroenterology*

World J Gastroenterol 2013 June 14; 19(22): 3371-3530

Baishideng Publishing Group
www.wjgnet.com

WJG World Journal of Gastroenterology

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

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World Journal of Gastroenterology

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Weekly Volume 19 Number 22 June 14, 2013

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World Journal of Gastroenterology
Volume 19 Number 22 June 14, 2013

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

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World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

NAME OF JOURNAL
World Journal of Gastroenterology

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

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

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PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F, Lucky Plaza, 315-321 Lockhart Road, Wanchai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
June 14, 2013

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

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

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3371

World J Gastroenterol 2013 June 14; 19(22): 3371-3374
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Controversy over the use of intraoperative blood salvage autotransfusion during liver transplantation for hepatocellular carcinoma patients

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Received: April 23, 2013 Revised: May 2, 2013
Accepted: May 9, 2013
Published online: June 14, 2013

Abstract

Intraoperative blood salvage autotransfusion (IBSA) is used in various surgical procedures. However, because of the risk of reinfusion of salvaged blood contaminated by tumor cells, the use of IBSA in hepatocellular carcinoma (HCC) patients undergoing liver transplantation (LT) is controversial. The critical points include whether tumor cells can be cleared by IBSA, whether IBSA increases the risk of recurrence or metastasis, and what are the indications for IBSA. Moreover, is it warranted to take the risk of tumor dissemination by using IBSA to avoid allogeneic blood transfusion? Do the remaining tumor cells after additional filtration by leukocyte depletion filters still possess potential tumorigenicity? Does IBSA always work well? We have reviewed the literature and tried to address these questions. The available data indicate that IBSA is safe in LT for HCC, but randomized, controlled and prospective trials are urgently required to clarify the uncertainty.

Key words: Intraoperative blood salvage autotransfusion; Liver transplantation; Hepatocellular carcinoma; Leukocyte depletion filters; Allogeneic blood transfusion

Core tip: The use of intraoperative blood salvage autotransfusion (IBSA) in hepatocellular carcinoma (HCC) patients undergoing liver transplantation is controversial as it may reinfuse salvaged blood contaminated by tumor cells. In this article, we reviewed the relevant literature and tried to address the critical questions about IBSA. The available data indicate that IBSA is safe in liver transplantation for HCC, but randomized, controlled and prospective trials are urgently required to clarify the uncertainty.

Zhai B, Sun XY. Controversy over the use of intraoperative blood salvage autotransfusion during liver transplantation for hepatocellular carcinoma patients. *World J Gastroenterol* 2013; 19(22): 3371-3374 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3371.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3371

COMMENTARY ON HOT TOPICS

We have read with great interest the recent article by Akbulut *et al*[1] describing the effects of intraoperative blood salvage autotransfusion (IBSA) during liver transplantation (LT) in patients with hepatocellular carcinoma (HCC) on tumor recurrence or metastasis, and would strongly recommend it to the readers.

HCC is often associated with chronic hepatitis B and C, particularly in East and Southeast Asia, Middle and Western Africa, and Northern and Eastern Europe, where as high as 85% of HCC patients simultaneously suffer from liver cirrhosis[2]. In view of the impaired liver function, elevated portal pressure and increased collateral circulation in these patients, LT has been recommended

as an optimal treatment for HCC as the tumor burden and the underlying liver disease are resolved at the same time[3]. However, the decreased synthesis of coagulation factors, elevated portal pressure and increased collateral circulation all increase the risk of hemorrhage during surgery. Intraoperative hemorrhage is currently recognized as a mortality risk and massive blood transfusion is necessary during LT[4].

As a result of the underlying risk of transfusion of banked blood, IBSA has been used in various surgery procedures[5,6]. With this technique, blood lost during surgery is recovered and processed through a pump system called cell saver, then transfused back into the patient[7]. This requires a system that suctions the wound, separates the blood cells from the other blood products and debris, washes the cells, and returns them to the patient[1]. It is estimated that IBSA reduces the intraoperative blood requirement by up to 60%[1]. The main complication is dilutional or disseminated coagulopathy[8,9]. Another rare complication is pulmonary injury probably linked to leukoagglutinins and transient hemoglobinuria[10]. At present, the risk of complications during IBSA has declined due to technical advances, and IBSA has significantly lower adverse events than allogeneic blood transfusion[11]. However, the use of IBSA in surgical oncology involving LT in HCC patients is controversial, as it may result in reinfusion of salvaged blood contaminated by tumor cells[11]. To date, a few studies have investigated the effects of IBSA system on tumor recurrence in HCC patients undergoing LT[4,6,11].

Can tumor cells be filtered away by IBSA?

The process of IBSA involves collection of blood, filtration, washing, and reinfusion. The use of IBSA for cancer patients is always performed with caution as blood collected from the operating site is at a high risk of contamination with tumor cells. Tumor cells are detected in 91%-93% of blood samples from surgical sites during various cancer surgical procedures including liver resection for liver metastasis[12,13]. During LT for HCC patients, the detection rate of tumor cells in samples from surgical sites was as high as 62.5%[6]. Moreover, Hansen *et al*[13] has reported that in one-third of cases examined, tumor cells in blood collected from surgical sites showed proliferative capacity by forming cell colonies, invasive capacity by passing the collagen coated membrane *in vitro*, and one cell line displayed tumorigenicity *in vivo*, indicating the underlying hazards of salvaged blood.

The crucial consideration is whether IBSA can effectively filter out the tumor cells. Obviously, the use of IBSA alone is not satisfactory, as tumor cells are detected in 62%-91.2% of blood samples after filtration[6,11,14,15]. Leukocyte depletion filters (LDF) with smaller-diameter (3-8 μm) holes have been recommended to further clear away tumor cells from the collected blood. The high efficacy of LDF in removing tumor cells from blood collected from surgical sites has been reported in patients with HCC and prostate, bladder, lung, breast, endometrial, cervical and ovarian cancers[11,14-18]. Catling *et al*[15] reported that LDF removed all the tumor cells in 91.2% of positive samples after LDF with IBSA. Considering the difference in diameters of tumor cells, Liang *et al*[6] further compared the positive cell rates by using the IBSA system with and without LDF in HCC patients undergoing LT, and the results showed that only 25% of the positive samples became negative after cell saver processing, while after additional filtration by LDF, only 2 out of 20 patients whose tumors were unexpectedly ruptured during surgery had the collected blood positive for α-fetoprotein mRNA, and one of these patients was still positive after the second LDF[6]. These data indicate that LDF can render IBSA more efficient in eliminating tumor cells from blood collected from surgical sites, and thus reduce the risk of tumor cell reinfusion.

Does IBSA increase the risk of recurrence or metastasis?

Although more evidence supports application of IBSA in surgical oncology, the fear of reinfusing tumor cells always troubles surgeons. However, in fact, a case report was the only evidence supporting the opinion up to now[19]. In 1975, a patient died from diffuse metastasis 4 wk after pneumonectomy. Because of the blood salvaged during surgery and tumor cells detected in salvaged blood, the metastasis was ascribed to the autotransfusion of blood[19]. Although the American Medical Association issued an alert about the use of IBSA in cancer surgery in 1986[11,15], some organizations, including the National Institute of Clinical Excellence, the Association of Anaesthetists of Great Britain and Ireland, the Obstetric Anaesthetists Association, the American College of Obstetricians and Gynecologists, and the British Confidential Enquiry of Maternal and Child Health have developed guidelines to support the use of IBSA alone or in combination with LDF in cancer surgery[11,15,20,21].

Notwithstanding the above facts, clinical investigations to clarify the correlation of IBSA with tumor recurrence or metastasis have been carefully conducted in the past few decades. The currently available results have failed to show that IBSA increases the risk of recurrence or metastasis; on the contrary, equivalent or even better outcomes have been reported in patients with various cancers who received IBSA during surgery[5,6,12,22-25].

A study aimed at evaluating the long-term safety of IBSA in hepatectomy for HCC was conducted by Hirano and collaborators[22]. Significantly higher 10-year cumulative overall survival and disease-free survival rates were demonstrated in the patients receiving IBSA, particularly patients with stage Ⅰ/Ⅱ HCC, but the differences in cumulative survival and cancer-free survival rates of patients with stage Ⅲ/Ⅳ HCC were not significant from those who did not receive IBSA[22]. Another study reported similar cumulative overall survival and recurrence rates in the IBSA group and IBSA-free group, but IBSA reduced the mean volume of infused allogeneic blood[26].

To date, three studies have investigated the use of IBSA in LT for HCC[4,6,11]. One was to evaluate the efficiency of additional LDF in eliminating tumor cells from IBSA[6]; the other two investigated whether IBSA

increased the risk of recurrence or metastasis[4,11]. In the study by Muscari *et al*[4], among the 47 HCC patients undergoing LT, 31 patients received IBSA while the other 16 did not. During a mean 34-mo follow-up period, both groups showed similar recurrence rates (6.4% in the IBSA group *vs* 6.3% in the IBSA-free group). In another study, Foltys *et al*[11] reported a similar recurrence rate and 5-year survival rate in the IBSA group of 40 patients compared with the IBSA-free group of 96 patients during a mean follow-up period of 1015 d. However, because the two studies lacked a randomized design, heterogeneities existed in age[11], Child score[4,11], the percentage of severe portal hypertension[4] and pretreatment with transarterial chemoembolization[11]. In the study by Akbulut *et al*[1], recurrence, overall survival and disease-free survival rates were comparable in the IBSA and non-IBSA groups, which were similar in age, gender, body mass index, underlying disease, donor type, graft-to-recipient weight ratio, Child-Pugh and model for end-stage liver disease scores, number of tumors, tumor size, alpha-fetoprotein level, Milan and University of California San Francisco (UCSF) criteria, tumor differentiation, macrovascular invasion, or median hospital stay.

Without results from prospective, randomized and controlled clinical trials in a large number of subjects, it is difficult at this stage to judge whether the use of IBSA during LT for HCC is more beneficial or not. However, the current available data may at least indicate that IBSA does not increase the risk of recurrence or metastasis.

What are the indications of IBSA?

Although the current studies partially reduced the fear of the theoretical risk of IBSA during cancer surgery, there are still a series of problems urgently requiring attention.

First, is it justified to take the risk of tumor dissemination during IBSA to avoid or reduce allogeneic blood transfusion? Allogeneic blood transfusion is not a cost-effective method as it is associated with increased risk of transfusion reactions and transfusion-transmitted infections, and induction of immunosuppression[27]. In hepatectomy for HCC, autologous blood transfusion has shown benefits in simulating liver synthetic function[28]. Moreover, allogeneic blood transfusion also increased the tumor recurrence rate by nearly 2-fold in a dose-dependent manner[28]. From the prevailing evidence, it was concluded that the correlation of cancer recurrence and allogeneic blood carried more weight than the theoretical risk of utilizing blood salvage in cancer surgery[28].

Second, do the remaining tumor cells after additional filtration by LDF still possess potential carcinogenicity? It is difficult to answer this question because of lacking of detailed and systemic studies, although the answer has been speculated by some authors[20,28]. Only 0.01%-0.000001% of circulating tumor cells have the potential to form metastatic lesions[20,25,28]. However, the effect of LDF in eliminating tumor cells is also limited[25]. In patients undergoing LT for HCC, a 10% remnant rate of tumor cells was reported by Liang *et al*[6]. However, another fact is that tumor cells ubiquitously exist in circulating blood of cancer patients[28]. Although the theoretical risk of the remnant tumor cells after reinfusion has not been verified[5,6], the correlation of circulating tumor cells and poor prognosis has been proved in various cancers[25,28]. In the study by Akbulut *et al*[1], more than 50% of patients were beyond the Milan and UCSF criteria in the IBSA group, but the metastasis rate in the IBSA group was similar to that in the non-IBSA group. Based on the above facts, it is difficult to distinguish whether the recurrence or metastasis is caused by the reinfusion or circulating tumor cells, but the risk indeed exists. There is the point of view that if tumor cells are already in circulation, is there any significance to adding a few more[20,28]?

Third, does IBSA always work well? Although the scavenging capacity of LDF is far beyond the amount of tumor cells remaining in the reinfusion blood[6,13], the remnant tumor cells appeared in all salvaged blood samples from the HCC patients with ruptured tumors[6]. Moreover, IBSA showed less benefit for patients with stage Ⅲ/Ⅳ HCC than for those with stage Ⅰ/Ⅱ HCC, when compared respectively with corresponding patients without IBSA[22]. The results warn against the application of IBSA in patients with more tumor cells in salvaged blood, such as patients with ruptured tumors or advanced HCC, which may exceed the filtering capacity of IBSA.

In conclusion, IBSA is a safe procedure of blood salvage in LT for HCC based on the available evidence to date. However, well-designed, randomized, controlled, prospective trials are urgently required to clarify the existing concerns.

REFERENCES

1 **Akbulut S**, Kayaalp C, Yilmaz M, Ince V, Ozgor D, Karabulut K, Eris C, Toprak HI, Aydin C, Yilmaz S. Effect of autotransfusion system on tumor recurrence and survival in hepatocellular carcinoma patients. *World J Gastroenterol* 2013; **19**: 1625-1631 [PMID: 23538988 DOI: 10.3748/wjg.v19.i10.1625]

2 **Alves RC**, Alves D, Guz B, Matos C, Viana M, Harriz M, Terrabuio D, Kondo M, Gampel O, Polletti P. Advanced hepatocellular carcinoma. Review of targeted molecular drugs. *Ann Hepatol* 2011; **10**: 21-27 [PMID: 21301005]

3 **Rahbari NN**, Mehrabi A, Mollberg NM, Müller SA, Koch M, Büchler MW, Weitz J. Hepatocellular carcinoma: current management and perspectives for the future. *Ann Surg* 2011; **253**: 453-469 [PMID: 21263310 DOI: 10.1097/SLA.0b013e31820d944f]

4 **Muscari F**, Suc B, Vigouroux D, Duffas JP, Migueres I, Mathieu A, Lavayssiere L, Rostaing L, Fourtanier G. Blood salvage autotransfusion during transplantation for hepatocarcinoma: does it increase the risk of neoplastic recurrence? *Transpl Int* 2005; **18**: 1236-1239 [PMID: 16221153 DOI: 10.1111/j.1432-2277.2005.00207.x]

5 **Waters JH**, Yazer M, Chen YF, Kloke J. Blood salvage and cancer surgery: a meta-analysis of available studies. *Transfusion* 2012; **52**: 2167-2173 [PMID: 22321196 DOI: 10.1111/j.1537-2995.2011.03555.x]

6 **Liang TB**, Li DL, Liang L, Li JJ, Bai XL, Yu W, Wang WL, Shen Y, Zhang M, Zheng SS. Intraoperative blood salvage during liver transplantation in patients with hepatocellular

carcinoma: efficiency of leukocyte depletion filters in the removal of tumor cells. *Transplantation* 2008; **85**: 863-869 [PMID: 18360269 DOI: 10.1097/TP.0b013e3181671f2e]
7 **Pasternak J**, Nikolic D, Milosevic D, Popovic V, Markovic V. An analysis of the influence of intra-operative blood salvage and autologous transfusion on reducing the need for allogeneic transfusion in elective infrarenal abdominal aortic aneurysm repair. *Blood Transfus* 2012; 1-6 [PMID: 23114525 DOI: 10.2450/2012.0069-12]
8 **Scrascia G**, Rotunno C, Nanna D, Rociola R, Guida P, Rubino G, de Luca Tupputi Schinosa L, Paparella D. Pump blood processing, salvage and re-transfusion improves hemoglobin levels after coronary artery bypass grafting, but affects coagulative and fibrinolytic systems. *Perfusion* 2012; **27**: 270-277 [PMID: 22440640 DOI: 10.1177/0267659112442236]
9 **Page P**. Perioperative autotransfusion and its correlation to hemostasis and coagulopathies. *J Extra Corpor Technol* 1991; **23**: 14-21 [PMID: 10149019]
10 **Ashworth A**, Klein AA. Cell salvage as part of a blood conservation strategy in anaesthesia. *Br J Anaesth* 2010; **105**: 401-416 [PMID: 20802228 DOI: 10.1093/bja/aeq244]
11 **Foltys D**, Zimmermann T, Heise M, Kaths M, Lautem A, Wisser G, Weiler N, Hoppe-Lotichius M, Hansen T, Otto G. Liver transplantation for hepatocellular carcinoma--is there a risk of recurrence caused by intraoperative blood salvage autotransfusion? *Eur Surg Res* 2011; **47**: 182-187 [PMID: 21986299 DOI: 10.1159/000330746]
12 **Oefelein MG**, Kaul K, Herz B, Blum MD, Holland JM, Keeler TC, Cook WA, Ignatoff JM. Molecular detection of prostate epithelial cells from the surgical field and peripheral circulation during radical prostatectomy. *J Urol* 1996; **155**: 238-242 [PMID: 7490843]
13 **Hansen E**, Wolff N, Knuechel R, Ruschoff J, Hofstaedter F, Taeger K. Tumor cells in blood shed from the surgical field. *Arch Surg* 1995; **130**: 387-393 [PMID: 7710337]
14 **Futamura N**, Nakanishi H, Hirose H, Nakamura S, Tatematsu M. The effect of storage on the survival of cancer cells in blood and efficient elimination of contaminating cancer cells by a leukocyte depletion filter. *Am Surg* 2005; **71**: 585-590 [PMID: 16089124]
15 **Catling S**, Williams S, Freites O, Rees M, Davies C, Hopkins L. Use of a leucocyte filter to remove tumour cells from intraoperative cell salvage blood. *Anaesthesia* 2008; **63**: 1332-1338 [PMID: 19032302 DOI: 10.1111/j.1365-2044.2008.05637.x]
16 **Raval JS**, Nelson JB, Woldemichael E, Triulzi DJ. Intraoperative cell salvage in radical prostatectomy does not appear to increase long-term biochemical recurrence, metastases, or mortality. *Transfusion* 2012; **52**: 2590-2593 [PMID: 22612661 DOI: 10.1111/j.1537-2995.2012.03682.x]
17 **Aning J**, Dunn J, Daugherty M, Mason R, Pocock R, Ridler B, Thompson J, McGrath JS. Towards bloodless cystectomy: a 10-year experience of intra-operative cell salvage during radical cystectomy. *BJU Int* 2012; **110**: E608-E613 [PMID: 22823412 DOI: 10.1111/j.1464-410X.2012.11338.x]
18 **Kongsgaard UE**, Wang MY, Kvalheim G. Leucocyte depletion filter removes cancer cells in human blood. *Acta Anaesthesiol Scand* 1996; **40**: 118-120 [PMID: 8904269]
19 **Yaw PB**, Sentany M, Link WJ, Wahle WM, GGlover JL. Tumor cells carried through autotransfusion. Contraindication to intraoperative blood recovery? *JAMA* 1975; **231**: 490-491 [PMID: 1172829]
20 **Esper SA**, Waters JH. Intra-operative cell salvage: a fresh look at the indications and contraindications. *Blood Transfus* 2011; **9**: 139-147 [PMID: 21251468 DOI: 10.2450/2011.0081-10]
21 **Bouras I**, Mingo O. Should cell salvage be used in oncological surgery? *Br J Hosp Med* (Lond) 2010; **71**: 57 [PMID: 20081649]
22 **Hirano T**, Yamanaka J, Iimuro Y, Fujimoto J. Long-term safety of autotransfusion during hepatectomy for hepatocellular carcinoma. *Surg Today* 2005; **35**: 1042-1046 [PMID: 16341484 DOI: 10.1007/s00595-005-3082-8]
23 **Ubee S**, Kumar M, Athmanathan N, Singh G, Vesey S. Intraoperative red blood cell salvage and autologous transfusion during open radical retropubic prostatectomy: a cost-benefit analysis. *Ann R Coll Surg Engl* 2011; **93**: 157-161 [PMID: 22041147 DOI: 10.1308/003588411X561044]
24 **MacIvor D**, Nelson J, Triulzi D. Impact of intraoperative red blood cell salvage on transfusion requirements and outcomes in radical prostatectomy. *Transfusion* 2009; **49**: 1431-1434 [PMID: 19320863 DOI: 10.1111/j.1537-2995.2009.02131.x]
25 **Trudeau JD**, Waters T, Chipperfield K. Should intraoperative cell-salvaged blood be used in patients with suspected or known malignancy? *Can J Anaesth* 2012; **59**: 1058-1070 [PMID: 22996966 DOI: 10.1007/s12630-012-9781-x]
26 **Fujimoto J**, Okamoto E, Yamanaka N, Oriyama T, Furukawa K, Kawamura E, Tanaka T, Tomoda F. Efficacy of autotransfusion in hepatectomy for hepatocellular carcinoma. *Arch Surg* 1993; **128**: 1065-1069 [PMID: 8396388]
27 **Ishizawa T**, Hasegawa K, Tsuno NH, Tanaka M, Mise Y, Aoki T, Imamura H, Beck Y, Sugawara Y, Makuuchi M, Takahashi K, Kokudo N. Predeposit autologous plasma donation in liver resection for hepatocellular carcinoma: toward allogenic blood-free operations. *J Am Coll Surg* 2009; **209**: 206-214 [PMID: 19632597 DOI: 10.1016/j.jamcollsurg.2009.03.004]
28 **Waters JH**, Donnenberg AD. Blood salvage and cancer surgery: should we do it? *Transfusion* 2009; **49**: 2016-2018 [PMID: 19903281 DOI: 10.1111/j.1537-2995.2009.02379.x]

P- Reviewer Dehghani SM **S- Editor** Zhai HH
L- Editor Cant MR **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3375

World J Gastroenterol 2013 June 14; 19(22): 3375-3384
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome?

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Received: November 8, 2012 Revised: January 16, 2013
Accepted: January 23, 2013
Published online: June 14, 2013

Abstract

Non-alcoholic fatty liver disease (NAFLD) is currently not a component of the diagnostic criteria for metabolic syndrome (MetS); however, the development of NAFLD has some common mechanisms with the development of MetS, as they share the pathophysiologic basis of insulin resistance. It is also recognized that NAFLD is the hepatic manifestation of MetS. To define MetS, the presence of at least three of the proposed criteria is required, and sometimes it is sufficient to have only one laboratory value, modified by diet or drugs, for the classification of MetS. Ultrasonographically-detected NAFLD (US-NAFLD) is more stable, only changing during the middle- to long-term. Although controversies over MetS continue, and considering that abdominal ultrasonography for diagnosing NAFLD has high specificity and guidelines to modify the natural course of NAFLD by diet composition or lifestyle have not yet been established, why should we not introduce US-NAFLD as a new criterion to define MetS?

Key words: Non-alcoholic fatty liver disease; Metabolic syndrome; Nonalcoholic steatohepatitis; Ultrasonography; Criteria

Tarantino G, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; 19(22): 3375-3384 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3375.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3375

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fat in the liver when it exceeds 5%-10% of its weight[1]. In addition to leading to major histopathological alterations, it may be associated with elevated liver enzymes and abnormal liver function, ranging from steatosis to steatohepatitis, fibrosis, and cirrhosis[2,3]. Although diagnosed worldwide, its prevalence varies, reaching approximately 20%-30% in western countries[4]. In the United States, where 25% of the adult population is obese, the disease occurs in more than two-thirds of these individuals and in more than 90% of class Ⅲ obese individuals[5]. It is estimated that 2% to 3% of the population has nonalcoholic steatohepatitis (NASH)[6,7].

Approximately 74%-90% of patients who undergo liver biopsy show alterations due to triacylglycerol accumulation[8]. The disease is highly prevalent (88.7%) in obese patients undergoing bariatric surgery[9], and the likelihood of developing steatohepatitis is increased in class Ⅲ obesity, with 15%-20% of these patients diagnosed with NASH[10].

Some studies have shown an increased prevalence and higher incidence of cardiovascular disease (CVD) in individuals with NAFLD. These studies have shown hepatic

Table 1 National Cholesterol Education Programme Adult Treatment Panel Ⅲ - 2001/American Heart Association - 2005 metabolic syndrome (diagnosis: 3 of 5)

Risk factor	Defining level (AHA 2005)
Abdominal obesity (waist, inches)	
Men	> 40
Women	> 35
Triglycerides (mg/dL)	150 (or Med)
HDL-C (mg/dL)	
Men	< 40 (or Med)
Women	< 50
BP (mmHg)	130/85 (or Med)
Fasting glucose (mg/dL)	110 (100)

AHA: American Heart Association; HDL-C: High-density lipoprotein-cholesterol; BP: Blood pressure; Med: Medium.

Table 2 International Diabetes Federation: Definitions of metabolic syndrome

Central obesity plus any two of the following four factors	
Raised triglyceride	150 mg/dL, or specific treatment for this lipid abnormality
Reduced HDL-C	< 40 mg/dL in men and < 50 mg/dL in women, or specific treatment for this lipid abnormality
Raised BP	Systolic BP 130 or diastolic BP 85 mmHg, or treatment of previously diagnosed hypertension
Raised FPG	100 mg/dL, or previously diagnosed type 2 diabetes. If above 100 mg/dL, OGTT is strongly recommended but is not necessary to define presence of the syndrome

OGTT: Oral glucose tolerance test; FPG: Fasting plasma glucose; HDL-C: High-density lipoprotein-cholesterol; BP: Blood pressure.

steatosis as an independent risk factor for the development of this disease[11,12].

Metabolic syndrome (MetS), which involves the combination of risk factors for CVD such as insulin resistance, abdominal fat, dyslipidaemia, glucose intolerance, and hypertension, has often been associated with more severe liver abnormalities[13].

NAFLD is now considered to be the hepatic component of the MetS[14,15].

Conventional radiology studies used in the diagnosis of fatty liver include ultrasonography (US), computed tomography, and magnetic resonance imaging. Other than these radiological studies, there are no sensitive and low invasive screening methods for NAFLD. Alanine aminotransferase (ALT) > 30 IU/L is usually used as the cut off level for screening NAFLD[16,17]. This threshold had a sensitivity of 0.92 for detecting the fatty-fibrotic pattern proven by ultrasound among obese children[18]. However, ALT was within normal levels in 69% of those who had increased liver fat[19]. Similarly, in the Dallas Heart Study, 79% of the subjects with a fatty liver (liver fat content > 5.6%) had normal serum ALT[20]. This implies that a normal ALT does not exclude steatosis. Aspartate aminotransferase and gamma glutamyltransferase also correlate with liver fat content independent of obesity[21], but are even less sensitive than serum ALT.

It is well known that NAFLD mirrors insulin resistance, and patients with NAFLD tend to have the abnormal components of the MetS. However, the target for correctly detecting MetS has not yet been met.

METABOLIC SYNDROME: DEFINITION, IMPORTANCE AND PATHOPHYSIOLOGY

Definition

MetS identifies patients at increased risk of developing CVD and type 2 diabetes mellitus. As it is a clustering of different risk factors, and its pathogenesis is not well understood, this has given rise to the development of multiple concurrent definitions. Central obesity and insulin resistance are acknowledged as important causative factors[22-24], together with other associated conditions, including physical inactivity[25], ageing[26] and hormonal imbalance[27,28] such as polycystic ovary syndrome or testosterone insufficiency.

The concept of clustering of risk factors was first described by Reaven[29], when the term "insulin resistance syndrome" was conceived. However, as the mechanisms underlying the link to CVD risk factors remain uncertain and insulin resistance is not easily measured in clinical practice, the more recent consensus, *e.g.*, 2001 National Cholesterol Education Programme (NCEP) Adult Treatment Panel Ⅲ (ATP Ⅲ)[30] (Table 1) and the 2006 International Diabetes Federation (IDF) criteria[31] (Table 2), favours focusing on other clinical parameters that are easier to measure. It is, therefore, imperative to bear in mind that as the newer criteria do not include insulin resistance as one of its diagnostic criteria, individuals diagnosed as having the syndrome using these criteria may not necessarily be insulin resistant. This is in contrast to the 1999 World Health Organization (WHO)[32] and the 1999 European Group for the Study of Insulin Resistance[33] criteria, which emphasise insulin resistance. The IDF consensus, however, takes into account the importance of gender and ethnic differences in predicting early cardiovascular risk and may indeed be a better predictor for risk in women[34] and specific ethnic groups, *e.g.*, South Asians (Indians), who appear to be more susceptible to the development of MetS at waist circumferences below that of the NCEP/ATP Ⅲ cutpoints[35] (Table 3). It is also worth noting that the NCEP/ATP Ⅲ criteria were revised in 2004, where the threshold for fasting glucose was lowered to ⩾ 100 mg/dL (5.6 mmol/L) in concordance with American Diabetes Association criteria for impaired fasting glucose (IFG)[36]. Hence, in view of the various diagnostic criteria used, care will need to be exercised when interpreting clinical studies related to MetS.

Importance

MetS continues to be highly prevalent and contributes to a rapidly growing problem globally. About 40% of adults in the US population are estimated to have MetS by the

Table 3 Central obesity defined according to the International Diabetes Federation

Country/ethnic group	WC
Europids (In the United States, the NCEP-ATP Ⅲ values[1] are likely to continue to be used for clinical purposes)	
Male	⩾ 94 cm (37 in)
Female	⩾ 80 cm (32 in)
South Asians (based on a Chinese, Malay, and Asian-Indian population)	
Male	⩾ 90 cm (35 in)
Female	⩾ 80 cm (32 in)
Chinese	
Male	⩾ 90 cm (35 in)
Female	⩾ 80 cm (32 in)
Japanese	
Male	⩾ 85 cm (34 in)
Female	⩾ 90 cm (32 in)

[1]102 cm, 40 in, male; 88 cm, 35 in, female. NCEP: National Cholesterol Education Programme; WC: Waist circumference; ATP: Adult Treatment Panel.

age of 60 years[26,37]. At least one-fourth of the adult European population may have MetS[38-40], with a similar prevalence in Latin America[41]. MetS is also considered an emerging epidemic in developing Asian countries, including Singapore, China, Japan and South Korea, with a prevalence of 8%-13% in men and 2%-18% in women, depending on the population and definitions used[42-44].

Pathophysiology

There have been several proposed hypotheses for the development of MetS. One such widely quoted hypothesis suggests that adipose tissue dysfunction is the underlying cause, resulting in abnormal metabolism of free fatty acids and the release of adipocytokines which are responsible for the observed inflammatory changes and insulin resistance[45-47]. Adipose tissue is in itself an endocrine organ that is metabolically active, rather than purely an energy storage organ[48-51]. Adiponectin is secreted exclusively by adipocytes in adipose tissue, and low levels in individuals have consistently predicted the presence of MetS and CVD risk[52-56]. In fact, adiponectin can be measured reliably in a clinical setting; its circulating values do not umdergo diurnal fluctuation as much as other markers such as insulin, glucose or triglycerides, and only a small amount is required for its measurement, making this a potentially suitable biomarker for MetS[57,58]. Resistin[23,59,60] and visfatin[61-64] are the other adipocytokines implicated in the pathogenesis of MetS.

An alternative proposed aetiology suggests an underlying state of chronic, low-grade inflammation[65-67], leading to endothelial dysfunction and the release of inflammatory cytokines, which induce insulin resistance in adipose tissue and muscle[67,68]. Indeed, insulin-resistant individuals manifest evidence of low-grade inflammation even without an increase in total body fat[69].

Excess visceral fat accumulation may be causally related to the features of insulin resistance, but might also be a marker of dysfunctional adipose tissue which is unable to appropriately store the energy excess (Figure 1). According to this model, the body's ability to cope with the surplus of calories (resulting from excess caloric consumption, a sedentary lifestyle or, as is often the case, a combination of both factors) might, ultimately, determine the individual's susceptibility to developing MetS. There is evidence to suggest that if the extra energy is channelled into insulin-sensitive subcutaneous adipose tissue, the individual, although in positive energy balance, will be protected against the development of MetS. However, in cases in which adipose tissue is absent, deficient or insulin resistant with a limited ability to store the energy excess, the triacylglycerol surplus will be deposited at undesirable sites such as the liver, the heart, the skeletal muscle and in visceral adipose tissue - a phenomenon described as ectopic fat deposition. Factors associated with the preferential accumulation of visceral fat and with features of insulin resistance include, among others, smoking, the well documented genetic susceptibility to visceral obesity[70] and a neuroendocrine profile related to a maladaptive response to stress[71]. The resulting metabolic consequences of this "defect" in energy partitioning include visceral obesity, insulin resistance, an atherogenic dyslipidaemia and a prothrombotic, inflammatory profile. These are defining features of MetS.

This constellation of abnormalities can be detected by the clinical criteria for MetS, the two simplest being the simultaneous presence of increased waist girth and fasting triacylglycerol levels, a condition that has been described as "hypertriglyceridaemic waist"[72].

It is noteworthy to stress that the identification of other risk factors might improve knowledge on the pathogenesis of NAFLD and open the way to new therapeutic approaches[73-75]. The debate surrounding the mechanisms inducing or favouring the presence/severity of NAFLD continues. In fact, some investigators have identified factors other that MetS to be associated with NAFLD[76-78].

RECENT CONTROVERSY

Although the MetS has existed in various forms and definitions for more than eight decades, only in the past 5 years has real controversy about its definition and significance emerged[79,80]. The main controversy is that the syndrome has had too many definitions and there is a lack of clarity about its role and value in clinical practice[81]. It is fair to say, with exceptions[82], that most of the published reports indicate that the syndrome does not predict cardiovascular events or disease progression any better than the sum of its components[3,83,84]. The relative value in predicting type 2 diabetes remains uncertain[85]. The controversy, however, drove the need for a single global definition. Thus, came the initiative of the IDF and the American Heart Association/National Heart, Lung, and Blood Institute, joined by the World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity[79] to develop one unified definition[86].

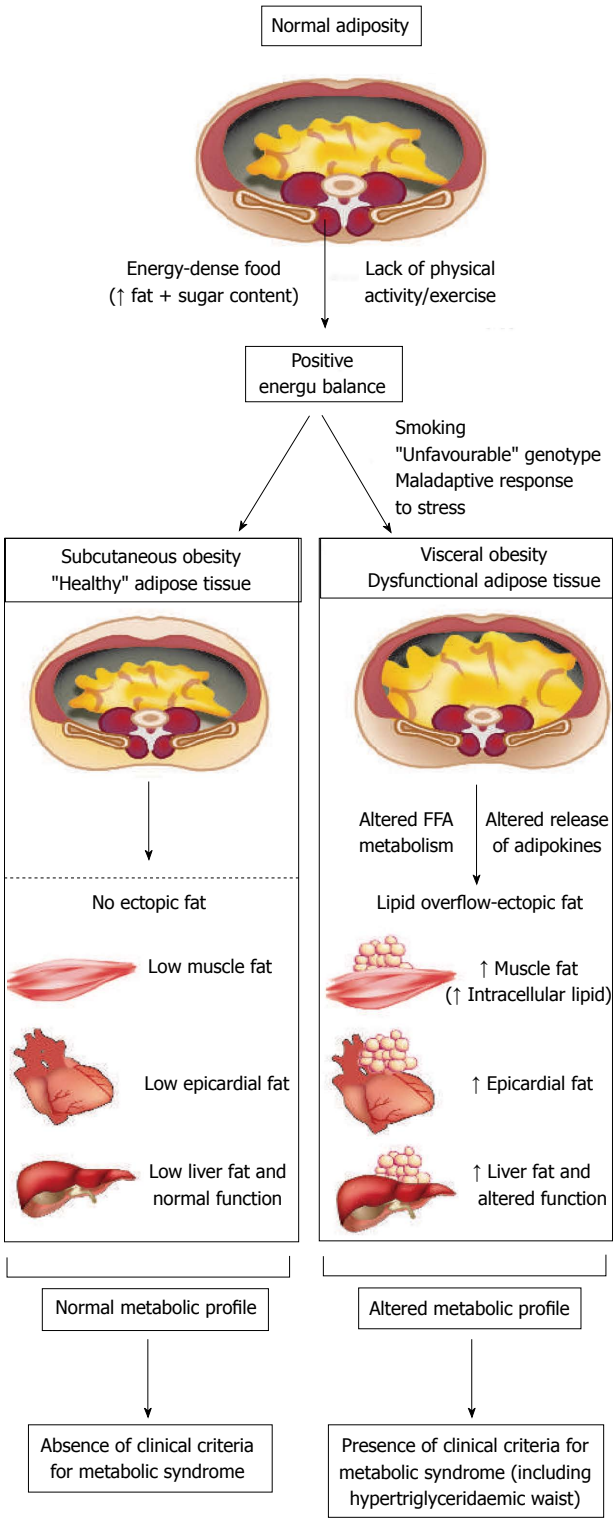

Figure 1 The lipid overflow-ectopic fat model. FFA: Free fatty acid.

The main difference between the NCEP ATP Ⅲ (National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults)[87] and the IDF definitions[88] was that the IDF had a threshold value for waist circumference as obligatory. As a major step in consensus, this obligation has been reversed so that we now have a platform for standardised reporting in epidemiological and clinical research[81]. Yet, because the relation between waist circumference and CVD and diabetes risk differs globally, the definition for the expanded waist circumference remains unsettled. In the meantime, national or regional cutpoints for waist circumference can be used.

Insulin resistance continues to explain most, if not all of the MetS. In fact, no other mechanisms have emerged that come close to justifying the individual components or their clustering. Evidence now indicates that the MetS begins with excess central adiposity[89]. When β-cell function is responsive, hyperinsulinaemia results but fasting and postprandial glycaemia often remain normal for years. However, in those genetically predisposed, defects in insulin secretion and IFG and/or impaired glucose tolerance follow[90].

Most controversial has been the mechanism of hypertension under the tent of insulin resistance. However, not only are the effects of insulin on sodium reabsorption and sympathetic nervous system activation maintained despite insulin resistance, but increases in angiotensinogen, resistin, and leptin secretion from adipose tissue have also been implicated in the pathophysiology of hypertension in the syndrome[91]. Moreover, insulin resistance is closely associated with abnormalities in nitric oxide (NO) bioavailability and reduced phosphatidylinositol 3-kinase/protein kinase B signalling in the vascular wall, both of which have a crucial role in mobilisation of endothelial progenitor cells from bone marrow[91]. Not only do higher levels of free fatty acids directly reduce NO-dependent vasodilatation, but insulin resistance itself also results in structural or functional damage to the endothelium and apoptosis[91]. Reparative processes that regenerate injured endothelium might be increased by agents such as peroxisome proliferator-activated receptor γ agonists that enhance insulin sensitivity, an effect mediated by endothelial progenitor cells[92].

Genetic predisposition also relates to the MetS. A recent study found that a polymorphism in the multi-PDZ domain-containing adaptor protein, a protein that regulates the high-density lipoprotein-receptor scavenger-receptor type B class 1, was associated with the MetS[93]. Shift work, sleep deprivation, and bright-light exposure at night also relate to increased adiposity and prevalence of the MetS; clock genes are expressed in adipose tissue, and both their levels of expression and their genetic variants correlate with different components of the syndrome[94].

Another area of recent interest is vitamin D. Increasing evidence indicates that vitamin D deficiency is associated with the risk of CVD. Particularly relevant is a study that examined the association of serum vitamin D concentrations with risk factors for CVD in US adolescents[95].

The hypothesis that the MetS is an outgrowth of insulin resistance provides a strategy for management. Weight loss often reduces insulin resistance; and caloric restriction, weight-loss drugs, and bariatric surgery have been proved to be effective.

Although long-term weight reduction through dietary and pharmacological means is theoretically possible, most dietary and weight-loss drug studies have only continued for a few years. In contrast, in one 10-year follow-up after bariatric surgery[96], weight loss of 25% and improvement in the MetS were achieved; total mortality was also reduced. Even in the absence of weight loss, long-term physical activity, as measured by cardiorespiratory fitness, prevents the MetS[97], reduces cancer incidence and related mortality, and all-cause mortality[98].

Finally, one class of drugs that reduces insulin resistance and many of the components of the syndrome is the thiazolidinediones. These drugs act mainly in adipose tissue to favourably modify secretions of products that contribute to the pathophysiology of the MetS, including free fatty acids and adipocytokines. The major effect of thiazolidinediones is on dysglycaemia, which accounts for their use in the treatment of diabetes, yet the class as a whole has anti-inflammatory effects. At present, however, drug therapy for the MetS largely requires separate agents for the treatment of dysglycaemia, dyslipidaemia, and hypertension[99].

The MetS is a widely accepted concept that identifies the centrally obese patient with increased risk for CVD and diabetes. A global definition has now been proposed, insights into aetiology and mechanisms have been furthered, and, despite the controversies, lifestyle interventions remain the primary therapy. After lifestyle, residual risk for CVD needs to be treated with appropriate drugs.

US AND NAFLD

US is currently the most common method for screening asymptomatic patients with elevated liver enzymes and suspected NAFLD[100]. US findings of fatty liver include hepatomegaly, diffuse increases in the echogenicity of the liver parenchyma, and vascular blunting.

Nonsteatotic hepatic parenchyma exhibits an echotexture similar to that of renal parenchyma, but becomes "brighter" when infiltrated with fat[101]. This hepatorenal contrast can be used to detect hepatosteatosis[102,103]. However, bright liver contrast associated with fibrosis is discussed in the literature[103]. US is easily performed and has a low cost, however, it also has limitations. It is operator dependent and subject to significant intra- and interobserver variability[104]. It is impossible for US to provide quantitative information about the degree of fat accumulation. The sensitivity of US to detect steatosis decreases with a degree of fat infiltration less than 30%[105]. In obese patients, sensitivity lower than 40% has been reported in the detection of hepatosteatosis[106]. Finally, US has failed to prove efficacious in the detection of inflammation and fibrosis, therefore, it cannot be utilized to diagnose NASH and hepatic fibrosis[107]. However, in a recent study, Iijima *et al*[108] used an ultrasound contrast agent (Levovist; Sherling, Berlin, Germany) to distinguish between simple steatosis and NASH. Levovist contains galactose and palmitic acid and is taken up by hepatocytes[109]. These moieties participate in sugar and fat metabolism[110]. The uptake of Levovist was observed to significantly decrease in NASH patients, thus correlating with fibrosis rather than steatosis[108]. Larger studies are needed to evaluate the use of contrast US in the diagnosis of NASH characterised by inflammation and fibrosis, although there is a no absolute consensus in separating NASH from simple fatty liver as two distinct entities.

FUTURE REMARKS

The MetS is associated with abdominal obesity and its criteria include waist circumference[30,31]. In addition, NAFLD has been reported to be associated with abdominal obesity[110].

The presence of multiple metabolic disorders such as diabetes mellitus, obesity, dyslipidaemia and hypertension is associated with a potentially progressive, severe liver disease[14,111-115]. Previous reports demonstrated that the prevalence of NAFLD increased to 10%-80% in individuals with obesity, 35%-90% in individuals with type 2 diabetes mellitus, 30%-56% in individuals with hypertension, and 26%-58% in individuals with dyslipidaemia[116-119].

It is clinically critical that a large number of patients with NAFLD were not diagnosed with the MetS, when we used today's definition of the MetS[120]. Why not change the approach and use the presence of NAFLD as a new criterion for detecting the MetS?

Recently, it was shown that ultrasonographically-detected NAFLD (US-NAFLD) is an independent predictor for identifying patients with insulin resistance in non-obese, non-diabetic middle-aged Asian adults[121]. Therefore, US-NAFLD may identify individuals with insulin resistance that cannot be identified by MetS in this population[121].

On this basis, we believe that this suggestion, *i.e.*, the inclusion of NAFLD could help initiate weight control at the "earliest possible time" in the progression of disease, *i.e.*, obesity/MetS, which means diagnosing NAFLD earlier rather than later, using the simplest method possible, *i.e.*, at US[110].

CONCLUSION

NAFLD is highly prevalent and is considered the hepatic component of the MetS. The WHO, the NCEP-ATP Ⅲ and the IDF have different criteria to define MetS. The MetS is associated with NAFLD, with the WHO definition being the best to determine its presence, probably due to the inclusion of insulin resistance as a main component. Unification of criteria is needed to adequately compare the prevalence of MetS and its relationship with NAFLD in different population, however, this is very hard task.

Further study will be needed to verify whether the inclusion of steatosis in the panel of MetS indicators will improve the predictive power of cardiovascular risk bet-

ter than the current MetS criteria.

To define MetS, the presence of at least three of the proposed criteria is required, however, sometimes it is sufficient to have only one laboratory value, modified by diet or drugs, for the classification. US-NAFLD detection is more stable, and changes in the middle-to-long term. Although the controversy surrounding the utility of the MetS continue, considering that abdominal US in the diagnosis of NAFLD has a sensitivity of 91.7% and a specificity of 100%[122] and guidelines to modify the natural course of NAFLD by diet composition or lifestyle have not been established[123], why should we not introduce US-NAFLD as a new criterion to define MetS?

REFERENCES

1 **Finelli C**, Tarantino G. Is visceral fat reduction necessary to favour metabolic changes in the liver? *J Gastrointestin Liver Dis* 2012; **21**: 205-208 [PMID: 22720311]

2 **Tarantino G**, Colao A, Capone D, Conca P, Tarantino M, Grimaldi E, Chianese D, Finelli C, Contaldo F, Scopacasa F, Savastano S. Circulating levels of cytochrome C, gamma-glutamyl transferase, triglycerides and unconjugated bilirubin in overweight/obese patients with non-alcoholic fatty liver disease. *J Biol Regul Homeost Agents* 2011; **25**: 47-56 [PMID: 21382273]

3 **Tarantino G**, Finelli C, Colao A, Capone D, Tarantino M, Grimaldi E, Chianese D, Gioia S, Pasanisi F, Contaldo F, Scopacasa F, Savastano S. Are hepatic steatosis and carotid intima media thickness associated in obese patients with normal or slightly elevated gamma-glutamyl-transferase? *J Transl Med* 2012; **10**: 50 [PMID: 22424154 DOI: 10.1186/1479-5876-10-50]

4 **Colicchio P**, Tarantino G, del Genio F, Sorrentino P, Saldalamacchia G, Finelli C, Conca P, Contaldo F, Pasanisi F. Non-alcoholic fatty liver disease in young adult severely obese non-diabetic patients in South Italy. *Ann Nutr Metab* 2005; **49**: 289-295 [PMID: 16088092 DOI: 10.1159/000087295]

5 **Lazo M**, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; **28**: 339-350 [PMID: 18956290 DOI: 10.1055/s-0028-1091978]

6 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

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 **De Ridder RJ**, Schoon EJ, Smulders JF, van Hout GC, Stockbrügger RW, Koek GH. Review article: Non-alcoholic fatty liver disease in morbidly obese patients and the effect of bariatric surgery. *Aliment Pharmacol Ther* 2007; **26** Suppl 2: 195-201 [PMID: 18081662 DOI: 10.1111/j.1365-2036.2007.03483.x]

9 **Chavez-Tapia NC**, Tellez-Avila FI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. *Cochrane Database Syst Rev* 2010; (1): CD007340 [PMID: 20091629]

10 **Scheen AJ**, Luyckx FH. Obesity and liver disease. *Best Pract Res Clin Endocrinol Metab* 2002; **16**: 703-716 [PMID: 12468416 DOI: 10.1053/beem.2002.0225]

11 **Liou I**, Kowdley KV. Natural history of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; **40** Suppl 1: S11-S16 [PMID: 16540761]

12 **Targher G**, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. *Atherosclerosis* 2007; **191**: 235-240 [PMID: 16970951 DOI: 10.1016/j.atherosclerosis.2006.08.021]

13 **Gupte P**, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, Patel N, Madan A, Amarapurkar A. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol* 2004; **19**: 854-858 [PMID: 15242486 DOI: 10.1111/j.1440-1746.2004.03312.x]

14 **Tarantino G**, Saldalamacchia G, Conca P, Arena A. Non-alcoholic fatty liver disease: further expression of the metabolic syndrome. *J Gastroenterol Hepatol* 2007; **22**: 293-303 [PMID: 17295757 DOI: 10.1111/j.1440-1746.2007.04824.x]

15 **Souza MR**, Diniz Mde F, Medeiros-Filho JE, Araújo MS. Metabolic syndrome and risk factors for non-alcoholic fatty liver disease. *Arq Gastroenterol* 2012; **49**: 89-96 [PMID: 22481692 DOI: 10.1590/S0004-28032012000100015]

16 **Fraser A**, Longnecker MP, Lawlor DA. Prevalence of elevated alanine aminotransferase among US adolescents and associated factors: NHANES 1999-2004. *Gastroenterology* 2007; **133**: 1814-1820 [PMID: 18054554 DOI: 10.1053/j.gastro.2007.08.077]

17 **Strauss RS**, Barlow SE, Dietz WH. Prevalence of abnormal serum aminotransferase values in overweight and obese adolescents. *J Pediatr* 2000; **136**: 727-733 [PMID: 10839867]

18 **Devadason CA**, Scheimann AO. Overview of screening methods for fatty liver disease in children. *World J Hepatol* 2012; **4**: 1-4 [PMID: 22312449 DOI: 10.4254/wjh.v4.i1.1]

19 **Reeder SB**, Cruite I, Hamilton G, Sirlin CB. Quantitative assessment of liver fat with magnetic resonance imaging and spectroscopy. *J Magn Reson Imaging* 2011; **34**: 729-749 [PMID: 21928307 DOI: 10.1002/jmri.22580]

20 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

21 **Thamer C**, Tschritter O, Haap M, Shirkavand F, Machann J, Fritsche A, Schick F, Häring H, Stumvoll M. Elevated serum GGT concentrations predict reduced insulin sensitivity and increased intrahepatic lipids. *Horm Metab Res* 2005; **37**: 246-251 [PMID: 15952086 DOI: 10.1055/s-2005-861411]

22 **Anderson PJ**, Critchley JA, Chan JC, Cockram CS, Lee ZS, Thomas GN, Tomlinson B. Factor analysis of the metabolic syndrome: obesity vs insulin resistance as the central abnormality. *Int J Obes Relat Metab Disord* 2001; **25**: 1782-1788 [PMID: 11781758 DOI: 10.1038/sj.ijo.0801837]

23 **Sadashiv S**, Paul BN, Kumar S, Chandra A, Dhananjai S, Negi MP. Over expression of resistin in adipose tissue of the obese induces insulin resistance. *World J Diabetes* 2012; **3**: 135-141 [PMID: 22816026]

24 **Indulekha K**, Surendar J, Mohan V. High sensitivity C-reactive protein, tumor necrosis factor-α, interleukin-6, and vascular cell adhesion molecule-1 levels in Asian Indians with metabolic syndrome and insulin resistance (CURES-105). *J Diabetes Sci Technol* 2011; **5**: 982-988 [PMID: 21880241]

25 **Golbidi S**, Mesdaghinia A, Laher I. Exercise in the metabolic syndrome. *Oxid Med Cell Longev* 2012; **2012**: 349710 [PMID: 22829955]

26 **Gombet T**, Longo-Mbenza B, Ellenga-Mbolla B, Ikama MS, Mokondjimobe E, Kimbally-Kaky G, Nkoua JL. Aging, female sex, migration, elevated HDL-C, and inflammation are associated with prevalence of metabolic syndrome among African bank employees. *Int J Gen Med* 2012; **5**: 495-503 [PMID: 22807636]

27 **Malik SM**, Traub ML. Defining the role of bariatric surgery in polycystic ovarian syndrome patients. *World J Diabetes* 2012; **3**: 71-79 [PMID: 22532886]

28 **Saad F**, Gooren LJ. The role of testosterone in the etiology

and treatment of obesity, the metabolic syndrome, and diabetes mellitus type 2. *J Obes* 2011; **2011**: 471584 [PMID: 20847893 DOI: 10.1155/2011/471584]
29 **Reaven GM**. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607 [PMID: 3056758 DOI: 10.2337/diabetes.37.12.1595]
30 **Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults**. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; **285**: 2486-2497 [PMID: 11368702]
31 **Alberti KG**, Zimmet P, Shaw J. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med* 2006; **23**: 469-480 [PMID: 16681555 DOI: 10.1111/j.1464-5491.2006.01858.x]
32 **Alberti KG**, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539-553 [PMID: 9686693]
33 **Balkau B**, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med* 1999; **16**: 442-443 [PMID: 10342346]
34 **Skilton MR**, Moulin P, Sérusclat A, Nony P, Bonnet F. A comparison of the NCEP-ATPIII, IDF and AHA/NHLBI metabolic syndrome definitions with relation to early carotid atherosclerosis in subjects with hypercholesterolemia or at risk of CVD: evidence for sex-specific differences. *Atherosclerosis* 2007; **190**: 416-422 [PMID: 16616756 DOI: 10.1016/j.atherosclerosis.2006.02.019]
35 **Ajjan R**, Carter AM, Somani R, Kain K, Grant PJ. Ethnic differences in cardiovascular risk factors in healthy Caucasian and South Asian individuals with the metabolic syndrome. *J Thromb Haemost* 2007; **5**: 754-760 [PMID: 17408409 DOI: 10.1111/j.1538-7836.2007.02434.x]
36 **Grundy SM**, Brewer HB, Cleeman JI, Smith SC, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109**: 433-438 [PMID: 14744958]
37 **Ford ES**, Li C, Zhao G. Prevalence and correlates of metabolic syndrome based on a harmonious definition among adults in the US. *J Diabetes* 2010; **2**: 180-193 [PMID: 20923483]
38 **Martínez MA**, Puig JG, Mora M, Aragón R, O'Dogherty P, Antón JL, Sánchez-Villares T, Rubio JM, Rosado J, Torres R, Marcos J, Pallardo LF, Banegas JR. Metabolic syndrome: prevalence, associated factors, and C-reactive protein: the MADRIC (MADrid RIesgo Cardiovascular) Study. *Metabolism* 2008; **57**: 1232-1240 [PMID: 18702949 DOI: 10.1016/j.metabol.2008.04.017]
39 **Zanchetti A**, Hennig M, Baurecht H, Tang R, Cuspidi C, Carugo S, Mancia G. Prevalence and incidence of the metabolic syndrome in the European Lacidipine Study on Atherosclerosis (ELSA) and its relation with carotid intima-media thickness. *J Hypertens* 2007; **25**: 2463-2470 [PMID: 17984668 DOI: 10.1097/HJH.0b013e3282f063d5]
40 **Anagnostis P**. Metabolic syndrome in the Mediterranean region: Current status. *Indian J Endocrinol Metab* 2012; **16**: 72-80 [PMID: 22276255]
41 **Escobedo J**, Schargrodsky H, Champagne B, Silva H, Boissonnet CP, Vinueza R, Torres M, Hernandez R, Wilson E. Prevalence of the metabolic syndrome in Latin America and its association with sub-clinical carotid atherosclerosis: the CARMELA cross sectional study. *Cardiovasc Diabetol* 2009; **8**: 52 [PMID: 19781089 DOI: 10.1186/1475-2840-8-52]
42 **Shen J**, Goyal A, Sperling L. The emerging epidemic of obesity, diabetes, and the metabolic syndrome in china. *Cardiol Res Pract* 2012; **2012**: 178675 [PMID: 21961074]
43 **Lee J**, Heng D, Ma S, Chew SK, Hughes K, Tai ES. The metabolic syndrome and mortality: the Singapore Cardiovascular Cohort Study. *Clin Endocrinol* (Oxf) 2008; **69**: 225-230 [PMID: 18208579 DOI: 10.1111/j.1365-2265.2008.03174.x]
44 **Park HS**, Kim SM, Lee JS, Lee J, Han JH, Yoon DK, Baik SH, Choi DS, Choi KM. Prevalence and trends of metabolic syndrome in Korea: Korean National Health and Nutrition Survey 1998-2001. *Diabetes Obes Metab* 2007; **9**: 50-58 [PMID: 17199718 DOI: 10.1111/j.1463-1326.2005.00569.x]
45 **Dodson MV**, Mir PS, Hausman GJ, Guan LL, Du M, Jiang Z, Fernyhough ME, Bergen WG. Obesity, metabolic syndrome, and adipocytes. *J Lipids* 2011; **2011**: 721686 [PMID: 21811683]
46 **Ye J**. Emerging role of adipose tissue hypoxia in obesity and insulin resistance. *Int J Obes* (Lond) 2009; **33**: 54-66 [PMID: 19050672]
47 **Lee K**, Villena JA, Moon YS, Kim KH, Lee S, Kang C, Sul HS. Inhibition of adipogenesis and development of glucose intolerance by soluble preadipocyte factor-1 (Pref-1). *J Clin Invest* 2003; **111**: 453-461 [PMID: 12588883]
48 **Galic S**, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* 2010; **316**: 129-139 [PMID: 19723556 DOI: 10.1016/j.mce.2009.08.018]
49 **Spiegelman BM**, Flier JS. Adipogenesis and obesity: rounding out the big picture. *Cell* 1996; **87**: 377-389 [PMID: 8898192 DOI: 10.1016/S0092-8674(00)81359-8]
50 **Frühbeck G**. The adipose tissue as a source of vasoactive factors. *Curr Med Chem Cardiovasc Hematol Agents* 2004; **2**: 197-208 [PMID: 15320786 DOI: 10.2174/1568016043356255]
51 **Rondinone CM**. Adipocyte-derived hormones, cytokines, and mediators. *Endocrine* 2006; **29**: 81-90 [PMID: 16622295]
52 **Garg MK**, Dutta MK, Mahalle N. Adipokines (adiponectin and plasminogen activator inhhibitor-1) in metabolic syndrome. *Indian J Endocrinol Metab* 2012; **16**: 116-123 [PMID: 22276262]
53 **Alikaşifoğlu A**, Gönç N, Özön ZA, Sen Y, Kandemir N. The relationship between serum adiponectin, tumor necrosis factor-alpha, leptin levels and insulin sensitivity in childhood and adolescent obesity: adiponectin is a marker of metabolic syndrome. *J Clin Res Pediatr Endocrinol* 2009; **1**: 233-239 [PMID: 21274300]
54 **Bai YM**, Chen TT, Yang WS, Chi YC, Lin CC, Liou YJ, Wang YC, Su TP, Chou P, Chen JY. Association of adiponectin and metabolic syndrome among patients taking atypical antipsychotics for schizophrenia: a cohort study. *Schizophr Res* 2009; **111**: 1-8 [PMID: 19409756 DOI: 10.1016/j.schres.2009.03.014]
55 **Ahonen TM**, Saltevo JT, Kautiainen HJ, Kumpusalo EA, Vanhala MJ. The association of adiponectin and low-grade inflammation with the course of metabolic syndrome. *Nutr Metab Cardiovasc Dis* 2012; **22**: 285-291 [PMID: 21093230 DOI: 10.1016/j.numecd.2010.07.001]
56 **Okamoto Y**. Adiponectin provides cardiovascular protection in metabolic syndrome. *Cardiol Res Pract* 2011; **2011**: 313179 [PMID: 21318102]
57 **Brooks NL**, Moore KS, Clark RD, Perfetti MT, Trent CM, Combs TP. Do low levels of circulating adiponectin represent a biomarker or just another risk factor for the metabolic syndrome? *Diabetes Obes Metab* 2007; **9**: 246-258 [PMID: 17391150 DOI: 10.1111/j.1463-1326.2006.00596.x]
58 **Hirose H**, Yamamoto Y, Seino-Yoshihara Y, Kawabe H, Saito I. Serum high-molecular-weight adiponectin as a marker for the evaluation and care of subjects with metabolic syndrome and related disorders. *J Atheroscler Thromb* 2010; **17**: 1201-1211 [PMID: 20948162 DOI: 10.5551/jat.6106]
59 **Povel CM**, Boer JM, Feskens EJ. Shared genetic variance between the features of the metabolic syndrome: heritability studies. *Mol Genet Metab* 2011; **104**: 666-669 [PMID: 21963081 DOI: 10.1016/j.ymgme.2011.08.035]
60 **Mojiminiyi OA**, Abdella NA. Associations of resistin with inflammation and insulin resistance in patients with type

2 diabetes mellitus. *Scand J Clin Lab Invest* 2007; **67**: 215-225 [PMID: 17366001 DOI: 10.1080/00365510601032532]
61 **Hajianfar H**, Bahonar A, Entezari MH, Askari G, Yazdani M. Lipid Profiles and Serum Visfatin Concentrations in Patients with Type II Diabetes in Comparison with Healthy Controls. *Int J Prev Med* 2012; **3**: 326-331 [PMID: 22708029]
62 **Taşkesen D**, Kirel B, Us T. Serum visfatin levels, adiposity and glucose metabolism in obese adolescents. *J Clin Res Pediatr Endocrinol* 2012; **4**: 76-81 [PMID: 22672864]
63 **Filippatos TD**, Derdemezis CS, Gazi IF, Lagos K, Kiortsis DN, Tselepis AD, Elisaf MS. Increased plasma visfatin levels in subjects with the metabolic syndrome. *Eur J Clin Invest* 2008; **38**: 71-72 [PMID: 18173555]
64 **Chang YH**, Chang DM, Lin KC, Shin SJ, Lee YJ. Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. *Diabetes Metab Res Rev* 2011; **27**: 515-527 [PMID: 21484978 DOI: 10.1002/dmrr.1201]
65 **Hanley AJ**, Festa A, D'Agostino RB, Wagenknecht LE, Savage PJ, Tracy RP, Saad MF, Haffner SM. Metabolic and inflammation variable clusters and prediction of type 2 diabetes: factor analysis using directly measured insulin sensitivity. *Diabetes* 2004; **53**: 1773-1781 [PMID: 15220201 DOI: 10.2337/diabetes.53.7.1773]
66 **Hu FB**, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. *Diabetes* 2004; **53**: 693-700 [PMID: 14988254 DOI: 10.2337/diabetes.53.3.693]
67 **Haring R**, Rosvall M, Völker U, Völzke H, Kroemer H, Nauck M, Wallaschofski H. A network-based approach to visualize prevalence and progression of metabolic syndrome components. *PLoS One* 2012; **7**: e39461 [PMID: 22724019 DOI: 10.1371/journal.pone.0039461]
68 **Sampey BP**, Freemerman AJ, Zhang J, Kuan PF, Galanko JA, O'Connell TM, Ilkayeva OR, Muehlbauer MJ, Stevens RD, Newgard CB, Brauer HA, Troester MA, Makowski L. Metabolomic profiling reveals mitochondrial-derived lipid biomarkers that drive obesity-associated inflammation. *PLoS One* 2012; **7**: e38812 [PMID: 22701716 DOI: 10.1371/journal.pone.0038812]
69 **Jeemon P**, Prabhakaran D, Ramakrishnan L, Gupta R, Ahmed F, Thankappan K, Kartha C, Chaturvedi V, Reddy K. Association of high sensitive C-reactive protein (hsCRP) with established cardiovascular risk factors in the Indian population. *Nutr Metab* (Lond) 2011; **8**: 19 [PMID: 21443784 DOI: 10.1186/1743-7075-8-19]
70 **Bouchard C**, Tremblay A. Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. *J Nutr* 1997; **127**: 943S-947S [PMID: 9164270]
71 **Björntorp P**. Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition* 1997; **13**: 795-803 [PMID: 9290093 DOI: 10.1016/S0899-9007(97)00191-3]
72 **Lemieux I**, Poirier P, Bergeron J, Alméras N, Lamarche B, Cantin B, Dagenais GR, Després JP. Hypertriglyceridemic waist: a useful screening phenotype in preventive cardiology? *Can J Cardiol* 2007; **23** Suppl B: 23B-31B [PMID: 17932584 DOI: 10.1016/S0828-282X(07)71007-3]
73 **Green RM**. NASH--hepatic metabolism and not simply the metabolic syndrome. *Hepatology* 2003; **38**: 14-17 [PMID: 12829980 DOI: 10.1053/jhep.2003.50325]
74 **Chitturi S**, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 2001; **21**: 27-41 [PMID: 11296694 DOI: 10.1055/s-2001-12927]
75 **Vanni E**, Bugianesi E, Kotronen A, De Minicis S, Yki-Järvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? *Dig Liver Dis* 2010; **42**: 320-330 [PMID: 20207596 DOI: 10.1016/j.dld.2010.01.016]
76 **Yilmaz Y**, Senates E, Ayyildiz T, Colak Y, Tuncer I, Ovunc AO, Dolar E, Kalayci C. Characterization of nonalcoholic fatty liver disease unrelated to the metabolic syndrome. *Eur J Clin Invest* 2012; **42**: 411-418 [PMID: 21913918 DOI: 10.1111/j.1365-2362.2011.02597.x]
77 **Xu L**, Xu CF, Yu CH, Miao M, Li YM. Haemoglobin and non-alcoholic fatty liver disease: further evidence from a population-based study. *Gut* 2009; **58**: 1706-1707 [PMID: 19923352 DOI: 10.1136/gut.2009.186668]
78 **Yu C**, Xu C, Xu L, Yu J, Miao M, Li Y. Serum proteomic analysis revealed diagnostic value of hemoglobin for nonalcoholic fatty liver disease. *J Hepatol* 2012; **56**: 241-247 [PMID: 21756851 DOI: 10.1016/j.jhep.2011.05.027]
79 **Kahn R**, Buse J, Ferrannini E, Stern M. The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2005; **28**: 2289-2304 [PMID: 16123508 DOI: 10.2337/diacare.28.9.2289]
80 **Saukkonen T**, Jokelainen J, Timonen M, Cederberg H, Laakso M, Härkönen P, Keinänen-Kiukaanniemi S, Rajala U. Prevalence of metabolic syndrome components among the elderly using three different definitions: a cohort study in Finland. *Scand J Prim Health Care* 2012; **30**: 29-34 [PMID: 22324547 DOI: 10.3109/02813432.2012.654192]
81 **Kassi E**, Pervanidou P, Kaltsas G, Chrousos G. Metabolic syndrome: definitions and controversies. *BMC Med* 2011; **9**: 48 [PMID: 21542944 DOI: 10.1186/1741-7015-9-48]
82 **Mottillo S**, Filion KB, Genest J, Joseph L, Pilote L, Poirier P, Rinfret S, Schiffrin EL, Eisenberg MJ. The metabolic syndrome and cardiovascular risk a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; **56**: 1113-1132 [PMID: 20863953 DOI: 10.1016/j.jacc.2010.05.034]
83 **Kang HM**, Kim DJ. Metabolic Syndrome versus Framingham Risk Score for Association of Self-Reported Coronary Heart Disease: The 2005 Korean Health and Nutrition Examination Survey. *Diabetes Metab J* 2012; **36**: 237-244 [PMID: 22737664]
84 **Koskinen J**, Kähönen M, Viikari JS, Taittonen L, Laitinen T, Rönnemaa T, Lehtimäki T, Hutri-Kähönen N, Pietikäinen M, Jokinen E, Helenius H, Mattsson N, Raitakari OT, Juonala M. Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: the cardiovascular risk in young Finns study. *Circulation* 2009; **120**: 229-236 [PMID: 19581494 DOI: 10.1161/CIRCULATIONAHA.108.845065]
85 **Ford ES**, Li C, Sattar N. Metabolic syndrome and incident diabetes: current state of the evidence. *Diabetes Care* 2008; **31**: 1898-1904 [PMID: 18591398 DOI: 10.2337/dc08-0423]
86 **Alberti KG**, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640-1645 [PMID: 19805654 DOI: 10.1161/CIRCULATIONAHA.109.192644]
87 **National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)**. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-3421 [PMID: 12485966]
88 **Alberti KG**, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; **366**: 1059-1062 [PMID: 16182882 DOI: 10.1016/S0140-6736(05)67402-8]
89 **Cameron AJ**, Boyko EJ, Sicree RA, Zimmet PZ, Söderberg S, Alberti KG, Tuomilehto J, Chitson P, Shaw JE. Central obesity as a precursor to the metabolic syndrome in the

AusDiab study and Mauritius. *Obesity* (Silver Spring) 2008; **16**: 2707-2716 [PMID: 18820650 DOI: 10.1038/oby.2008.412]
90 **Tabák AG**, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet* 2009; **373**: 2215-2221 [PMID: 19515410 DOI: 10.1016/S0140-6736(09)60619-X]
91 **Zhang Y**, Sowers JR, Ren J. Pathophysiological insights into cardiovascular health in metabolic syndrome. *Exp Diabetes Res* 2012; **2012**: 320534 [PMID: 22844270]
92 **Kahn MB**, Yuldasheva NY, Cubbon RM, Smith J, Rashid ST, Viswambharan H, Imrie H, Abbas A, Rajwani A, Aziz A, Baliga V, Sukumar P, Gage M, Kearney MT, Wheatcroft SB. Insulin resistance impairs circulating angiogenic progenitor cell function and delays endothelial regeneration. *Diabetes* 2011; **60**: 1295-1303 [PMID: 21317296 DOI: 10.2337/db10-1080]
93 **Povel CM**, Boer JM, Reiling E, Feskens EJ. Genetic variants and the metabolic syndrome: a systematic review. *Obes Rev* 2011; **12**: 952-967 [PMID: 21749608 DOI: 10.1111/j.1467-789X.2011.00907.x]
94 **Garaulet M**, Madrid JA. Chronobiology, genetics and metabolic syndrome. *Curr Opin Lipidol* 2009; **20**: 127-134 [PMID: 19276891 DOI: 10.1097/MOL.0b013e3283292399]
95 **Reis JP**, von Mühlen D, Miller ER, Michos ED, Appel LJ. Vitamin D status and cardiometabolic risk factors in the United States adolescent population. *Pediatrics* 2009; **124**: e371-e379 [PMID: 19661053 DOI: 10.1542/peds.2009-0213]
96 **Sjöström L**, Peltonen M, Jacobson P, Sjöström CD, Karason K, Wedel H, Ahlin S, Anveden Å, Bengtsson C, Bergmark G, Bouchard C, Carlsson B, Dahlgren S, Karlsson J, Lindroos AK, Lönroth H, Narbro K, Näslund I, Olbers T, Svensson PA, Carlsson LM. Bariatric surgery and long-term cardiovascular events. *JAMA* 2012; **307**: 56-65 [PMID: 22215166 DOI: 10.1001/jama.2011.1914]
97 **LaMonte MJ**, Barlow CE, Jurca R, Kampert JB, Church TS, Blair SN. Cardiorespiratory fitness is inversely associated with the incidence of metabolic syndrome: a prospective study of men and women. *Circulation* 2005; **112**: 505-512 [PMID: 16009797 DOI: 10.1161/CIRCULATIONAHA.104.503805]
98 **Kodama S**, Saito K, Tanaka S, Maki M, Yachi Y, Asumi M, Sugawara A, Totsuka K, Shimano H, Ohashi Y, Yamada N, Sone H. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: a meta-analysis. *JAMA* 2009; **301**: 2024-2035 [PMID: 19454641 DOI: 10.1001/jama.2009.681]
99 **Grundy SM**. Drug therapy of the metabolic syndrome: minimizing the emerging crisis in polypharmacy. *Nat Rev Drug Discov* 2006; **5**: 295-309 [PMID: 16582875 DOI: 10.1038/nrd2005]
100 **Obika M**, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. *Exp Diabetes Res* 2012; **2012**: 145754 [PMID: 22110476]
101 **Osawa H**, Mori Y. Sonographic diagnosis of fatty liver using a histogram technique that compares liver and renal cortical echo amplitudes. *J Clin Ultrasound* 1996; **24**: 25-29 [PMID: 8655663]
102 **Sanyal AJ**. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 1705-1725 [PMID: 12404245 DOI: 10.1053/gast.2002.36572]
103 **Zardi EM**, Caturelli E. May sonography distinguish between liver fibrosis and liver steatosis? *Dig Liver Dis* 2007; **39**: 790 [PMID: 17604239 DOI: 10.1016/j.dld.2007.05.001]
104 **Strauss S**, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. *AJR Am J Roentgenol* 2007; **189**: W320-W323 [PMID: 18029843]
105 **Ryan CK**, Johnson LA, Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. *Liver Transpl* 2002; **8**: 1114-1122 [PMID: 12474149 DOI: 10.1053/jlts.2002.36740]
106 **Mottin CC**, Moretto M, Padoin AV, Swarowsky AM, Toneto MG, Glock L, Repetto G. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. *Obes Surg* 2004; **14**: 635-637 [PMID: 15186630 DOI: 10.1381/096089204323093408]
107 **Wieckowska A**, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Semin Liver Dis* 2008; **28**: 386-395 [PMID: 18956295 DOI: 10.1055/s-0028-1091983]
108 **Iijima H**, Moriyasu F, Tsuchiya K, Suzuki S, Yoshida M, Shimizu M, Sasaki S, Nishiguchi S, Maeyama S. Decrease in accumulation of ultrasound contrast microbubbles in nonalcoholic steatohepatitis. *Hepatol Res* 2007; **37**: 722-730 [PMID: 17559420 DOI: 10.1111/j.1872-034X.2007.00130.x]
109 **Iijima H**, Moriyasu F, Miyahara T, Yanagisawa K. Ultrasound contrast agent, Levovist microbubbles are phagocytosed by Kupffer cells-In vitro and in vivo studies. *Hepatol Res* 2006; **35**: 235-237 [PMID: 16831566 DOI: 10.1016/j.hepres.2006.04.016]
110 **Tarantino G**, Colicchio P, Conca P, Finelli C, Di Minno MN, Tarantino M, Capone D, Pasanisi F. Young adult obese subjects with and without insulin resistance: what is the role of chronic inflammation and how to weigh it non-invasively? *J Inflamm* (Lond) 2009; **6**: 6 [PMID: 19291292 DOI: 10.1186/1476-9255-6-6]
111 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]
112 **Tarantino G**. Should nonalcoholic fatty liver disease be regarded as a hepatic illness only? *World J Gastroenterol* 2007; **13**: 4669-4672 [PMID: 17729388]
113 **Tarantino G**, Pizza G, Colao A, Pasanisi F, Conca P, Colicchio P, Finelli C, Contaldo F, Di Somma C, Savastano S. Hepatic steatosis in overweight/obese females: new screening method for those at risk. *World J Gastroenterol* 2009; **15**: 5693-5699 [PMID: 19960566 DOI: 10.3748/wjg.15.5693]
114 **Labruna G**, Pasanisi F, Nardelli C, Tarantino G, Vitale DF, Bracale R, Finelli C, Genua MP, Contaldo F, Sacchetti L. UCP1 -3826 AG+GG genotypes, adiponectin, and leptin/adiponectin ratio in severe obesity. *J Endocrinol Invest* 2009; **32**: 525-529 [PMID: 19474520]
115 **Tarantino G**, Caputi A. JNKs, insulin resistance and inflammation: A possible link between NAFLD and coronary artery disease. *World J Gastroenterol* 2011; **17**: 3785-3794 [PMID: 21987620 DOI: 10.3748/wjg.v17.i33.3785]
116 **Tarantino G**. Non-alcoholic fatty liver disease, obesity and other illnesses. *Clin Invest Med* 2008; **31**: E290-E295 [PMID: 18980719]
117 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-10 [PMID: 16540768]
118 **Speliotes EK**, Massaro JM, Hoffmann U, Vasan RS, Meigs JB, Sahani DV, Hirschhorn JN, O'Donnell CJ, Fox CS. Fatty liver is associated with dyslipidemia and dysglycemia independent of visceral fat: the Framingham Heart Study. *Hepatology* 2010; **51**: 1979-1987 [PMID: 20336705 DOI: 10.1002/hep.23593]
119 **Fan JG**, Peng YD. Metabolic syndrome and non-alcoholic fatty liver disease: Asian definitions and Asian studies. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 572-578 [PMID: 18086620]
120 **Hamaguchi M**, Takeda N, Kojima T, Ohbora A, Kato T, Sarui H, Fukui M, Nagata C, Takeda J. Identification of individuals with non-alcoholic fatty liver disease by the diagnostic criteria for the metabolic syndrome. *World J Gastroenterol* 2012; **18**: 1508-1516 [PMID: 22509083 DOI: 10.3748/wjg.v18.i13.1508]

121 **Sinn DH**, Gwak GY, Park HN, Kim JE, Min YW, Kim KM, Kim YJ, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC. Ultrasonographically detected non-alcoholic fatty liver disease is an independent predictor for identifying patients with insulin resistance in non-obese, non-diabetic middle-aged Asian adults. *Am J Gastroenterol* 2012; **107**: 561-567 [PMID: 22108448 DOI: 10.1038/ajg.2011.400]

122 **Hamaguchi M**, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, Kato T, Takeda N, Okuda J, Ida K, Kawahito Y, Yoshikawa T, Okanoue T. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* 2007; **102**: 2708-2715 [PMID: 17894848 DOI: 10.1111/j.1572-0241.2007.01526.x]

123 **Finelli C**, Tarantino G. Is there any consensus as to what diet or lifestyle approach is the right one for NAFLD patients? *J Gastrointestin Liver Dis* 2012; **21**: 293-302 [PMID: 23012671]

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World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3385

World J Gastroenterol 2013 June 14; 19(22): 3385-3396
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Thinking outside the liver: Induced pluripotent stem cells for hepatic applications

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Received: August 15, 2011 Revised: December 6, 2011
Accepted: December 15, 2011
Published online: June 14, 2013

Abstract

The discovery of induced pluripotent stem cells (iPSCs) unraveled a mystery in stem cell research, after identification of four re-programming factors for generating pluripotent stem cells without the need of embryos. This breakthrough in generating iPSCs from somatic cells has overcome the ethical issues and immune rejection involved in the use of human embryonic stem cells. Hence, iPSCs form a great potential source for developing disease models, drug toxicity screening and cell-based therapies. These cells have the potential to differentiate into desired cell types, including hepatocytes, under *in vitro* as well as under *in vivo* conditions given the proper microenvironment. iPSC-derived hepatocytes could be useful as an unlimited source, which can be utilized in disease modeling, drug toxicity testing and producing autologous cell therapies that would avoid immune rejection and enable correction of gene defects prior to cell transplantation. In this review, we discuss the induction methods, role of reprogramming factors, and characterization of iPSCs, along with hepatocyte differentiation from iPSCs and potential applications. Further, we discuss the location and detection of liver stem cells and their role in liver regeneration. Although tumor formation and genetic mutations are a cause of concern, iPSCs still form a promising source for clinical applications.

Key words: Liver stem cells; Hepatocytes; Disease modeling; Drug toxicity; Clinical applications; Patient-specific induced pluripotent stem cell-derived hepatocytes

Subba Rao M, Sasikala M, Reddy DN. Thinking outside the liver: Induced pluripotent stem cells for hepatic applications. *World J Gastroenterol* 2013; 19(22): 3385-3396 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3385.htm
DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3385

INTRODUCTION

Patients suffering from chronic end-stage liver disease are currently receiving inadequate treatment due to the lack of organ donors for transplantation[1]. Alternatively, cell-based therapies are gaining importance as supportive therapy. Hepatocytes (adult, fetal) and liver stem cells form promising sources for cellular therapies in the treatment of liver diseases. However, inadequate proliferation, ethical issues and scanty numbers limit their applicability[2-5]. Therefore, it is essential to think outside the liver in favor of generating hepatocytes for drug screening, disease modeling and cell therapy applications. Identification of four reprogramming transcription factors revolutionized stem cell research in generating induced pluripotent stem cells (iPSCs). iPSCs generated from somatic cells can be utilized not only for cell-based therapies, but also for disease modeling and drug toxicity screening. Patient-specific iPSCs can be generated by reprogramming and differentiating somatic cells from the patient into the desired cell type. Key advantages of iPSCs over current transplantation approaches are that

they form an unlimited potential source and are patient-specific. In addition, the possibility of correcting genetic defects in liver diseases is currently under investigation[6].

The identification of patient-specific pluripotent stem cells has long been an important goal for scientists working in the field of stem cells. In 2006, Takahashi *et al*[7] first reported that forced expression of four transcription factors [octamer-binding transcription factor (Oct) 3/4, SRY box-containing gene 2 (Sox2), Kruppel-like factor 4 (Klf4) and c-Myc] reprogrammed mouse somatic fibroblasts into embryonic stem cell (ESC)-like colonies, which were termed iPSCs. Later, human induced pluripotent stem cells (hiPSCs) were generated from embryonic, neonatal and adult fibroblasts[8-10]. In addition, derivation of patient-specific iPSCs for various diseases/disorders has also been reported[11-15]. Recently, several groups have investigated the possibilities of disease modeling using patient-derived iPSCs[6,16-23]. Apart from all these applications, hepatocytes derived from iPSCs will have larger implications in drug toxicity studies. Before iPSCs, several approaches were used to reprogram the differentiated cells to a pluripotent state. In the beginning, patient-specific human embryonic stem cells (hESCs) were derived using somatic cell nuclear transfer or therapeutic cloning. This technique requires the introduction of a nucleus from an adult donor cell into an enucleated oocyte to generate a nuclear transfer embryo. The objective of this technique is to produce pluripotent hESCs that carry the nuclear genome of the patient and then induce them to differentiate into cells which may be transplanted back into the patient[24-28]. Another method is the fusion of fibroblasts with ESCs[29,30]. However, the therapeutic application of either approach has been experiencing both ethical and technical difficulties, summarized in Table 1.

METHODS TO GENERATE iPSCs

It was demonstrated that somatic cells can be re-programmed into pluripotent stem cells by ectopic expression of four transcription factors, namely Oct4, Klf4, Sox2, and c-Myc, using four independent retroviral vectors[7]. This achievement revolutionized stem cell research. Initially, iPSCs were derived from somatic cells by the retroviral or lentiviral transduction of transcription factors in which transgenes are randomly inserted into the genome of the hosts. Such integration of transgenes has the risk of tumorigenicity[31]. Later, trials to omit transgenic insertion of c-Myc resulted in low reprogramming efficiency and did not eliminate the risk of tumor formation[32], as overexpression of Oct3/4 and Klf4 can also cause tumor formation[33]. In Table 2, we have summarized the advantages and disadvantages of various strategies used for inducing iPSCs generation[7,8,9,32,34-49]. Furthermore, combining all four factors (Oct4, Klf4, Sox2, and c-Myc) into a single vector allowed derivation of iPSCs with a single lentiviral stem cell cassette containing a loxP sequence in the long terminal repeat (LTR)[43]. Following this, transgenes were removed using Cre-mediated excision. Although it left an incomplete LTR in the iPS genome, this method minimized the genomic alteration[44]. A transposon system encoding a reprogramming cassette has also been used for iPSC induction. The transduction of a plasmid-based transposon vector can integrate into the host genome with the help of transposase, and induces iPSC colony formation. The re-expression of the transposase after the establishment of iPSCs recognizes the terminal repeat of the integrated transposon vector, and excises it from the genome. The excision of the transposon does not leave a footprint in most cases, so it maintains the original endogenous sequences[45,46,50,51]. Several techniques have been used for obtaining transgene-free iPSCs.

The first integration-free iPSCs were generated from adult mouse hepatocytes using non integrating adenoviral vectors. However, this required repeated transduction to maintain transgene expression[34,38]. Another technique used is transduction with the Sendai virus, an RNA virus, to deliver the reprogramming factors[35]. The Sendai virus does not integrate into the genome, but working with this system requires more than 15 passages to eliminate viral transgene expression. This complexity limits the general use of this method[48]. Transient transfection of plasmids, episome-based DNA vectors and minicircle vectors has been used to generate transgene-free iPSCs. Mouse embryonic fibroblasts were reprogrammed by repeated transfection with two plasmid constructs carrying the reprogramming factors; the first plasmid expressed c-Myc, while the second expressed the other three factors Oct4, Klf4 and Sox2[36]. Furthermore, experiments with non-integrating episomal vectors have also been successful in iPSC generation[16]. Similarly, minicircle vectors lack the bacterial origin of replication and antibiotic resistance gene and offer higher transfection efficiencies and more prolonged transgene expression as compared to regular plasmids[52]. Moreover, iPSCs have been established by the direct delivery of recombinant reprogramming proteins[38] and small molecules[39]. More recently, one research group has utilized synthetic mRNA molecules to reprogram human fibroblasts to pluripotency and stimulate them into myogenic cells[32]. However, reprogramming using modified RNAs is technically difficult, sensitive to reagents and requires labor-intensive procedures. The efficiency of iPSC induction using transgene-free methods is lower than that with retrovirus vectors, possibly due to low transduction efficiency and unstable expression. Therefore, it is essential to develop methods that require less time and have higher efficiency of reprogramming involving viral and transgene-free techniques to generate iPSCs.

REPROGRAMMING FACTORS

Takahashi *et al*[7] used a combination of four nuclear reprogramming factors, such as Oct4, Sox2, c-Myc and Klf4, for generating iPSCs from mice and reported an efficiency of 0.02%. Simultaneously, the Thomson group used a slightly different combination of factors, namely Oct4, Sox2, Nanog and Lin28, to reprogram human somatic cells at a similar efficiency (0.02%)[9]. Subsequently,

Table 1 New approaches to reprogramming of differentiated cells to a pluripotent state

Method	Results of reprogramming	Drawbacks	Ref.
Transfer of the nucleus from a somatic cell to an enucleated oocyte	The somatic cell nucleus is reprogrammed in the oocyte, and a whole organism develops as a result. Patient-specific hESCs can be derived	Low efficiency. Developmental abnormalities in cloned animals. Ethical and legal restrictions	[24-28]
Fusion of ESCs with differentiated cells	Hybrids of differentiated cells and ESCs display all properties of pluripotent cells	Cell hybrids lack a normal diploid chromosome set	[29,30]
Reprogramming of somatic cells to a pluripotent state can be generated by the ectopic expression of 4 transcription factors, Oct4, Klf4, Sox2 and c-Myc	Somatic cells regain a pluripotent state and become similar in properties to ESCs	Low efficiency of iPSC derivation. Viral integration. Tumor formation	[7]

ESCs: Embryonic stem cells; hESCs: Human embryonic stem cells; iPSC: Induced pluripotent stem cell; Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4.

Table 2 Various induction methods to generate induced pluripotent stem cells

Methods	Advantages	Disadvantages	Ref.
Retroviral vectors	High efficiency	Genome integration, dividing target cells needed	[7-9,32,41,42]
Lentiviral vectors	High efficiency, target cells need not be dividing	Genome integration	[47-49]
Lentiviral vectors with Cre/Lox	High efficiency	Minimize genomic integration	[43,44]
Piggyback transposon	Precise deletion is possible	Minimize genomic integration, laborious	[45,46]
Viral vectors	No genome integration	Low efficiency	[34-37]
Adenoviral vectors			
Sendai vectors			
DNA vectors			
Plasmid vectors			
Episomal vectors			
Minicircle vectors			
Protein transduction	No genome integration	Low efficiency	[38]
Small molecules	No genetic modification	Low efficiency	[39]
Synthetic mRNA	No genetic modification, high efficiency	Multiple rounds of transfection are needed	[40]

researchers have started to identify new reprogramming factors and usage of minimum factors for generating safe iPSCs. iPSCs have been established by 3 transcriptional factors without c-Myc (Oct3/4, Klf4, SOX2) at an efficiency of 0.002%[53,54]. It was also shown that using only Oct4 and Klf4 was enough to reprogram murine NSCs at an efficiency of 0.11%[55]. More recently, the forced expression of Oct4 alone was shown sufficient to reprogram murine NSCs, at a low efficiency of 0.014%[56]. However, the efficiency of iPSC generation has been significantly reduced with usage of minimum factors for generating safe iPSCs. The *Oct4*, *Sox2*, and *Nanog* genes code for transcription factors that activate the genes and signaling pathways responsible for the establishment and maintenance of the pluripotent state and repress the genes responsible for differentiation[57,58]. Others have reported that the expression of *Oct4* and *Sox2* genes is absolutely essential for iPSC generation. In addition, the products of the *Nanog, c-Myc, Klf4* and *Lin28* genes seem to act as catalysts which accelerate the reprogramming[59]. In Table 3, we have summarized the role of various reprogramming factors for iPSC generation[60-66].

Recently, molecules have been used in combination with reprogramming factors to improve the efficiency of iPSC generation, including cotransduction of the catalytic subunit of human telomerase, human telomerase reverse transcriptase, along with SV40 large T antigen, or the repression of the *Ink4a/Arf* locus (encoding cell cycle-dependent kinase inhibitors), or repression of the p53/p21 pathway. These efforts have led to dramatic increases in the efficiency of reprogramming[10,67-69].

CHARACTERIZATION OF iPSCs

The hiPSCs generated can be characterized for their pluripotency, as shown in Figure 1. In addition, assessment of their epigenetic status, silencing of transgene expression and DNA fingerprinting need to be established for confirmation. Assessment of pluripotency of iPSCs can be performed by checking the expression of protein and genes of Oct4, Sox2, Nanog, as well as for SSEA-1 (mouse) or SSEA-3/-4 and TRA-1-60/-81 (human) using flow cytometry, immunocytochemistry and reverse transcription-polymerase chain reaction (PCR) methods[70]. The pluripotent nature of iPSCs is routinely tested by two methods. The first is to determine the *in vitro* differentiation ability of iPSCs, where iPSCs can be allowed to differentiate spontaneously *in vitro* to form embryoid bodies. These embryoid bodies can be assessed for three embryonic germ layers, *i.e.*, mesoderm, endoderm and ectoderm. The second is to determine the *in vivo* differentiation ability of iPSCs[71], where iPSCs can be injected into adult immune-deficient mice (SCID mice). In the host animal, injected iPSCs can form tumors called teratomas.

Table 3 Role of reprogramming factors for induced pluripotent stem cell generation

Reprogramming factors	Description	Function	Ref.
Oct4	Octamer binding transcription factor 4	This transcription factor plays a role in embryonic development, especially during early embryogenesis, and it is necessary for embryonic stem cell pluripotency	[7]
Sox2	SRY box 2	In embryonic stem cells, Sox2 and Oct3/4 often co-occupy target genes, including own promoters. These proteins cooperate regulatory feedback loops to maintain pluripotency	[60]
Klf4	Kruppel-like factor 4	This transcription factor plays a role in upregulation of pluripotency gene *Nanog* and the modification of chromatin structure to facilitate the binding of Oct3/4 and Sox2 to their sequences. Klf4 itself is an oncogenic factor. This gene is over expressed in a variety of tumor types associated with advanced cancer	[61-63]
c-Myc	Proto oncogene protein	An oncogene that induces global histone acetylation, allowing Oct3/4 and Sox2 to bind to their specific target loci	[60,63]
Nanog	Homeo box transcription factor	A transcription factor critically involved with self-renewal of undifferentiated embryonic stem cells	[64]
Lin28	RNA binding protein Lin28	The *Lin28* gene codes for an RNA-binding protein that selectively blocks the processing of microRNAs of the let-7 family, and possibly certain other microRNAs in ESCs, to prevent their differentiation	[65,66]

ESCs: Embryonic stem cells; Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4.

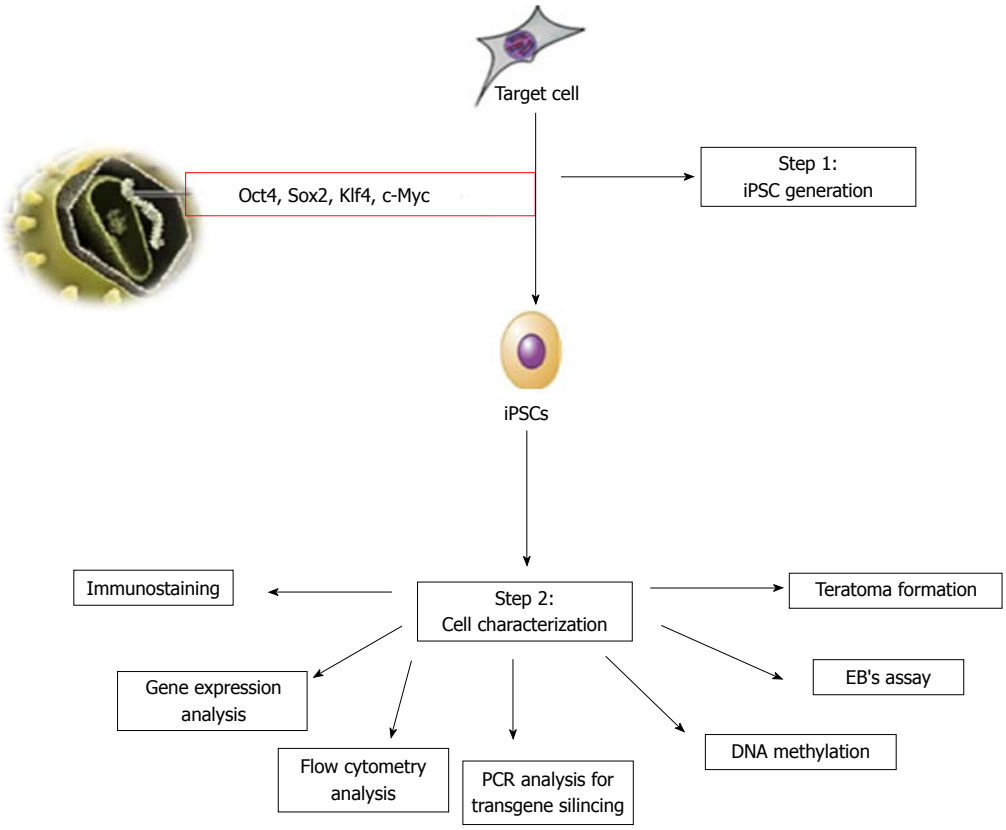

Figure 1 Flow diagram of generation and characterization of human induced pluripotent stem cells. Induced pluripotent stem cells (iPSCs) are derived through the introduction of stem cell factors into fibroblasts. After that, assessment of pluripotency of iPSCs can be studied by expression of protein and genes using various techniques such as immunocytochemistry, flow cytometry and reverse transcription-polymerase chain reaction (PCR) methods, respectively. *In vitro* and *in vivo* differentiation ability of iPSCs can be studied by embryoid body assay (EB assay) and teratoma formation assay, respectively. In addition, PCR analysis is required to demonstrate silencing of transgene expression in iPSCs and DNA methylation to confirm reprogramming of somatic cells. Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4.

In addition to pluripotency assessment, it is important to confirm the silencing of exogenous transgene expression. PCR analysis can be used to demonstrate silencing of retro/lentiviral transgene expression using virus-specific primers[70]. Further, DNA fingerprinting can be performed to confirm iPSCs are genetically matched to their parental somatic cells. DNA methylation analysis of the *Oct4*, *Sox2* and *Nanog* promoter regions using bisulfite sequencing can be used to reveal the different epigenetic states of the cells. Thus, the methylation status of promoter regions of pluripotency genes confirms successful reprogramming[70].

Table 4 Differentiation protocols for induced pluripotent stem cell-derived hepatocytes

Ref.	Species	Differentiation protocol	Remarks
Sullivan *et al*[78]	Human	Activin A, Wnt3a (3 d), Activin A (2 d), DMSO (3 d), HGF, OSM (6 d)	Generated functional hepatocyte-like cells from human-iPSCs
Song *et al*[79]	Human	Activin A (3 d), FGF4, BMP-2 (4 d), HGF, KGF (6 d), OSM, Dex (5 d) then OSM, Dex, N2B27 (3 d)	iPSCs had fewer expressed liver-enriched genes compared with human hepatocytes
Si-Tayeb *et al*[80]	Human	Activin A (5 d), bFGF, BMP-4 (5 d), HGF (5 d), OSM (5 d)	Transplanted hepatocyte-like cells into the lobe of newborn mice and demonstrated homing of donor cells
Liu *et al*[81]	Human	Activin A (5 d), FGF4, HGF (5 d), Single Quotes (lonza), FGF4, HGF, OSM, Dex (10 d)	Human hepatocyte-derived iPSCs are able to differentiate into functional hepatocytes
Takata *et al*[82]	Human	Activin A (3 d), HGF (5 d), OSM (5 d)	Generated hepatocyte-like cells from iPSCs using three growth factors in a short time
Gai *et al*[83]	Mouse	Activin A, Wnt3 (6 d), bFGF, DMSO (3 d), HGF, DMSO (9 d), HGF, OSM, DMSO (7 d)	Generated hepatocytes from iPSCs
Iwamuro *et al*[84]	Mouse	Activin A, bFGF (3 d), HGF (5 d)	Generated hepatocyte-like cells from iPSCs

iPSCs: Induced pluripotent stem cells; DMSO: Dimethyl sulfoxide; HGF: Hepatocyte growth factor; OSM: Oncostatin M; Dex: Dexamethasone; FGF4: Fibroblast growth factor-4; BMP: Bone morphogenetic protein; KGF: Keratinocyte growth gactor; bFGF: Basic fibroblast growth factor.

GENERATION OF HEPATOCYTES FROM iPSCs

To date, many protocols have been used to differentiate iPSCs into desired cell types. However, different iPSC lines have different outcomes under identical culture conditions. iPSC lines have a propensity to produce certain lineages or cell types when allowed to differentiate spontaneously, indicating that choosing a proper clone is also essential in differentiating iPSCs into a specific lineage[72-74]. A major issue in differentiation is to obtain hepatocytes from pluripotent stem cells that have an adult phenotype, and which stably express liver-like functions and reflect those *in vivo* functions[75]. Recently, a number of protocols have been developed to derive hepatocytes from hiPSCs. These protocols for hepatocyte generation are hampered by inefficient differentiation and maturation that lead to low yield and heterogeneous cell populations in cultures[76]. Recently, a homogenous population of hepatocytes from pluripotent stem cells has been isolated by sorting for surface asialoglycoprotein receptor marker; however, these enriched cells are found to retain immature fetal liver characteristics[77]. In Table 4, we have summarized various protocols used to differentiate hepatocytes from iPSCs[78-84]. Even after enriching the hepatocytes from culture prior to transplantation, the risk of teratoma formation may arise due to the presence of a few undifferentiated iPSCs. Therefore, further enriching hepatocytes using negative selection against pluripotent cells could be useful to avoid teratoma formation. Figure 2 summarizes the strategy on differentiation of human iPSCs into hepatocytes. Figure 3 depicts the hepatocytes generated from hiPSCs in our laboratory.

POTENTIAL APPLICATIONS OF iPSC-DERIVED HEPATOCYTES

iPSCs represent a promising source of hepatocytes for a wide range of applications, including disease modeling, drug toxicity testing and cell transplantation (Figure 4).

Disease modeling

iPSCs represent a novel tool for *in vitro* disease modeling. Traditionally, researchers rely on animal models, hepatic immortalized cell lines, or short-lived primary hepatocyte cultures to understand the mechanisms and pathogenesis of diseases and testing of drug candidates[85-87]. Each of these has limitations in functionality, reproducibility and availability. Disease-specific iPSCs derived from patients suffering from specific diseases may provide a more relevant model system because their properties closely resemble those found in the patient's own system, without the need for genetic manipulation. Several groups have successfully derived a wide range of iPSCs from patients with diseases[88] and inherited liver diseases[21]. These cells can be used as models to study the pathogenesis, disease mechanism(s) and possible cure for liver disorders. Therefore, human iPSC-derived hepatocytes could generate more accurate predictions of human physiological responses than animal models. iPSC-derived hepatocytes will overcome these limitations and provide a reliable source of highly reproducible and readily available human hepatocytes for disease modeling in pre-clinical drug development.

Drug toxicity screening

Hepatotoxicity is the most common side effect of new candidate drugs under clinical trial, and is the leading cause of post approval drug recalls; for example, bromfenac and troglitazone[89]. The development of liver toxicity screening technologies utilizing iPSC-derived hepatocytes would allow investigation into the effects of single nucleotide polymorphisms on drug metabolism and toxicity[90]. An example of this is warfarin, a drug for which polymorphisms in cytochrome P-450 2C9 create problems with obtaining an appropriate pharmacotherapeutic range[91]. iPSC-derived hepatocytes could remain viable in culture for several months, enabling the assessment of acute and chronic toxicity of drugs due to their pluripotent ability. Drug toxicity assays will be performed in petri dishes which require small amounts of compound for a hepatic cytotoxicity profile. Terminally differentiated he-

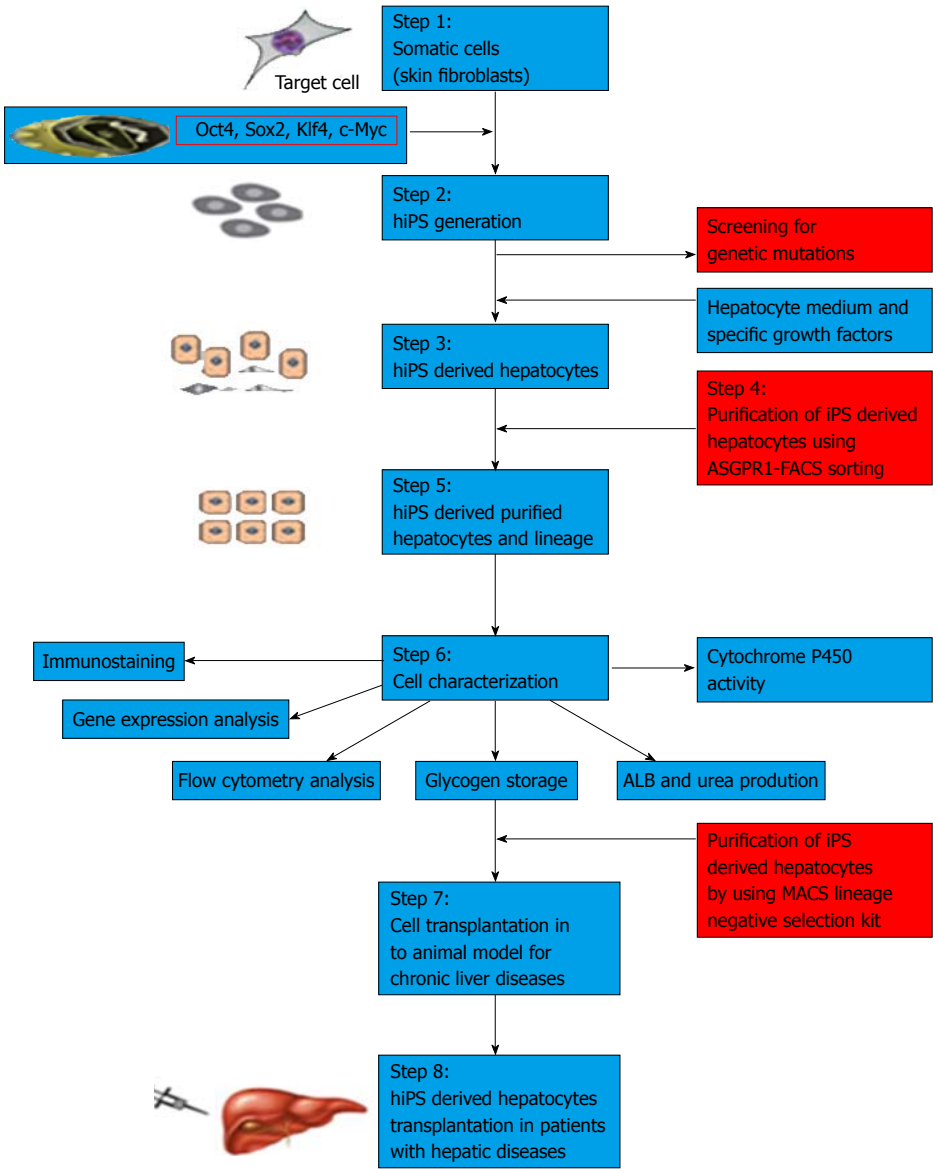

Figure 2 Flow diagram showing the strategy for human induced pluripotent stem cell-derived hepatocyte clinical applications. Steps 1 and 2, human induced pluripotent stem cells (hiPSCs) are generated from somatic cells using reprogramming techniques and screened for mutations; Step 3, hiPSCs are differentiated into hepatocytes using specific growth factors and medium; Step 4, enrichment of hiPSC-derived hepatocytes; Steps 5 and 6, characterization of enriched iPSC-derived hepatocytes for protein expression, gene expression and functional assays. Before clinical transplantation, hiPSC-derived hepatocytes are enriched again using negative selection against pluripotent cells to avoid teratoma formation; Step 7, transplantation of enriched hiPSC-derived hepatocytes into chronic liver failure animal model; Step 8, hiPSC-derived enriched hepatocytes could be transplanted into liver disease patients. Oct: Octamer-binding transcription factor; Sox2: SRY box-containing gene 2; Klf4: Kruppel-like factor 4; ASGPR: Asialoglycoprotein receptor; FACS: Fluorescence activated cell sorting; MACS: Magnetic-activated cell sorting; ALB: Albumin.

patocytes with cytochrome P-3A4 functional activity and scale-up of iPSC-derived hepatocytes will help in pharmaceutical industry drug toxicity applications.

Patient-specific iPSC-derived hepatocytes for cell transplantation

Liver transplantation represents the only way to treat patients suffering from chronic liver failure, but this is associated with numerous problems, including shortage of donors, high cost, rejection and complications. Transplantation of hepatocytes derived from hiPSCs could represent an alternative cell source for liver failure and inborn liver diseases. The important issue is the generation of safe and functional cell types for therapy. Indeed, the cell sources of iPSCs influence the safety of the established iPSCs. It has been demonstrated that hiPSCs retain certain gene expressions of the parent cells, and this suggests that iPSCs of different origins may possess different capacities to differentiate. A complete study using various mouse iPSCs has demonstrated that the origin of the iPSCs has a profound influence on the tumor-forming propensities in a cell transplantation therapy model[92]. Mouse tail-tip fibroblast iPSCs (mesoderm origin) have shown the highest tumorigenic propensity, whereas gastric epithelial cells and hepatocyte iPSCs (both are endoderm origin) have shown lower propensities[93]. The recent evidence suggests that epigenetic memory of the somatic cell of origin is retained in the iPSCs, and

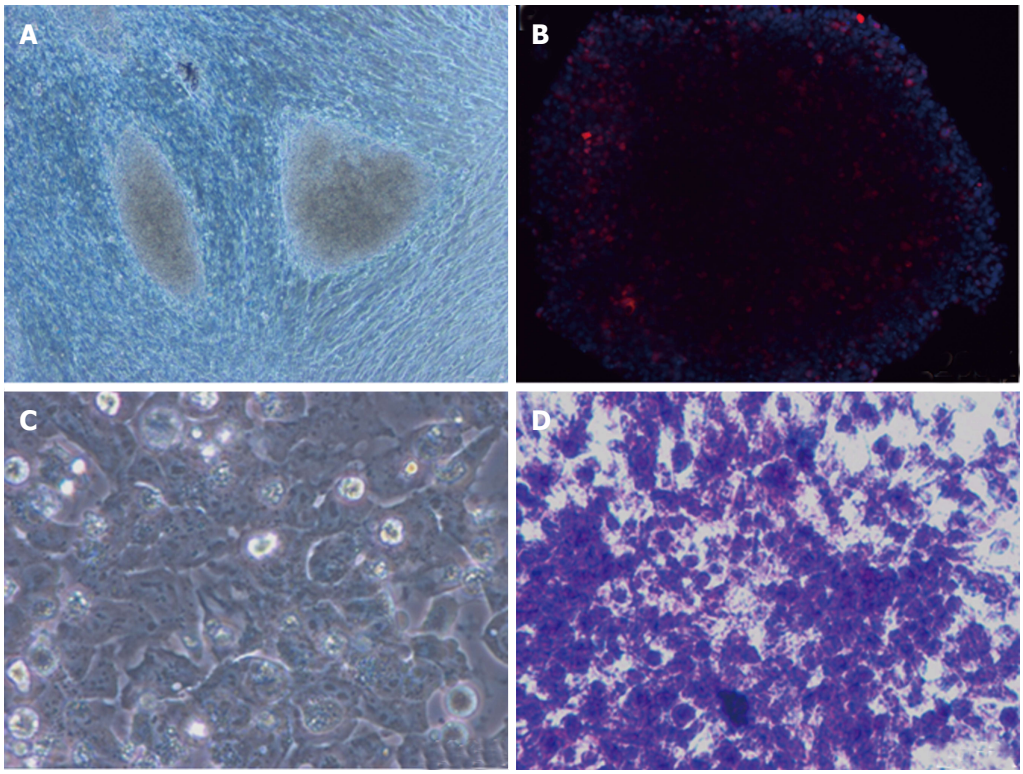

Figure 3 Human induced pluripotent stem cells generated from human foreskin fibroblasts using single lentiviral stem cell cassette kit (Millipore, United States) method. A, B: Human induced pluripotent stem cell (hiPSC) colonies resembling embryonic stem cells in morphology were observed, and iPSC with a flat, packed, tight colony morphology and a high nucleus to cytoplasm ratio (A, × 40) were positive for Oct4 marker on immunocytochemistry (B, × 200); C, D: hiPSCs were differentiated into hepatocytes. At day 13, these differentiated cells exhibited polygonal morphology (C, × 400) and showed pink color (glycogen storage) on periodic acid schiff staining (D, × 200).

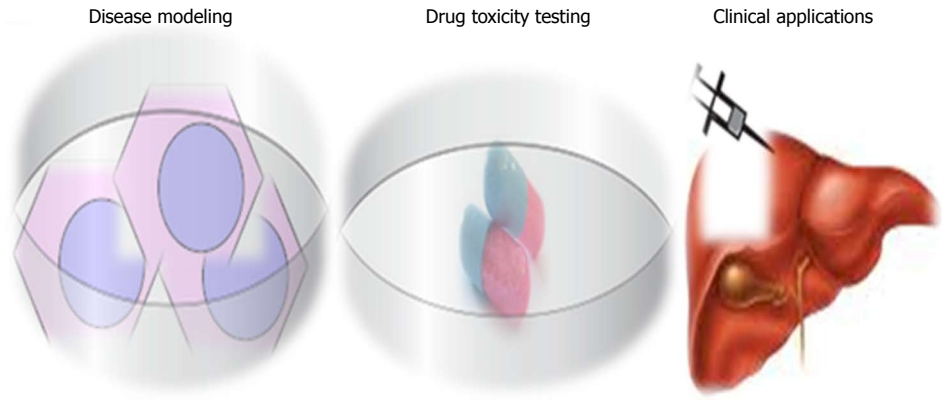

Figure 4 Flow diagram of potential applications of induced pluripotent stem cell-derived hepatocytes. Induced pluripotent stem cells (iPSCs) are capable of self-renewal and are able to differentiate into hepatocytes *in vitro*. iPSC-derived hepatocytes can be applied to disease modeling, drug toxicity screening assays, and clinical applications.

that may influence their directed differentiation potential into blood cells[94,81] or hepatocytes[92]. In the mouse, iPSCs have been generated from derivatives of all three embryonic germ layers, including mesodermal fibroblasts, epithelial cells of endodermal origin and ectodermal keratinocytes, whereas human iPSCs have been produced from mesoderm (fibroblasts and blood cells) or ectoderm (keratinocytes and neural stem cells) and endoderm (hepatocytes)[81]. It is therefore extremely important to establish human iPSC lines of multiple origins and thoroughly examine the source impact on both the safety issues and their differentiation potentials.

Recently, it has been demonstrated that iPSC-derived hepatocytes can restore liver function in an animal model of liver failure[95]. These results indicate the utility of hiPSC-derived hepatocytes as an alternative treatment for patients with end-stage liver disease. Researchers investigated and analyzed the potential of hiPSC-derived hepatocytes to model inborn liver diseases such as α_1-antitrypsin deficiency, familial hypercholesterolemia, glycogen storage disease type 1a, hereditary tyrosinemia, and Crigler-Najjar syndrome[6]. Genetic diseases of the liver modeled in hiPSC-

Table 5 Direct conversion approaches for specific cell types

Ref.	Key factors	Direct converted cell type
Vierbuchen *et al*[109]	Brn2, Ascl1, and Myt1l	Transdifferentiated mouse fibroblasts into functional neuronal cells
Ieda *et al*[110]	Gata4, Mef2c, and Tbx5	Transdifferentiated mouse dermal fibroblasts into cardiomyocyte-like cells
Szabo *et al*[111]	Oct4	Transdifferentiated human fibroblast cells into hematopoietic progenitors
Huang *et al*[112]	Gata4, Hnf1α and Foxa3, and inactivation of p19Arf	Transdifferentiated mouse tail-tip fibroblasts into hepatocyte-like cell

Brn2: Brain-2; Ascl1: Achaete-scute homolog 1; Myt1l: Myelin transcription factor 1; Gata4: GATA binding protein 4; Mef2c: Myocyte enhancer factor 2; Tbx5: T-box protein 5; Hnf1α: Hepatocyte nuclear factor 1α; Foxa3: Forkhead box protein A3; p19: protein p19; Oct: Octamer-binding transcription factor.

derived human hepatocytes create new opportunities to develop autologous cell transplantation therapy to correct genetic defects in liver diseases.

Liver stem cells

The liver has a massive regenerative capacity. When liver regeneration is impaired, oval shaped cells emerge and are implicated in liver tissue repair[96]. These cells are derived from the canals of Hering, which are located in the periportal region of the liver and account for 0.3%-0.7% of the liver mass[97]. In rodents, these liver progenitor cells are called oval cells, while in humans they are known as hepatic progenitor cells[98]. These cells are phenotypically similar to fetal hepatoblasts and also have a bipotent differentiation potential. Oval cells or hepatic progenitors are difficult to isolate because of the lack of definitive markers. Various markers have been used to identify oval cells in adult liver, including liver stem cell and hematopoietic markers, such as OV6, Thy-1, CD34, c-kit, and Sca-1[99]. Hepatic progenitors have been isolated from fetal liver using the specific surface marker, epithelial cell adhesion molecule (EPCAM). These $EPCAM^+$ cells showed positive for hepatic progenitor markers such as CD29, CD49f and CD90[86]. Clinical studies have identified and confirmed the efficacy of fetal liver hepatic progenitors in end-stage liver diseases[100]. However, the clinical application of this cell source is limited due to the difficulty in obtaining large numbers of fetal liver cells, as well as ethical and immune rejection issues. Another stem cell population found in the fetal liver is side population cells which represents another potential source of liver progenitor cells[101], but these cell numbers are very much fewer in fetal liver. There is increasing evidence in the literature suggesting that bone marrow is another source of hepatic progenitor cells[102,103]. Autologous bone marrow-derived stem cell transplantations have been performed in patients with liver diseases but it is difficult to assess overall clinical benefit from these therapies[104].

In support of a role in liver regeneration, oval cell activation has been detected in chronic liver injury caused by inflammation, chronic hepatic necrosis, chronic alcoholism induced cirrhosis and hepatitis models[105,106]. Although the full complements of signals required for oval cell activation are still unknown, both continuous metabolic stress and chemical hepatotoxic substances have been implicated as potential oval cell activators when hepatocyte proliferation is inhibited[105,106]. A recent study reported the production of a chemokine known as stromal derived factor-1α in the liver following tissue damage[107]. The role of liver stem cells in physiology, pathophysiology and therapy is not yet exactly known; therefore, it needs to be further investigated[108]. Although a number of successful techniques have been developed, stem cell-derived hepatocytes from adult, fetal and embryonic sources are found to retain immature fetal liver characteristics, which are not similar in primary hepatocyte functionality. Therefore, the elucidation of other key developmental factors and tissue culture environments, together with iPSC technology, are essential in order to obtain functional hepatocytes for hepatic applications.

DIRECT CONVERSION

Apart from the methods discussed above, overexpression of lineage-specific transcription factors in somatic cells is a new approach (direct conversion) to generate specific cell types including neurons, cardiomyocytes, blood progenitors and hepatocyte-like cells; as summarized in Table 5[109-112]. This method could be useful as an alternative approach for autologous cell-replacement therapies. Unlike iPSCs and ESCs, directly converted cells may not easily multiply in the lab since they do not have pluripotency properties. Therefore, this approach may have limitations. Choosing highly proliferative starting somatic cells is essential in the direct conversion approach.

CONCLUSION

Thinking outside the liver explores the potential of iPSCs as an unlimited source for *in vitro* disease modeling and for drug toxicity studies and clinical applications. Patient-specific iPSCs or custom-made iPSCs may have future promising implications without immuno rejection. However, iPSC technology has several technical issues to be addressed such as generation of iPSCs without viral integration, elimination of tumor formation and genetic mutations that need to be eliminated before the cells are put to clinical applications. Despite limitations, iPSC-derived hepatocytes are a very promising population for cell therapies in hepatology.

REFERENCES

1 **Jozefczuk J**, Prigione A, Chavez L, Adjaye J. Comparative analysis of human embryonic stem cell and induced pluripotent stem cell-derived hepatocyte-like cells reveals current drawbacks and possible strategies for improved differentia-

tion. *Stem Cells Dev* 2011; **20**: 1259-1275 [PMID: 21162674 DOI: 10.1089/scd.2010.0361]
2 **Herrera MB**, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G. Isolation and characterization of a stem cell population from adult human liver. *Stem Cells* 2006; **24**: 2840-2850 [PMID: 16945998 DOI: 10.1634/stemcells.2006-0114]
3 **Lázaro CA**, Rhim JA, Yamada Y, Fausto N. Generation of hepatocytes from oval cell precursors in culture. *Cancer Res* 1998; **58**: 5514-5522 [PMID: 9850088]
4 **Sahin MB**, Schwartz RE, Buckley SM, Heremans Y, Chase L, Hu WS, Verfaillie CM. Isolation and characterization of a novel population of progenitor cells from unmanipulated rat liver. *Liver Transpl* 2008; **14**: 333-345 [PMID: 18306374]
5 **Czyz J**, Wiese C, Rolletschek A, Blyszczuk P, Cross M, Wobus AM. Potential of embryonic and adult stem cells in vitro. *Biol Chem* 2003; **384**: 1391-1409 [PMID: 14669982 DOI: 10.1515/BC.2003.155]
6 **Rashid ST**, Corbineau S, Hannan N, Marciniak SJ, Miranda E, Alexander G, Huang-Doran I, Griffin J, Ahrlund-Richter L, Skepper J, Semple R, Weber A, Lomas DA, Vallier L. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J Clin Invest* 2010; **120**: 3127-3136 [PMID: 20739751 DOI: 10.1172/JCI43122]
7 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]
8 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]
9 **Yu J**, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**: 1917-1920 [PMID: 18029452 DOI: 10.1126/science.1151526]
10 **Park IH**, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008; **451**: 141-146 [PMID: 18157115 DOI: 10.1038/nature06534]
11 **Park IH**, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ. Disease-specific induced pluripotent stem cells. *Cell* 2008; **134**: 877-886 [PMID: 18691744 DOI: 10.1016/j.cell.2008.07.041]
12 **Dimos JT**, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 2008; **321**: 1218-1221 [PMID: 18669821 DOI: 10.1126/science.1158799]
13 **Ye Z**, Zhan H, Mali P, Dowey S, Williams DM, Jang YY, Dang CV, Spivak JL, Moliterno AR, Cheng L. Human-induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders. *Blood* 2009; **114**: 5473-5480 [PMID: 19797525 DOI: 10.1182/blood-2009-04-217406]
14 **Raya A**, Rodríguez-Pizà I, Guenechea G, Vassena R, Navarro S, Barrero MJ, Consiglio A, Castellà M, Río P, Sleep E, González F, Tiscornia G, Garreta E, Aasen T, Veiga A, Verma IM, Surrallés J, Bueren J, Izpisúa Belmonte JC. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* 2009; **460**: 53-59 [PMID: 19483674 DOI: 10.1038/nature08129]
15 **Ku S**, Soragni E, Campau E, Thomas EA, Altun G, Laurent LC, Loring JF, Napierala M, Gottesfeld JM. Friedreich's ataxia induced pluripotent stem cells model intergenerational GAA·TTC triplet repeat instability. *Cell Stem Cell* 2010; **7**: 631-637 [PMID: 21040903]
16 **Lee G**, Papapetrou EP, Kim H, Chambers SM, Tomishima MJ, Fasano CA, Ganat YM, Menon J, Shimizu F, Viale A, Tabar V, Sadelain M, Studer L. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 2009; **461**: 402-406 [PMID: 19693009 DOI: 10.1038/nature08320]
17 **Ebert AD**, Yu J, Rose FF, Mattis VB, Lorson CL, Thomson JA, Svendsen CN. Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 2009; **457**: 277-280 [PMID: 19098894 DOI: 10.1038/nature07677]
18 **Soldner F**, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, Isacson O, Jaenisch R. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 2009; **136**: 964-977 [PMID: 19269371 DOI: 10.1016/j.cell.2009.02.013]
19 **Ye L**, Chang JC, Lin C, Sun X, Yu J, Kan YW. Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases. *Proc Natl Acad Sci USA* 2009; **106**: 9826-9830 [PMID: 19482945 DOI: 10.1073/pnas.0904689106]
20 **Carvajal-Vergara X**, Sevilla A, D'Souza SL, Ang YS, Schaniel C, Lee DF, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B, Waghray A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature* 2010; **465**: 808-812 [PMID: 20535210 DOI: 10.1038/nature09005]
21 **Ghodsizadeh A**, Taei A, Totonchi M, Seifinejad A, Gourabi H, Pournasr B, Aghdami N, Malekzadeh R, Almadani N, Salekdeh GH, Baharvand H. Generation of liver disease-specific induced pluripotent stem cells along with efficient differentiation to functional hepatocyte-like cells. *Stem Cell Rev* 2010; **6**: 622-632 [PMID: 20821352 DOI: 10.1007/s12015-010-9189-3.]
22 **Marchetto MC**, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* 2010; **143**: 527-539 [PMID: 21074045 DOI: 10.1016/j.cell.2010.10.016]
23 **Zhang N**, An MC, Montoro D, Ellerby LM. Characterization of Human Huntington's Disease Cell Model from Induced Pluripotent Stem Cells. *PLoS Curr* 2010; **2**: RRN1193 [PMID: 21037797 DOI: 10.1371/currents.RRN1193]
24 **Stojkovic M**, Lako M, Strachan T, Murdoch A. Derivation, growth and applications of human embryonic stem cells. *Reproduction* 2004; **128**: 259-267 [PMID: 15333777 DOI: 10.1530/rep.1.00243]
25 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]
26 **Drukker M**, Benvenisty N. The immunogenicity of human embryonic stem-derived cells. *Trends Biotechnol* 2004; **22**: 136-141 [PMID: 15036864 DOI: 10.1016/j.tibtech.2004.01.003]
27 **Rhind SM**, Taylor JE, De Sousa PA, King TJ, McGarry M, Wilmut I. Human cloning: can it be made safe? *Nat Rev Genet* 2003; **4**: 855-864 [PMID: 14634633 DOI: 10.1038/nrg1205]
28 **Jaenisch R**. Human cloning - the science and ethics of nuclear transplantation. *N Engl J Med* 2004; **351**: 2787-2791 [PMID: 15625328]
29 **Cowan CA**, Atienza J, Melton DA, Eggan K. Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* 2005; **309**: 1369-1373 [PMID: 16123299 DOI: 10.1126/science.1116447]
30 **Tada M**, Takahama Y, Abe K, Nakatsuji N, Tada T. Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. *Curr Biol* 2001; **11**: 1553-1558 [PMID: 11591326 DOI: 10.1016/S0960-9822(01)00459-6]
31 **Yoshida Y**, Yamanaka S. iPS cells: a source of cardiac regeneration. *J Mol Cell Cardiol* 2011; **50**: 327-332 [PMID: 21040726

DOI: 10.1016/j.yjmcc.2010.10.026]
32 **Okita K**, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007; **448**: 313-317 [PMID: 17554338 DOI: 10.1038/nature05934]
33 **Hacein-Bey-Abina S**, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JI, de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A, Cavazzana-Calvo M. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science* 2003; **302**: 415-419 [PMID: 14564000]
34 **Stadtfeld M**, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 2008; **322**: 945-949 [PMID: 18818365]
35 **Fusaki N**, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc Jpn Acad Ser B Phys Biol Sci* 2009; **85**: 348-362 [PMID: 19838014 DOI: 10.2183/pjab.85.348]
36 **Okita K**, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008; **322**: 949-953 [PMID: 18845712 DOI: 10.1126/science.1164270]
37 **Yu J**, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II, Thomson JA. Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 2009; **324**: 797-801 [PMID: 19325077 DOI: 10.1126/science.1172482]
38 **Zhou H**, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y, Siuzdak G, Schöler HR, Duan L, Ding S. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 2009; **4**: 381-384 [PMID: 19398399 DOI: 10.1016/j.stem.2009.04.005]
39 **Huangfu D**, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol* 2008; **26**: 795-797 [PMID: 18568017 DOI: 10.1038/nbt1418]
40 **Warren L**, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 2010; **7**: 618-630 [PMID: 20888316 DOI: 10.1016/j.stem.2010.08.012]
41 **Maherali N**, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, Stadtfeld M, Yachechko R, Tchieu J, Jaenisch R, Plath K, Hochedlinger K. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 2007; **1**: 55-70 [PMID: 18371336 DOI: 10.1016/j.stem.2007.05.014]
42 **Wernig M**, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 2007; **448**: 318-324 [PMID: 17554336 DOI: 10.1038/nature05944]
43 **Sommer CA**, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G. Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells* 2009; **27**: 543-549 [PMID: 19096035 DOI: 10.1634/stemcells.2008-1075]
44 **Sommer CA**, Sommer AG, Longmire TA, Christodoulou C, Thomas DD, Gostissa M, Alt FW, Murphy GJ, Kotton DN, Mostoslavsky G. Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector. *Stem Cells* 2010; **28**: 64-74 [PMID: 19904830 DOI: 10.1002/stem.255]
45 **Kaji K**, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* 2009; **458**: 771-775 [PMID: 19252477 DOI: 10.1038/nature07864]
46 **Woltjen K**, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R, Cowling R, Wang W, Liu P, Gertsenstein M, Kaji K, Sung HK, Nagy A. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* 2009; **458**: 766-770 [PMID: 19252478 DOI: 10.1038/nature07863]
47 **Brambrink T**, Foreman R, Welstead GG, Lengner CJ, Wernig M, Suh H, Jaenisch R. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* 2008; **2**: 151-159 [PMID: 18371436 DOI: 10.1016/j.stem.2008.01.004]
48 **Maherali N**, Ahfeldt T, Rigamonti A, Utikal J, Cowan C, Hochedlinger K. A high-efficiency system for the generation and study of human induced pluripotent stem cells. *Cell Stem Cell* 2008; **3**: 340-345 [PMID: 18786420 DOI: 10.1016/j.stem.2008.08.003]
49 **Wernig M**, Lengner CJ, Hanna J, Lodato MA, Steine E, Foreman R, Staerk J, Markoulaki S, Jaenisch R. A drug-inducible transgenic system for direct reprogramming of multiple somatic cell types. *Nat Biotechnol* 2008; **26**: 916-924 [PMID: 18594521 DOI: 10.1038/nbt1483]
50 **Okita K**, Yamanaka S. Induction of pluripotency by defined factors. *Exp Cell Res* 2010; **316**: 2565-2570 [PMID: 20420827]
51 **Hussein SM**, Nagy K, Nagy A. Human induced pluripotent stem cells: the past, present, and future. *Clin Pharmacol Ther* 2011; **89**: 741-745 [PMID: 21430659 DOI: 10.1038/clpt.2011.37]
52 **Jia F**, Wilson KD, Sun N, Gupta DM, Huang M, Li Z, Panetta NJ, Chen ZY, Robbins RC, Kay MA, Longaker MT, Wu JC. A nonviral minicircle vector for deriving human iPS cells. *Nat Methods* 2010; **7**: 197-199 [PMID: 20139967 DOI: 10.1038/nmeth.1426]
53 **Nakagawa M**, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K, Mochiduki Y, Takizawa N, Yamanaka S. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 2008; **26**: 101-106 [PMID: 18059259 DOI: 10.1038/nbt1374]
54 **Wernig M**, Meissner A, Cassady JP, Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2008; **2**: 10-12 [PMID: 18371415 DOI: 10.1016/j.stem.2007.12.001]
55 **Kim JB**, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Araúzo-Bravo MJ, Ruau D, Han DW, Zenke M, Schöler HR. Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 2008; **454**: 646-650 [PMID: 18594515 DOI: 10.1038/nature07061]
56 **Kim JB**, Sebastiano V, Wu G, Araúzo-Bravo MJ, Sasse P, Gentile L, Ko K, Ruau D, Ehrich M, van den Boom D, Meyer J, Hübner K, Bernemann C, Ortmeier C, Zenke M, Fleischmann BK, Zaehres H, Schöler HR. Oct4-induced pluripotency in adult neural stem cells. *Cell* 2009; **136**: 411-419 [PMID: 19203577 DOI: 10.1016/j.cell.2009.01.023]
57 **Medvedev SP**, Shevchenko AI, Mazurok NA, Zakiian SM. [OCT4 and NANOG are the key genes in the system of pluripotency maintenance in mammalian cells]. *Genetika* 2008; **44**: 1589-1608 [PMID: 19178078 DOI: 10.1134/S1022795408120016]
58 **Masui S**, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov AA, Ko MS, Niwa H. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat Cell Biol* 2007; **9**: 625-635 [PMID: 17515932 DOI: 10.1038/ncb1589]
59 **Shevchenko AI,** Medvedev SP, Mazurok NA, Zakiian SM. Induced pluripotent stem cells. *Russ J Genet* 2009; **45:** 139-146 [DOI: 10.1134/S1022795409020021]
60 **Avilion AA**, Nicolis SK, Pevny LH, Perez L, Vivian N, Lovell-Badge R. Multipotent cell lineages in early mouse develop-

ment depend on SOX2 function. *Genes Dev* 2003; **17**: 126-140 [PMID: 12514105 DOI: 10.1101/gad.224503]
61 **Rowland BD**, Bernards R, Peeper DS. The KLF4 tumour suppressor is a transcriptional repressor of p53 that acts as a context-dependent oncogene. *Nat Cell Biol* 2005; **7**: 1074-1082 [PMID: 16244670 DOI: 10.1038/ncb1314]
62 **Lin T**, Chao C, Saito S, Mazur SJ, Murphy ME, Appella E, Xu Y. p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat Cell Biol* 2005; **7**: 165-171 [PMID: 15619621 DOI: 10.1038/ncb1211]
63 **Yamanaka S**. Strategies and new developments in the generation of patient-specific pluripotent stem cells. *Cell Stem Cell* 2007; **1**: 39-49 [PMID: 18371333 DOI: 10.1016/j.stem.2007.05.012]
64 **Field M**, Alvarez A, Bushnev S, Sugaya K. Embryonic stem cell markers distinguishing cancer stem cells from normal human neuronal stem cell populations in malignant glioma patients. *Clin Neurosurg* 2010; **57**: 151-159 [PMID: 21280509]
65 **Büssing I**, Slack FJ, Grosshans H. let-7 microRNAs in development, stem cells and cancer. *Trends Mol Med* 2008; **14**: 400-409 [PMID: 18674967 DOI: 10.1016/j.molmed.2008.07.001]
66 **Viswanathan SR**, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. *Science* 2008; **320**: 97-100 [PMID: 18292307]
67 **Banito A**, Rashid ST, Acosta JC, Li S, Pereira CF, Geti I, Pinho S, Silva JC, Azuara V, Walsh M, Vallier L, Gil J. Senescence impairs successful reprogramming to pluripotent stem cells. *Genes Dev* 2009; **23**: 2134-2139 [PMID: 19696146 DOI: 10.1101/gad.1811609]
68 **Li H**, Collado M, Villasante A, Strati K, Ortega S, Cañamero M, Blasco MA, Serrano M. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature* 2009; **460**: 1136-1139 [PMID: 19668188 DOI: 10.1038/nature08290]
69 **Utikal J**, Polo JM, Stadtfeld M, Maherali N, Kulalert W, Walsh RM, Khalil A, Rheinwald JG, Hochedlinger K. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature* 2009; **460**: 1145-1148 [PMID: 19668190 DOI: 10.1038/nature08285]
70 **Zaehres H**, Kim JB, Schöler HR. Induced pluripotent stem cells. *Methods Enzymol* 2010; **476**: 309-325 [PMID: 20691874]
71 **Ohnuki M**, Takahashi K, Yamanaka S. Generation and characterization of human induced pluripotent stem cells. *Curr Protoc Stem Cell Biol* 2009; **Chapter 4**: Unit 4A.2 [PMID: 19536759 DOI: 10.1002/9780470151808.sc04a02s9]
72 **Chang KH**, Nelson AM, Fields PA, Hesson JL, Ulyanova T, Cao H, Nakamoto B, Ware CB, Papayannopoulou T. Diverse hematopoietic potentials of five human embryonic stem cell lines. *Exp Cell Res* 2008; **314**: 2930-2940 [PMID: 18692044 DOI: 10.1016/j.yexcr.2008.07.019]
73 **Mikkola M**, Olsson C, Palgi J, Ustinov J, Palomaki T, Horelli-Kuitunen N, Knuutila S, Lundin K, Otonkoski T, Tuuri T. Distinct differentiation characteristics of individual human embryonic stem cell lines. *BMC Dev Biol* 2006; **6**: 40 [PMID: 16895598 DOI: 10.1186/1471-213X-6-40]
74 **Buchholz DE**, Hikita ST, Rowland TJ, Friedrich AM, Hinman CR, Johnson LV, Clegg DO. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. *Stem Cells* 2009; **27**: 2427-2434 [PMID: 19658190 DOI: 10.1002/stem.189]
75 **Andersson TB,** Sundberg MI. Livers cells derived from human embryonic stem cells Progress and potential use in ADMET applications. *Drug Discov Today: Technol* 2008; **5:** e143-e147 [DOI: 10.1016/j.ddtec.2008.09.001]
76 **Agarwal S**, Holton KL, Lanza R. Efficient differentiation of functional hepatocytes from human embryonic stem cells. *Stem Cells* 2008; **26**: 1117-1127 [PMID: 18292207 DOI: 10.1634/stemcells.2007-1102]
77 **Basma H**, Soto-Gutiérrez A, Yannam GR, Liu L, Ito R, Yamamoto T, Ellis E, Carson SD, Sato S, Chen Y, Muirhead D, Navarro-Alvarez N, Wong RJ, Roy-Chowdhury J, Platt JL, Mercer DF, Miller JD, Strom SC, Kobayashi N, Fox IJ. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. *Gastroenterology* 2009; **136**: 990-999 [PMID: 19026649 DOI: 10.1053/j.gastro.2008.10.047]
78 **Sullivan GJ**, Hay DC, Park IH, Fletcher J, Hannoun Z, Payne CM, Dalgetty D, Black JR, Ross JA, Samuel K, Wang G, Daley GQ, Lee JH, Church GM, Forbes SJ, Iredale JP, Wilmut I. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. *Hepatology* 2010; **51**: 329-335 [PMID: 19877180 DOI: 10.1002/hep.23335]
79 **Song Z**, Cai J, Liu Y, Zhao D, Yong J, Duo S, Song X, Guo Y, Zhao Y, Qin H, Yin X, Wu C, Che J, Lu S, Ding M, Deng H. Efficient generation of hepatocyte-like cells from human induced pluripotent stem cells. *Cell Res* 2009; **19**: 1233-1242 [PMID: 19736565 DOI: 10.1038/cr.2009.107]
80 **Si-Tayeb K**, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, North PE, Dalton S, Duncan SA. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. *Hepatology* 2010; **51**: 297-305 [PMID: 19998274 DOI: 10.1002/hep.23354]
81 **Liu H**, Ye Z, Kim Y, Sharkis S, Jang YY. Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. *Hepatology* 2010; **51**: 1810-1819 [PMID: 20432258 DOI: 10.1002/hep.23626]
82 **Takata A,** Otsuka M, Kogiso T, Kojima K, Yoshikawa T, Tateishi R, Kato N, Shiina S, Yoshida H, Omata M, Koike K. Direct differentiation of hepatic cells from human induced pluripotent stem cells using a limited number of cytokines. *Hepatol Int* 2011 Feb 6 [Epub ahead of print] [PMID: 21484132 DOI: 10.1007/s12072-011-9251-5]
83 **Gai H**, Nguyen DM, Moon YJ, Aguila JR, Fink LM, Ward DC, Ma Y. Generation of murine hepatic lineage cells from induced pluripotent stem cells. *Differentiation* 2010; **79**: 171-181 [PMID: 20106584 DOI: 10.1016/j.diff.2010.01.002]
84 **Iwamuro M**, Komaki T, Kubota Y, Seita M, Kawamoto H, Yuasa T, Shahid JM, Hassan RA, Hassan WA, Nakaji S, Nishikawa Y, Kondo E, Yamamoto K, Fox IJ, Kobayashi N. Hepatic differentiation of mouse iPS cells in vitro. *Cell Transplant* 2010; **19**: 841-847 [PMID: 20955659 DOI: 10.3727/096368910X508960]
85 **Gómez-Lechón MJ**, Donato MT, Castell JV, Jover R. Human hepatocytes as a tool for studying toxicity and drug metabolism. *Curr Drug Metab* 2003; **4**: 292-312 [PMID: 12871046]
86 **Rao MS**, Khan AA, Parveen N, Habeeb MA, Habibullah CM, Pande G. Characterization of hepatic progenitors from human fetal liver during second trimester. *World J Gastroenterol* 2008; **14**: 5730-5737 [PMID: 18837092 DOI: 10.3748/wjg.14.5730]
87 **Wilkening S**, Stahl F, Bader A. Comparison of primary human hepatocytes and hepatoma cell line Hepg2 with regard to their biotransformation properties. *Drug Metab Dispos* 2003; **31**: 1035-1042 [PMID: 12867492 DOI: 10.1124/dmd.31.8.1035]
88 **Seifinejad A**, Tabebordbar M, Baharvand H, Boyer LA, Salekdeh GH. Progress and promise towards safe induced pluripotent stem cells for therapy. *Stem Cell Rev* 2010; **6**: 297-306 [PMID: 20180049 DOI: 10.1007/s12015-010-9121-x]
89 **Andrade RJ**, Robles M, Fernández-Castañer A, López-Ortega S, López-Vega MC, Lucena MI. Assessment of drug-induced hepatotoxicity in clinical practice: a challenge for gastroenterologists. *World J Gastroenterol* 2007; **13**: 329-340 [PMID: 17230599]
90 **Dekant W**. The role of biotransformation and bioactivation in toxicity. *EXS* 2009; **99**: 57-86 [PMID: 19157058 DOI: 10.1007/978-3-7643-8336-7_3]
91 **Greenhough S**, Medine CN, Hay DC. Pluripotent stem cell derived hepatocyte like cells and their potential in toxicity screening. *Toxicology* 2010; **278**: 250-255 [PMID: 20674645 DOI: 10.1016/j.tox.2010.07.012]
92 **Miura K**, Okada Y, Aoi T, Okada A, Takahashi K, Okita K,

Nakagawa M, Koyanagi M, Tanabe K, Ohnuki M, Ogawa D, Ikeda E, Okano H, Yamanaka S. Variation in the safety of induced pluripotent stem cell lines. *Nat Biotechnol* 2009; **27**: 743-745 [PMID: 19590502 DOI: 10.1038/nbt.1554]

93 **Kim K**, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, Yabuuchi A, Takeuchi A, Cunniff KC, Hongguang H, McKinney-Freeman S, Naveiras O, Yoon TJ, Irizarry RA, Jung N, Seita J, Hanna J, Murakami P, Jaenisch R, Weissleder R, Orkin SH, Weissman IL, Feinberg AP, Daley GQ. Epigenetic memory in induced pluripotent stem cells. *Nature* 2010; **467**: 285-290 [PMID: 20644535 DOI: 10.1038/nature09342]

94 **Polo JM**, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol* 2010; **28**: 848-855 [PMID: 20644536 DOI: 10.1038/nbt.1667]

95 **Espejel S**, Roll GR, McLaughlin KJ, Lee AY, Zhang JY, Laird DJ, Okita K, Yamanaka S, Willenbring H. Induced pluripotent stem cell-derived hepatocytes have the functional and proliferative capabilities needed for liver regeneration in mice. *J Clin Invest* 2010; **120**: 3120-3126 [PMID: 20739754 DOI: 10.1172/JCI43267]

96 **Fausto N**. Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. *Hepatology* 2004; **39**: 1477-1487 [PMID: 15185286 DOI: 10.1002/hep.20214]

97 **Schmelzer E**, Wauthier E, Reid LM. The phenotypes of pluripotent human hepatic progenitors. *Stem Cells* 2006; **24**: 1852-1858 [PMID: 16627685 DOI: 10.1634/stemcells.2006-0036]

98 **Roskams TA**, Theise ND, Balabaud C, Bhagat G, Bhathal PS, Bioulac-Sage P, Brunt EM, Crawford JM, Crosby HA, Desmet V, Finegold MJ, Geller SA, Gouw AS, Hytiroglou P, Knisely AS, Kojiro M, Lefkowitch JH, Nakanuma Y, Olynyk JK, Park YN, Portmann B, Saxena R, Scheuer PJ, Strain AJ, Thung SN, Wanless IR, West AB. Nomenclature of the finer branches of the biliary tree: canals, ductules, and ductular reactions in human livers. *Hepatology* 2004; **39**: 1739-1745 [PMID: 15185318 DOI: 10.1002/hep.20130]

99 **Terrace JD**, Currie IS, Hay DC, Masson NM, Anderson RA, Forbes SJ, Parks RW, Ross JA. Progenitor cell characterization and location in the developing human liver. *Stem Cells Dev* 2007; **16**: 771-778 [PMID: 17999598]

100 **Khan AA**, Shaik MV, Parveen N, Rajendraprasad A, Aleem MA, Habeeb MA, Srinivas G, Raj TA, Tiwari SK, Kumaresan K, Venkateswarlu J, Pande G, Habibullah CM. Human fetal liver-derived stem cell transplantation as supportive modality in the management of end-stage decompensated liver cirrhosis. *Cell Transplant* 2010; **19**: 409-418 [PMID: 20447340]

101 **Challen GA**, Little MH. A side order of stem cells: the SP phenotype. *Stem Cells* 2006; **24**: 3-12 [PMID: 16449630 DOI: 10.1634/stemcells.2005-0116]

102 **Petersen BE**, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. *Science* 1999; **284**: 1168-1170 [PMID: 10325227]

103 **Theise ND**, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS. Liver from bone marrow in humans. *Hepatology* 2000; **32**: 11-16 [PMID: 10869283 DOI: 10.1053/jhep.2000.9124]

104 **Houlihan DD**, Newsome PN. Critical review of clinical trials of bone marrow stem cells in liver disease. *Gastroenterology* 2008; **135**: 438-450 [PMID: 18585384 DOI: 10.1053/j.gastro.2008.05.040]

105 **He ZP**, Tan WQ, Tang YF, Zhang HJ, Feng MF. Activation, isolation, identification and in vitro proliferation of oval cells from adult rat livers. *Cell Prolif* 2004; **37**: 177-187 [PMID: 15030551 DOI: 10.1111/j.1365-2184.2004.00293.x]

106 **Petersen BE**, Goff JP, Greenberger JS, Michalopoulos GK. Hepatic oval cells express the hematopoietic stem cell marker Thy-1 in the rat. *Hepatology* 1998; **27**: 433-445 [PMID: 9462642 DOI: 10.1002/hep.510270218]

107 **Hatch HM**, Zheng D, Jorgensen ML, Petersen BE. SDF-1alpha/CXCR4: a mechanism for hepatic oval cell activation and bone marrow stem cell recruitment to the injured liver of rats. *Cloning Stem Cells* 2002; **4**: 339-351 [PMID: 12626097 DOI: 10.1089/153623002321025014]

108 **Sharma AD**, Cantz T, Manns MP, Ott M. The role of stem cells in physiology, pathophysiology, and therapy of the liver. *Stem Cell Rev* 2006; **2**: 51-58 [PMID: 17142887 DOI: 10.1007/s12015-006-0009-8]

109 **Vierbuchen T**, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* 2010; **463**: 1035-1041 [PMID: 20107439 DOI: 10.1038/nature08797]

110 **Ieda M**, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 2010; **142**: 375-386 [PMID: 20691899 DOI: 10.1016/j.cell.2010.07.002]

111 **Szabo E**, Rampalli S, Risueño RM, Schnerch A, Mitchell R, Fiebig-Comyn A, Levadoux-Martin M, Bhatia M. Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature* 2010; **468**: 521-526 [PMID: 21057492 DOI: 10.1038/nature09591]

112 **Huang P**, He Z, Ji S, Sun H, Xiang D, Liu C, Hu Y, Wang X, Hui L. Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* 2011; **475**: 386-389 [PMID: 21562492 DOI: 10.1038/nature10116]

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World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3397

World J Gastroenterol 2013 June 14; 19(22): 3397-3403
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Endoscopic ultrasound-guided ethanol ablation therapy for tumors

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Received: February 9, 2013 Revised: May 14, 2013
Accepted: May 18, 2013
Published online: June 14, 2013

Abstract

Endoscopic ultrasonography (EUS) has evolved into a useful therapeutic tool for treating a broad range of tumors since being introduced into clinical practice as a diagnostic modality nearly three decades ago. In particular, EUS-guided fine-needle injection has proven a successful minimally invasive approach for treating benign lesions such as pancreatic cysts, relieving pancreatic pain through celiac plexus neurolysis, and controlling local tumor growth of unresectable malignancies by direct delivery of anti-tumor agents. One such ablative agent, ethanol, is capable of safely ablating solid or cystic lesions in hepatic tissues *via* percutaneous injection. Recent research and clinical interest has focused on the promise of EUS-guided ethanol ablation as a safe and effective method for treating pancreatic tumor patients with small lesions or who are poor operative candidates. Although it is not likely to replace radical resection of localized lesions or systemic treatment of metastatic tumors in all patients, EUS-guided ablation is an ideal method for patients who refuse or are not eligible for surgery. Moreover, this treatment modality may play an active role in the development of future pancreatic tumor treatments. This article reviews the most recent clinical applications of EUS-guided ethanol ablation in humans for treating pancreatic cystic tumors, pancreatic neuroendocrine tumors, and metastatic lesions.

Key words: Endoscopic ultrasonography; Ethanol; Tumor ablation; Pancreas cancer; Cystic tumor; Neuroendocrine tumors; Celiac plexus neurolysis

Core tip: Ethanol, a commonly used ablative agent, has been used to successfully and safely ablate solid and cystic hepatic lesions *via* percutaneous injection. Endoscopic ultrasonography (EUS)-guided ethanol ablation, a minimally invasive approach, was recently developed and has been successfully applied as treatment of pancreatic cysts, pancreatic neuroendocrine tumors, and abdominal metastatic lesions. Although it is not likely to replace radical resection for treating localized lesions or systemic therapy for managing metastatic tumors, EUS-guided ablation therapies represent an attractive alternative treatment modality for patients who refuse or are not eligible for surgery.

Zhang WY, Li ZS, Jin ZD. Endoscopic ultrasound-guided ethanol ablation therapy for tumors. *World J Gastroenterol* 2013; 19(22): 3397-3403 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3397.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3397

INTRODUCTION

Endoscopic ultrasonography (EUS) has been used in clinical practice for more than 30 years[1,2] since its development for the original purpose of diagnosing pancre-

atic disease and carrying out malignancy staging. In the early 1990s, the introduction of curvilinear array echoendoscope made it possible to perform fine-needle aspiration (FNA) biopsy under direct endosonographic visualization. The minimally invasive and safe nature of FNA led to a boon in use of therapeutic EUS techniques, including EUS-guided fine needle injection (EUS-FNI). More recently, EUS-FNI has been applied as a pancreatic cancer treatment aimed at controlling pain through nerve blockade, as a solid tumor therapy for introduction of brachytherapy seeds and viral vectors, and as a tool for ablation therapy[3-6].

Ethanol, a commonly used ablative agent, gained popularity according to its advantageous features of cost effectiveness, ready availability, and rapidly ablation function. The mechanism of ethanol ablation mainly involves cell death by causing cell membrane lysis, protein denaturation, and vascular occlusion[7]. Percutaneous ethanol injection has been used in the ablation of renal cysts, hepatic cysts, and solid tumors, such as liver or adrenal tumors[8-12]. EUS-guided ethanol injection is superior to the percutaneous application because it offers real-time image monitoring of the lesions located deep in the pancreas. In addition, EUS can provide precise measurement of lesions and identification of surrounding structures, and can readily deliver therapeutic agents to a target site, thereby minimizing damage to non-tumor tissue. Some EUS-guided ethanol ablation procedures for pancreatic tissue have been shown to be safe and feasible in animal models[13,14].

In this review, the recent applications, features, and outcomes of EUS-guided ethanol injection treatment for pancreatic cystic tumors, pancreatic neuroendocrine tumors (pNETs), celiac plexus, and metastatic lesions in humans are summarized.

EUS-GUIDED ETHANOL ABLATION THERAPY FOR PANCREATIC CYSTIC TUMORS

Pancreatic cysts are common, with the incidence of asymptomatic cysts being reported at about 2.5% in the general population[15]. The advances of various imaging modalities, such as computed tomography (CT) scanning, magnetic resonance imaging, and EUS, have been accompanied by an increase in detection of pancreatic cystic neoplasms (PCNs). Pancreatic cystic tumors are mainly classified as either mucinous cystic neoplasms (MCNs), serous cystic neoplasms (SCNs), or intraductal papillary mucinous neoplasms (IPMNs), according to histopathological features[16]. Some cystic tumors of the pancreas, such as MCNs and IPMNs, have the potential to become malignant and are often treated by surgical resection when malignancy is suspected. Although surgical resection is generally recommended, pancreatic cyst ablation is an increasingly popular treatment option, especially *via* injection of ethanol or other ablative agents into the cyst cavity under EUS guidance.

Recent studies have shown that EUS-guided ethanol injection into pancreatic cysts is safe and feasible, slows or inhibits growth, and avoids the risks associated with surgical resection. Gan *et al*[17] published the first report of safety and feasibility for the ethanol lavage and ablation of pancreatic cystic lesions using EUS-FNI. In that study, 25 patients with pancreatic cysts (MCNs, n = 13; IPMNs, n = 4; SCNs, n = 3; pseudocysts, n = 3; uncertain etiology, n = 2) were treated with escalating doses (5%-80%) of ethanol for 3-5 min under EUS guidance. The results demonstrated that this procedure effectively reduced tumors without inducing complications, such as pancreatitis, in the short- and long-term follow-up periods. Eight patients (35%) had complete resolution of the cysts, and five patients undergoing surgical resection showed histological evidence of epithelial ablation.

To further determine whether EUS-guided ethanol lavage can decrease pancreatic cyst size, a multicenter, randomized, prospective trial was performed in 2009[18]. The primary aim of that study was to compare the change of pancreatic cyst size after EUS-guided lavage with 80% ethanol or saline solution alone. Of the 42 patients enrolled, 25 patients were initially treated with 80% ethanol lavage and 17 patients were administered saline solution lavage. Three months after the first lavage, a second pancreatic cystic lavage was performed. Patients who initially received an ethanol lavage received a second ethanol lavage, and those who received an initial saline solution lavage were then given an 80% ethanol lavage. The results indicated that EUS-guided 80% ethanol injection resulted in a greater decrease in size of pancreatic cystic tumors compared with the saline solution injection, and that the overall resolution rate of pancreatic cystic tumors was 33.3%. Incidences of major complications, such as abdominal pain, intracystic bleeding during lavage, and acute pancreatitis, were similar in the two groups. Furthermore, nine of the study patients (75%) were followed-up for two years after initial cyst resolution, and no evidence of cyst recurrence was found in any patient[19].

To improve the effect of ethanol ablation therapy, Oh *et al*[20-22] performed EUS-guided ethanol lavage with a paclitaxel injection to treat cystic tumors of the pancreas. An initial study found that complete resolution of pancreatic cystic tumors was achieved in 11 of 14 patients after treatment with ethanol and paclitaxel injection; in addition, minor complications, including hyperamylasemia and abdominal pain, were observed in one patient[20], but no cases of acute pancreatitis occurred. The study's collective findings demonstrated that ethanol lavage with paclitaxel injection is a safe, feasible, and effective method to treat pancreatic cystic tumors. However, only a small number of patients and only short-term outcomes were assessed in this study. A subsequent study by this group was carried out involving a larger population (n = 52) with a longer-term follow-up period (n = 47 for > 12 mo). All patients underwent 99% pure ethanol injection into the collapsed cyst for a 3-5 min lavage. After the injected ethanol was aspirated, paclitaxel solution was injected into the cyst cavity. Ultimately,

complete resolution was achieved in 29 of the patients (62%). No treatment-related complications, including bleeding, bowel perforation, or severe pancreatitis, were observed[22].

DiMaio *et al*[23] retrospectively analyzed the effectiveness of multiple EUS-guided ethanol lavages for pancreatic cystic tumor treatment. The results showed that multiple ethanol lavage treatments resulted in a greater decrease in the size and surface area of PCNs compared with only one ethanol lavage treatment. Complete cyst resolution was not seen in any patient after the first EUS-ethanol lavage, but was achieved in 5 of the 13 patients (38%) who underwent two sequential EUS-ethanol lavage treatments. Again, no treatment-related complications were observed, with the exception of one patient complaining of minor abdominal pain.

Collectively, these preliminary studies suggest that ethanol ablation is relatively safe and feasible for clinical use in humans. Therefore, EUS-guided ablation of pancreatic cystic tumors *via* ethanol injection, a minimally invasive technique, may be an attractive choice to treat patients who refuse or are not eligible for surgery. However, there are a number of limitations in these studies. First, some studies only included a treatment group, and no control group was included. Second, there was not a definitive diagnosis made prior to application of the ethanol ablation procedure. Inclusive criteria were based on the imaging findings and some benign lesions might have been treated unnecessarily. Third, the patients were followed-up for a relatively short period of time for documentation of cyst resolution. Thus, EUS-guided cyst ablation is an experimental therapy modality that should be used with caution.

EUS-guided pancreatic cyst ablation is mainly used currently for the following indications: (1) patients who refuse surgery or are high-risk surgical candidates; (2) cysts that appear to be benign and are morphologically indeterminate; and (3) asymptomatic cysts that increase in size during the follow-up period. Before ethanol ablation for pancreatic cysts can be widely accepted, a prospective, randomized, controlled trial with a long follow-up period is required to determine the clinical efficacy of ethanol ablation therapy compared with surgical resection.

EUS-GUIDED ETHANOL ABLATION THERAPY FOR PANCREATIC NEUROENDOCRINE TUMORS

pNET account for a small percentage of all pancreatic tumors (1.3%), but their incidence is increasing[24]. Although surgical enucleation or resection is considered as the treatment of choice for pNET, a few patients are not suitable candidates for surgery because of old age or comorbidities. Recently, successful application of EUS-guided ablation therapy using ethanol has been reported for the treatment of pNET. Jürgensen *et al*[25] reported successful resolution of a 13 mm insulinoma by EUS-guided ethanol ablation therapy in a 78-year-old woman (diagnosed by laboratory findings and EUS-FNA). Because the patient was in poor general condition and refused surgical resection, EUS-guided ethanol injection was performed. Although mild procedure-associated pancreatitis was experienced after the ethanol ablation procedure, complete resolution was achieved in the patient. The patient had durable glycemic control throughout the recovery and follow-up and no indications of insulinoma were observed by follow-up EUS.

Muscatiello *et al*[26,27] described another case of successful ethanol ablation involving a patient with a pancreatic endocrine tumor. A female patient with multiple endocrine neoplasia type 1 (11 and 7 mm, respectively) underwent 40% ethanol ablation therapy. Two months after the ethanol ablation, EUS with contrast enhancement revealed areas of fibrosis. However, subsequent resolution of the accompanying perturbed levels of vasoactive intestinal peptide and chromogranin A was observed. The main complication in this case was a small pancreatic necrotic lesion, which was presumably caused by minimal ethanol effusion and managed by laparoscopic necrosectomy.

Deprez *et al*[28] reported treatment of a patient with insulinoma of the pancreas head involving a 98% ethanol injection under EUS guidance. The patient achieved complete resolution (evidenced by imaging) at 3 mo after the procedure and remained asymptomatic and normoglycemic for more than two years later. Vleggaar *et al*[29] reported an ethanol ablation operation for an 82-year-old patient with a pNET, for which 96% ethanol was delivered to under EUS guidance. Two months after the ablation therapy, EUS examination indicated that a significant reduction in the diameter of the tumor had been achieved. Finally, Levy *et al*[30] retrospectively reviewed eight patients with insulinomas who received US-guided ethanol ablation. Five of those patients underwent EUS-guided ethanol injection and the remaining three underwent intra-operative ultrasound (IOUS)-guided ethanol injection. No complications occurred during or after the EUS-guided procedure. The IOUS-guided ethanol injection, however, was associated with minor peritumoral bleeding ($n = 1$), pseudocyst ($n = 1$), and pancreatitis with peri-pancreatic fluid ($n = 1$). Hypoglycemia-related symptoms completely disappeared in five patients who underwent EUS-guided ethanol injection, and significantly improved in the three patients who underwent IOUS-guided ethanol injection.

Based on these case reports, EUS-guided ethanol ablation seems to be an alternative treatment option for pancreatic solid tumors. However, some issues remain that require further study, such as the choice of target area and adequate ethanol dose to achieve successful ablation without causing serious complications. In addition, EUS-guided ethanol ablation therapy for pNETs appears to harbor the possibility of late relapse requiring re-intervention, as well as incomplete ablation and risk of metastasis. While currently the strongest indication for intratumoral therapy is patients who refuse surgery

or are poor surgical candidates, a clinical trial enrolling more patients with longer follow-up is required to more definitively determine the particular physiological indications.

EUS-GUIDED ETHANOL ABLATION THERAPY FOR CELIAC PLEXUS ABLATION

Pain is one of the major complications of advanced pancreatic cancer. It is estimated that pain is present in 80%-85% of pancreatic cancer patients at the time of diagnosis, and it can be difficult to control, even with high doses of analgesics[31,32]. Celiac plexus neurolysis (CPN), a chemical splanchnicectomy of the celiac plexus, has been well established as an effective method for controlling pain and decreasing morphine consumption in patients with locally advanced or unresectable pancreatic cancer[32,33]. Before the advent of EUS, CPN was performed by radiological guidance or intraoperative method, both of which are associated with a risk of the serious complications of paraesthesia, paraplegia, and pneumothorax[34,35]. With the development of the EUS technique, EUS-guided CPN (EUS-CPN) has become a novel option to treat cancer-related pain in patients with inoperable pancreatic cancer.

EUS-CPN consists of an injection of absolute alcohol, a neurolytic agent, into the celiac ganglia to permanently destroy neural tissue of celiac ganglia. The first applications of EUS-CPN were reported by Wiersema *et al*[36]. Thirty patients with pain due to intra-abdominal malignancies were injected with 98% dehydrated absolute alcohol under EUS guidance into the celiac plexus *via* the transgastric route. The majority of patients (79%-88%) experienced a significant decrease in pain scores at the 10 wk post-treatment (median follow-up time).

Subsequent studies have demonstrated that EUS-CPN is a safe and effective method to relieve severe pain from advanced pancreatic cancer[3,37-39]. In a meta-analysis of these EUS-CPN studies, pain relief was observed in about 80% of 289 patients with pain due to pancreatic cancer[40]. While EUS-CPN can be delivered on either or both sides of the aorta, a recent study showed that bilateral injection was more effective than a central single injection[41]. The common complications of EUS-CPN, transient diarrhea and hypotension, were observed in 9% and 10%-15% of cases, respectively, and both complications were self-limiting in most cases[37].

EUS-GUIDED ETHANOL ABLATION THERAPY FOR OTHER TUMORS

EUS-guided injection of alcohol is also used for ablation of other abdominal tumors, such as gastrointestinal stromal tumor (GIST) and intra-abdominal metastatic lesions located in the liver, adrenal glands, and pelvic lymph nodes. Günter *et al*[42] reported a case of EUS-guided ethanol ablation of a GIST involving a 59-year-old man with a 4 cm lesion in the muscularis propria of the stomach that was diagnosed by EUS and EUS-guided FNA. Severe comorbidity precluded surgery and the patient was treated with an injection of 1.5 mL of 95% ethanol under EUS guidance. Seven weeks after the injection, no endosonographic evidence of residual tumor was seen, but a 1.5-cm ulcer at the treatment site was present, which was resolved by acid suppression therapy. Two years after the ethanol ablation treatment, a follow-up examination showed complete remission of the tumor. No severe complications were observed, except for self-limiting pain in the upper abdomen.

Recently, two cases of EUS-guided ethanol ablation of a solid hepatic metastasis carcinoma have been reported[43,44]. In the first, a 65-year-old man with a 3.3-cm lesion in the left liver and a markedly elevated carcinoembryonic antigen level was considered as having metastatic cancer from either a pancreatic adenocarcinoma or prior colorectal cancer. Multiple EUS-guided injections of absolute alcohol were administered over several years. The lesion size decreased over time, as well as the levels of corresponding tumor markers, indicating that this approach was effective in controlling tumor growth. Furthermore, the multiple EUS-guided hepatic tumor injections did not result in major complications, except for a small subcapsular hematoma that resolved spontaneously[43].

Our group also reported a case of a 47-year-old man with a hepatic metastatic carcinoma from a pancreatic adenocarcinoma treated by injection of anhydrous ethanol under EUS guidance (Figure 1). One month later, an abdominal CT follow-up scan revealed a decrease in size of the lesion (Figure 2). No significant procedure-related complications were observed, except for a transient low fever[44].

Artifon *et al*[45] described a case of EUS-guided alcohol ablation of a 5 cm left adrenal metastatic carcinoma in a 52-year-old man. For this patient, the main presenting complaint was abdominal pain and the diagnosis of left adrenal metastasis from non-small-cell lung carcinoma was made by EUS-FNA. Three days after the alcohol ablation therapy treatment (15 mL of 98% absolute alcohol), the symptom of abdominal pain disappeared. At the one-month follow-up, EUS revealed a hyperechoic nodule between the pancreas and the left kidney, which was presumed to represent alcohol ablation-induced fibrosis.

More recently, DeWitt *et al*[46] reported the successful EUS-guided alcohol ablation of metastatic pelvic lymph nodes in a patient with rectal cancer. The two injected metastatic pelvic lymph nodes received 4 and 2 mL of ethanol, respectively, which achieved local complete resolution at 10 mo post-treatment. No procedure-related complications were reported.

The basic characteristics and outcomes of the studies described herein of EUS-guided ethanol ablation therapy for pancreatic cystic tumors, pNETs, and other tumors are summarized in Tables 1-3, respectively.

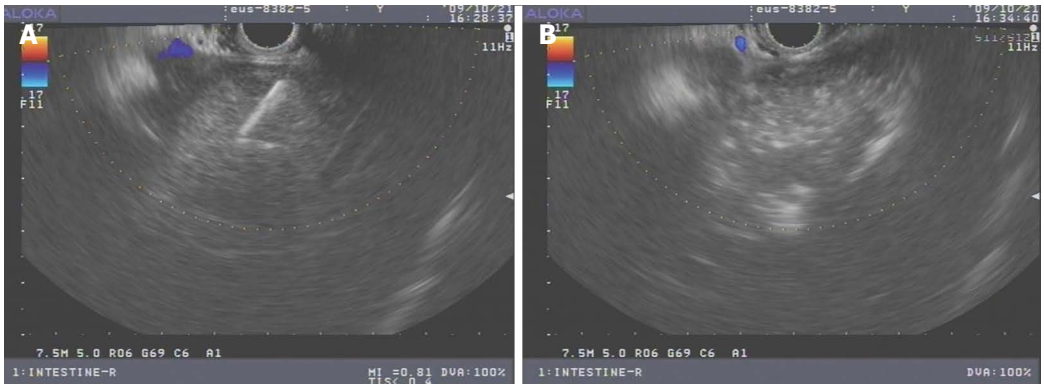

Figure 1 Endoscopic ultrasonography image of hepatic tumor. A: During ethanol injection; B: Hyperechoic appearance after ethanol injection.

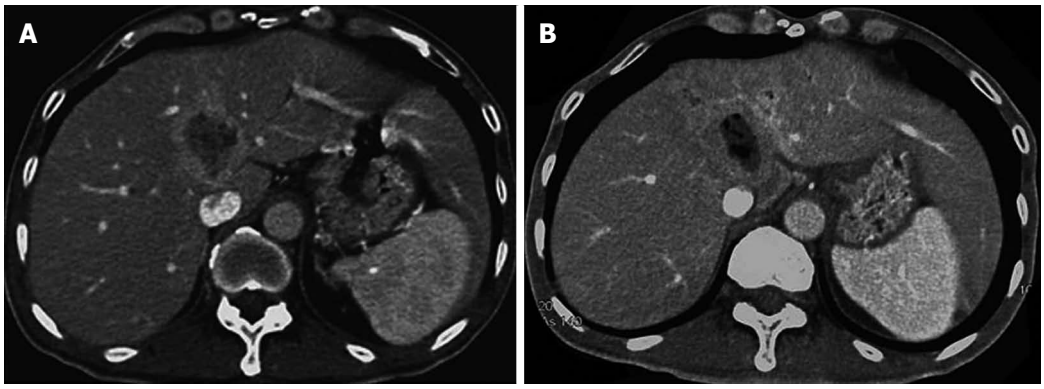

Figure 2 Computer tomography scans of hepatic metastatic carcinoma. A: Before ethanol injection; B: After ethanol injection.

Table 1 Summary of endoscopic ultrasonography-guided ethanol ablation for pancreatic cystic tumor

Ref.	*n*	Median size, mm (range)	Ablative agents	Complete resolution	Complications
Gan *et al*[17]	25	19.4 (6-30)	Ethanol	35%	None
Oh *et al*[20]	14	25.5 (17-52)	Ethanol and paclitaxel	79%	Acute pancreatitis (n = 1) Hyperamylasemia (n = 6) Vague abdominal pain (n = 1)
Oh *et al*[21]	10	29.5 (20-68)	Ethanol and paclitaxel	60%	Mild pancreatitis (n = 1)
DeWitt *et al*[18]	42	22.4 (10-58)	Saline *vs* ethanol	33%	Abdominal pain at 2 h (n = 2) Abdominal pain at 7 d (n = 5) Pancreatitis (n = 1) Acystic bleeding (n = 1)
Oh *et al*[22]	52	31.8 (17-68)	Ethanol and paclitaxel	62%	Fever (n = 1) Vague abdominal discomfort (n = 1) Mild pancreatitis (n = 1) Splenic vein obliteration (n = 1)

CONCLUSION

With the advent of curvilinear EUS, therapeutic EUS has emerged as an important approach to manage malignancies. While prominent advances have been made in applications of EUS-FNI for specific antitumor therapy, the field of EUS-guided ethanol ablation therapy for malignancies continues to evolve. Currently, our understanding of the safety and efficacy of EUS-guided ethanol ablation as a tumor therapy is primarily limited by the small sample sizes and short-term follow-up periods of related case studies. Prospective, large trials should be performed to better evaluate this technique: its indications and complications before it is recommended for widespread use in clinical practice. For example, particular patient populations may receive more benefit than others from the EUS-guided ethanol ablation with or without systemic therapies, and prospective clinical trials will help to define these patients.

ACKNOWLEDGMENTS

We thank Medjaden Bioscience Limited for assisting in the preparation of this manuscript.

Table 2 Summary of endoscopic ultrasound-guided ethanol ablation for pancreatic neuroendocrine tumor

Ref.	*n*	Maximum diameter (mm)	Ethanol	Volume (mL)	Complications
Jurgensen *et al*[25]	1	13	95%	8.0	Pain in the upper abdomen A mild increase of serum lipase activity
Muscatiello *et al*[26]	1	11 and 7	40%	2.0	A small pancreatic necrotic lesion
Deprez *et al*[28]			98%	3.5	A mild elevation of pancreatic enzymes Hematoma ulceration of the duodenal wall
Vleggaar *et al*[29]	1	10	96%	0.3	None
Levy *et al*[30]	5	18	95%	0.1	None
				0.4	
				0.1	
		20	98%	0.1	
				0.3	
		21	98%	1.0	
				0.3	
		8	98%	3.0	
				1.5	
		16	99%	0.7	
				1.0	

Table 3 Cases of endoscopic ultrasound-guided ethanol ablation for other tumor

Target tumor	Maximum diameter (mm)	Ethanol	Volume (mL)	Complications
Gastrointestinal stromal tumor[42]	40	95%	1.5	None
Hepatic metastasis carcinoma from colorectal carcinoma[43]	33	98%	6.0	A tiny subcapsular hematoma
Hepatocellular carcinoma from pancreatic adenocarcinoma[44]	35	100%	10.0	Low-grade fever
Left adrenal metastasis carcinoma from non-small-cell lung carcinoma[45]	50	98%	15.0	None
Pelvic metastatic lymph nodes from rectal cancer[46]	11 and 10	Not stated	4.0 and 2.0	None

REFERENCES

1 **DiMagno EP**, Buxton JL, Regan PT, Hattery RR, Wilson DA, Suarez JR, Green PS. Ultrasonic endoscope. *Lancet* 1980; **1**: 629-631 [PMID: 6102631 DOI: 10.1016/S0140-6736(80)91122-8]
2 **Strohm WD**, Phillip J, Hagenmüller F, Classen M. Ultrasonic tomography by means of an ultrasonic fiberendoscope. *Endoscopy* 1980; **12**: 241-244 [PMID: 7428729 DOI: 10.1055/s-2007-1021752]
3 **Wiechowska-Kozłowska A**, Boer K, Wójcicki M, Milkiewicz P. The efficacy and safety of endoscopic ultrasound-guided celiac plexus neurolysis for treatment of pain in patients with pancreatic cancer. *Gastroenterol Res Pract* 2012; **2012**: 503098 [PMID: 22474439 DOI: 10.1155/2012/503098]
4 **Jin Z**, Du Y, Li Z, Jiang Y, Chen J, Liu Y. Endoscopic ultrasonography-guided interstitial implantation of iodine 125-seeds combined with chemotherapy in the treatment of unresectable pancreatic carcinoma: a prospective pilot study. *Endoscopy* 2008; **40**: 314-320 [PMID: 18283622 DOI: 10.1055/s-2007-995476]
5 **Hecht JR**, Bedford R, Abbruzzese JL, Lahoti S, Reid TR, Soetikno RM, Kirn DH, Freeman SM. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. *Clin Cancer Res* 2003; **9**: 555-561 [PMID: 12576418]
6 **Yoon WJ**, Brugge WR. Endoscopic ultrasonography-guided tumor ablation. *Gastrointest Endosc Clin N Am* 2012; **22**: 359-69, xi [PMID: 22632957 DOI: 10.1016/j.giec.2012.04.017]
7 **Gelczer RK**, Charboneau JW, Hussain S, Brown DL. Complications of percutaneous ethanol ablation. *J Ultrasound Med* 1998; **17**: 531-533 [PMID: 9697961]
8 **Bean WJ**. Renal cysts: treatment with alcohol. *Radiology* 1981; **138**: 329-331 [PMID: 7455112]
9 **Omerović S**, Zerem E. Alcohol sclerotherapy in the treatment of symptomatic simple renal cysts. *Bosn J Basic Med Sci* 2008; **8**: 337-340 [PMID: 19125704]
10 **Larssen TB**, Jensen DK, Viste A, Horn A. Single-session alcohol sclerotherapy in symptomatic benign hepatic cysts. Long-term results. *Acta Radiol* 1999; **40**: 636-638 [PMID: 10598853 DOI: 10.3109/02841859909175601]
11 **Livraghi T**, Bolondi L, Lazzaroni S, Marin G, Morabito A, Rapaccini GL, Salmi A, Torzilli G. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992; **69**: 925-929 [PMID: 1310435]
12 **Xiao YY**, Tian JL, Li JK, Yang L, Zhang JS. CT-guided percutaneous chemical ablation of adrenal neoplasms. *AJR Am J Roentgenol* 2008; **190**: 105-110 [PMID: 18094300 DOI: 10.2214/AJR.07.2145]
13 **Aslanian H**, Salem RR, Marginean C, Robert M, Lee JH, Topazian M. EUS-guided ethanol injection of normal porcine pancreas: a pilot study. *Gastrointest Endosc* 2005; **62**: 723-727 [PMID: 16246687 DOI: 10.1016/j.gie.2005.06.048]
14 **Matthes K**, Mino-Kenudson M, Sahani DV, Holalkere N, Brugge WR. Concentration-dependent ablation of pancreatic tissue by EUS-guided ethanol injection. *Gastrointest Endosc* 2007; **65**: 272-277 [PMID: 17258986 DOI: 10.1016/j.gie.2006.04.043]
15 **Laffan TA**, Horton KM, Klein AP, Berlanstein B, Siegelman SS, Kawamoto S, Johnson PT, Fishman EK, Hruban RH. Prevalence of unsuspected pancreatic cysts on MDCT. *AJR Am J Roentgenol* 2008; **191**: 802-807 [PMID: 18716113 DOI: 10.2214/AJR.07.3340]
16 **Salvia R**, Festa L, Butturini G, Tonsi A, Sartori N, Biasutti C, Capelli P, Pederzoli P. Pancreatic cystic tumors. *Minerva Chir* 2004; **59**: 185-207 [PMID: 15238892]
17 **Gan SI**, Thompson CC, Lauwers GY, Bounds BC, Brugge WR. Ethanol lavage of pancreatic cystic lesions: initial pilot study. *Gastrointest Endosc* 2005; **61**: 746-752 [PMID: 15855986 DOI: 10.1016/S0016-5107(05)00320-2]

18 **DeWitt J**, McGreevy K, Schmidt CM, Brugge WR. EUS-guided ethanol versus saline solution lavage for pancreatic cysts: a randomized, double-blind study. *Gastrointest Endosc* 2009; **70**: 710-723 [PMID: 19577745 DOI: 10.1016/j.gie.2009.03.1173]

19 **DeWitt J**, DiMaio CJ, Brugge WR. Long-term follow-up of pancreatic cysts that resolve radiologically after EUS-guided ethanol ablation. *Gastrointest Endosc* 2010; **72**: 862-866 [PMID: 20883866 DOI: 10.1016/j.gie.2010.02.039]

20 **Oh HC**, Seo DW, Lee TY, Kim JY, Lee SS, Lee SK, Kim MH. New treatment for cystic tumors of the pancreas: EUS-guided ethanol lavage with paclitaxel injection. *Gastrointest Endosc* 2008; **67**: 636-642 [PMID: 18262182 DOI: 10.1016/j.gie.2007.09.038]

21 **Oh HC**, Seo DW, Kim SC, Yu E, Kim K, Moon SH, Park do H, Lee SS, Lee SK, Kim MH. Septated cystic tumors of the pancreas: is it possible to treat them by endoscopic ultrasonography-guided intervention? *Scand J Gastroenterol* 2009; **44**: 242-247 [PMID: 18949629 DOI: 10.1080/00365520802495537]

22 **Oh HC**, Seo DW, Song TJ, Moon SH, Park do H, Soo Lee S, Lee SK, Kim MH, Kim J. Endoscopic ultrasonography-guided ethanol lavage with paclitaxel injection treats patients with pancreatic cysts. *Gastroenterology* 2011; **140**: 172-179 [PMID: 20950614 DOI: 10.1053/j.gastro.2010.10.001]

23 **DiMaio CJ**, DeWitt JM, Brugge WR. Ablation of pancreatic cystic lesions: the use of multiple endoscopic ultrasound-guided ethanol lavage sessions. *Pancreas* 2011; **40**: 664-668 [PMID: 21562447 DOI: 10.1097/MPA.0b013e3182128d06]

24 **Yao JC**, Eisner MP, Leary C, Dagohoy C, Phan A, Rashid A, Hassan M, Evans DB. Population-based study of islet cell carcinoma. *Ann Surg Oncol* 2007; **14**: 3492-3500 [PMID: 17896148 DOI: 10.1245/s10434-007-9566-6]

25 **Jürgensen C**, Schuppan D, Neser F, Ernstberger J, Junghans U, Stölzel U. EUS-guided alcohol ablation of an insulinoma. *Gastrointest Endosc* 2006; **63**: 1059-1062 [PMID: 16733126 DOI: 10.1016/j.gie.2005.10.034]

26 **Muscatiello N**, Salcuni A, Macarini L, Cignarelli M, Prencipe S, di Maso M, Castriota M, D'Agnessa V, Ierardi E. Treatment of a pancreatic endocrine tumor by ethanol injection guided by endoscopic ultrasound. *Endoscopy* 2008; **40** Suppl 2: E258-E259 [PMID: 19090457 DOI: 10.1055/s-2007-966962]

27 **Muscatiello N**, Nacchiero M, Della Valle N, Di Terlizzi F, Verderosa G, Salcuni A, Macarini L, Cignarelli M, Castriota M, D'Agnessa V, Ierardi E. Treatment of a pancreatic endocrine tumor by ethanol injection (PEI) guided by endoscopic ultrasound. *Endoscopy* 2008; **40** Suppl 2: E83 [PMID: 18633893 DOI: 10.1055/s-2007-995540]

28 **Deprez PH**, Claessens A, Borbath I, Gigot JF, Maiter D. Successful endoscopic ultrasound-guided ethanol ablation of a sporadic insulinoma. *Acta Gastroenterol Belg* 2008; **71**: 333-337 [PMID: 19198582]

29 **Vleggaar FP**, Bij de Vaate EA, Valk GD, Leguit RJ, Siersema PD. Endoscopic ultrasound-guided ethanol ablation of a symptomatic sporadic insulinoma. *Endoscopy* 2011; **43** Suppl 2 UCTN: E328-E329 [PMID: 22020710 DOI: 10.1055/s-0030-1256775]

30 **Levy MJ**, Thompson GB, Topazian MD, Callstrom MR, Grant CS, Vella A. US-guided ethanol ablation of insulinomas: a new treatment option. *Gastrointest Endosc* 2012; **75**: 200-206 [PMID: 22078104 DOI: 10.1016/j.gie.2011.09.019]

31 **Moore JC**, Adler DG. Celiac plexus neurolysis for pain relief in pancreatic cancer. *J Support Oncol* 2009; **7**: 83-87, 90 [PMID: 19507453]

32 **Yan BM**, Myers RP. Neurolytic celiac plexus block for pain control in unresectable pancreatic cancer. *Am J Gastroenterol* 2007; **102**: 430-438 [PMID: 17100960 DOI: 10.1111/j.1572-0241.2006.00967.x]

33 **Wyse JM**, Carone M, Paquin SC, Usatii M, Sahai AV. Randomized, double-blind, controlled trial of early endoscopic ultrasound-guided celiac plexus neurolysis to prevent pain progression in patients with newly diagnosed, painful, inoperable pancreatic cancer. *J Clin Oncol* 2011; **29**: 3541-3546 [PMID: 21844506 DOI: 10.1200/JCO.2010.32.2750]

34 **O'Toole TM**, Schmulewitz N. Complication rates of EUS-guided celiac plexus blockade and neurolysis: results of a large case series. *Endoscopy* 2009; **41**: 593-597 [PMID: 19588286 DOI: 10.1055/s-0029-1214868]

35 **Eisenberg E**, Carr DB, Chalmers TC. Neurolytic celiac plexus block for treatment of cancer pain: a meta-analysis. *Anesth Analg* 1995; **80**: 290-295 [PMID: 7818115]

36 **Wiersema MJ**, Wiersema LM. Endosonography-guided celiac plexus neurolysis. *Gastrointest Endosc* 1996; **44**: 656-662 [PMID: 8979053 DOI: 10.1016/S0016-5107(96)70047-0]

37 **Gunaratnam NT**, Sarma AV, Norton ID, Wiersema MJ. A prospective study of EUS-guided celiac plexus neurolysis for pancreatic cancer pain. *Gastrointest Endosc* 2001; **54**: 316-324 [PMID: 11522971 DOI: 10.1067/mge.2001.117515]

38 **Kaufman M**, Singh G, Das S, Concha-Parra R, Erber J, Micames C, Gress F. Efficacy of endoscopic ultrasound-guided celiac plexus block and celiac plexus neurolysis for managing abdominal pain associated with chronic pancreatitis and pancreatic cancer. *J Clin Gastroenterol* 2010; **44**: 127-134 [PMID: 19826273 DOI: 10.1097/MCG.0b013e3181bb854d]

39 **Levy MJ**, Topazian MD, Wiersema MJ, Clain JE, Rajan E, Wang KK, de la Mora JG, Gleeson FC, Pearson RK, Pelaez MC, Petersen BT, Vege SS, Chari ST. Initial evaluation of the efficacy and safety of endoscopic ultrasound-guided direct Ganglia neurolysis and block. *Am J Gastroenterol* 2008; **103**: 98-103 [PMID: 17970834 DOI: 10.1111/j.1572-0241.2007.01607.x]

40 **Puli SR**, Reddy JB, Bechtold ML, Antillon MR, Brugge WR. EUS-guided celiac plexus neurolysis for pain due to chronic pancreatitis or pancreatic cancer pain: a meta-analysis and systematic review. *Dig Dis Sci* 2009; **54**: 2330-2337 [PMID: 19137428 DOI: 10.1007/s10620-008-0651-x]

41 **Sahai AV**, Lemelin V, Lam E, Paquin SC. Central vs. bilateral endoscopic ultrasound-guided celiac plexus block or neurolysis: a comparative study of short-term effectiveness. *Am J Gastroenterol* 2009; **104**: 326-329 [PMID: 19174816 DOI: 10.1038/ajg.2008.64]

42 **Günter E**, Lingenfelser T, Eitelbach F, Müller H, Ell C. EUS-guided ethanol injection for treatment of a GI stromal tumor. *Gastrointest Endosc* 2003; **57**: 113-115 [PMID: 12518147 DOI: 10.1067/mge.2003.39]

43 **Barclay RL**, Perez-Miranda M, Giovannini M. EUS-guided treatment of a solid hepatic metastasis. *Gastrointest Endosc* 2002; **55**: 266-270 [PMID: 11818938 DOI: 10.1067/mge.2002.120784]

44 **Hu YH**, Tuo XP, Jin ZD, Liu Y, Guo Y, Luo L. Endoscopic ultrasound (EUS)-guided ethanol injection in hepatic metastatic carcinoma: a case report. *Endoscopy* 2010; **42** Suppl 2: E256-E257 [PMID: 20931470 DOI: 10.1055/s-0030-1255653]

45 **Artifon EL**, Lucon AM, Sakai P, Gerhardt R, Srougi M, Takagaki T, Ishioka S, Bhutani MS. EUS-guided alcohol ablation of left adrenal metastasis from non-small-cell lung carcinoma. *Gastrointest Endosc* 2007; **66**: 1201-1205 [PMID: 18061721 DOI: 10.1016/j.gie.2007.05.051]

46 **DeWitt J**, Mohamadnejad M. EUS-guided alcohol ablation of metastatic pelvic lymph nodes after endoscopic resection of polypoid rectal cancer: the need for long-term surveillance. *Gastrointest Endosc* 2011; **74**: 446-447 [PMID: 21481867 DOI: 10.1016/j.gie.2011.01.064]

P- Reviewers Nakajima N, Oh HC, ReshetnyakVI, Tanno S
S- Editor Zhai HH **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3404

World J Gastroenterol 2013 June 14; 19(22): 3404-3414
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Fluctuations in butyrate-producing bacteria in ulcerative colitis patients of North India

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Supported by Council of Scientific and Industrial Research, New Delhi; Government of India
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Received: November 24, 2012 Revised: January 1, 2013
Accepted: February 2, 2013
Published online: June 14, 2013

Abstract

AIM: To study the interplay between butyrate concentration and butyrate-producing bacteria in fecal samples of ulcerative colitis (UC) patients *vs* control individuals.

METHODS: Fecal samples were collected from 14 control individuals (hemorrhoid patients only) and 26 UC patients (severe: n = 12, moderate: n = 6, remission: n = 8), recruited by the gastroenterologist at the Department of Gastroenterology, All India Institute of Medical Sciences, New Delhi, India. Disease activity in UC patients was determined by clinical colitis activity index. We employed fluorescent *in situ* hybridization in combination with flow cytometry to enumerate the clostridium cluster population targeted by *16S rRNA* gene probe. Major butyrate-producing species within this cluster were quantified to see if any change existed in control *vs* UC patients with different disease activity. This observed change was further validated by quantitative polymerase chain reaction. In addition to this, we carried out gas chromatography to evaluate the changes in concentration of major short chain fatty acids (SCFAs), namely acetate, *n*-butyrate, *iso*-butyrate, in the above samples. Student t test and Graph pad prism-6 were used to compare the data statistically.

RESULTS: There was a significant decrease of *Clostridium coccoides* (control, 25.69% ± 1.62% *vs* severe, 9.8% ± 2.4%, P = 0.0001) and *Clostridium leptum* clusters (control, 13.74% ± 1.05% *vs* severe, 6.2% ± 1.8%, P = 0.0001) in fecal samples of UC patients. Furthermore, we demonstrated that some butyrate-producing members of the clostridial cluster, like *Fecalibacterium prausnitzii* (control, 11.66% ± 1.55% *vs* severe, 6.01% ± 1.6%, P = 0.0001) and *Roseburia intestinalis* (control, 14.48% ± 1.52% *vs* severe, 9% ± 1.83%, P = 0.02) were differentially present in patients with different disease activity. In addition, we also demonstrated decreased concentrations of fecal SCFAs, especially of *n*-butyrate (control, 24.32 ± 1.86 mmol/μL *vs* severe, 12.74 ± 2.75 mmol/μL, P = 0.003), *iso*-butyrate (control, 1.70 ± 0.41 mmol/μL *vs* severe, 0.68 ± 0.24 mmol/μL, P = 0.0441) and acetate (control, 39.51 ± 1.76 mmol/μL *vs* severe, 32.12 ± 2.95 mmol/μL, P = 0.047), in the fecal samples of UC patients. The observed decrease of predominant butyrate producers of clostridial clusters correlated with the reduced SCFA levels in active UC patients. This was further confirmed by the restoration in the population of some butyrate producers with simultaneous increase in the level of SCFA in remission samples.

CONCLUSION: Our observations indicate that decreases in members of the clostridial cluster resulting in reduced butyrate levels contribute to the etiology of UC.

Key words: Fecal microbiota; Ulcerative colitis; Short chain fatty acids; Clostridial cluster; Fluorescent *in situ* hybridization-flow cytometry; Quantitative polymerase chain reaction

Kumari R, Ahuja V, Paul J. Fluctuations in butyrate-producing bacteria in ulcerative colitis patients of North India. *World J Gastroenterol* 2013; 19(22): 3404-3414 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3404.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3404

INTRODUCTION

Inflammatory bowel disease (IBD), comprising of Crohn's disease and ulcerative colitis (UC), is a class of chronic inflammatory disorders of the intestine. An increasing trend in the incidence and prevalence of IBD in the Asian population has been recognized for the past two decades[1]. The dynamic balance between commensal microbiota and host defensive responses at the mucosal frontier has a pivotal role in the initiation and pathogenesis of chronic IBD[2]. Whether the exaggerated immune response is exerted to all commensal bacteria, or to a subset or a single strain of bacteria, is not known[3]. Differences in fecal microbiota of healthy subjects and IBD patients have been enumerated using different techniques[4-6]. Impaired cellular metabolism, such as butyrate oxidation and short chain fatty acid (SCFA) fermentation, has shown strong association with altered gut microbiota in UC patients[7,8].

SCFAs, such as acetate, propionate and butyrate, are produced by intestinal microbial fermentation of mainly undigested dietary carbohydrates, specifically resistant starches and dietary fiber, but also in a minor part generated by dietary and endogenous proteins in the intestine[9]. SCFAs are important for normal intestinal biology[10]. They also stimulate colonic sodium and fluid absorption and exert proliferative effects on the colonocytes[11]. Therefore, monitoring the fluctuations in SCFA concentration may help in understanding the relation of dysbiosis with UC.

Members of *Clostridium leptum* (*C. leptum*) and *Clostridium coccoides* (*C. coccoides*) groups together constitute the majority of Firmicutes (low G + C content bacteria)[12], producing large amounts of butyrate that function as an energy source for colonic epithelial cells and inhibit mRNA expression of proinflammatory cytokines in the mucosa by inhibiting nuclear factor-κB (NF-κB) activation[13]. Butyrate has been reported to help in prevention of colorectal cancer[14]. Evidence indicates that bacteria related to *Eubacterium hallii* (*E. hallii*), *Roseburia* species and *Eubacterium rectale* (*E. rectale*) within cluster XIVa and *Faecalibacterium prausnitzii* (*F. prausnitzii*)-related bacteria within cluster IV are normally the two most abundant groups of human fecal bacteria that produce butyrate[15]. These species-level probes account for a mean of 7.7% of the population of the total human fecal microbiota[16]. However, both the clostridial clusters harbor a diverse collection of several species of butyrate producers and non-butyrate producers as well[17].

Dynamics of predominant butyrate producers at the species level during disease activity and their correlation with the fluctuation in SCFA have not been established clearly. Here we explored the alteration in population of dominant butyrate-producing bacterial species present in fecal samples of UC patients at different disease stages. Abundance of the butyrate-producing clostridial cluster group was estimated by fluorescent *in situ* hybridization (FISH) in combination with flow cytometry and real time polymerase chain reaction (PCR). Further enumeration was carried out for known predominant members of butyrate-producing bacteria such as (1) *F. prausnitzii* as a member of the *C. leptum* group; and (2) *E. hallii* and *Roseburia intestinalis* (*R. intestinalis*) as members of the *C. coccoides* group. We also checked the level of butyrate, *iso*-butyrate and acetate in fecal samples to see if any correlation exists with butyrate-producing bacteria during different disease conditions.

MATERIALS AND METHODS

Fecal sample collection and processing

Disease activity in UC patients was determined by simple clinical colitis activity index[18] and the patients with total index score of 7-10, > 10 and 0-2 were assigned moderate, severe and remission category, respectively[19]. The patients were recruited by the gastroenterologist at the Department of Gastroenterology of the All India Institute of Medical Sciences, New Delhi, India. Clinical and demographic features of UC patients and controls are shown in Table 1. Patients who had hemorrhoids only and showed no evidence of small and large intestinal disease were enrolled as control individuals in this study. No study patient had received any antibiotic treatment in the past three months before sample collection. Patients under any antibiotic or probiotic treatments were excluded from this study. The fecal samples were collected in sterile stool specimen containers and stored at -80 ℃ within 3 h of sample collection until further processing.

Probes and oligonucleotides

All probes were designed from the *16S rRNA* gene. EUB 338 conserved within the bacterial domain was used as a positive control probe[20]; conversely NONEUB338 (reverse of EUB)[21] was used as a negative control probe. The positive control probe was double labeled with fluorescein isothiocyanate (FITC) at both the 5' and 3' end[22], whereas the negative control probe was labeled with FITC at the 5' end and Cy5 at the 3' end. We used two group-specific probes labeled with Cy5 at their 5' end (Sigma, India) and three species-specific probes labeled with FITC at their 5' end (Sigma, India). Two competitors (unlabeled) and 5 helper oligonucleotides (unlabeled) were used to increase the accessibility of the Clep 1156 probe[23] (Table 2).

Analysis of fecal samples by FISH-flow cytometry

About 1 g of fecal sample was suspended in 9 mL of phosphate buffered saline (PBS) and vortexed with 10-15 glass beads for 5 min to homogenize the sample. This suspension was centrifuged at 1500 rpm for 1 min

Table 1 Clinical and demographic features of ulcerative colitis patients and controls *n* (%)

Feature	UC (*n* = 26)	Control (*n* = 14)
Sex (F/M)	11 (42.30)/15 (57.69)	7 (50)/7(50)
Age at diagnosis (yr)		
mean ± SD	38.35 ± 11.49	35.00 ± 14.14
15-40	18 (69.23)	8 (57.14)
> 40	8 (30.76)	6 (42.85)
Disease behavior		
Severe	12 (46.15)	-
Moderate	6 (23.07)	-
Remission	8 (30.76)	-
Disease extent		
Proctitis	4 (15.38)	-
Left sided colitis	6 (23.00)	-
Pancolitis	6 (23.00)	-
None of the above	10 (38.46)	-
Smoking history		
Yes	3 (11.53)	2 (14.28)
No	23 (88.46)	12 (85.70)
Treatment history		
Immunosuppressant	16 (61.53)	-
Steroids	14 (53.84)	-
Appendectomy Y/N	4 (15.38)/22 (84.61)	0/14
Family history Y/N	2 (7.69)/24 (92.30)	0/14

UC: Ulcerative colitis; F: Female; M: Male; Y: Yes; N: No.

to pellet down the debris and the supernatant was collected. To fix the cells, 1 mL of this supernatant was incubated with 4% paraformaldehyde (1:3 ratio) at 4 ℃, overnight. The fixed cells were washed twice with PBS and incubated in ethanol-PBS solution (1:1 ratio) at -20 ℃ for 2 h. For each hybridization reaction, 60 μL of fixed cells were used. The fixed cells were washed twice with PBS and resuspended in 50 μL hybridization buffer (900 mmol/L NaCl, 20 mmol/L Tris-HCl of pH 8, and 0.01% sodium dodecyl sulfate at pH 7.2). All hybridizations were performed in the dark at 50 ℃ for 16 h in the hybridization solution containing 4 ng/μL of the appropriate labeled probe. One hundred and fifty microliter of hybridization solution (without probe) was added to stop the reaction and cells were pelleted at 1610 *g* for 10 min. Hybridized cells were further resuspended in prewarmed washing buffer [65 mmol/L NaCl, 20 mmol/L Tris-HCl, 5 mmol/L diaminoethanetetraacetic acid (EDTA), 0.01% sodium dodecyl sulfate, pH 7.2] and incubated at 50 ℃ for 20 min to remove non-specific binding of the probe. Finally, cells were pelleted down at 6000 rpm for 10 min and suspended in 200 μL PBS[5,24,25]. An aliquot of 100 μL was added to 0.5 mL of flow sheath solution (Becton Dickinson) for flow cytometry analysis.

Data acquisition by flow cytometry

Data acquisition was performed with a FACS calibur flow cytometer (Becton Dickinson) which is equipped with an air-cooled argon-ion laser providing 15 mW at 488 nm light combined with a 635 nm red-diode laser. The 488 nm laser was used to measure the forward angle light scatter (FSC, in the 488/10 nm band pass filter), the side angle light scatter (SSC, in the 488/10 nm band pass) and the green fluorescence intensity conferred by FITC labeled probes (filter 1 in the 530/30 nm band pass filter). The red-diode laser was used to detect the red fluorescence conferred by Cy5 labeled probes (filter 4 in a 661/16 nm band pass filter). The acquisition threshold was set in the side scatter channel. All the parameters were collected as logarithmic signals. The rate of events in the flow was set at low (12 μL/s). A total of 25000 events were collected and subsequent analyses were conducted using the Cell Quest Software (Becton Dickinson).

Enumeration of bacterial groups in fecal samples

Enumeration of bacterial groups was performed by a double staining method in the same reaction tube where the hybridization of the EUB338 probe labeled with FITC and the genus-specific probe labeled with Cy5 were combined. This led us to estimate the abundance of bacterial groups targeted by the respective Cy5-labeled probe as a proportion of total bacteria labeled with the EUB338 FITC probe. Next, the abundance of known butyrate producers was enumerated as cells hybridized with the FITC-labeled species-specific probe as a proportion of total cells hybridized with the respective genus-specific Cy5-labeled probe. Each time, the proportion of hybridized bacteria was corrected by subtracting the background fluorescence obtained with hybridization of the negative control probe NONEUB338.

Quantitative polymerase chain reaction

Genomic DNA from human fecal samples (220 mg) was extracted using the Qiagen stool DNA kit and eluted in 50 μL of Tris-EDTA buffer. About 20 ng of DNA from each sample was used to analyze the bacterial population. All primer sets used in the study were designed from the *16S rRNA* gene as shown in Table 2. Genus-specific primers were used to amplify respective genus and species from genomic DNA of the fecal samples of healthy individuals. The amplified product was cloned and sequenced and sequences were deposited in the EMBL database to obtain the accession numbers (Table 3). These *16S rRNA* gene fragments containing plasmids were used as reference strains. The standard curves were constructed by serial dilutions of each reference clone prepared from 0.05 to 500000 pg/tube, corresponding to 1 × 10 to 1 × 10^7 copy numbers. The standard curve of the reference clones was used to extrapolate the numbers of bacteria present in the fecal samples. With the molecular mass of the plasmid and insert known, the copy number was calculated as follows: mass in Daltons (g/molecule) = [size of double-stranded (ds) product in base pairs (bp)] (330 Da × 2 nucleotides (nt)/bp]/Avogadro's number.

Thus, the precise number of molecules (molecules/μL) = Conc./mass in Daltons[26].

Gas chromatography analysis of fecal SCFA

Fecal SCFAs were analyzed using gas chromatography/flame ionization detection (GC-FID). An aliquot of fecal

Table 2 Probes and oligonucleotides employed in the study

Primer or probe	Target (phylogenetic group)	Sequence (5′-3′) from *16S rRNA* gene	Used in FISH-flow cytometry or qPCR	Ref.
NON338	No bacteria	ACATCCTACGGGAGGC	Probe[1]	[21]
EUB338	Most bacteria	GCTGCCTCCCGTAGGAGT	Probe[1]	[20]
Erec 482	*C. coccoides*/*E.rectale* cluster	GCTTCTTAGTCARGTACCG	Probe[1]	[34]
Clep 1156	*Clostridium leptum* subgroup	GTTTTRTCAACGGCAGTC	Probe[1]	[35]
Fpra-655	*F. prausnitzii*	CGCCTACCTCTGCACTAC	Probe[1]	[16]
Rint-623	*R. intestinalis* subcluster	TTCCAATGCAGTACCGGG	Probe[1]	[16]
Ehal-057	*E. halliii* L2-7/*E. halliii*	TTGCACTGCCACCTACGC	Probe[1]	[16]
Cp1	Competitor 1	GRTTTRTCAYCGGCAGTC	Competitor[1]	[23]
Cp2	Competitor 1	GTVTTRTCBACGGCAGTC	Competitor[1]	[23]
H1174	Helper oligonucleotide	TTGACGTCRTCCCCACCTTCCTCC	Helper[1]	[23]
H1129	Helper oligonucleotide	TAGAGTGMTCTTGCGTA	Helper[1]	[23]
H1090	Helper oligonucleotide	GGTTGCGCTCGTTGCGGGACTTAA	Helper[1]	[23]
H750	Helper oligonucleotide	TCGHGCCTCAGCGTCAG	Helper[1]	[23]
H122	Helper oligonucleotide	GAAGGCAGGTTACTCACGC	Helper[1]	[23]
Clep FP	*C. leptum* subgroup	CGTCAGCTCGTGTCGTGAGAT	Primer set[2]	[36]
Clep RP		CGTCATCCCCACCTTCCTCC		
C.cocci FP	*C. coccoides* subgroup	GCCACATTGGGACTGAGA	Primer set[2]	[36]
C.cocci RP		GCTTCTTAGTCAGGTACCG		
Fpraus FP	*F. prausnitzii*	GATGGCCTCGCGTCCGATTAG	Primer set[2]	[37]
Fpraus RP		CCGAAGACCTTCTTCCTC		
Rint FP	*Roseburia*/*E. rectale* cluster	CKGCAAGTCTGATGTGAAAG	Primer set[2]	This study
Rint RP		GCGGGTCCCCGTCAATTCC		
Ehal FP	*E. hallii* L2-7/*E. hallii* members	GCGTAGGTGGCAGTGCAA	Primer set[2]	[38]
Ehal RP		GCACCGRAGCCTATACGG		

[1]Respective probe or oligonucleotide was used in fluorescent *in situ* hybridization (FISH)-flow cytometry; [2]Respective primer was used in quantitative polymerase chain reaction (qPCR). FP: Forward primer; RP: Reverse primer; *R. intestinalis*: *Roseburia intestinalis*; *E. hallii*: *Eubacterium hallii*; *F. prausnitzii*: *Fecalibacterium prausnitzii*; *C. coccoides*: *Clostridium coccoides*; *C. leptum*: *Clostridium leptum*; *E. rectale*: *Eubacterium rectale*.

Table 3 Accession number of reference strain used in the study

Bacteria	Source	Accession No.
C. leptum	Healthy human fecal sample	AM042697
F. prausnitzii	Healthy human fecal sample	JX556686
R. intestinalis	Healthy human fecal sample	JX556688
E. hallii	Healthy human fecal sample	JX556687

C. leptum: *Clostridium leptum*; *F. prausnitzii*: *Fecalibacterium prausnitzii*; *R. intestinalis*: *Roseburia intestinalis*; *E. hallii*: *Eubacterium hallii*.

content (250 mg) was extracted with 1 mL of extraction buffer [0.1% (w/v) $HgCl_2$ and 1% (v/v) H_3PO_4] supplemented with 0.045 mg/mL 2,2-dimethylbutyrate (as internal standard). The resulting slurry was centrifuged for 30 min at 5000 *g* at 4 ℃, and the supernatant was filtered through a 0.2-μm filter. SCFAs in the supernatant collected were analyzed using a GC (Shimadzu-2010) equipped with FID and a stabilwax column (Restek, United States) of 30 m length, 530 μm diameter and 1 μm film thickness. The system was run with nitrogen as carrier gas at an inlet constant pressure of 18.1 kPa. Samples were run at an initial temperature of 120 ℃ for 0.5 min, and then with 8 ℃/min change in temperature till it reached 220 ℃ and was held at 220 ℃ for 8 min for a total program time of 20.5 min[27]. SCFAs were identified using external standards consisting of acetate, *iso*-butyrate, *n*-butyrate (Sigma, India) and the concentration was calculated using the area percentage method.

Ethics statement

Ethical clearance for the study was obtained from the Institute Ethics Committee, All India Institute of Medical Sciences, New Delhi. Written informed consent was obtained from all the participants.

Statistical analysis

The mean cell proportion and number of bacteria in fecal samples were estimated by FISH and qPCR in triplicate, and the results were expressed as a percentage of bacteria and number of bacteria, respectively. SCFA level was determined by GC, and the results were expressed in mmol/μL. Student's *t* test was employed to check any significant changes in the SCFA concentrations with the changes in the disease activity. Graphpad prism-6 was used to analyze FISH and qPCR data.

RESULTS

Analysis of *C. leptum* and *C. coccoides* groups

FISH-flow cytometry: In Figure 1A, the region R1 corresponds to relative size (FSC) with granularity (SSC) of the bacteria during flow cytometry with NONEUB338 hybridized cells. This region R1 was gated for further dot plots. Flow cytometric analysis of the hybridized samples gave a shift in signal of 1 log unit compared to the nonhybridized cells, enabling the specific detection and enumeration of the different bacterial groups (Figure 1B-D). Scoring of bacteria could not be achieved uni-

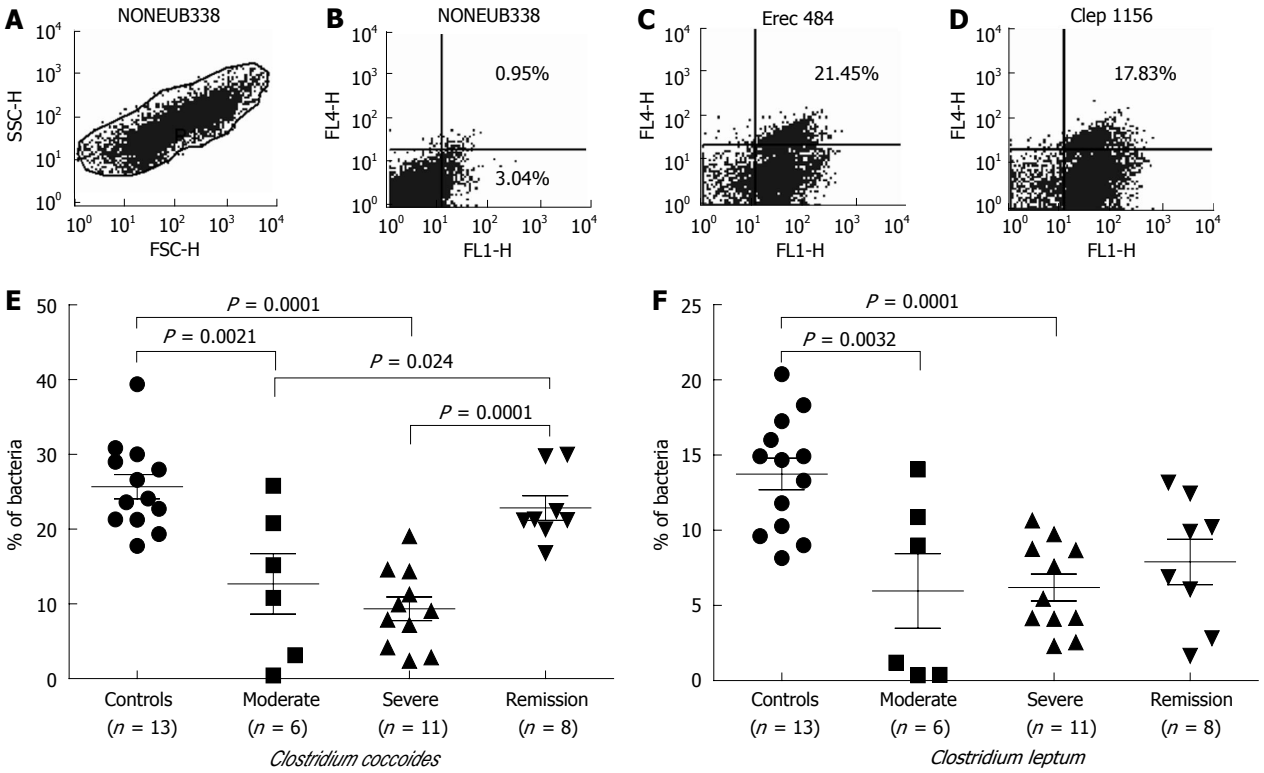

Figure 1 Flow cytometric analysis of fecal microflora using 16S rRNA targeted probes. A: The region R1 corresponding to relative size (the forward angle light scatter)/granularity (the side angle light scatter) of the bacteria was delineated. This region R1 was gated for further dot plots; B: Bacteria from fecal samples were hybridized with NONEUB338 probe; C: EUB338 and Erec 482; D: EUB338 and Clep 1156. A shift in fluorescence to higher intensities was obtained upon hybridization with positive control or group-specific probe: right lower and upper quadrant compared to the lower left quadrant. The signal in lower left quadrant represents debris. The events in upper right quadrant represent the proportion of bacterial cells hybridized with the group-specific probe within the total bacterial cells hybridized with the universal bacterial probe EUB338-fluorescein isothiocyanate (FITC). The enumeration of targeted cells was corrected by subtracting the background fluorescence, which was measured using the negative control NONEUB338 probe. Fluorescent *in situ* hybridization (FISH)-flow cytometry data were expressed as the mean % group-specific probe/EUB338 ± SE as enumerated by FISH-flow cytometry in control, moderate, severe and remission samples of ulcerative colitis; E: Erec 482; F: Clep 1156.

formly; therefore, sample size differed in each category.

In UC patients belonging to the moderate and severe disease categories, we observed significant decreases in members of the *C. coccoides* and *C. leptum* groups compared to control individuals (Figure 1E and F). Among the *C. coccoides* cluster, decreases in moderate disease samples attained the *P* value of 0.0021, while there was a *P* value of 0.0001 in the case of severe patient samples in comparison to controls. However, in the case of *C. leptum,* the *P* values were 0.0032 in moderate and 0.0001 in severe categories of samples, respectively, as compared to controls. Samples in the remission stage showed significant restoration in the population of the *C. coccoides* group ($P = 0.0001$); although an increasing trend was observed in the members of the *C. leptum* group, this did not attain a significant value.

Quantitative polymerase chain reaction: In order to validate our FISH-flow cytometry data, we carried out a qPCR study (Figure 2A and B), where we observed significant decreases in both the members of *C. coccoides group* ($P = 0.027$) and *C. leptum* group ($P = 0.041$) in samples of moderate and severe disease stages. Members of both the clusters showed restoration of bacteria in samples of remission category; however, this did not attain a significant *P* value.

SCFA quantification by GC: We further quantified the change in concentration of fecal SCFAs, namely acetate, *n*-butyrate and *iso*-butyrate, in control *vs* UC patient samples by GC. The concentrations of butyrate ($P = 0.003$), *iso*-butyrate ($P = 0.044$) and acetate ($P = 0.047$), were significantly reduced in severe UC samples when compared with control samples (Figure 3). As expected, during the remission stage *n*-butyrate level significantly restored back to normal level ($P = 0.05$) as seen in control individuals, confirming that the decrease observed during disease conditions reflects the loss of butyrate-producing bacteria (Figure 3C).

Evaluation of predominant butyrate producers

FISH-flow cytometry: Next, we evaluated the concentration of predominant members of both clostridial clusters (XIVa and IV). The population of *F. prausnitzii,* a member of the *C. leptum* group, was significantly low in UC samples of severe ($P = 0.0001$) category of disease in comparison to control samples (Figure 4A). Samples from the remission stage did not show significant restora-

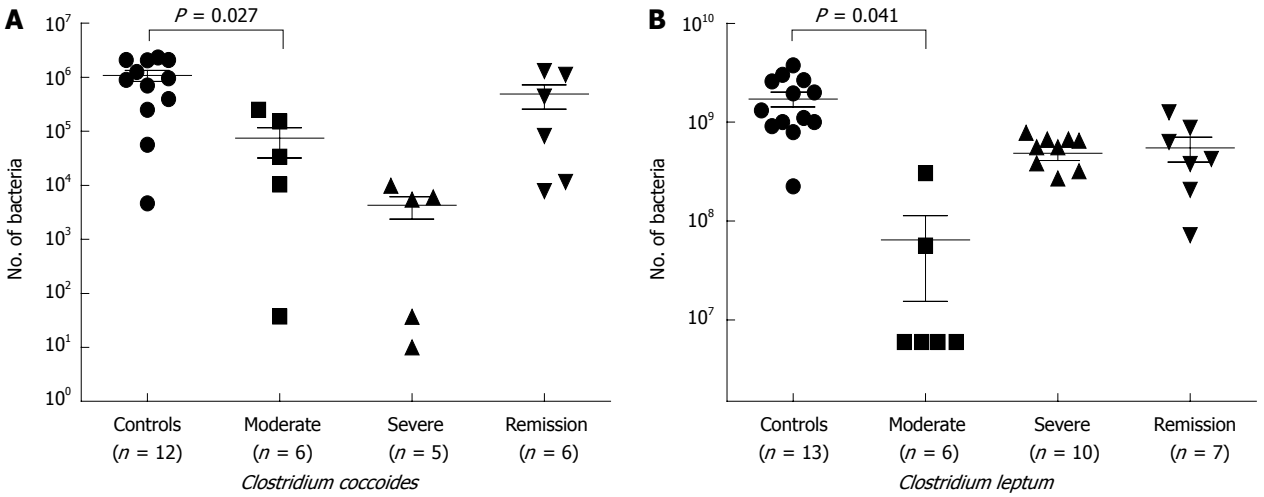

Figure 2 Quantitative polymerase chain reaction data showing the numbers of bacteria (± SE) in fecal samples of control *vs* ulcerative colitis patients. A: *Clostridium coccoides;* B: *Clostridium leptum*. The *Y* axis represents number of bacteria and *X* axis represents the sample category.

tion of *F. prausnitzii* in our FISH-flow cytometry experiment, as was observed for the *C. leptum* cluster during remission (Figures 1F and 2B).

R. intestinalis, a member of the *C. coccoides* group, was also significantly low (P = 0.02) in patient samples of severe category disease (Figure 4B); however, this did not show significant restoration in the samples of remission stage. The population of *E. halii,* another member of the *C. coccoides* group, decreased in disease conditions, but not significantly (Figure 4C). We failed to detect *E. hallii* in the majority of samples either by flow cytometry or qPCR, indicating low abundance of these bacteria in our study population. An increase in the population of *R. intestinalis* was recorded during remission stage in comparison to severe stage of the disease (Figure 4B and C). This was in agreement with our FISH-flow cytometry data of *C. coccoides* group. Abundance of *R. intestinalis, E. hallii* and *F. prausnitzii* was calculated out of total microbiota, as shown in Table 4. We detected a higher representation of *R. intestinalis* compared to *E. hallii* and *F. prausnitzii*. Therefore, we can infer that at the species level, abundance of the members of the *C. coccoides* cluster was higher in comparison to the members of the *C. leptum* cluster.

Quantitative polymerase chain reaction

As expected, the population of *F. prausnitzii* was significantly reduced in the samples of severe category of UC disease (P = 0.045) (Figure 4D) as compared to control. In addition, abundance of the same species was restored at remission stage when compared to severe stage (P = 0.041) (Figure 4D). Similarly, the *R. intestinalis* population showed significant reduction in samples of moderate (P = 0.015) and severe (P = 0.001) stage of the disease in comparison to control (Figure 4E). The recovery in the population of *R. intestinalis* was also seen in the remission category of samples when compared to severe category (P = 0.018) (Figure 4E). However, qPCR analysis in the case of *E. hallii* did not show any significant reduction; in addition, *E. hallii* was undetected in the majority of samples observed by FISH-flow cytometry (Figure 4F).

DISCUSSION

Our study revealed significant reduction in the members of both *C. coccoides* and *C. leptum* groups in fecal samples obtained from the severe disease category of UC patients in comparison to controls. FISH-flow cytometry and qPCR analysis of fecal samples belonging to the above groups supported the observations made by Takaishi *et al*[4] and Sokol *et al*[5].

We quantified the abundance of predominant butyrate-producing species of clostridial clusters to see their association with acetate and butyrate. Since in most cases butyryl-CoA:acetate CoA-transferase rather than butyrate kinase appears to perform the final step in butyrate synthesis, we targeted bacteria possessing butyryl CoA:acetate CoA-transferase for butyrate synthesis, *e.g.*, *F. prausnitzii* and *Roseburia spp*/*E. rectale* which apparently lack butyrate kinase activity[15,28]. It is known that *Roseburia spp.* and *F. prausnitzii* strains contribute in butyrate production and many strains are associated with the net consumption of both acetate and carbohydrate[15]. Our study revealed that both the above species exhibited low abundance in the samples of severe category of UC and tended to restore their population during remission.

F. prausnitzii has been shown to exhibit anti-inflammatory effects on cellular and trinitrobenzenesulphonic acid-induced colitis models, partly due to secreted metabolites that are able to block NF-κB activation and interleukin 8 production[29]. In our observations (Table 4), *F. prausnitzii* accounted for 11.66% ± 1.55% of the *C. leptum* group, and *R. intestinalis* and *E. hallii* accounted for 14.48% ± 1.52% and 5.93% ± 0.54% of the *C. coccoides* group, respectively, in control individuals. In total these three species accounted for 6.83% of the total fecal flora. Previous studies have already reported low counts of *F. prausnitzii* in UC patients[30].

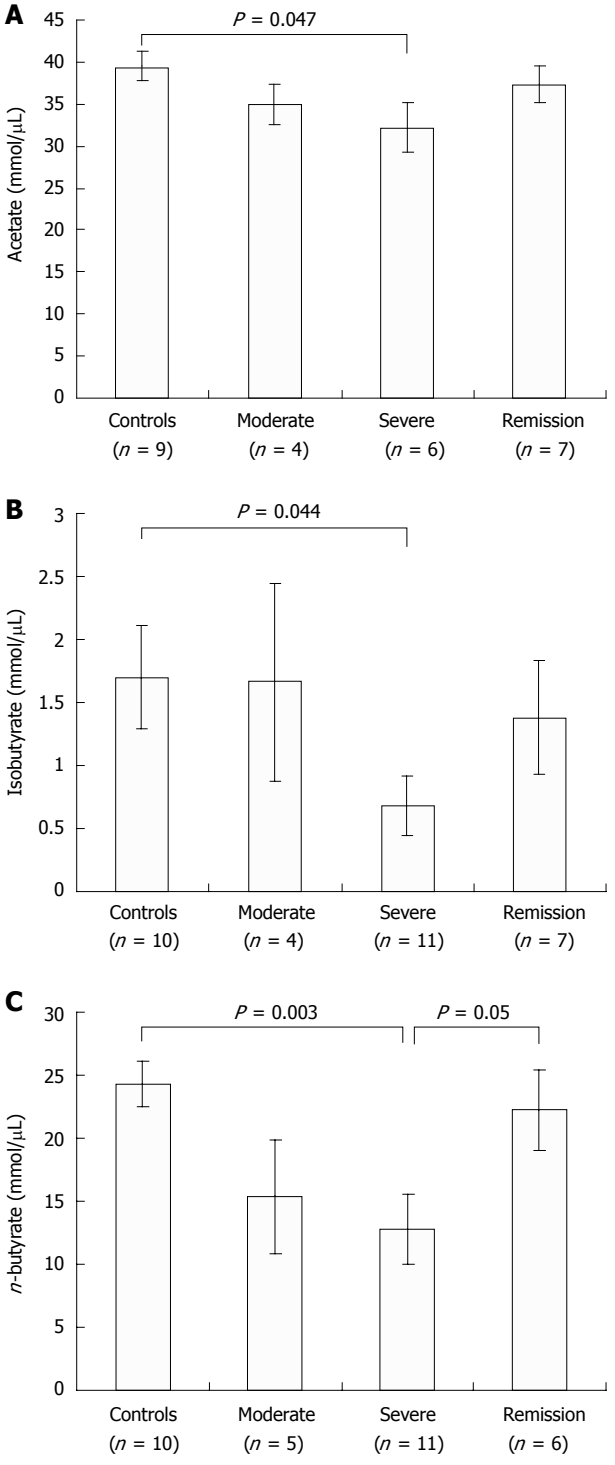

Figure 3 Short chain fatty acids in fecal samples of ulcerative colitis patients *vs* control samples, analyzed by gas chromatography. A: Acetate; B: *Iso*-butyrate; C: Butyrate. The *Y* axis represents concentration of respective short chain fatty acid and *X* axis represents the sample category.

We observed a significant decrease in concentrations of butyrate, *iso*-butyrate and acetate in UC fecal samples compared to controls through SCFA quantification by GC analysis, supporting earlier observations[4,31].

An intriguing link between the level of SCFA and an intracellular energy sensor for the maintenance of intestinal barrier function has been suggested[10]. Our study further substantiates a link between the fluctuations of butyrate production and the changes in the numbers of butyrate producers during UC. Lack of butyrate availability may lead to compromised intestinal barrier function resulting in increased exposure of luminal content to the host immune system, thus exaggerating the immune response[10].

The reduced population of dominant butyrate producers and decreases in concentrations of butyrate and acetate in diseased samples indicate impaired butyrate supply in the colon, which may lead to energy deficiency for colonocytes. The resurgence of butyrate-producing bacteria at the remission stage and simultaneous increases in butyrate concentration, as observed here, show an association with UC and support the energy deficiency hypothesis of IBD[32]. Earlier studies have shown a requirement of acetate by *F. prausnitzii* and *R. intestinalis* for survival and other activities like production of butyrate[15,33]. Thus, major butyrogenic species depend on other bacteria, including net producers of acetate (*e.g.*, amylolytic bifidobacteria) and other bacterial species capable of degrading a variety of complex carbohydrates[15]. These bacteria play an important role in net butyrate production and may be associated with dysbiosis during UC.

Acetate and butyrate are major SCFAs and decreases in their concentration may affect the overall SCFA concentration. SCFAs in the colon maintain mild acidic conditions which boost the formation of butyrate by favoring growth of butyrate-producing bacteria and allowing them to compete against gram-negative bacteria such as *Bacteroides spp* to maintain the homeostasis[9]. Thus, a reduced butyrate level may lead to an increased Gram-negative bacteria population due to reduced competition. This was validated by our observation of increased representation of *Bacteroides* in UC samples (data not shown).

Our data showing reduced abundance of three predominant butyrate-producing species indicate that during UC, butyrate deficiency may not be solely due to reduced uptake of butyrate by the inflamed mucosa as reported by Canani *et al*[9], but also due to reduced abundance of predominant butyrate producers and conversely lower production of butyrate.

The limitation of our findings is that most of the microbial data are not presented in absolute values but in percentages; therefore, it is difficult to critically assess the real changes in the microbial composition. The study is performed in a very restricted number of subjects due to the exclusion criteria followed during sample collection.

In conclusion, our study shows a decreased abundance of predominant butyrate producers like *R. intestinalis* and *F. prausnitzii* belonging to clostridial clusters in the UC disease condition. This decrease was found to correlate with reduced SCFA concentration in UC patients. We thus provide evidence that reduced butyrate levels during the diseased state are due to less abundance of these species in UC. It is evident from our data that decreased levels of these species resulting in reduced butyrate levels may be associated with the etiology of UC.

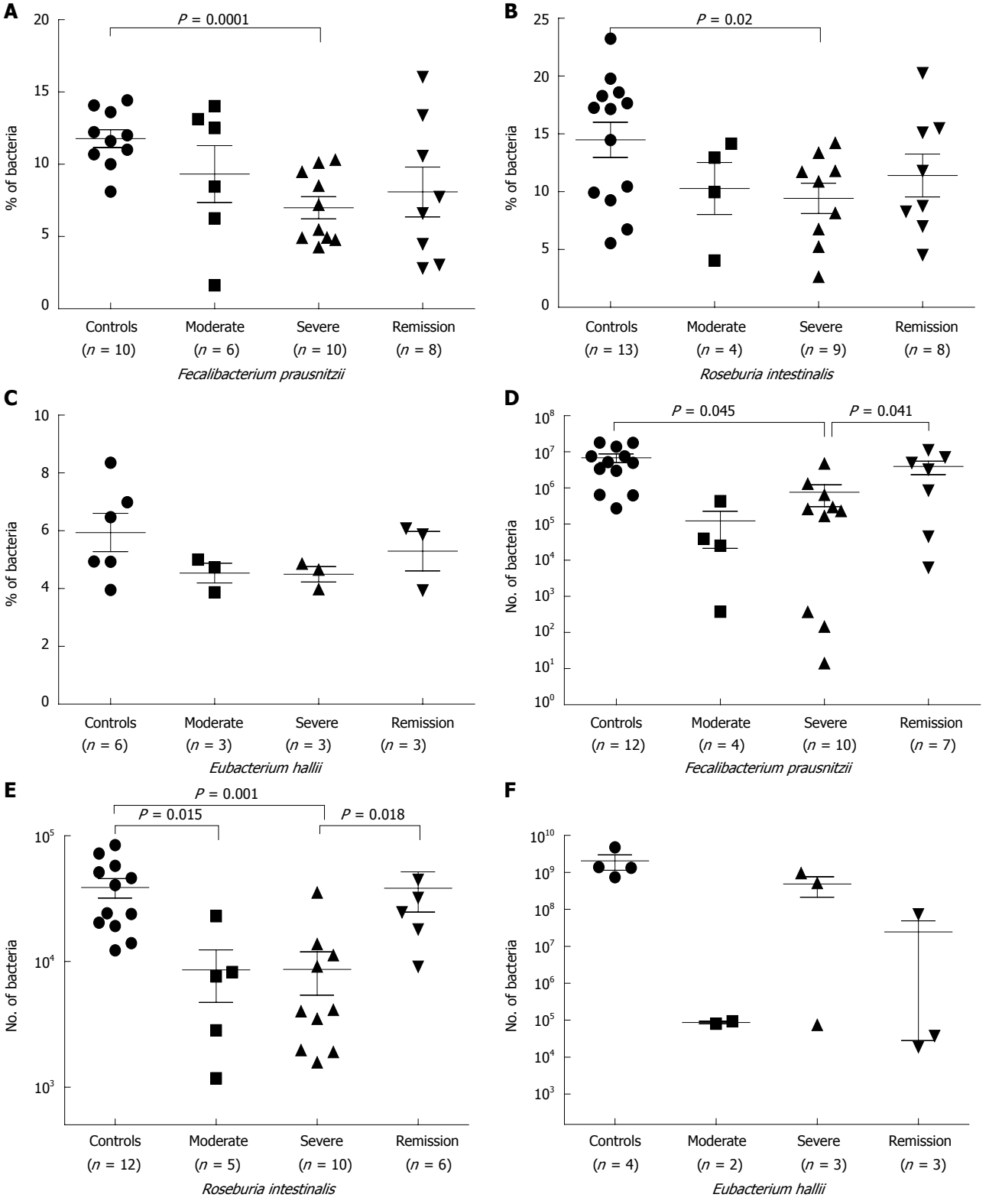

Figure 4 Analysis of predominant butyrate-producing bacteria. A-C: *Fecalibacterium prausnitzii* (A), *Roseburia intestinalis* (B), and *Eubacterium rectale* (C) by fluorescent *in situ* hybridization-flow cytometry; D-F: *Fecalibacterium prausnitzii* (D), *Roseburia intestinalis* (E), and *Eubacterium hallii* (F) by quantitative polymerase chain reaction. The *Y* axis represents number of bacteria and *X* axis represents the status of patients. Horizontal bars with asterisks represent comparison between ulcerative colitis and control conditions.

This was further demonstrated in our analysis of the samples from remission patients, where the targeted organisms tended to revert back to normal level. It would be of interest in the future to extend this approach to studying the diversity of each member of the clostridial cluster, including changes in non-butyrate producers.

Table 4 Enumeration of butyrate producers in fecal samples through fluorescent *in situ* hybridization-flow cytometry (mean ± SE)

Cluster or species	Out of total microbiota	Species	Out of respective cluster
Control fecal samples			
C. coccoides cluster	25.69% ± 1.62%	*R. intestinalis*	14.48% ± 1.52%
		E. hallii	5.93% ± 0.54%
C. leptum cluster	13.74% ± 1.05%	*F. prausnitzii*	11.66% ± 1.55%
R .intestinalis[1]	3.71% ± 0.39%		
E. hallii[1]	1.52% ± 0.13%		
F. prausnitzii[1]	1.60% ± 0.21%		
UC fecal samples (moderate)			
C. coccoides cluster	12.70% ± 4.60%	*R. intestinalis*	10.00% ± 2.25%
		E. hallii	4.03% ± 0.3%
C. leptum cluster	6.40% ± 3.40%	*F. prausnitzii*	7.5% ± 2.3%
R .intestinalis[1]	1.27% ± 0.10%		
E. hallii[1]	0.51% ± 0.01%		
F. prausnitzii[1]	0.48% ± 0.14%		
UC fecal samples (severe)			
C. coccoides cluster	9.80% ± 2.40%	*R. intestinalis*	9% ± 1.83%
		E. hallii	4.2% ± 0.2%
C. leptum cluster	6.20% ± 1.80%	*F. prausnitzii*	6.01% ± 1.6%
R .intestinalis[1]	0.82% ± 0.04%		
E. hallii[1]	0.41% ± 0.01%		
F. prausnitzii[1]	0.37% ± 0.02%		
UC fecal samples (remission)			
C. coccoides cluster	12.99% ± 2.65%	*R. intestinalis*	11.40% ± 1.83%
		E. hallii	5.07% ± 0.6%
C. leptum cluster	7.89% ± 1.50%	*F. prausnitzii*	7.40% ± 2.32%
R .intestinalis[1]	1.48% ± 0.04%		
E. hallii[1]	0.65% ± 0.01%		
F. prausnitzii[1]	0.58% ± 0.03%		

[1]Abundance of *Roseburia intestinalis* (*R. intestinalis*), *Eubacterium hallii* (*E. hallii*) and *Fecalibacterium prausnitzii* (*F. prausnitzii*) were calculated out of total microbiota. *Clostridium coccoides* (*C. coccoides*) and *Clostridium leptum* (*C. leptum*) were enumerated as proportion of total bacterial cell hybridized with group-specific probe. *R. intestinalis, E. hallii* and *F. prausnitzii* were enumerated as proportion of cell hybridized with species-specific probe. UC: Ulcerative colitis.

COMMENTS

Background

Inflammatory bowel disease (IBD), comprising of Crohn's disease and ulcerative colitis (UC), is a class of chronic inflammatory disorders of the intestine and involves impaired barrier function. A rising trend in the incidence and prevalence of inflammatory bowel IBD in the Asian population has been recognized for the past two decades. IBD is considered as an exaggerated immune response, exerted either to all commensal bacteria or to a subset or to a single strain of bacteria.

Research frontiers

Differences in fecal microbiota of healthy subjects and IBD patients have been identified. Commensal gut microbiota have been reported to exert various multiple beneficial effects on host gut epithelium. short chain fatty acids (SCFAs), like butyrate, produced by microbial fermentation of undigested carbohydrates have been depicted to regulate transepithelial transport, colonocyte proliferation and differentiation, mucosal inflammation, intestinal motility and barrier function. The homeostasis between commensal micobiota and host defensive responses at the mucosal level has a pivotal role in the initiation and pathophysiology of chronic IBD. Therefore, monitoring the fluctuations in butyrogenic bacteria and SCFA level may help in understanding the relation of dysbiosis with UC.

Innovations and breakthroughs

This work was carried out to study the fluctuations in butyrate-producing bacteria and simultaneous measurement of butyrate concentration in fecal samples of UC *vs* control individuals belonging to the northern part of India. The data were also correlated with patients differing in disease activity. Decreases in the level of butyrate producers in UC patients coincided with the changes in butyrate concentration during active stage disease. However, the levels reverted back to normal during remission stage showing the role of these bacteria in the disease etiology. Such comparisons have not been made so far to show the above relationship with disease activity.

Applications

It is speculated that by measuring the level of these species and concentration of SCFA in stool samples (a non-invasive method), authors can indicate the status of UC patients.

Terminology

Dysbiosis (also called dysbacteriosis) refers to a condition with microbial imbalances on or within the body. Dysbiosis is most prominent in the digestive tract or on the skin, but can also occur on any exposed surface or mucous membrane such as the vagina, lungs, mouth, nose, sinuses, ears, nails or eyes. It has been associated with different illnesses, such as IBD, as imbalances in the intestinal microbiome may be associated with bowel inflammation and chronic fatigue syndrome.

Peer review

This is a methodologically well performed, interesting report, addressing the potential role of gut commensal microbiota in the pathophysiology of UC.

REFERENCES

1 **Thia KT**, Loftus EV, Sandborn WJ, Yang SK. An update on the epidemiology of inflammatory bowel disease in Asia. *Am J Gastroenterol* 2008; **103**: 3167-3182 [PMID: 19086963 DOI: 10.1111/j.1572-0241.2008.02158.x]

2 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434 [PMID: 17653185 DOI: 10.1038/nature06005]

3 **Stenson WF**, Snapper SB. Challenges in IBD research: Assessing progress and rethinking the research agenda. *Inflamm Bowel Dis* 2008; **14**: 687-708 [PMID: 18260124 DOI: 10.1002/

ibd.20371]
4 **Takaishi H**, Matsuki T, Nakazawa A, Takada T, Kado S, Asahara T, Kamada N, Sakuraba A, Yajima T, Higuchi H, Inoue N, Ogata H, Iwao Y, Nomoto K, Tanaka R, Hibi T. Imbalance in intestinal microflora constitution could be involved in the pathogenesis of inflammatory bowel disease. *Int J Med Microbiol* 2008; **298**: 463-472 [PMID: 17897884 DOI: 10.1016/j.ijmm.2007.07.016]
5 **Sokol H**, Seksik P, Rigottier-Gois L, Lay C, Lepage P, Podglajen I, Marteau P, Doré J. Specificities of the fecal microbiota in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 106-111 [PMID: 16432374 DOI: 10.1097/01.MIB.0000200323.38139.c6]
6 **Manichanh C**, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 2006; **55**: 205-211 [PMID: 16188921 DOI: 10.1136/gut.2005.073817]
7 **Vernia P**, Marcheggiano A, Caprilli R, Frieri G, Corrao G, Valpiani D, Di Paolo MC, Paoluzi P, Torsoli A. Short-chain fatty acid topical treatment in distal ulcerative colitis. *Aliment Pharmacol Ther* 1995; **9**: 309-313 [PMID: 7654893 DOI: 10.1111/j.1365-2036.1995.tb00386.x]
8 **Gibson GR**. Physiology and ecology of the sulphate-reducing bacteria. *J Appl Bacteriol* 1990; **69**: 769-797 [PMID: 2286579 DOI: 10.1111/j.1365-2672.1990.tb01575.x]
9 **Canani RB**, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J Gastroenterol* 2011; **17**: 1519-1528 [PMID: 21472114 DOI: 10.3748/wjg.v17.i12.1519]
10 **Peng L**, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr* 2009; **139**: 1619-1625 [PMID: 19625695 DOI: 10.3945/jn.109.104638]
11 **Scheppach W**. Effects of short chain fatty acids on gut morphology and function. *Gut* 1994; **35**: S35-S38 [PMID: 8125387 DOI: 10.1136/gut.35.1_Suppl.S35]
12 **Lay C**, Rigottier-Gois L, Holmstrøm K, Rajilic M, Vaughan EE, de Vos WM, Collins MD, Thiel R, Namsolleck P, Blaut M, Doré J. Colonic microbiota signatures across five northern European countries. *Appl Environ Microbiol* 2005; **71**: 4153-4155 [PMID: 16000838 DOI: 10.1128/AEM.71.7.4153-4155.2005]
13 **Segain JP**, Raingeard de la Blétière D, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottière HM, Galmiche JP. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* 2000; **47**: 397-403 [PMID: 10940278 DOI: 10.1136/gut.47.3.397]
14 **Scheppach W**, Bartram HP, Richter F. Role of short-chain fatty acids in the prevention of colorectal cancer. *Eur J Cancer* 1995; **31A**: 1077-1080 [PMID: 7576995 DOI: 10.1016/0959-8049(95)00165-F]
15 **Duncan SH**, Holtrop G, Lobley GE, Calder AG, Stewart CS, Flint HJ. Contribution of acetate to butyrate formation by human faecal bacteria. *Br J Nutr* 2004; **91**: 915-923 [PMID: 15182395 DOI: 10.1079/BJN20041150]
16 **Hold GL**, Schwiertz A, Aminov RI, Blaut M, Flint HJ. Oligonucleotide probes that detect quantitatively significant groups of butyrate-producing bacteria in human feces. *Appl Environ Microbiol* 2003; **69**: 4320-4324 [PMID: 12839823 DOI: 10.1128/AEM.69.7.4320-4324.2003]
17 **Louis P**, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA: acetate CoA-transferase gene. *Environ Microbiol* 2010; **12**: 304-314 [PMID: 19807780 DOI: 10.1111/j.1462-2920.2009.02066.x]
18 **Walmsley RS**, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. *Gut* 1998; **43**: 29-32 [PMID: 9771402]
19 **Tursi A**, Brandimarte G, Papa A, Giglio A, Elisei W, Giorgetti GM, Forti G, Morini S, Hassan C, Pistoia MA, Modeo ME, Rodino' S, D'Amico T, Sebkova L, Sacca' N, Di Giulio E, Luzza F, Imeneo M, Larussa T, Di Rosa S, Annese V, Danese S, Gasbarrini A. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2010; **105**: 2218-2227 [PMID: 20517305 DOI: 10.1038/ajg.2010.218]
20 **Amann RI**, Krumholz L, Stahl DA. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J Bacteriol* 1990; **172**: 762-770 [PMID: 1688842]
21 **Wallner G**, Amann R, Beisker W. Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry* 1993; **14**: 136-143 [PMID: 7679962 DOI: 10.1002/cyto.990140205]
22 **Stoecker K**, Dorninger C, Daims H, Wagner M. Double labeling of oligonucleotide probes for fluorescence in situ hybridization (DOPE-FISH) improves signal intensity and increases rRNA accessibility. *Appl Environ Microbiol* 2010; **76**: 922-926 [PMID: 19966029 DOI: 10.1128/AEM.02456-09]
23 **Saunier K**, Rougé C, Lay C, Rigottier-Gois L, Doré J. Enumeration of bacteria from the Clostridium leptum subgroup in human faecal microbiota using Clep1156 16S rRNA probe in combination with helper and competitor oligonucleotides. *Syst Appl Microbiol* 2005; **28**: 454-464 [PMID: 16094872 DOI: 10.1016/j.syapm.2005.02.010]
24 **Zoetendal EG**, Ben-Amor K, Harmsen HJ, Schut F, Akkermans AD, de Vos WM. Quantification of uncultured Ruminococcus obeum-like bacteria in human fecal samples by fluorescent in situ hybridization and flow cytometry using 16S rRNA-targeted probes. *Appl Environ Microbiol* 2002; **68**: 4225-4232 [PMID: 12200269 DOI: 10.1128/AEM.68.9.4225-4232.2002]
25 **Langendijk PS**, Schut F, Jansen GJ, Raangs GC, Kamphuis GR, Wilkinson MH, Welling GW. Quantitative fluorescence in situ hybridization of Bifidobacterium spp. with genus-specific 16S rRNA-targeted probes and its application in fecal samples. *Appl Environ Microbiol* 1995; **61**: 3069-3075 [PMID: 7487040]
26 **Whelan JA**, Russell NB, Whelan MA. A method for the absolute quantification of cDNA using real-time PCR. *J Immunol Methods* 2003; **278**: 261-269 [PMID: 12957413]
27 **Martin FP**, Dumas ME, Wang Y, Legido-Quigley C, Yap IK, Tang H, Zirah S, Murphy GM, Cloarec O, Lindon JC, Sprenger N, Fay LB, Kochhar S, van Bladeren P, Holmes E, Nicholson JK. A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Mol Syst Biol* 2007; **3**: 112 [PMID: 17515922 DOI: 10.1038/msb4100153]
28 **Louis P**, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 2009; **294**: 1-8 [PMID: 19222573 DOI: 10.1111/j.1574-6968.2009.01514.x]
29 **Sokol H**, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottière HM, Doré J, Marteau P, Seksik P, Langella P. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; **105**: 16731-16736 [PMID: 18936492 DOI: 10.1073/pnas.0804812105]
30 **Sokol H**, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009; **15**: 1183-1189 [PMID: 19235886 DOI:

10.1002/ibd.20903]
31 **Huda-Faujan N**, Abdulamir AS, Fatimah AB, Anas OM, Shuhaimi M, Yazid AM, Loong YY. The impact of the level of the intestinal short chain Fatty acids in inflammatory bowel disease patients versus healthy subjects. *Open Biochem J* 2010; **4**: 53-58 [PMID: 20563285 DOI: 10.2174/1874091X01004010053]
32 **Roediger WE**. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* 1980; **2**: 712-715 [PMID: 6106826 DOI: 10.1016/S0140-6736(80)91934-0]
33 **Duncan SH**, Barcenilla A, Stewart CS, Pryde SE, Flint HJ. Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl Environ Microbiol* 2002; 68: 5186-5190 [PMID:12324374 DOI: 10.1128/AEM.68.10.5186-5190.2002]
34 **Franks AH**, Harmsen HJ, Raangs GC, Jansen GJ, Schut F, Welling GW. Variations of bacterial populations in human feces measured by fluorescent in situ hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. *Appl Environ Microbiol* 1998; **64**: 3336-3345 [PMID: 9726880]
35 **Sghir A**, Gramet G, Suau A, Rochet V, Pochart P, Dore J. Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. *Appl Environ Microbiol* 2000; **66**: 2263-2266 [PMID: 10788414]
36 **Verma AK**, Verma R, Ahuja V, Paul J. Real-time analysis of gut flora in Entamoeba histolytica infected patients of Northern India. *BMC Microbiol* 2012; **12**: 183 [PMID: 22913622 DOI: 10.1186/1471-2180-12-183]
37 **Wang RF**, Cao WW, Cerniglia CE. PCR detection and quantitation of predominant anaerobic bacteria in human and animal fecal samples. *Appl Environ Microbiol* 1996; **62**: 1242-1247 [PMID: 8919784]
38 **Ramirez-Farias C**, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. *Br J Nutr* 2009; **101**: 541-550 [PMID: 18590586 DOI: 10.1017/S0007114508019880]

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doi:10.3748/wjg.v19.i22.3415

World J Gastroenterol 2013 June 14; 19(22): 3415-3422
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Disruption of interstitial cells of Cajal networks after massive small bowel resection

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Supported by Grants from the Program for Innovative Research Team of Shanghai Municipal Education Commission and Special Foundation of Shanghai Municipal Public Health Bureau, LJ06021; the National Natural Science Foundation of China, No. 30772270, 30972427; and the Scientific Foundation of Nantong University, No. 10Z046
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Received: December 15, 2012 Revised: February 22, 2013
Accepted: April 13, 2013
Published online: June 14, 2013

Abstract

AIM: To investigate the disruptions of interstitial cells of Cajal (ICC) in the remaining bowel in rats after massive small bowel resection (mSBR).

METHODS: Thirty male Sprague-Dawley rats fitting entry criteria were divided randomly into three experimental groups (n = 10 each): Group A rats underwent bowel transection and re-anastomosis (sham) and tissue samples were harvested at day 7 post-surgery. Group B and C rats underwent 80% small bowel resection with tissue harvested from Group B rats at day 7 post-surgery, and from Group C rats at day 14 post-surgery. The distribution of ICC at the site of the residual small bowel was evaluated by immunohistochemical analysis of small intestine samples. The ultrastructural changes of ICC in the remnant ileum of model rats 7 and 14 d after mSBR were analyzed by transmission electron microscopy. Intracellular recordings of slow wave oscillations were used to evaluate electrical pacemaking. The protein expression of c-kit, ICC phenotypic markers, and membrane-bound stem cell factor (mSCF) in intestinal smooth muscle of each group were detected by Western blotting.

RESULTS: After mSBR, immunohistochemical analysis indicated that the number of c-kit-positive cells was dramatically decreased in Group B rats compared with sham tissues. Significant ultrastructural changes in ICC with associated smooth muscle hypertrophy were also observed. Disordered spontaneous rhythmic contractions with reduced amplitude (8.5 ± 1.4 mV *vs* 24.8 ± 1.3 mV, P = 0.037) and increased slow wave frequency (39.5 ± 2.1 cycles/min *vs* 33.0 ± 1.3 cycles/min, P = 0.044) were found in the residual intestinal smooth muscle 7 d post mSBR. The contractile function and electrical activity of intestinal circular smooth muscle returned to normal levels at 14 d post mSBR (amplitude, 14.9 ± 1.6 mV *vs* 24.8 ± 1.3 mV; frequency, 30.7 ± 1.7 cycles/min *vs* 33.0 ± 1.3 cycles/min). The expression of Mscf and c-kit protein was decreased at 7 d (P = 0.026), but gradually returned to normal levels at 14 d. The ICC and associated neural networks were disrupted, which was associated with the phenotype alterations of ICC.

CONCLUSION: Massive small bowel resection in rats triggered damage to ICC networks and decreased the number of ICC leading to disordered intestinal rhythmicity. The mSCF/c-kit signaling pathway plays a role in the regulation and maintenance of ICC phenotypes.

Key words: Interstitial cells of Cajal; c-kit; Slow wave; Massive small bowel resection; Intestinal dysfunction

Core tip: Several gastrointestinal motility diseases are associated with altered numbers of interstitial cells of Cajal (ICC). Short bowel syndrome is also characterized by disordered intestinal motility immediately after surgery. We have investigated the alterations in numbers and functional changes of ICC that occur as a result of short bowel syndrome. In summary, our study showed modifications of the ultrastructure morphology of ICC, altered numbers of ICC and subsequent altered electrophysiological functional activity in the ileum after massive small bowel resection. However, the association between motility disorders and the changes of ICC should be further evaluated.

Chen J, Du L, Xiao YT, Cai W. Disruption of interstitial cells of Cajal networks after massive small bowel resection. *World J Gastroenterol* 2013; 19(22): 3415-3422 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3415.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3415

INTRODUCTION

Short bowel syndrome (SBS) is characterized by disordered intestinal motility immediately after surgery[1]. After massive small bowel resection (mSBR), the remaining bowel undergoes a compensatory process termed adaptation. During the process of adaptation, the small intestine smooth muscle cells undergo dramatic changes in gross morphology and ultrastructure. Adaptation is associated with the hypertrophy of smooth muscle, which is a physiological response to the increased functional requirement placed on the residual small bowel[2]. In addition, hypertrophy of smooth muscle tissue produces distinct motility disorders in the intestinal remnant, resulting in malabsorption and loss of nutrients because of diarrhea. Therefore, there is a critical need for further investigation of the mechanisms regulating resection-induced adaptation and potential targets that could be developed as therapeutic strategies. In a previous study, we found that resection-induced intestinal adaptation in a rat SBR model involved both the mucosal and intestinal smooth muscle layers. However, the mechanisms regulating adaptation remain unclear[3].

Interstitial cells of Cajal (ICC) reside in the tunica muscularis of the gastrointestinal tract[4]. ICC play a crucial role in gastrointestinal motility in concert with the enteric nervous system, which is composed of both the myenteric (inter-muscular) plexus and the submucosal plexus[5]. ICC are present in organs containing smooth muscle tissue and are pacemaker cells that provide the basal electrical rhythm, which controls peristalsis in the gastrointestinal tract[6]. After receiving inputs from motor neurons, ICC generate and propagate electrical activity[7,8]. ICC are specialized cells in the gastrointestinal tract smooth muscle organs that express the c-kit receptor tyrosine kinase[9]. ICC can be identified by the expression of CD117 (c-kit), which is a membrane receptor with tyrosine kinase activity[5,10,11].

Intracellular signaling *via* c-kit plays a key role in the development and maintenance of the ICC phenotype and functional activity of ICC in the gastrointestinal tract[7,12]. Maintenance of the ICC phenotype requires membrane-bound stem cell factor (mSCF) produced locally within the tunica muscularis[13-16]. To date, remarkably few studies have investigated the functional changes of ICC that occur as a result of SBS. We hypothesize that the disruption to ICC activity involves the downregulation of the mSCF/c-kit signaling pathway following massive mSBR. In the current study, alterations in ICC phenotype and pacemaker activity were evaluated in a model of mSBR.

MATERIALS AND METHODS

Experimental design and animal model

Thirty male Sprague-Dawley rats weighing 250-300 g were obtained from the Experimental Animal Center of Shanghai Jiaotong University School of Medicine. All animals were housed in metabolic cages with free access to food and water and acclimated to their environment for 5 d before experimentation. Animals were maintained under standardized temperature, humidity and 12 h light-dark cycles. The rats were divided randomly into three experimental groups (n = 10 each): Group A rats underwent bowel transection and re-anastomosis (sham); Groups B and C rats underwent 80% small bowel resection; Group A and Group B (SBS1W) bowel tissues were harvested at day 7 post-surgery; Group C (SBS2W) bowel tissues were harvested at day 14 post-surgery.

Animals were fasted for 16 h prior to laparotomy, and intestinal surgery was performed the following morning, as previously described[17]. All operative procedures were performed under anesthesia by intraperitoneal (*ip*) injection of pentobarbital sodium (30 mg/mL), which was administered at doses of 33-40 mg/kg body weight. Briefly, during surgery, the abdomen was opened by a midline incision and the ligament of Treitz and the ileal-cecal junction was identified and marked. For SBR rats, enterectomy was performed by removing approximately 80% of the small intestine, leaving approximately 10 cm of the terminal ileum and 5 cm of the proximal jejunum, which were anastomosed. For sham-operated control rats, the laparotomy and all surgical manipulations were the same as above, but the resection procedure was not carried out. All animals received fluid resuscitation by *ip* injection of saline (10 mL 0.9% NaCl) before the abdominal wall was closed and surgery concluded. After recovery, rats were transferred back to individual cages and given water *ad libitum* overnight, after which their regular diet was reinstated.

All experimental protocols were approved by the local Animal Care Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the Science and Technology Commission of the People's Republic of China (STCC Publication No. 2, revised 1988).

Immunohistochemistry

Immunohistochemical analysis was performed on tissue samples of smooth muscle at the same site of the ileum from both control and mSBR rats. The bowel was opened along the mesenteric border and the lumen contents were washed away with Krebs Ringer buffer. Segments of the bowel were pinned to the base of a Sylgard silicone elastomer dish and the mucosa was removed by sharp dissection. After dissection, the tunica muscularis were embedded in Tissue-Tek® OCT compound medium (Sakura Finetek United States, Inc., Torrance, CA, United States). The embedded tunica muscularis was cut at a thickness of 30 μm with a freezing microtome (Leica Microsystems GmbH, Wetzlar, Germany) and stored at -20 ℃ until use. The sections were fixed with iced acetone for 10 min. After endogenous peroxidase activity was quenched with 3% hydrogen peroxide, the sections were preincubated in 10% goat serum/0.2% Triton X-100/0.1 mol/L phosphate buffered saline (PBS) for 1 h at room temperature. The sections were incubated with antibodies against c-kit protein (1:50 dilution, rabbit polyclonal c-kit antibody, Santa Cruz Technologies, Santa Cruz, CA, United States) in 0.1 mol/L PBS containing 1% goat serum for 2 h. The sections were then incubated for 1 h at room temperature with biotinylated anti-rabbit immunoglobulin G (1:200; Vector Labs, Burlingame, CA, United States). Positive staining was visualized using an avidin-biotin-peroxidase complex system (Vectastain ABC Elite Kit, Vector Labs). The degree of expression of c-kit antibody was analyzed using image analysis software (Zeiss).

Transmission electron microscopy

Tissue samples from intestinal smooth muscle at the same site of the ileum from both control and mSBR rats were fixed with 3% glutaraldehyde at room temperature. After fixation, the tissues were washed overnight in 0.1 mol/L sodium cacodylate buffer [6% sucrose and 1.25 mmol/L $CaCl_2$ (pH 7.4)] at 4 ℃ and postfixed with 1% osmium tetroxide in 0.05 mol/L sodium cacodylate buffer (pH 7.4) at 4 ℃ for 2 h. The tissues were stained with saturated uranyl acetate for 3.5 h at room temperature, dehydrated in graded alcohol and embedded in Eponate 12 resin (Ted Pella, Inc., United States). The tissue was sectioned parallel and transverse to the long axis of the circular muscle layer. At suitable sites, 3 μm sections were cut and stained with 2% toluidine blue. After examination of the toluidine blue stained sections, ultrathin sections of selected areas were obtained with the ultramicrotome using a diamond knife, mounted on 200-mesh grids, and stained with uranyl acetate and lead citrate. The grids were observed with a JEM1200EX electron microscope.

Electrophysiological experiments

Following euthanasia, a 50 mm mid-segment of the remaining small bowel was removed and placed in Krebs solution (mmol/L; NaCl 117, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, *D*-glucose 11, $CaCl_2$ 2.6). The bowel was opened along the mesenteric border, and the luminal contents were washed away with Krebs solution. Segments of the bowel were pinned to the base of a Sylgard silicone elastomeric dish (Dow Corning, Midland, MI, United States), and the mucosa was removed by sharp dissection[18]. Strips of smooth muscle tissue (8 mm × 4 mm) were cut parallel to the longitudinal muscle oral to the site of the occlusion clips. The muscle was placed in a recording chamber with the submucosal aspect of the muscle facing upwards at 37 ℃ in an atmosphere of 95% O_2 and 5% CO_2. Cells were impaled with KCl-filled glass microelectrodes with resistances of 50-90 MΩ. Electrical responses were recorded and amplified through a high input impedance amplifier (SYS-773 Duo 773 Electrometer, WPI, United States). Experiments were performed in the presence of nifedipine (1 μmol/L; Sigma, St Louis, MO, United States) in order to reduce contractions and facilitate the extended period of cell impalement. Slow waves in mouse intestine have been previously shown to be unaffected by nifedipine[19].

Western blotting

Smooth muscle tissues were lysed in radioimmunoprecipitation assay buffer [25 mmol/L Tris-HCl pH 7.6, 150 mmol/L NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS)] for the detection of protein expression levels. The Bradford method (Pierce, Rockford, IL, United States) was used to determine protein concentrations. Tissue lysates (20 μg of total protein per lane) were subjected to electrophoretic separation by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Hybond, GE Healthcare Biosciences, Pittsburgh, PA, United States). Nonspecific binding was reduced by incubation of the membrane in 5% milk. Western blots were performed using antibodies directed against c-kit (1:200 dilution, rabbit polyclonal c-kit antibody, Santa Cruz Technologies, Santa Cruz, CA, United States), murine stem cell factor (mSCF) (1:200 dilution, mouse monoclonal SCF antibody, Santa Cruz Technologies), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:600 dilution, rabbit monoclonal antibody, CWbiotech company, Beijing, China). Alkaline phosphatase conjugated secondary antibodies (CWbiotech Company Beijing, China) were used to detect protein bands. TIFF images were captured by Adobe Photoshop and analyzed by Quantity one image software (NIH, MA, United States).

Statistical analysis

The results are presented as mean ± SE. Statistical differences between the groups were determined using a one-way analysis of variance with the SigmaStat program (SPSS, United States). $P < 0.05$ was considered statistically significant.

RESULTS

Distribution of c-kit(+) cells

Representative pictures of c-kit immunopositivity at the myenteric plexus are shown in Figure 1. In sham-operated rats, c-kit-positive cells were predominantly present

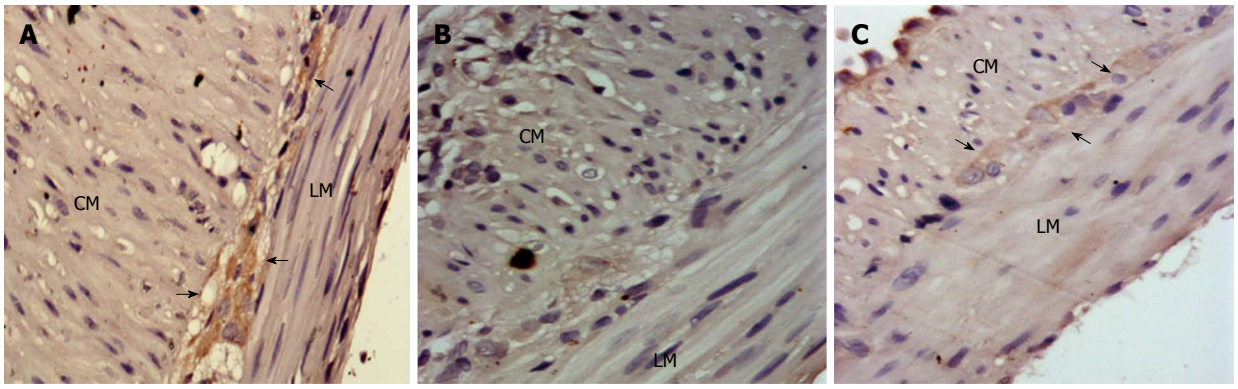

Figure 1 c-kit immunopositivity at the level of the myenteric plexus in ileal whole-mount preparations. A: In the sham-operated group, c-kit-positive cells were predominantly present at the myenteric plexus level; B: Compared with sham-operated tissues, the number and density of c-kit immunopositive cells were significantly decreased in the short bowel syndrome (SBS) 1W group; C: In the SBS2W group, the number of interstitial cells of Cajal had returned to normal levels. The arrows showed c-kit immunoexpression is positive (original magnification × 400). LM: Longitudinal muscle; CM: Circular muscle.

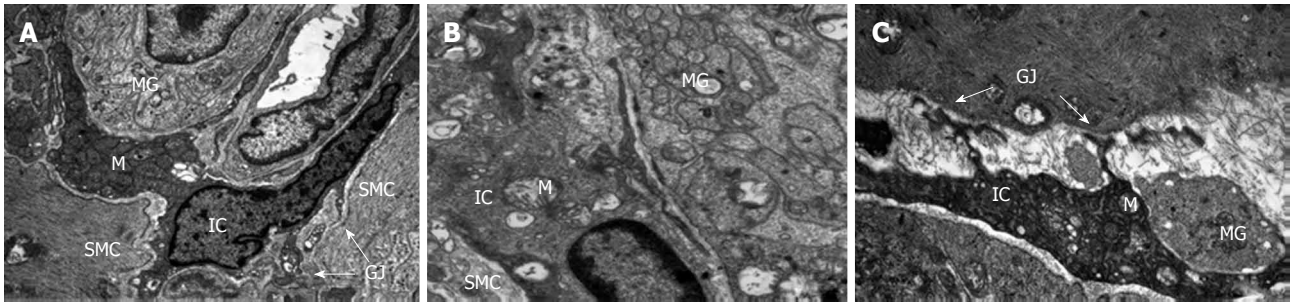

Figure 2 Ultrastructural morphological changes in interstitial cells of Cajal. A: At the ultrastructural morphological level, interstitial cells of Cajal (ICC) showed typical myofilaments and organelles of ICC, such as mitochondria and smooth sarcoplasmic reticulum in the sham-operated group; B: Ultrastructural changes of ICC were observed in the short bowel syndrome (SBS) 1W group, such as clear cytoplasm, sparse mitochondria, scarce smooth endoplasmic reticulum and a reduced number of contacts between nerves and ICC; C: In the SBS2W group, ultrastructural changes of ICC were ameliorated (original magnification × 3750, 2.5 K). SMC: Smooth muscle cell; GJ: Gap junction; M: Mitochondria; MG: Myenteric ganglion.

at the myenteric plexus level. However, the number and density of c-kit immunopositive cells were significantly decreased in SBS1W rats ($P < 0.05$) compared with sham tissues. However, after 2 wk, the number and density of ICC were clearly increased and approached sham levels, as indicated by c-kit positive cells (Figure 1).

Ultrastructural morphological changes

In sham rats, ultrastructural features of ICC in the small intestine were characterized by a less electron-dense cytoplasm and abundant mitochondria in sham rats (Figure 2A). However, the basal lamina or caveolae were not present. Intermediate filaments and thin filaments were apparent as thin processes. Along the length of overlapping processes, ICC predominantly formed large gap junctions between adjoining cells (Figure 2A). In certain areas, slender cytoplasmic processes of ICC were in close contact with a varicosity of the myenteric ganglion. Another part of the same cellular process was connected to a muscle cell *via* a gap junction, suggesting a functional relationship between these cell types.

Altered ICC ultrastructure was observed in the small intestine of SBS1W rats including clear cytoplasm, sparse mitochondria, scarce smooth endoplasmic reticulum, and a reduced number of contacts between nerves and ICC (Figure 2B). The ultrastructural abnormalities of ICC in the small intestine of SBS2W rats exhibited some improvement compared to SBS1W rats, but the ultrastructural morphology had not returned to normal as observed in the sham-operated rats (Figure 2C).

Electrophysiological studies

Electrophysiological studies were performed on circular muscles of the ileum in the remaining small bowel at day 7 or 14 following mSBR. The second component of slow waves was absent in SBS1W rats (Figure 3A). Circular muscle cells from the ileum of sham-operated rats (n = 7) exhibited resting membrane potentials (RMP) averaging -63.7 ± 1.8 mV and slow waves of 24.8 ± 1.3 mV in amplitude and a frequency of 33 ± 1.3 cycles/min. The electrical activities of ileum circular muscle cells were markedly different in animals 7 d after mSBR. The slow waves had considerably reduced RMP and amplitude and increased in frequency (RMP, -53.5 ± 2.1 mV; amplitude, 8.5 ± 1.4 mV; frequency, 39.5 ± 2.1 cycles/min; Figure 3B-D, respectively; $P < 0.05$) compared with sham-operated tissues. However, 14 d after mSBR, the electrical activity of the circular layer showed no significant chang-

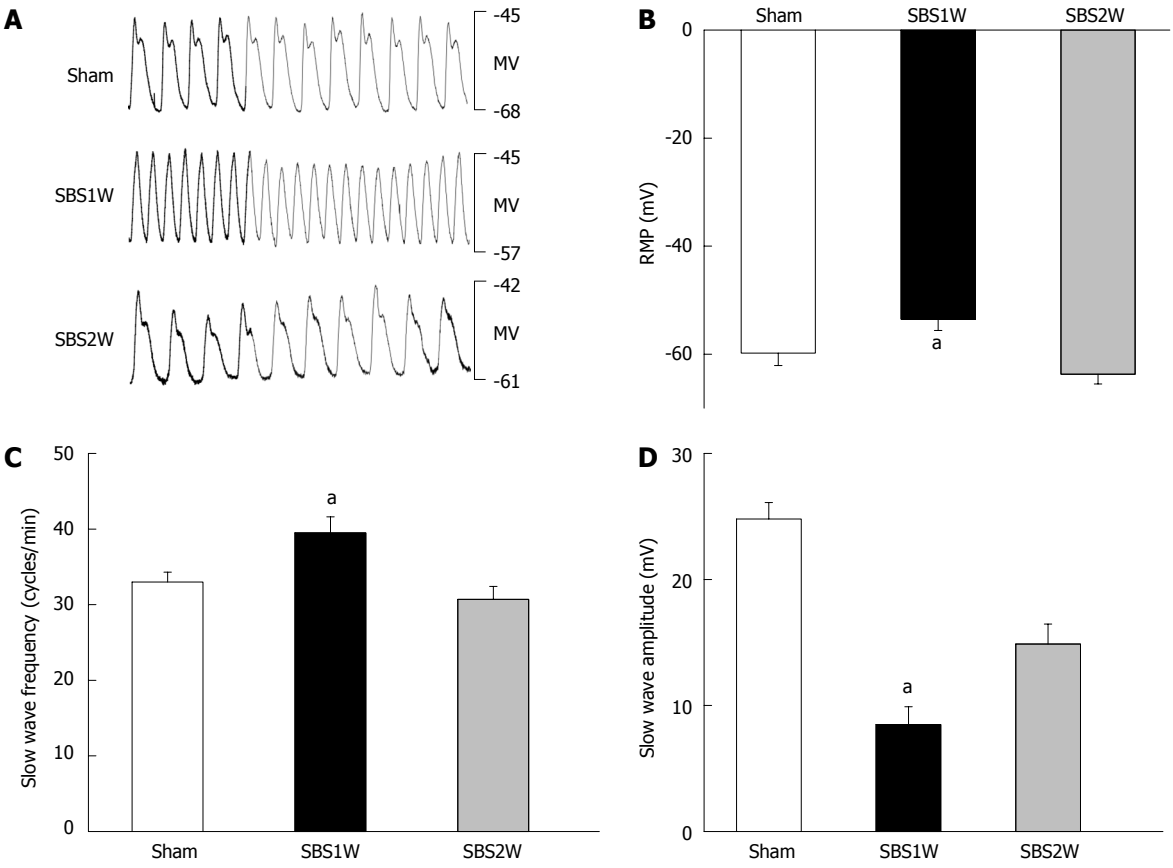

Figure 3 Electrical activity recorded from the small intestines (ileum) from sham-operated and short bowel syndrome rats. A: Slow waves in sham-operated group were biphasic, consisting of an upstroke and plateau component. Slow waves short bowel syndrome (SBS) 1W rats lacked an obvious secondary component; B: Resting membrane potentials (RMP) in each group were recorded, and a depolarized membrane potential appears to be a common feature of intestine smooth muscle in SBS rats; C: Slow wave frequency; D: Slow wave amplitude. [a]$P < 0.05$ *vs* sham group ($n = 7$).

es ($P > 0.05$) in RMP, amplitude and frequency (RMP, -59.8 ± 2.3 mV; slow wave amplitude, 14.87 ± 1.6 mV; frequency, 30.7 ± 1.7 cycles/min) compared with sham-operated tissues.

Expression levels of mSCF and c-kit are involved in the maintenance of the ICC phenotype and function after mSBR

In order to investigate the roles of mSCF and c-kit in the recovery process of the electrophysiological function of smooth muscle, we determined the expression of these two proteins in mSBR and sham rat intestinal smooth muscle tissues by Western blot (Figure 4A and C). The protein expression levels of mSCF and c-kit were normalized to the internal control GAPDH (Figure 4B and D). The protein expression levels of mSCF and c-kit were significantly decreased with mSBR in the SBS1W group ($n = 4$; $P < 0.05$). The protein expression levels returned to levels found in sham-operated rats by day 14 after mSBR, which coincided with the recovery of the electrical activities of ileal circular muscle cells.

DISCUSSION

After mSBR, adaptive alterations in the function of the remaining bowel are often accompanied by disorders of intestinal motility. However, the mechanisms regulating the adaptation and motility disorders are not clear. A number of factors have been implicated in the pathogenesis of intestinal dysfunction, including changes in the number, density and ultrastructural morphology of ICC[11,20-22]. Alterations in the normal function of ICC have been reported in many intestinal disorders. In our previous studies, the unexpected finding of contractile dysfunction after mSBR prompted the investigation of whether intestinal dysfunction after mSBR was also mediated by ICC depletion[3].

ICC are found between and within smooth muscle layers of the gastrointestinal tract from the esophagus to the internal anal sphincter[23,24]. ICC specifically express the proto-oncogene c-kit that encodes a receptor tyrosine kinase. *c-kit* expression can be clearly detected with the immunohistochemistry method and is a valuable tool for determining ICC structure, localization and distribution of cell networks.

Two separate functional groups of ICC exist in the lumen of the gastrointestinal tract: myenteric ICC (ICC-MY) and intramuscular ICC (ICC-IM). Networks of ICC-MY are located within the intermuscular space at the level of the myenteric plexus between the circular and longitudinal muscle layers. The plexus of ICC-MY, like the sino-atrial node, is the dominant pacemaker center that triggers the generation of slow waves, which are

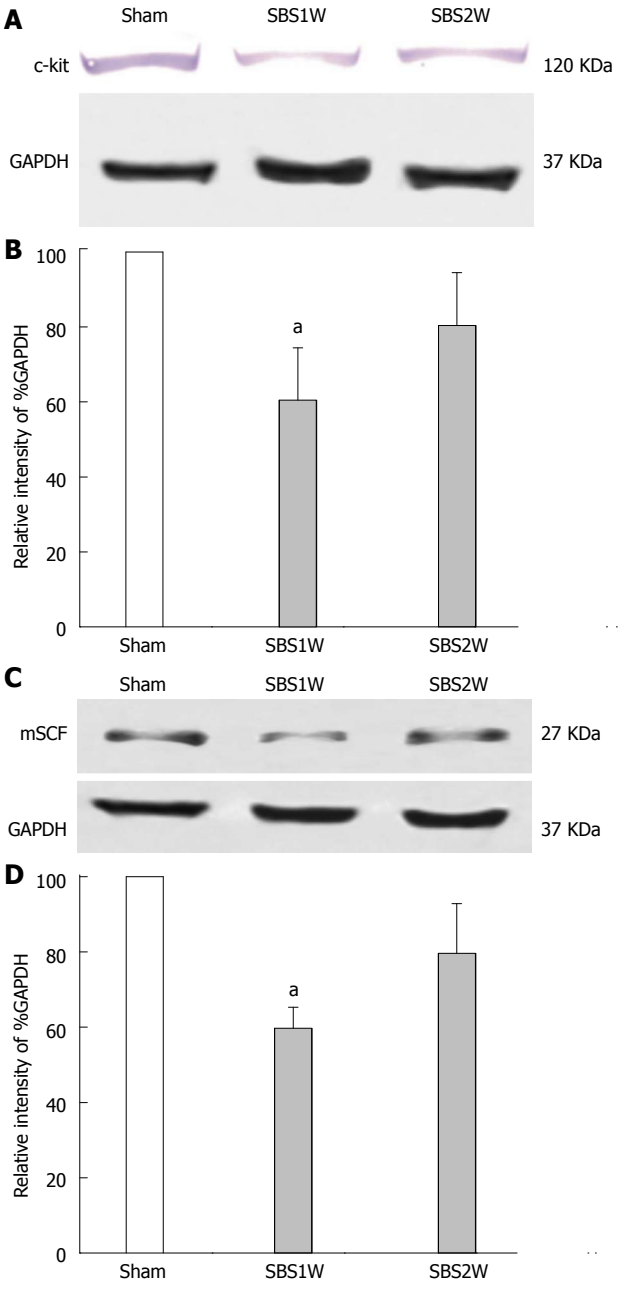

Figure 4 Changes in the expression profile of c-kit and membrane-bound stem cell factor in response to massive small bowel resection. A, C: The protein expression levels of c-kit and membrane-bound stem cell factor (mSCF) were downregulated in the first week after massive small bowel resection in the short bowel syndrome (SBS) 1W group and increased in the second week of the SBS2W group; B, D: Relative expression was determined by normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). $^{a}P < 0.05$ *vs* sham group ($n = 4$).

essential for orderly segmenting and peristaltic contractions in the tunica muscularis[25,26]. A second population of cells, ICC-IM, is localized within the muscle layers of the gastrointestinal tract and is innervated preferentially by enteric motor nerves. ICC-IM are closely associated with not only enteric motor nerves but also vagal afferent nerves. Elongated ICC-IM mediate efferent inputs to smooth muscle cells and the pacemaker apparatus, as well as relay afferent mechanical signals[27,28].

We hypothesize that ICC depletion is central to the pathogenesis of many intestinal disorders. ICC are reduced or otherwise dysfunctional in several gastrointestinal tract dysmotilities, including achalasia, diabetic and idiopathic gastroparesis, mechanical ileus, intestinal pseudo-obstructions, slow-transit constipation, inflammations and malformations[29-31]. Rolle *et al*[30] reported that intestinal neuronal dysplasia was associated with loss or deficiency of ICC networks in the neonatal period. In addition, Chang *et al*[32] reported that electrical slow waves were significantly disrupted and accompanied by disruption of ICC function and network in the obstructed ileum of mice.

In the current study, an absence or reduction in the number of ICCs was observed in the remaining bowel of an animal model of SBS. Utilizing intracellular recording of smooth muscle cells in isolated segments of the remnant ileal tissues, a reduction of rhythmic contractions and disruption of electrical slow waves were associated with the disruption of ICC and their network in the remnant ileum at 7 d in SBS rats. Slow waves are composed of two components: one produced by electrogenic propagation of driving potentials from the ICC-MY and the other formed by slow potentials from the ICC-IM[32,33]. In the current study, the frequency of slow waves changed in response to RMP changes as depolarization increased the frequency. Slow waves detected in SBS1W rats were lacking the secondary wave component compared with those in sham-operated and SBS2W rats. These differences suggested that alterations in the function of ICC account for the disruption of slow waves. This finding supports the concept that when ICC and their network were disrupted from the SBS ileum, the amplitude of slow waves decreased and the shape of slow waves and neural responses were lost due to the loss of ICC[12,32].

Furthermore, c-kit signaling is essential for normal development of ICC and is required for long-term ICC survival and function[7,8]. This was first demonstrated by the blockade of c-kit signaling postnatally with an antagonistic anti-kit antibody. Inhibition of c-kit signaling resulted in a severe anomaly of gut motility with depletion of c-kit-positive ICC-MY cells in the myenteric regions of the small intestine[33]. Albertí *et al*[34] also reported a deficient population of ICC in Ws/Ws rats harboring mutations in c-kit. In the gastrointestinal tract, c-kit expression on the cell surface of ICC is activated by mSCF produced by surrounding smooth muscle cells. Maintenance of ICC requires mSCF produced locally within the tunica muscularis. Another study reported a reduction in the content of mSCF in the stomach of non-obese diabetic mice[35]. These mice also exhibited marked depletion of ICC and a reduction in expression level of mSCF to one-third of that in normal mice. These studies support the hypothesis that reduced mSCF/c-kit signaling may underlie the decrease in ICC numbers and its network in obstructed mice[36].

In our study, there was a reduction in mSCF/c-kit in the remnant ileum of SBS1W rats. The correlation

between the decrease in ICC numbers and reduction in mSCF/c-kit protein expression levels suggest that reduced mSCF/c-kit in the gastrointestinal tract may be involved in the disruption of ICC function in the remnant ileum of rats with SBS. Although previous work has shown that inflammatory diseases can induce the disruption of ICC and their network, the underlying mechanism by which the expression of mSCF in intestinal smooth muscle is decreased during SBS remains unclear. Additional studies are warranted to determine the role of mSCF and c-kit in the disruption of ICC.

In conclusion, the current study showed modifications of the ultrastructure morphology of ICC, phenotypic changes in ICC and subsequent altered electrophysiological functional activity in the ileum after mSBR, which may be associated with the mechanical alterations. In addition, the association between motility disorders after mSBR and the changes in ICC should be further evaluated.

COMMENTS

Background

Motility disorders are a prevalent condition observed in the residual small bowel after massive small bowel resection (mSBR). A number of factors have been implicated in the pathogenesis of intestinal dysfunction. A potential role for interstitial cells of Cajal (ICC) networks in intestinal disorders after mSBR has not previously been reported.

Research frontiers

ICC are organized in distinct networks and serve several different functions. ICC generate the electrical slow wave and also set the smooth muscle membrane potential, are mechanosensors and modulate neuronal input to smooth muscle. Moreover, ICC play a protective role in gastrointestinal motility, although disruption of ICC networks are certainly not the only reason for intestinal dysfunction in some cases.

Innovations and breakthroughs

The authors show that disruption of ICC networks is associated with intestinal dysfunction in rats after mSBR. This role of the ICC has not previously been reported and identifies a potential new therapeutic target intestinal disorders after mSBR.

Applications

A greater understanding of mechanism of intestinal dysfunction after mSBR will help in utilizing its diverse effects more efficiently. Pharmacotherapy (such as glucagon-like peptide-2, which is supposed to improve the function of intestinal ileum in rats with short bowel syndrome) holds promise as an adjuvant treatment modality for short bowel syndrome.

Terminology

Phenotypic changes in ICC refers to changes in the structure and function of ICC.

Peer review

The authors investigate an attractive subject of Cajal network involvement in intestinal dysfunction after small bowel resection, using an experimental rat model. Experimental setup and methodology are appropriate and the study hypothesis and overall manuscript are well presented.

REFERENCES

1 **Kinoshita K**, Hori M, Fujisawa M, Sato K, Ohama T, Momotani E, Ozaki H. Role of TNF-alpha in muscularis inflammation and motility disorder in a TNBS-induced colitis model: clues from TNF-alpha-deficient mice. *Neurogastroenterol Motil* 2006; **18**: 578-588 [PMID: 16771773 DOI: 10.1111/j.1365-2982.2006.00784.x]

2 **Martin CA**, Bernabe KQ, Taylor JA, Nair R, Paul RJ, Guo J, Erwin CR, Warner BW. Resection-induced intestinal adaptation and the role of enteric smooth muscle. *J Pediatr Surg* 2008; **43**: 1011-1017 [PMID: 18558175 DOI: 10.1016/j.jpedsurg.2008.02.015]

3 **Chen J**, Wen J, Cai W. Smooth muscle adaptation and recovery of contractility after massive small bowel resection in rats. *Exp Biol Med* (Maywood) 2012; **237**: 578-584 [PMID: 22581812 DOI: 10.1258/ebm.2012.011338]

4 **Ordög T**, Redelman D, Horváth VJ, Miller LJ, Horowitz B, Sanders KM. Quantitative analysis by flow cytometry of interstitial cells of Cajal, pacemakers, and mediators of neurotransmission in the gastrointestinal tract. *Cytometry A* 2004; **62**: 139-149 [PMID: 15536638 DOI: 10.1002/cyto.a.20078]

5 **Takaki M**. Gut pacemaker cells: the interstitial cells of Cajal (ICC). *J Smooth Muscle Res* 2003; **39**: 137-161 [PMID: 14695026 DOI: 10.1540/jsmr.39.137]

6 **Huizinga JD**, Lammers WJ. Gut peristalsis is governed by a multitude of cooperating mechanisms. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1-G8 [PMID: 18988693 DOI: 10.1152/ajpgi.90380.2008]

7 **Sanders KM**, Ward SM. Kit mutants and gastrointestinal physiology. *J Physiol* 2007; **578**: 33-42 [PMID: 17095561 DOI: 10.1113/jphysiol.2006.122473]

8 **Huizinga JD**, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 1995; **373**: 347-349 [PMID: 7530333 DOI: 10.1038/373347a0]

9 **Sanders KM**. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* 1996; **111**: 492-515 [PMID: 8690216 DOI: 10.1053/gast.1996.v111.pm8690216]

10 **Ward SM**. Interstitial cells of Cajal in enteric neurotransmission. *Gut* 2000; **47** Suppl 4: iv40-iv43; discussion iv52 [PMID: 11076909 DOI: 10.1136/gut.47.suppl_4.iv40]

11 **Long QL**, Fang DC, Shi HT, Luo YH. Gastro-electric dysrhythm and lack of gastric interstitial cells of cajal. *World J Gastroenterol* 2004; **10**: 1227-1230 [PMID: 15069732]

12 **Won KJ**, Suzuki T, Hori M, Ozaki H. Motility disorder in experimentally obstructed intestine: relationship between muscularis inflammation and disruption of the ICC network. *Neurogastroenterol Motil* 2006; **18**: 53-61 [PMID: 16371083 DOI: 10.1111/j.1365-2982.2005.00718.x]

13 **Lorincz A**, Redelman D, Horváth VJ, Bardsley MR, Chen H, Ordög T. Progenitors of interstitial cells of cajal in the postnatal murine stomach. *Gastroenterology* 2008; **134**: 1083-1093 [PMID: 18395089 DOI: 10.1053/j.gastro.2008.01.036]

14 **Horváth VJ**, Vittal H, Lörincz A, Chen H, Almeida-Porada G, Redelman D, Ordög T. Reduced stem cell factor links smooth myopathy and loss of interstitial cells of cajal in murine diabetic gastroparesis. *Gastroenterology* 2006; **130**: 759-770 [PMID: 16530517 DOI: 10153/j.gastro.2005.12.027]

15 **Wang XY**, Albertí E, White EJ, Mikkelsen HB, Larsen JO, Jiménez M, Huizinga JD. Igf1r+/CD34+ immature ICC are putative adult progenitor cells, identified ultrastructurally as fibroblast-like ICC in Ws/Ws rat colon. *J Cell Mol Med* 2009; **13**: 3528-3540 [PMID: 19220583 DOI: 10.1111/j.1582-4934.2009.00689.x]

16 **He CL**, Soffer EE, Ferris CD, Walsh RM, Szurszewski JH, Farrugia G. Loss of interstitial cells of cajal and inhibitory innervation in insulin-dependent diabetes. *Gastroenterology* 2001; **121**: 427-434 [PMID: 11487552 DOI: 10.1053/gast.2001.26264]

17 **Washizawa N**, Gu LH, Gu L, Openo KP, Jones DP, Ziegler TR. Comparative effects of glucagon-like peptide-2 (GLP-2), growth hormone (GH), and keratinocyte growth factor (KGF) on markers of gut adaptation after massive small bowel resection in rats. *JPEN J Parenter Enteral Nutr* 2004; **28**: 399-409 [PMID: 15568286 DOI: 10.1177/014860710402800399]

18 **Chen J**, Chen H, Sanders KM, Perrino BA. Regulation of SRF/CArG-dependent gene transcription during chronic partial obstruction of murine small intestine. *Neurogastroen-*

terol Motil 2008; **20**: 829-842 [PMID: 18557893 DOI: 10.1111/j.1365-2982.2008.01149.x]
19 **Suzuki H**, Hirst GD. Regenerative potentials evoked in circular smooth muscle of the antral region of guinea-pig stomach. *J Physiol* 1999; **517** (Pt 2): 563-573 [PMID: 10332102 DOI: 10.1111/j.1469-7793.1999.0563t.x]
20 **Hudson N**, Mayhew I, Pearson G. A reduction in interstitial cells of Cajal in horses with equine dysautonomia (grass sickness). *Auton Neurosci* 2001; **92**: 37-44 [PMID: 11570702 DOI: 10.1016/S1566-0702(01)00316-2]
21 **Rolle U**, Piotrowska AP, Nemeth L, Puri P. Altered distribution of interstitial cells of Cajal in Hirschsprung disease. *Arch Pathol Lab Med* 2002; **126**: 928-933 [PMID: 12171490]
22 **Zárate N**, Mearin F, Wang XY, Hewlett B, Huizinga JD, Malagelada JR. Severe idiopathic gastroparesis due to neuronal and interstitial cells of Cajal degeneration: pathological findings and management. *Gut* 2003; **52**: 966-970 [PMID: 12801952 DOI: 10.1136/gut.52.7.966]
23 **Daniel EE**, Posey-Daniel V. Neuromuscular structures in opossum esophagus: role of interstitial cells of Cajal. *Am J Physiol* 1984; **246**: G305-G315 [PMID: 6703058]
24 **Hagger R**, Gharaie S, Finlayson C, Kumar D. Distribution of the interstitial cells of Cajal in the human anorectum. *J Auton Nerv Syst* 1998; **73**: 75-79 [PMID: 9862380 DOI: 10.1016/S0165-1838(98)00038-1]
25 **Ward SM**, Beckett EA, Wang X, Baker F, Khoyi M, Sanders KM. Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. *J Neurosci* 2000; **20**: 1393-1403 [PMID: 10662830]
26 **Sanders KM**, Koh SD, Ward SM. Interstitial cells of cajal as pacemakers in the gastrointestinal tract. *Annu Rev Physiol* 2006; **68**: 307-343 [PMID: 16460275 DOI: 10.1146/annurev.physiol.68.040504.094718]
27 **Ward SM**, Sanders KM. Interstitial cells of Cajal: primary targets of enteric motor innervation. *Anat Rec* 2001; **262**: 125-135 [PMID: 11146435 DOI: 10.1002/1097-0185(20010101)262]
28 **Fox EA**, Phillips RJ, Martinson FA, Baronowsky EA, Powley TL. C-Kit mutant mice have a selective loss of vagal intramuscular mechanoreceptors in the forestomach. *Anat Embryol* (Berl) 2001; **204**: 11-26 [PMID: 11506430 DOI: 10.1007/s004290100184]
29 **Burns AJ**. Disorders of interstitial cells of Cajal. *J Pediatr Gastroenterol Nutr* 2007; **45** Suppl 2: S103-S106 [PMID: 18185068 DOI: 10.1097/MPG.0b013e31812e65e0]
30 **Rolle U**, Piaseczna-Piotrowska A, Puri P. Interstitial cells of Cajal in the normal gut and in intestinal motility disorders of childhood. *Pediatr Surg Int* 2007; **23**: 1139-1152 [PMID: 17968564 DOI: 10.1007/s00383-007-2022-7]
31 **Farrugia G**. Interstitial cells of Cajal in health and disease. *Neurogastroenterol Motil* 2008; **20** Suppl 1: 54-63 [PMID: 18402642 DOI: 10.1111/j.1365-2982.2008.01109.x]
32 **Chang IY**, Glasgow NJ, Takayama I, Horiguchi K, Sanders KM, Ward SM. Loss of interstitial cells of Cajal and development of electrical dysfunction in murine small bowel obstruction. *J Physiol* 2001; **536**: 555-568 [PMID: 11600689 DOI: 10.1111/j.1469-7793.2001.0555c.xd]
33 **Torihashi S**, Nishi K, Tokutomi Y, Nishi T, Ward S, Sanders KM. Blockade of kit signaling induces transdifferentiation of interstitial cells of cajal to a smooth muscle phenotype. *Gastroenterology* 1999; **117**: 140-148 [PMID: 10381920 DOI: 10.1016/S0016-5085(99)70560-3]
34 **Albertí E**, Mikkelsen HB, Wang XY, Díaz M, Larsen JO, Huizinga JD, Jiménez M. Pacemaker activity and inhibitory neurotransmission in the colon of Ws/Ws mutant rats. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1499-G1510 [PMID: 17322067 DOI: 10.1152/ajpgi.00136.2006]
35 **Takeda M**, Takayama I, Terada N, Baba T, Ward SM, Ohno S, Fujino MA. Immunoelectron-microscopic study of Kit-expressing cells in the jejunum of wildtype and Ws/Ws rats. *Cell Tissue Res* 2001; **304**: 21-30 [PMID: 11383883 DOI: 10.1007/s004410000333]
36 **Guo X**, Huang X, Wu YS, Liu DH, Lu HL, Kim YC, Xu WX. Down-regulation of hydrogen sulfide biosynthesis accompanies murine interstitial cells of Cajal dysfunction in partial ileal obstruction. *PLoS One* 2012; **7**: e48249 [PMID: 23133623 DOI: 10.1371/journal.pone.0048249]

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World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3423

World J Gastroenterol 2013 June 14; 19(22): 3423-3432
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Human liver tissue metabolic profiling research on hepatitis B virus-related hepatocellular carcinoma

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Received: December 13, 2012 Revised: March 21, 2013
Accepted: April 3, 2013
Published online: June 14, 2013

Abstract

AIM: To select characteristic endogenous metabolites in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) patients and to identify their molecular mechanism and potential clinical value.

METHODS: An ultra performance liquid chromatography and linear trap quadrupole-Orbitrap XL-mass spectrometry platform was used to analyze endogenous metabolites in the homogenate of central tumor tissue, adjacent tissue and distant tissue obtained from 10 HBV-related HCC patients. After pretreatment with Mzmine software, including peak detection, alignment and normalization, the acquired data were treated with Simca-P+software to establish multivariate statistical analysis based on a pattern recognition technique and characteristic metabolites highly correlated with changing trends in metabolic profiling were selected and further identified.

RESULTS: Based on data acquired using Mzmine software, a principal component analysis model (R2X = 66.9%, Q2 = 21.7%) with 6 principal components and an orthogonal partial least squares discriminant analysis model (R2X = 76.5%, R2Y = 93.7%, Q2 = 68.7%) with 2 predicted principal components and 5 orthogonal principal components were established in the three tissue groups. Forty-nine ions were selected, 33 ions passed the 2 related samples nonparametric test ($P < 0.05$) and 14 of these were further identified as characteristic metabolites that showed significant differences in levels between the central tumor tissue group and distant tumor tissue group, including 9 metabolites (*L*-phenylalanine, glycerophosphocholine, lysophosphatidylcholines, lysophosphatidylethanolamines and chenodeoxycholic acid glycine conjugate) which had been reported as serum metabolite biomarkers for HCC diagnosis in previous research, and 5 metabolites (beta-sitosterol, quinaldic acid, arachidyl carnitine, tetradecanal, and oleamide) which had not been reported before.

CONCLUSION: Characteristic metabolites and metabolic pathways highly related to HCC pathogenesis and progression are identified through metabolic profiling analysis of HCC tissue homogenates.

Key words: Hepatocellular carcinoma; Metabolomics; Characteristic metabolites; Potential biomarker; Ultra performance liquid chromatography-mass spectrometry

Core tip: An ultra performance liquid chromatography-mass spectrometry platform was used in the present study to identify characteristic metabolites in hepatitis B virus-related hepatocellular carcinoma tumor tissues. From an orthogonal partial least squares discriminant analysis model established to determine metabolic profiling in the central tumor tissue group, adjacent tissue group and distant tissue group, 49 ions were selected and 14 of these were identified as characteristic metabolites. The detection of these metabolites in tumor tissue not only confirmed the targeted traceability of previously reported serum biomarkers related to cancer diagnosis, but also provided novel targets for anticancer research.

Liu SY, Zhang RL, Kang H, Fan ZJ, Du Z. Human liver tissue metabolic profiling research on hepatitis B virus-related hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(22): 3423-3432 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3423.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3423

INTRODUCTION

Hepatocellular carcinoma (HCC) is currently an important research topic in basic and clinical investigations due to its high malignancy, fast development, high mortality, complicated pathogenesis and significant individual differences. There have been a number of previous investigations on the molecular biology and proteomics of HCC[1-3]. However, the occurrence and development of this disease are not simply determined by innate genetic differences. The introduction of metabolomics has provided more information in HCC investigations. Metabolomics is the study of biological systems where changes in metabolites after specific stimulation or interference are determined[4-7]. This type of study focuses on the end products of biological systems, which are a reflection of both the physiological and biochemical status, and are affected by both genotype and environment and are close to biological characteristics. Thus it has the advantage of investigating the objective basis and generative mechanism of individual differences in metabolites.

Current metabolomic investigations in HCC are mainly focused on identifying characteristic metabolites in serum or urine, revealing changes in the metabolic network, and finally selecting potential biomarkers with clinical application and exploring pathologic mechanisms[8,9]. For example, Wu *et al*[10] used gas chromatography/mass spectrometry (MS) to analyze metabolic profiling in 20 male HCC patients and 20 healthy male volunteers. One hundred and three ions were detected and 66 ions were identified. Following *t* test analysis, 18 metabolites were significantly different between the central tumor tissue group and the distant tumor tissue group ($P < 0.05$). The ability to distinguish these 18 ions combined with alpha-fetoprotein was analyzed by the receiver operating characteristic curve (0.9275). This non-invasive method was considered quite promising. Tan *et al*[11] also used metabolic analytical methods to select serum biomarkers for small HCC diagnosis. Metabolic profiling was performed in a diethylnitrosamine-induced rat HCC model, which is similar to human HCC in the histopathology of liver disease development. The alterations in three metabolites, taurocholic acid, lysophosphoethanolamine 16:0 and lysophosphatidylcholine 22:5, were reported to be correlated with disease progression and considered as potential biomarkers. In addition, serum metabolic profiling was performed in 262 HCC patients, 76 liver cirrhosis patients and 74 HBV patients. The results showed that the sensitivity and specificity of these ions all reached 80%, which was better than alpha-fetoprotein in distinguishing HCC-liver cirrhosis. This research laid the foundation for the clinical application of metabolic biomarkers. At the same time, Soga *et al*[12] research group reported that gamma-glutamyl dipeptides were used as biomarkers for liver disease diagnosis. The metabolic profiling of 248 serum samples, including patients with 9 types of liver disease, was performed using a capillary electrophoresis-MS platform. Gamma-glutamyl dipeptides were selected and found to be predictive of a decrease in glutathione. Multiple regression analysis showed that the selected biomarkers had the ability to distinguish different liver diseases.

In animal models, serum and urine analysis and sample collection are easy to perform, and HCC serum and urine metabolic profiling can reflect specific signs and reveal pathological mechanisms, however, the targeting ability of these analyses is still limited. The present study used a ultra performance liquid chromatography and linear trap quadrupole (UPLC-LTQ)-Orbitrap XL MS analytical platform to perform metabolic profiling analyses on homogenates of hepatitis B virus (HBV)-related HCC tumors removed by surgery. A metabolic profiling model was established and metabolic pathways highly related to HCC were characterized for further investigations on the genesis and progression of HCC and the potential clinical value of characteristic metabolites.

MATERIALS AND METHODS

Chemicals and instruments

All solvents were high performance liquid chromatography (HPLC) grade and used without modification. Formic acid and acetonitrile (ACN) were obtained from Merck (KGaA Merck, Germany). Distilled water was produced using a Milli-Q Reagent Water System (Millipore, Billerica, MA, United States). All standard [*L*-phenylalanine, glycerophosphocholine, chenodeoxycholic acid glycine conjugate and lysophosphatidylcholine (lysoPC) (18:0)] preparations were purchased from Sigma-Aldrich (St. Louis, MO, United States). Ultra performance liquid chromatography was performed on a Thermo Fisher Accela system (Thermo Fisher Scientific, Franklin, MA, United States). MS was performed on a Thermo Fisher (Thermo Fisher Scientific, Franklin, MA, United States) LTQ Orbitrap XL hybrid mass spectrometer. Other equipment included a Multifuge X1R high-speed centrifuge (Thermo Fisher Scientific, United States).

Sample collection

The 10 HBV-related HCC patients included in this study were all from the Department of Hepatobiliary Surgery, Tianjin Third Central Hospital, and included 5 females and 5 males with average age of 54.2 ± 9.2 years. All patients voluntarily joined this study and gave informed consent. Tissue from the central area of the tumor, adjacent tissue (1-2 cm from the tumor) and distant tissue (5 cm from the tumor) were collected. Thirty samples (10 samples in each group) were washed in hypothermic normal saline, dried using neutral filter paper and then stored at -80 ℃.

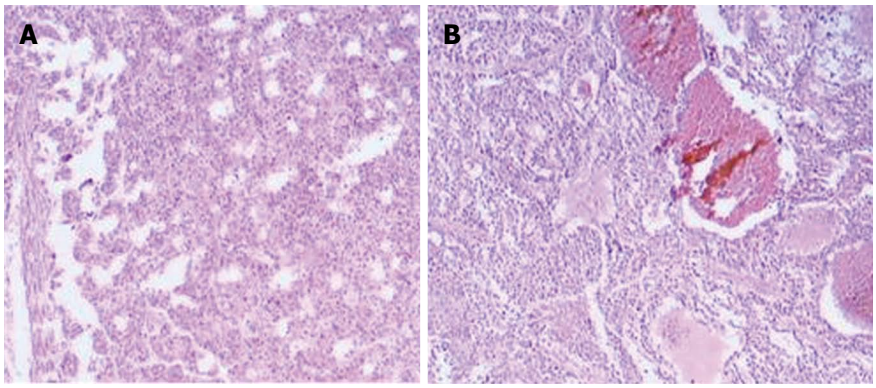

Figure 1 Pathological image of hepatocellular carcinoma tissues (hematoxylin-eosin staining, × 100). A: Moderately differentiated hepatocellular carcinoma (HCC), cancer emboli were observable in vessels; B: Moderately differentiated HCC, focal carcinoma tissue was observed in adjacent liver tissue.

Collection of samples was performed according to the following instructions: (1) all 10 patients had chronic persistent HBV (no other hepatotropic virus infection, no long-term drinking behavior, no autoimmune hepatitis, no schistosomiasis infection and no genetic metabolic liver disease); (2) all 10 patients had solitary tumors < 5 cm in diameter, and Barcelona Clinic Liver Cancer stage A1; (3) tissue pathological immunohistochemistry examination diagnosed HCC using hepatitis B surface antigen (+); the pathological section is shown in Figure 1. Samples were all HCC grade Ⅱ-Ⅲ differentiation. Invasive growth was detected using the appropriate specific stain. No cancer cells were detected at the incisional edge; (4) no radiotherapy or chemotherapy was performed before surgery; (5) no evidence of endocrine or metabolic disease; (6) renal function, liver function, blood routine and hydropower solution pH were all in the normal range; (7) no severe infection was detected and parenteral nutrition was used; and (8) patients' dietary requirements were managed by the Nutrition Department of Tianjin Third Central Hospital to a relatively uniform standard, as a result, exogenous dietary influence on metabolic profiling was limited to the lowest level.

Sample pretreatment

Samples were thawed at room temperature and then weighed, 1:3 ultrapure water was added to each sample according to their weight before being homogenized. Tissue homogenate was subjected to ultrasonication at 130 W × 60% for 3 cycles. One cycle included 10 s of ultrasonication and a 10 s interval (ultrasonication was performed in an ice-bath). Homogenate (400 μL) was centrifuged (4 ℃, 15000 *g*, 30 min). Supernatant (100 μL) was mixed with 400 μL methanol to precipitate proteins. The mixture was centrifuged at 4 ℃, 15000 *g* for 30 min. The supernatant was filtered using a 0.22 μm membrane. Quality control (QC) was the equivalent volume mixture in each sample.

Sample analysis

Chromatography conditions were as follows: Chromatography was performed on a Thermo Fisher Accela system (Thermo Fisher Scientific, Franklin, MA, United States) which was equipped with a binary solvent delivery manager, and a sample manager. The analytical column was a Thermo Hypersil GOLD (2.1 mm *id* × 50 mm 1.9 μm) C18 reversed phase column.

Mobile phase: Phase A: 0.1% formic acid (volume ratio), 1 mL of formic acid was added to a 1 L bottle of HPLC-grade water; Phase B: 95% ACN and 0.1% formic acid. And 950 mL ACN, 50 mL HPLC-grade water, and 1 mL formic acid were combined.

Chromatographic separation: Chromatographic separation was performed isocratically within 15 min and the injection volume was 10 μL. The flow rate was set at 200 μL/min. The sample manager and column oven temperature were set at 4 ℃ and 20 ℃, respectively. The chromatographic elution gradient was initialized at 5% Phase B and held for 3 min. In consecutive 10 min periods, Phase B was gradually escalated to 50%, and then a rapid increase in Phase B to 95% was completed within 3 min. After 4 min of maintaining the high volume of organic phase gradient, Phase B was immediately reduced to 5% and this elution gradient was used to balance the analytical column for the final 4 min.

MS was performed on a Thermo Fisher (Thermo Fisher Scientific, Franklin, MA, United States) LTQ Orbitrap XL hybrid mass spectrometer[13], operating in the positive ion mode with an ion source voltage of 4.5 kV, a capillary voltage of 30 V, cone voltage of 150 V, desolvation temperature of 275 ℃, sheath gas flow of 30 arb and assistant gas flow of 5 arb (99.999% nitrogen). Data were collected over 15 min in centroid mode over the mass range 50-1000 m/z. The MS resolution was at 100000 full width half maximum (FMHW) and the calibration standards were provided by Thermo Fisher Scientific (caffeine, Ultramark 1621 and *L*-methionyl-arginyol-phenylalanylalanine acetate H_2O). MS/MS analysis was carried out with collision-induced dissociation (CID) collision energy 35 (normalization collision energy) and the collision gas was 99.999% helium.

There were 14 QC samples throughout the test (equal

volume mixture of each analyzed sample). Before the samples were tested, 8 QC samples were analyzed continuously and the remaining QC samples were inserted into the sequence after every 5 samples were analyzed.

The sequence of samples was randomly generated by the excel function before and after sample analysis (including QC), and cross-contamination was avoided by inserting a blank between adjacent samples. The whole experiment lasted 780 min.

Ethics statement

All samples were collected in accordance with the ethical guidelines and written consent protocols mandated by the Tianjin Third Central Hospital. The Institutional Review Board approved the collection of serum for comprehensive metabolite characterization. All patients and all control individuals were approached using approved ethical guidelines and those who agreed to participate in this study, were required to sign consent forms. Patients could refuse entry, discontinue participation, or withdraw from the study at any time without prejudice for further treatment or management. All participants provided written consent.

Statistical analysis

MZmine 2.0 was used for peak detection, alignment and normalization. The filter conditions were: chromatography peak intensity signal/noise > 30, retention time tolerance = ± 0.1 min, and m/z tolerance = ± 0.01.

One of the aims of this study was to establish a model to predict the tissue metabolic profile in HCC. With the help of Simca-P+12.0.1.0 (Umetrics, Sweden), software based on chemometrics methods, the OPLS-DA supervised model was established. The detailed process was as follows: The variables were firstly traded by Pareto scaling. The principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) model of all the samples were established and checked by cross validation[14,15]. The preliminary selection of characteristic metabolites was accomplished using the corresponding variable importance (VIP) value, confidence interval and coefficient plot generated by the OPLS-DA model. The selected metabolites were then preliminarily confirmed by the S and SUS diagram. Finally, the variances were evaluated by SPSS 16.0 software (SPSS, United States), using the 2 related samples nonparametric test.

RESULTS

Data pretreatment and QC analysis

The total ion chromatogram of the central tumor tissue group, adjacent tissue group and distant tissue group acquired by the UPLC-MS platform are shown in Figure 2. After pretreatment and standardization using MZmine 2.0, there were 211 integral peaks following extraction ion chromatography detected in QC samples and 215 peaks in test samples.

The stability of the UPLC-MS system was adequately assessed by the analysis of QC samples during the entire experimental period[16]. Through PCA of 14 data sets of QC samples, a PCA model with 2 principal components was established (Figure 3). Figure 3 shows the score plot of QC sample sequence versus first principal component (the most influential factor which varied with time). From the QC principal component score plot we found that the UPLS-MS system was stable after the first 8 continuous QC injections. In the test sample sequence, a QC sample was inserted after every 5 test samples to evaluate the stability of the system during the entire analytical process. The results showed that the detection system was stable throughout the experiment after the first 8 QC samples were injected (no outliers exceeding ± 2 SD were detected in the QC samples). According to a related article[17], the QC standard was set as follows: (1) ion peaks were defined as reliable peaks when their intensity was in the range of ± 30% average ion intensity; (2) a QC sample was qualified if its 70% ion peaks were reliable; and (3) experimental data were accepted only when 60% QC was qualified. In the present experiment, 11 of 14 QC samples (reliable ion peaks distributed among 70.5%-86.3%) inserted into the test sample sequence qualified and the qualified ratio was 78.5%, which meant that the analytical results were trusted.

Ability of the metabolic profile to distinguish disease states

A PCA model with 6 principal components was established (R2X = 66.9%, Q2 = 21.7%) and the score plot of its first two principal components is shown in Figure 4A. It can be seen that the central tumor tissue group and distant tissue group showed a clustering tendency in the direction of first predictive principal component (X axis). The adjacent tissue group was located between the central tumor tissue and distant tissue groups. Although the clustering tendency was not significant, its distribution could assess the development of HCC. An OPLS-DA model was established using all 30 samples. The model had 2 predictive principal components and 5 orthogonal principal components (R2X = 76.5%, R2Y = 93.7%, Q2 = 68.7%). As shown in Figure 4B, the score plot of the first predictive principal component and first orthogonal principal component showed significant clustering tendency. In the X axis direction, the changing tendency of metabolic profiling in the three groups reflected the development of HCC, at the same time differences between the three groups in metabolic profiling were significant. As a result, it was concluded that the first principal component could reflect the development of HCC.

Selection of characteristic metabolites

Through metabolic profiling analysis of the homogenates of HCC tumor tissue, endogenous metabolites highly related to HCC were detected. The main focus of the present study was the metabolites which had an obvious impact on clustering tendency of the central tumor tissue group and distant tissue group. First, the OPLS-DA

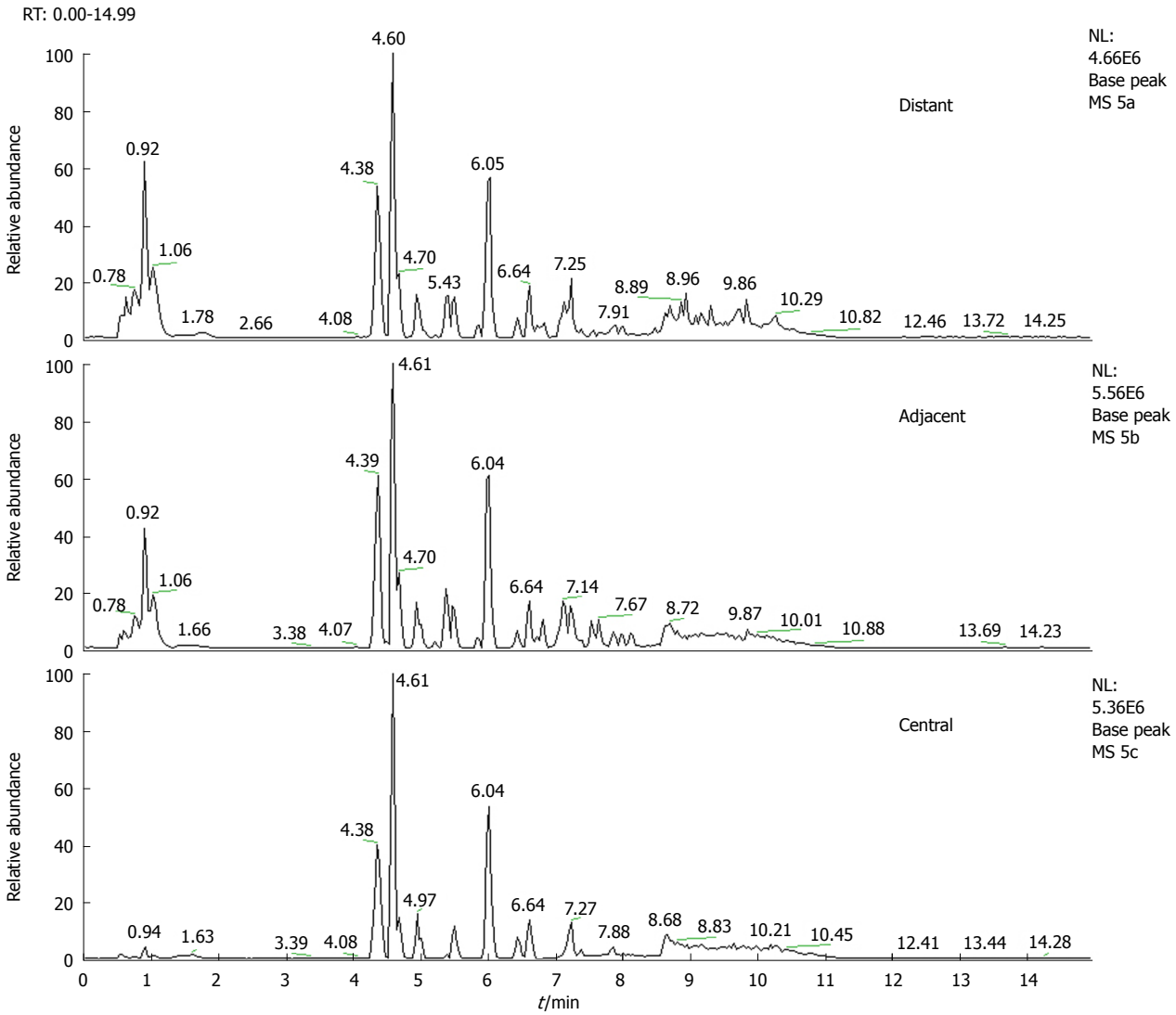

Figure 2 Total ion chromatogram of tissue metabolic profiling. This was the total ion chromatogram of one single sample chosen randomly. Distant: Distant tissue group; Adjacent: Adjacent tissue group; Central: Central tumor tissue group; RT: Retention time.

model was established for the central tumor tissue group and distant tissue group (Figure 5A), which had 1 predictive principal component and 3 orthogonal principal components (R2Y = 97.6%, Q2 = 83.8%). The characteristic metabolites with the ability to distinguish disease were selected in the established OPLS-DA model after the following two processes: (1) Among ions with VIP > 1, excluded ions which included zero in the confidence interval in the VIP diagram (Figure 5B) and excluded ions which included zero in the confidence interval in the coefficient plot (Figure 5C, excluded ions were marked by black arrows)[18]; and (2) From the S-plot (Figure 5B), ions with a high degree of variation (high horizontal ordinate value) and reliability (high vertical ordinate value) were selected. After these two steps, 14 ions were selected. To limit the cover-up effect, a "blank" OPLS-DA model was established which excluded the 14 selected ions. The OPLS-DA model had 1 predictive principal component and 3 orthogonal principal components (R2Y = 97.1%, Q2 = 74.2%). Because the ability of the "blank" model to distinguish disease was still high, other characteristic metabolites were selected following the two steps previously mentioned, and 35 ions were selected. Among the total 49 ions selected, 33 ions passed the 2 related samples nonparametric test ($P < 0.05$).

Identification of characteristic metabolites

Some characteristic metabolites were identified by MS graphs when compared with the chromatographic peaks and mass spectrographic peaks of the standard (including MS1 and MS2). Identification of other selected ions was performed as follows: Firstly, because of the high resolution of the Orbitrap XL mass spectrometer (resolution set as 100000 FMHW), characteristic metabolites were preliminarily identified by checking accurate m/z on the Human Metabolome DataBase (http://hmdb.ca/). The matching metabolites were retained for further identification according to the rules that m/z deviation was below 0.01, with equal charge number and with suitable ionization mode. Secondly, after MS/MS scanning, MS2 graphs

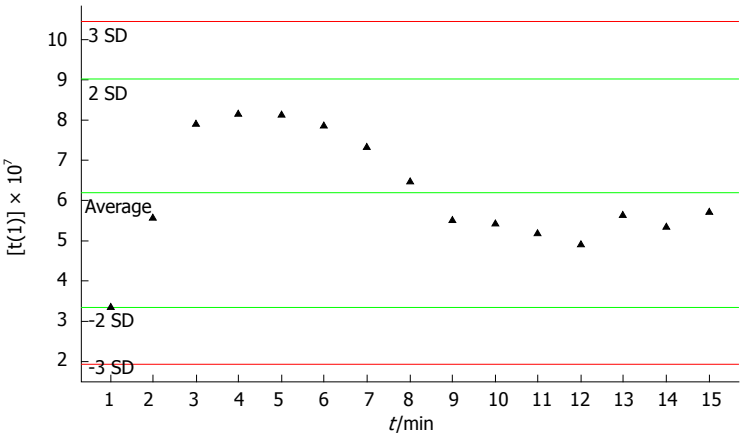

Figure 3 Score plot of principal component [t(1)] for quality control principal component analysis model. Each point represents a quality control sample.

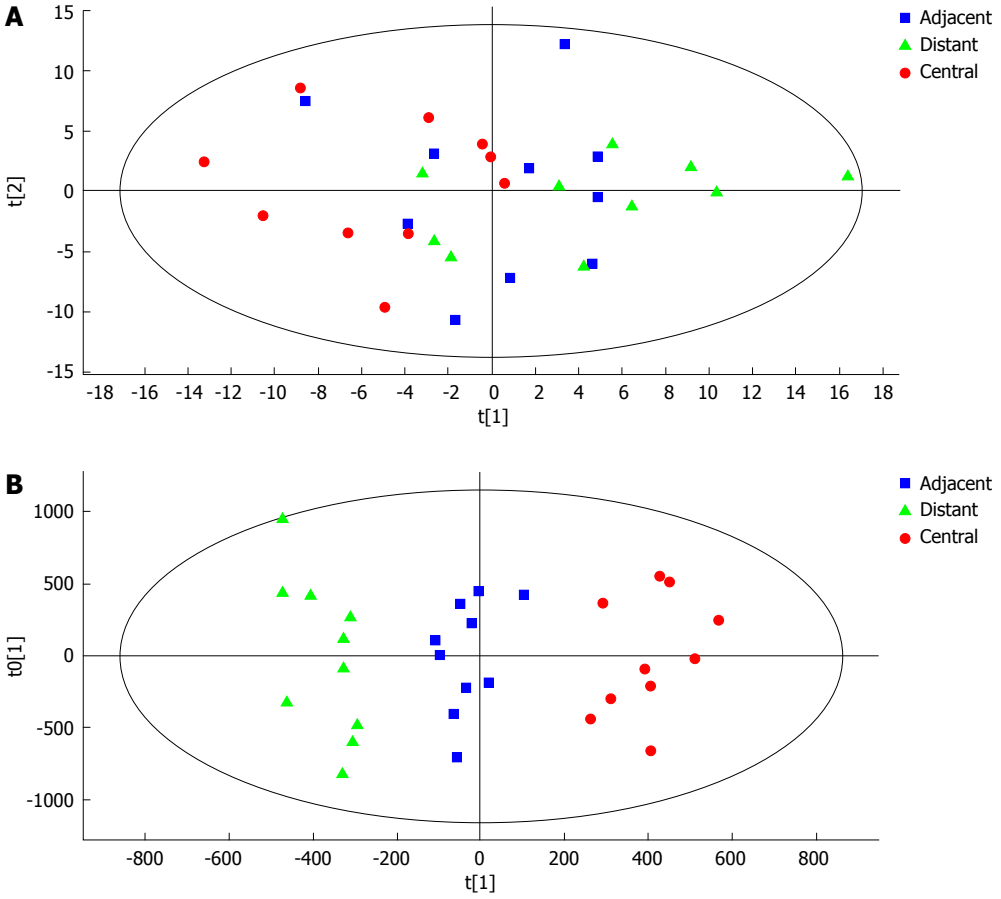

Figure 4 Ability of metabolic profiling to distinguish disease in tissue samples. A: Score plot of the first two components [t(1)/t(2)] of the tissue metabolic profiling principal component analysis model; B: Score plot of metabolic profiling orthogonal partial least squares discriminant analysis model. Each point in the figure represents a sample. Distant: Distant tissue group; Adjacent: Adjacent tissue group; Central: Central tumor tissue group.

of the characteristic ions were obtained and then compared with theoretical fragments of previous preliminary results according to the rules that MS2 m/z deviation was below 0.2, matching the top three peaks and at least 80% of secondary MS graphs (secondary MS fragments were generated by the ion trap CID model). The theoretical fragments were derived using Mass Frontier 6.0. The final identified results and statistical differences among the three groups are shown in Table 1 (only 14 identified metabolites are listed).

DISCUSSION

HCC, the fifth commonest cancer and the third most common cause of cancer-related death, accounts for 6% of all cancers worldwide[19,20]. Fifty percent of diagnosed

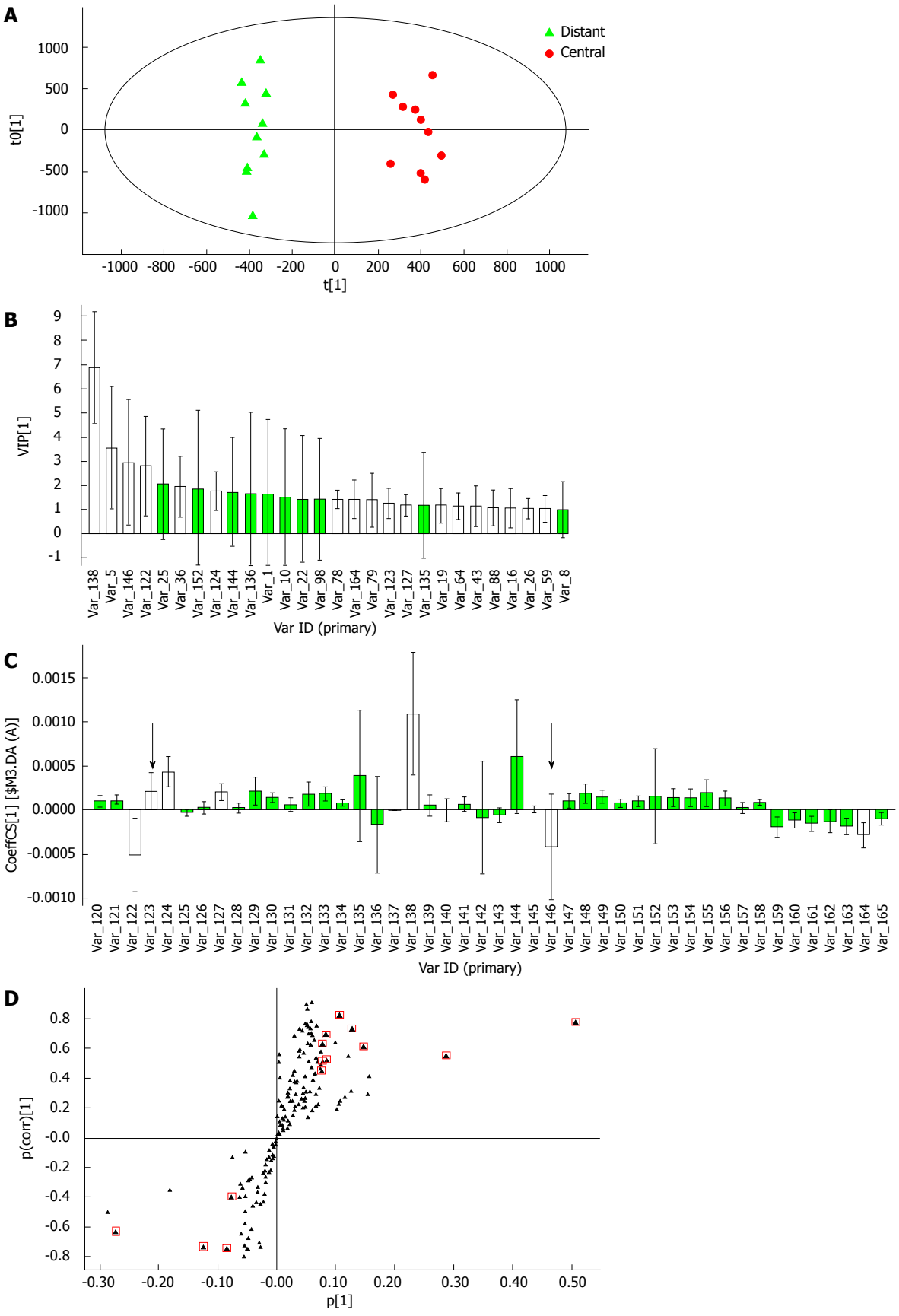

Figure 5 Selecting process of characteristic metabolites. A: Orthogonal partial least squares discriminant analysis model of the central tumor tissue group and distant tissue group, each point in the figure represents a sample; B: Variable importance (VIP) diagram with confidence interval (green bars were excluded metabolites, the others were target metabolites); C: Coefficient plot of included variants in the VIP diagram (variants marked by arrows were excluded metabolites); D: S-plot of selected ions passed last two steps of filtering (points marked by red frame represent characteristic metabolites). Distant: Distant tissue group; Central: Central tumor tissue group.

Table 1 Metabolite identification results

m/z	Retention time (min)	Metabolite	Adduct[2]	Content[3]		
				Normal/adjacent	Adjacent/tumor	Normal/tumor
453.345	4.39632	Sitosterol-β	M + K [1+]	Up[a]		Up[a]
166.086	1.05638	*L*-phenylalanine[1]	M + H [1+]		Up[a]	Up[b]
520.341	6.84401	LysoPC [18:2 (9Z, 12Z)]	M + H [1+]		Up[a]	Up[b]
258.111	0.77819	Glycerophosphocholine[1]	M + H [1+]		Up[b]	Up[b]
476.276	7.10121	LysoPE [18:3 (9Z, 12Z, 15Z)/0:0]	M + H [1+]		Up[a]	Up[b]
450.322	5.88844	Chenodeoxycholic acid glycine conjugate[1]	M + H [1+]		Up[b]	Up[b]
568.342	6.78248	LysoPC [22:6 (4Z, 7Z, 10Z, 13Z, 16Z, 19Z)]	M + H [1+]		Up[a]	Up[b]
212.02	5.54796	Quinaldic acid	M + K [1+]	Up[b]		Up[a]
456.406	8.78787	Arachidyl carnitine	M + H [1+]		Down[a]	Down[b]
504.308	7.89672	LysoPE (18:0/0:0)	M + Na [1+]		Up[a]	Up[a]
546.357	7.08546	LysoPC (18:0)[1]	M + Na [1+]		Up[a]	Up[b]
566.323	6.82403	LysoPC [20:4 (5Z, 8Z, 11Z, 14Z)]	M + Na [1+]		Up[b]	Up[b]
230.249	6.09076	Tetradecanal	M + NH4 [1+]		Down[b]	Down[b]
282.28	9.02044	Oleamide	M + H [1+]	Down[b]		Down[b]

[1]Metabolites identified by standard comparison; [2]Ionospheric models of mass spectrometry cationic scanning; [3]Comparison of characteristic metabolites' integral peak area in the three groups. [a]$P < 0.05$, [b]$P < 0.01$ *vs* samples related non-parametric test. lysoPC: Lysophosphatidylcholine; lysoPE: Lysophosphatidylethanolamines.

HCC cases occur in China[21-23]. Because it takes a long time for HCC to occur in liver cirrhosis patients, early diagnosis of HCC is of great importance. Lots of effort has gone in discovering early biomarkers in human fluid, especially in serum, to monitor the occurrence and development of HCC[11,24,25]. Unfortunately, although the biomarkers discovered in serum have great potential value in clinical and subclinical areas, their targeting ability and specificity are still limited. The present study determined serum metabolic profiling of HCC and provided new research targets for molecular biology.

Tissue metabolic profiling, especially human tissue metabolic profiling has several advantages in metabolomics investigations[26,27]. For example, compared with serum metabolic profiling, tissue metabolic profiling reflects the metabolic changes in certain target lesions in tissues or organs instead of changes in the whole metabolic system. Thus, it has higher targeting ability, specificity and is affected by fewer exogenous influencing factors, such as psychological changes in patients. Compared with animal tissue metabolic profiling, where the model is generally induced by a single factor, human tissue metabolic profiling reflects the natural physiological changes of lesions and is trusted in investigations on the occurrence and development of certain diseases.

Following tissue metabolite profiling of HBV-related HCC, 215 ions were detected and after multivariate statistical analysis of their relative levels (integral peak area of extraction ion), 14 ions were selected as characteristic metabolites and then identified. Of these characteristic metabolites, 9 had been suggested as serum metabolite biomarkers for HCC diagnosis in previous reports, including *L*-phenylalanine, glycerophosphocholine, lysoPCs, lysophosphatidylethanolamines (lysoPEs) and chenodeoxycholic acid glycine conjugate[11,28-30]. The present results enhanced the targeting ability of these characteristic metabolites, as alterations in these metabolites occurred in HCC cells.

The remaining 5 identified characteristic metabolites have seldom been reported, including Beta-Sitosterol, Quinaldic acid, Arachidyl carnitine, Tetradecanal, and Oleamide. This was possibly due to alterations in these metabolites which were too small to be observed in serum or were masked by alterations in other metabolites. Although these metabolites did not show great value in clinical application as potential biomarkers, they still provided new targets for anticancer research.

Previous research has shown that the levels of 11 metabolites including beta-sitosterol, *L*-phenylalanine, lysoPCs, glycerophosphocholine, lysoPEs, chenodeoxycholic acid glycine conjugate and quinaldic acid were significantly high in the distant tissue group compared with the central tumor tissue group. In contrast, the levels of Arachidyl carnitine, tetradecanal and oleamide were significantly lower in the distant tissue group compared with the central tumor tissue group. A comparison of the levels of these metabolites in the adjacent tissue group showed that beta-sitosterol, quinaldic acid and oleamide were significantly lower in the central tumor tissue group, but relatively close to those in the central tumor tissue group. From these changes in metabolite levels, we can speculate that these endogenous metabolites correlate with the progression of adjacent tissue to central tumor tissue and contain important information on the occurrence and development of HCC. Further attention should be focused on these metabolites which will hopefully lead to the discovery of novel potential biomarkers for cancer diagnosis and disease course monitoring and provide new targets for tumor therapy.

In summary, the establishment of a metabolic profiling model and the analysis of the characteristic metabolites provided new perspectives for further research into the pathophysiology of HCC. Following investigations on homogenates of tumor tissue obtained during radical surgery of HCC, detection and identification of these previously reported potential biomarkers in human tissue

enhanced their targeted traceability and a comparison of the changes in these metabolites in the central tumor tissue group, adjacent tissue group and distant tissue group revealed metabolic information highly related to HCC development. More attention should be focused on these characteristic metabolites which will provide more potential biomarkers for HCC diagnosis and development. In addition, further research into the pathways related to these metabolites would provide new clinical targets for HCC therapy.

ACKNOWLEDGMENTS

We would like to express my gratitude to Jie Gao and Yi-Jun Wang who helped with the sample collection and to Li Zhang who helped with the data collection. We also acknowledge the Liver Surgery Department of Tianjin Third Central Hospital for sample submission.

COMMENTS

Background

Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) is the most common type of liver cancer in Asian populations with an extremely high incidence and poor survival rate. Unfortunately, there are no satisfactory definitive positive and negative markers for HCC diagnosis in clinical application at the present time.

Research frontiers

Recently, metabolomics research provided a series of novel biomarkers in serum or urine with clinical value in the diagnosis and prognosis of HCC. However, the application of these biomarkers is still limited due to the lack of target traceability in the diseased organ. The detection of these previously reported characteristic metabolites in tumor tissue in the present research provided supporting proof for these novel biomarkers.

Innovations and breakthroughs

The targeting ability of animal models, serum and urine analysis are limited although experiments and sample collection are easier to perform. The present research used homogenates of central, adjacent and distant HBV-related HCC tumor tissue removed by surgery to identify these metabolites. Thus, this method had higher targeting ability, specificity and was less affected by exogenous influencing factors. In addition, the analysis detected alterations in these metabolites that were barely observed in serum or urine.

Applications

By detecting characteristic metabolites in tumor tissue, this research confirmed the traceability of 9 previously reported biomarkers detected in serum or urine provided supporting evidence for their potential clinical applications. The introduction of homogenates also suggested new avenues of research in metabolic analysis.

Terminology

Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) analysis are data analysis methods based on projection technology. PCA can visually represent sample correlations (results distribution) such as clustering and migration. However, this method is easily affected by noise signals such as instrumental bias and operation error. In this situation, OPLS-DA analysis is often introduced in data analysis to focus experimental data in a particular direction according to a priori knowledge.

Peer review

The manuscript selects 14 characteristic metabolites in liver tumor tissue that not only supports the clinical application of previous reported serum or urine biomarkers but also provides novel research targets for anticancer investigations.

REFERENCES

1 **Masuzaki R**, Karp SJ, Omata M. New serum markers of hepatocellular carcinoma. *Semin Oncol* 2012; **39**: 434-439 [PMID: 22846860 DOI: 10.1053/j.seminoncol.2012.05.009]
2 **Zhong DN**, Ning QY, Wu JZ, Zang N, Wu JL, Hu DF, Luo SY, Huang AC, Li LL, Li GJ. Comparative proteomic profiles indicating genetic factors may involve in hepatocellular carcinoma familial aggregation. *Cancer Sci* 2012; **103**: 1833-1838 [PMID: 22726459]
3 **Fu WM**, Zhang JF, Wang H, Tan HS, Wang WM, Chen SC, Zhu X, Chan TM, Tse CM, Leung KS, Lu G, Xu HX, Kung HF. Apoptosis induced by 1,3,6,7-tetrahydroxyxanthone in Hepatocellular carcinoma and proteomic analysis. *Apoptosis* 2012; **17**: 842-851 [PMID: 22610480]
4 **Nicholson JK**, Connelly J, Lindon JC, Holmes E. Metabonomics: a platform for studying drug toxicity and gene function. *Nat Rev Drug Discov* 2002; **1**: 153-161 [PMID: 12120097 DOI: 10.1038/nrd728]
5 **Verma M**, Khoury MJ, Ioannidis JP. Opportunities and challenges for selected emerging technologies in cancer epidemiology: mitochondrial, epigenomic, metabolomic, and telomerase profiling. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 189-200 [PMID: 23242141]
6 **Monteiro MS**, Carvalho M, Bastos ML, Guedes de Pinho P. Metabolomics analysis for biomarker discovery: advances and challenges. *Curr Med Chem* 2013; **20**: 257-271 [PMID: 23210853]
7 **Davis VW**, Schiller DE, Eurich D, Sawyer MB. Urinary metabolomic signature of esophageal cancer and Barrett's esophagus. *World J Surg Oncol* 2012; **10**: 271 [PMID: 23241138 DOI: 10.1186/1477-7819-10-271]
8 **Xiao JF**, Varghese RS, Zhou B, Nezami Ranjbar MR, Zhao Y, Tsai TH, Di Poto C, Wang J, Goerlitz D, Luo Y, Cheema AK, Sarhan N, Soliman H, Tadesse MG, Ziada DH, Ressom HW. LC-MS based serum metabolomics for identification of hepatocellular carcinoma biomarkers in Egyptian cohort. *J Proteome Res* 2012; **11**: 5914-5923 [PMID: 23078175 DOI: 10.1021/pr300673x]
9 **Wang X**, Zhang A, Sun H. Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. *Hepatology* 2013; **57**: 2072-2077 [PMID: 23150189 DOI: 10.1002/hep.26130]
10 **Wu H**, Xue R, Dong L, Liu T, Deng C, Zeng H, Shen X. Metabolomic profiling of human urine in hepatocellular carcinoma patients using gas chromatography/mass spectrometry. *Anal Chim Acta* 2009; **648**: 98-104 [PMID: 19616694 DOI: 10.1016/j.aca.2009.06.033]
11 **Tan Y**, Yin P, Tang L, Xing W, Huang Q, Cao D, Zhao X, Wang W, Lu X, Xu Z, Wang H, Xu G. Metabolomics study of stepwise hepatocarcinogenesis from the model rats to patients: potential biomarkers effective for small hepatocellular carcinoma diagnosis. *Mol Cell Proteomics* 2012; **11**: M111.010694 [PMID: 22084000 DOI: 10.1074/mcp.M111.010694]
12 **Soga T**, Sugimoto M, Honma M, Mori M, Igarashi K, Kashikura K, Ikeda S, Hirayama A, Yamamoto T, Yoshida H, Otsuka M, Tsuji S, Yatomi Y, Sakuragawa T, Watanabe H, Nihei K, Saito T, Kawata S, Suzuki H, Tomita M, Suematsu M. Serum metabolomics reveals γ-glutamyl dipeptides as biomarkers for discrimination among different forms of liver disease. *J Hepatol* 2011; **55**: 896-905 [PMID: 21334394 DOI: 10.1016/j.jhep.2011.01.031]
13 **Makarov A**, Scigelova M. Coupling liquid chromatography to Orbitrap mass spectrometry. *J Chromatogr A* 2010; **1217**: 3938-3945 [PMID: 20299023 DOI: 10.1016/j.chroma.2010.02.022]
14 **Trygg J**, Holmes E, Lundstedt T. Chemometrics in metabonomics. *J Proteome Res* 2007; **6**: 469-479 [PMID: 17269704]
15 **Eriksson L**, Johansson E, Kettaneh-Wold N, Wold S. Multi- and megavariate data analysis: principles and applications. Umetrics: Umea, 2001
16 **Gika HG**, Theodoridis GA, Wingate JE, Wilson ID. Within-day reproducibility of an HPLC-MS-based method for metabonomic analysis: application to human urine. *J Pro-*

teome Res 2007; **6**: 3291-3303 [PMID: 17625818 DOI: 10.1021/pr070183p]
17 **Dunn WB**, Broadhurst D, Brown M, Baker PN, Redman CW, Kenny LC, Kell DB. Metabolic profiling of serum using Ultra Performance Liquid Chromatography and the LTQ-Orbitrap mass spectrometry system. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; **871**: 288-298 [PMID: 18420470 DOI: 10.1016/j.jchromb.2008.03.021]
18 **Yin P**, Wan D, Zhao C, Chen J, Zhao X, Wang W, Lu X, Yang S, Gu J, Xu G. A metabonomic study of hepatitis B-induced liver cirrhosis and hepatocellular carcinoma by using RP-LC and HILIC coupled with mass spectrometry. *Mol Biosyst* 2009; **5**: 868-876 [PMID: 19603122 DOI: 10.1039/b820224a]
19 **Patel M**, Shariff MI, Ladep NG, Thillainayagam AV, Thomas HC, Khan SA, Taylor-Robinson SD. Hepatocellular carcinoma: diagnostics and screening. *J Eval Clin Pract* 2012; **18**: 335-342 [PMID: 21114800 DOI: 10.1111/j.1365-2753.2010.01599.x]
20 **Patterson AD**, Maurhofer O, Beyoglu D, Lanz C, Krausz KW, Pabst T, Gonzalez FJ, Dufour JF, Idle JR. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. *Cancer Res* 2011; **71**: 6590-6600 [PMID: 21900402 DOI: 10.1158/0008-5472.CAN-11-0885]
21 **Llovet JM**, Beaugrand M. Hepatocellular carcinoma: present status and future prospects. *J Hepatol* 2003; **38** Suppl 1: S136-S149 [PMID: 12591191]
22 **Pei Y**, Zhang T, Renault V, Zhang X. An overview of hepatocellular carcinoma study by omics-based methods. *Acta Biochim Biophys Sin* (Shanghai) 2009; **41**: 1-15 [PMID: 19129945]
23 **Khwaja A**, Silverman D, Sloan F, Wang Y. Are mature smokers misinformed? *J Health Econ* 2009; **28**: 385-397 [PMID: 19178971 DOI: 10.1016/j.jhealeco.2008.12.004]
24 **Ressom HW**, Xiao JF, Tuli L, Varghese RS, Zhou B, Tsai TH, Ranjbar MR, Zhao Y, Wang J, Di Poto C, Cheema AK, Tadesse MG, Goldman R, Shetty K. Utilization of metabolomics to identify serum biomarkers for hepatocellular carcinoma in patients with liver cirrhosis. *Anal Chim Acta* 2012; **743**: 90-100 [PMID: 22882828 DOI: 10.1016/j.aca.2012.07.013]
25 **Zhou L**, Wang Q, Yin P, Xing W, Wu Z, Chen S, Lu X, Zhang Y, Lin X, Xu G. Serum metabolomics reveals the deregulation of fatty acids metabolism in hepatocellular carcinoma and chronic liver diseases. *Anal Bioanal Chem* 2012; **403**: 203-213 [PMID: 22349331 DOI: 10.1007/s00216-012-5782-4]
26 **York B**, Sagen JV, Tsimelzon A, Louet JF, Chopra AR, Reineke EL, Zhou S, Stevens RD, Wenner BR, Ilkayeva O, Bain JR, Xu J, Hilsenbeck SG, Newgard CB, O'Malley BW. Research resource: tissue- and pathway-specific metabolomic profiles of the steroid receptor coactivator (SRC) family. *Mol Endocrinol* 2013; **27**: 366-380 [PMID: 23315938 DOI: 10.1210/me.2012-1324]
27 **Budczies J**, Denkert C, Müller BM, Brockmöller SF, Klauschen F, Györffy B, Dietel M, Richter-Ehrenstein C, Marten U, Salek RM, Griffin JL, Hilvo M, Orešič M, Wohlgemuth G, Fiehn O. Remodeling of central metabolism in invasive breast cancer compared to normal breast tissue - a GC-TOFMS based metabolomics study. *BMC Genomics* 2012; **13**: 334 [PMID: 22823888 DOI: 10.1186/1471-2164-13-334]
28 **Yang Y**, Li C, Nie X, Feng X, Chen W, Yue Y, Tang H, Deng F. Metabonomic studies of human hepatocellular carcinoma using high-resolution magic-angle spinning 1H NMR spectroscopy in conjunction with multivariate data analysis. *J Proteome Res* 2007; **6**: 2605-2614 [PMID: 17564425 DOI: 10.1021/pr070063h]
29 **Chen F**, Xue J, Zhou L, Wu S, Chen Z. Identification of serum biomarkers of hepatocarcinoma through liquid chromatography/mass spectrometry-based metabonomic method. *Anal Bioanal Chem* 2011; **401**: 1899-1904 [PMID: 21833635 DOI: 10.1007/s00216-011-5245-3]
30 **Chen T**, Xie G, Wang X, Fan J, Qiu Y, Zheng X, Qi X, Cao Y, Su M, Wang X, Xu LX, Yen Y, Liu P, Jia W. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteomics* 2011; **10**: M110.004945 [PMID: 21518826 DOI: 10.1074/mcp.M110.004945]

P- Reviewer Grizzi F **S- Editor** Zhai HH
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3433

World J Gastroenterol 2013 June 14; 19(22): 3433-3438
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Polysomnographic sleep aspects in liver cirrhosis: A case control study

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Supported by Grants from the Associação Fundo de Incentivo a Pesquisa and FAPESP-CEPID-Proc. 95/14303-3
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Received: September 19, 2012 Revised: January 22, 2013
Accepted: February 5, 2013
Published online: June 14, 2013

Abstract

AIM: To study sleep aspects and parameters in cirrhotic patients and assess the role of liver dysfunction severity in polysomnographic results.

METHODS: This was a case-control study. Patients with a diagnosis of liver cirrhosis were consecutively enrolled in the study. Clinical examinations and laboratory liver tests were performed in all patients, and disease severity was assessed using the Child-Pugh score. The control group consisted of age- and gender-matched healthy volunteers. All individuals answered a questionnaire about habits, behaviors, and complaints related to sleep and were submitted to polysomnography. Sleep parameters were compared between the two groups, and separate analyses were performed among classes of Child-Pugh classification in the cirrhotic group.

RESULTS: Forty-two cirrhotic patients and forty-two controls were enrolled. Compared to the control group, the cirrhotic group exhibited lower sleep efficiency (mean ± SD: 73.89% ± 14.99% *vs* 84.43% ± 8.55%, $P < 0.01$), increased latency (151.27 ± 93.24 min *vs* 90.62 ± 54.74 min, $P < 0.01$) and a lower percentage of rapid eye movement (REM) sleep (14.04% ± 5.64% *vs* 20.71% ± 6.77%, $P < 0.05$) as well as a higher frequency of periodic limb movements (10.56 ± 2.85/h *vs* 2.79 ± 0.61/h, $P < 0.01$). The comparison of sleep parameters among Child A, B and C cirrhotic patients revealed a significant reduction of REM sleep stage occurrence in individuals with severe liver disease (Child C patients) compared to Child A/B patients (polysomnography percentage of REM sleep stage of patients Child A: 16.1% ± 1.2%; Child B: 14.9% ± 1.2%; Child C: 8.6% ± 1.6%, $P < 0.05$).

CONCLUSION: Cirrhosis was associated with shorter sleep time, reduced sleep efficiency, increased sleep latency, increased REM latency and reduced REM sleep. Additionally, disease severity influences sleep parameters.

Key words: Liver cirrhosis; Sleep; Child-Pugh classification; Polysomnography; Rapid eye movement sleep; Periodic limb movements in sleep; Apnea-hypopnea index; Obstructive sleep apnea syndrome

Teodoro VV, Júnior MAB, Lucchesi LM, Cavignolli D, de Mello MT, Kondo M, Tufik S. Polysomnographic sleep aspects in liver cirrhosis: A case control study. *World J Gastroenterol* 2013; 19(22): 3433-3438 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3433.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3433

INTRODUCTION

Although modern sleep research has a short forty-year history, interest in the impact of sleep quality on bio-regulatory functions and human health dates back to ancient times[1-3]. The development of polysomnography (PSG) provided the ability to assess sleep structure and led to thorough characterizations of different sleep stages and sleep disorders[4]. The rapid eye movement (REM) sleep stage has acquired increasing relevance; it is considered fundamental to the maintenance of important intellectual functions such as memory, attention and mood[5]. The REM stage is characterized by cortical activation; this is evidenced by a rapid transition to higher frequency rhythms with rapid, low-voltage, irregular activity on electroencephalogram (EEG). It is also characterized by irregular breathing, heart rate and muscle atonia[2]. Time spent in REM sleep is markedly reduced in several organic dysfunctions and is associated with cardiovascular adverse events, such as systemic arterial hypertension[6-8]. Similarly, spontaneous or induced REM sleep deprivation was previously correlated with higher death rates[9].

Sleep disturbances are commonly reported in liver cirrhosis (LC), particularly in patients with hepatic encephalopathy (HE)[10,11]. There are few clinical[12,13] and experimental[14] studies assessing the sleep patterns of LC patients without overt HE; however, to our knowledge, an approach based on full-night PSG (level 1) has never been performed[15-17].

A study that used sleep questionnaires, neuropsychological tests and actigraph monitoring suggested that sleep complaints are an early sign of HE[17] and that insomnia and excessive daytime sleepiness (EDS) are often described in patients with liver disease[10,11,13,17]. Conversely, Vignatelli *et al*[18] found only a small percentage of cirrhotic patients with EDS. Although clinical reports vary, one possible explanation for sleep dysfunction in LC patients is a disruption in melatonin circadian rhythms[13,14]; one study reported a delayed onset and peak of melatonin secretion, which caused a sleep phase delay and possibly EDS[19,20].

Detailed information regarding PSG sleep aspects in LC patients is lacking. Moreover, no study to date has attempted to determine the influence of disease severity on sleep structure. This study aimed to characterize sleep patterns of LC patients using a full-night PSG-based approach focusing on the following: (1) sleep structure in LC; (2) sleep pattern variations associated with liver disease severity; and (3) detection of possible sleep disorders linked to LC. We then conducted a case-control study to compare PSG sleep aspects between LC patients and healthy volunteers.

MATERIALS AND METHODS

Clinical and laboratory assessment

Patients with a diagnosis of LC by either liver biopsy or analysis of clinical and laboratory data were enrolled in the cirrhotic group. They were invited to participate in the study upon reporting to the Gastroenterology Outpatient Clinic of the Universidade Federal de São Paulo (UNIFESP). The exclusion criteria were the following: younger than 18 years, alcohol consumption or gastrointestinal bleeding in the last 6 mo, serum creatinine levels higher than 2.0 mg/dL, psychoactive drug intake in the last 2 wk and overt clinical HE in the initial assessment. Those who fulfilled the selection criteria had venous blood drawn, and a clinical evaluation was performed to determine liver disease severity according to the Child-Pugh score. At the same time, arterial blood was collected to determine arterial ammonia levels. LC etiology was determined in all patients. Selected patients were submitted to a full-night PSG examination in the same week as the initial assessment.

The control group was composed of age- and gender-matched volunteers from the UNIFESP-Instituto do Sono laboratories, who met the criteria listed above and were considered healthy according to clinical and laboratorial evaluations. They also completed a PSG examination.

The research protocol was reviewed and approved by the institutional research ethics committee (protocol number 1503/04), and all participants provided informed consent before enrollment in the study.

PSG

All PSG recordings were performed in the Instituto do Sono. Before PSG, all study participants completed a questionnaire about habits, behaviors, and complaints related to sleep. The questionnaire was developed by the internal staff of the Instituto do Sono[21] and validated for the local population.

Full-night PSGs were performed in all participants using the sleep laboratory digital system EMBLA S7000® (Embla Systems Inc., Broomfield, CO, United States). The subjects were instructed to go to sleep at their usual bedtime. The following physiological variables were simultaneously and continuously recorded during PSG: (1) four channels to EEG; (2) two channels to electro-oculogram; (3) channels placed in the submentonian region, the anterior tibial muscle, the masseter region and the seventh intercostal space to the electromyogram; (4) electrocardiogram; (5) two channels for airflow monitoring (one for the thermocouple and the other for nasal pressure measurement); (6) the detection of respiratory efforts of the thorax (one channel) and the abdomen (one channel) for inductance plethysmography; (7) snore monitoring; (8) one channel for monitoring body position; and (9) oxy-hemoglobin saturation measurement.

All PSG sessions were monitored by trained technicians and visually scored according to standardized criteria[22]. EEG arousals and leg movement episodes were scored according to the "Manual for Scoring Sleep and Associated Events"[23,24]; apnea and hypopnea episodes as well as other detected sleep events were also scored and classified using recognized rules[25].

The following PSG sleep parameters were recorded and systematically evaluated: (1) total recording time

Table 1 General characteristics of the two groups evaluated *n* (%) or mean ± SD

Variables	Control group (*n* = 42)	Cirrhotic group (*n* = 42)	*P* value
Age (yr)	48.4 ± 8.3	50.0 ± 8.5	0.32
Males	29 (69.0)	33 (78.5)	0.70
BMI (kg/m^2)	25.3 ± 3.4	26.3 ± 4.4	0.40
LC etiology			
Alcohol	-	15 (38)	-
HCV	-	12 (30)	-
Alcohol + HCV	-	8 (20)	-
HBV	-	2 (4)	-
Alcohol + HBV	-	1 (2)	-
Cryptogenic	-	4 (6)	-
Child-Pugh			
Child A	-	16 (38)	-
Child B	-	17 (40)	-
Child C	-	9 (22)	-

BMI: Body mass index; LC: Liver cirrhosis; HCV: Hepatitis C virus; HBV: Hepatitis B virus.

Table 2 Polysomnographic parameters of the two groups evaluated (mean ± SD)

Variables	Control group (*n* = 42)	Cirrhotic group (*n* = 42)	*P* value
TRT (min)	421.98 ± 38.20	445.65 ± 50.64	0.07
TST (min)	357.18 ± 53.42	329.67 ± 76.62	< 0.05
Wake (min)	51.86 ± 29.91	115.05 ± 67.92	< 0.01
SE	84.43% ± 8.55%	73.89% ± 14.99%	< 0.01
Sleep Lat (min)	13.39 ± 14.40	28.41 ± 29.28	< 0.01
REM Lat (min)	90.62 ± 54.74	151.27 ± 93.24	< 0.01
S1	5.14% ± 3.26%	5.90% ± 3.12%	0.77
S2	57.00% ± 8.73%	61.72% ± 7.38%	0.29
S3 + 4	17.31% ± 7.00%	18.32% ± 6.64%	0.73
REM	20.71% ± 6.77%	14.04% ± 5.64%	< 0.05
AHI	7.33 ± 1.01	5.16 ± 0.80	0.09
PLMS/h	2.79 ± 0.61	10.56 ± 2.85	< 0.01
Arousals/h	15.88 ± 5.46	11.78 ± 7.06	< 0.01
Mean SpO_2	94.80% ± 1.62%	94.38% ± 2.05%	0.06
Nadir SpO_2	87.45% ± 5.24%	85.87% ± 8.98%	< 0.05

TRT: Total recording time; TST: Total sleep time; Wake: Minutes awake after sleep onset; SE: Sleep efficiency; Sleep Lat: Latency to sleep onset; REM: Rapid eye movement; REM Lat: REM sleep latency; S1: Stage 1; S2: Stage 2; S3: Stage 3; S3 + 4: Stages 3 and 4; AHI: Apnea-hypopnea index; PLMS/h: Number of periodic limbs movements of sleep per hour; Arousals/h: Number of arousals per hour of sleep; Mean SpO_2: Mean oxy-hemoglobin saturation; Nadir SpO_2: Minimal oxy-hemoglobin saturation.

(TRT): the entire period under PSG monitoring; (2) total sleep time (TST): the entire PSG recorded while sleeping; (3) wake: the entire PSG recorded while the patient was awake; (4) sleep efficiency: the TST/TRT ratio, expressed as percentage; (5) sleep latency: the length of time to sleep onset; (6) latency to REM sleep: the latency of REM sleep stage onset; (7) sleep stages 1, 2, 3 + 4 and REM sleep stage (S1, S2, S3 + 4 and REM): the percentage (%) of time patients spent in sleep stages 1, 2, 3 + 4 or REM sleep stage, respectively; (8) apnea-hypopnea index (AHI): an index to express the mean number of apneas or hypopneas in a 1-h period; (9) periodic leg movements of sleep per hour (PLMS/h): the average number of PLM events in a one hour period; (10) arousals/h: the average number of arousals in a one hour period; (11) mean SpO_2: the mean oxy-hemoglobin saturation; and (12) nadir SpO_2: minimal oxy-hemoglobin saturation recorded during PSG. These parameters were analyzed and scored by a blinded specialist before between-groups comparisons were made.

Statistical analysis

Statistical analyses were performed using STATISTICA software, version 5.1. The Student's *t*-test was used to compare the quantitative PSG parameters between groups. Analysis of variance and Tukey's *post-hoc* test were used to compare the sleep parameters among the three classes of liver disease according to the Child-Pugh score. Results were considered statistically significant if $P < 0.05$.

RESULTS

Forty-two cirrhotic patients and forty-two controls who satisfied the selection criteria were evaluated between May 2003 and August 2005. The cirrhotic group consisted of 29 males (69%) with a mean age of 50.0 ± 8.5 years, and the general demographic characteristics of cirrhotic patients did not differ significantly from those of the control group (Table 1). LC etiology and Child-Pugh scores are also shown in Table 1. The arterial ammonia measurements in cirrhotic patients were (mean ± SD) 166.38 ± 26.10 mol/L (normal value: 9-33 mol/L).

LC patients reported more sleep difficulties on the questionnaire, including trouble initiating sleep, non-restorative sleep and more episodes of napping during the day. Although the TRT and AHI were similar in both groups, a significant reduction in the TST, sleep efficiency and wake time were observed in the cirrhotic group. Increased sleep latency, REM latency, PLMS index and a decreased REM sleep percentage were also observed in the cirrhotic group (Table 2).

Comparison of sleep parameters among Child A, B and C cirrhotic patients revealed a significant reduction of REM sleep stage occurrence in individuals with severe liver disease (Child C patients) (Figure 1).

To assess the influence of alcoholism on sleep parameters, we divided the patients into two groups: those who had alcoholic etiology (alone or associated with viral hepatitis; $n = 24$) and those who had no history of alcoholism ($n = 18$). No significant differences were detected between the two groups in our study for any sleep parameter except sleep latency, which was longer in the group without alcoholic etiology (21.68 ± 15.38 min *vs* 40.53 ± 42.74 min for the alcoholic etiology group and without alcoholic etiology group respectively, $P = 0.04$).

DISCUSSION

The sleep parameters evaluated by PSG in this study indicated worsening sleep in the cirrhotic group compared to the control group. This was due to decreased sleep

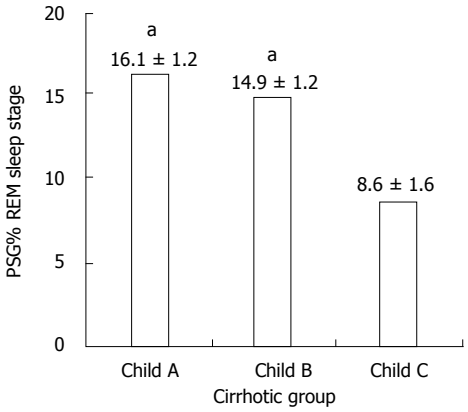

Figure 1 Rapid eye movement sleep stage percentage comparison among different Child-Pugh score system classes. [a]*P* < 0.05 *vs* Child C group. Child A, B and C: Liver disease severity classes; PSG: Polysomnography; REM: Rapid eye movement.

efficiency, increased time to initiate sleep, increased latency of REM sleep, reduced REM sleep percentage and higher PLMS indices. As our data suggest, disease severity influenced sleep parameters, especially when data were classified by the patients' Child scale rating.

The sleep questionnaire results showed that the cirrhotic patients had more complaints about their sleep, such as difficulty initiating sleep and non-restorative sleep. These findings are consistent with the PSG results indicating lower sleep efficiency and higher sleep latency. Other studies have also described a higher number of sleep complaints in LC patients[12,26]. Moreover, LC patients report more episodes of napping during the day[10,19,27]. EDS appears to be attributable to a dysfunction of the neural circuit responsible for the maintenance of wakefulness and sleep states. The monoaminergic system, the systems of the locus coeruleus (noradrenergic) and the raphe nuclei (serotonergic) are important for directing attention and recruiting the cortex for processing external sensory stimuli[28]. High levels of ammonia can reduce serotonin and noradrenaline levels in the central nervous system, resulting in low alertness and attention[29,30].

The higher incidence of PLMS in our sample of LC patients was not related to any clinical disorder or laboratory finding commonly associated with this phenomenon. This would include conditions such as anemia, renal failure, or low levels of iron or plasma transferritin[31]. PLMS is also associated sleep complaints, including difficulty falling asleep, multiple arousals and EDS[32,33]. These complaints were also reported by our group of patients and objectively confirmed by the PSG findings of higher sleep latency and higher arousal index.

This study did not find statistically significant differences between groups regarding AHI. This is in contrast with two previous studies[15,26]; however, it is consistent with the findings of Nikaina *et al*[16]. Differing results may be explained by the presence or absence of ascites[34]. In fact, Nikaina *et al*[16] found no significant differences in the index of respiratory events between controls and patients with compensated cirrhosis without ascites.

It is widely recognized that chronic alcohol abuse influences sleep parameters[35] and many patients in this study experienced cirrhosis of alcoholic etiology. However, in our study, we did not confirm this influence, perhaps due to the fact that patients had been in withdrawal for at least six months. The absence of differences suggests that LC may be seen as a determining factor of the sleep parameters observed for study in this group. The only difference that was found, in sleep latency, which was longer in the group without alcoholic etiology, cannot be easily explained; considering the effects of alcohol, the expected result would be the opposite of what was actually observed[35].

The intense electrical and metabolic activity observed in REM sleep is the best argument supporting sleep as an active phenomenon[2,3]. The system responsible for the generation of REM sleep encompasses several specific nerve structures and follows a model of reciprocal interaction between the co-organizers and suppressor structures, which are located mainly in the brainstem and midbrain[2,3]. In the current study, both the latency and percentage of REM sleep of LC patients differed from those of controls, suggesting the hyperfunction of REM sleep suppressor mechanisms. Evidence from sleep deprivation studies suggests a role for dopamine during REM sleep[36], and these studies have described the relationship of REM sleep in terms of dopamine D2 receptors[37]. Specific changes in the dopamine receptors of the brain of LC patients have been previously described[38,39]. Dopamine receptor dysfunction is associated with low concentrations of serotonin, which may suppress REM sleep and constitute a possible explanation for the PSG findings in our LC group.

It is possible that our findings of sleep impairment in subjects with cirrhosis are common to all forms of metabolic encephalopathy. However, our study's strength resides in the fact that we found differences in both subjective and objective parameters. In our observational study, LC patients had longer sleep latencies, shorter sleep time, worse sleep efficiency, increased REM sleep latencies and lower REM sleep percentages. The latter finding was negatively related to the severity of the disease. Therefore, we need to draw clinician`s attention to the importance of sleep complaints and parameters regarding prognosis in cirrhotic patients.

ACKNOWLEDGMENTS

The authors would like to thank Altay Lino de Souza and Ana Amelia Benedito Silva for their valuable suggestions and statistical analyses. All the efforts of the Associação Fundo de Incentivo a Pesquisa staff, particularly those of the sleep technicians, are deeply appreciated.

COMMENTS

Background

Liver cirrhosis (LC) is currently a serious clinical problem, and is considered a very common disease with a major impact on public health worldwide. It is a

chronic and irreversible process characterized by progressive replacement of the normal structure of the liver by fibrosis as a response to a continuous injury to this organ, leading to various clinical consequences, as alterations in sleep. In fact, sleep disturbances are commonly reported in LC.

Research frontiers

Polysomnography provided the ability to assess sleep structure and led to a thorough characterization of sleep stages and sleep disorders. Sleep is divided into rapid eye movement (REM) and non-REM sleep stages. REM sleep has acquired increasing relevance because it is considered fundamental to the maintenance of important intellectual functions, such as memory, attention and mood. Time spent in REM is markedly reduced in several organic dysfunctions and is associated with cardiovascular adverse events, such as systemic arterial hypertension.

Innovations and breakthroughs

There is a lack of detailed information regarding polysomnographic sleep aspects in patients with LC. Moreover, no study has attempted to determine the influence of disease severity on sleep structure. This study aimed to characterize the sleep patterns of patients with LC with a full-night polysomnographic based approach focusing on the following: (1) sleep structure in LC; (2) sleep pattern variations associated with liver disease severity; and (3) detection of possible sleep disorders linked to LC. Authors then compared polysomnographic sleep aspects between patients with LC and healthy volunteers.

Applications

The study results suggest that hepatic cirrhosis was associated with shorter sleep time, reduced sleep efficiency, increased sleep latency, increased REM latency and reduced REM sleep. Additionally, disease severity influenced sleep parameters. Therefore, authors need to draw clinician's attention to the importance of sleep complaints and parameters regarding prognosis in cirrhotic patients.

Peer review

In this paper, the authors study sleep aspects and parameters in cirrhotic patients and assess the role of liver dysfunction severity on polysomnographic results. This study is interesting and suggests that cirrhosis is associated with sleep disturbances and that disease severity influences sleep parameters.

REFERENCES

1 **Dement WC.** History of sleep physiology and medicine. In: Kriger MH, Roth T, Dement WC, editors. Principles and practice of sleep medicine. 5th ed. Philadelphia: WB Saunders, 2011

2 **Swick TJ.** The neurobiology of sleep. *Sleep Med Clin* 2011; **6:** 1-15 [DOI: 10.1016/j.jsmc.2010.12.009]

3 **España RA**, Scammell TE. Sleep neurobiology for the clinician. *Sleep* 2004; **27**: 811-820 [PMID: 15283019]

4 **Carskadon MA.** Basics for polygraphic monitoring of sleep. In: Guilleminault C, editor. Sleeping and walking disorders: indications and techniques. Boston: Butterworth Publishers, 1982: 1-16

5 **Wamsley EJ,** Stickgold R. Memory, Sleep and Dreaming: Experiencing Consolidation. *Sleep Med Clin* 2011; **6**: 97-108 [PMID: 21516215 DOI: 10.1016/j.jsmc.2010.12.008]

6 **Radulovacki M**, Trbovic SM, Carley DW. Cardiopulmonary interactions following REM sleep deprivation in Sprague-Dawley rats. *Exp Neurol* 1997; **145**: 371-375 [PMID: 9217073 DOI: 10.1006/exnr.1997.6460]

7 **Endeshaw YW**, Bloom HL, Bliwise DL. Sleep-disordered breathing and cardiovascular disease in the Bay Area Sleep Cohort. *Sleep* 2008; **31**: 563-568 [PMID: 18457244]

8 **Wolk R**, Somers VK. Cardiovascular consequences of obstructive sleep apnea. *Clin Chest Med* 2003; **24**: 195-205 [PMID: 12800778 DOI: 10.1016/S0272-5231(03)00020-0]

9 **Rechtschaffen A**, Gilliland MA, Bergmann BM, Winter JB. Physiological correlates of prolonged sleep deprivation in rats. *Science* 1983; **221**: 182-184 [PMID: 6857280 DOI: 10.1126/science.6857280]

10 **Baldy-Moulinier M**, Besset A, Calvet B, Michel H. [24 hour polygraphic study of the waking-up and falling asleep periods in patients with hepatic encephalopathy (author's transl)]. *Rev Electroencephalogr Neurophysiol Clin* 1981; **11**: 123-132 [PMID: 7313246]

11 **Groeneweg M**, Quero JC, De Bruijn I, Hartmann IJ, Essinkbot ML, Hop WC, Schalm SW. Subclinical hepatic encephalopathy impairs daily functioning. *Hepatology* 1998; **28**: 45-49 [PMID: 9657095 DOI: 10.1002/hep.510280108]

12 **Córdoba J**, Cabrera J, Lataif L, Penev P, Zee P, Blei AT. High prevalence of sleep disturbance in cirrhosis. *Hepatology* 1998; **27**: 339-345 [PMID: 9462628 DOI: 10.1002/hep.510270204]

13 **Tarter RE**, Hegedus AM, Van Thiel DH, Schade RR, Gavaler JS, Starzl TE. Nonalcoholic cirrhosis associated with neuropsychological dysfunction in the absence of overt evidence of hepatic encephalopathy. *Gastroenterology* 1984; **86**: 1421-1427 [PMID: 6714571]

14 **Jiménez-Anguiano A**, Díaz-Medina V, Farfán-Labonne BE, Giono-Chiang G, Kersenobich D, García-Lorenzana M, Gutiérrez-Ruiz MC, Velázquez-Moctezuma J. Modification of sleep architecture in an animal model of experimental cirrhosis. *World J Gastroenterol* 2009; **15**: 5176-5180 [PMID: 19891016 DOI: 10.3748/wjg.15.5176]

15 **Ogata T**, Nomura M, Nakaya Y, Ito S. Evaluation of episodes of sleep apnea in patients with liver cirrhosis. *J Med Invest* 2006; **53**: 159-166 [PMID: 16538010 DOI: 10.2152/jmi.53.159]

16 **Nikaina I**, Pastaka C, Zachou K, Dalekos GN, Gourgoulianis K. Sleep apnoea syndrome and early stage cirrhosis: a pilot study. *Eur J Gastroenterol Hepatol* 2006; **18**: 31-35 [PMID: 16357616 DOI: 10.1097/00042737-20060100000006]

17 **Quero JC**, Schalm SW. Subclinical hepatic encephalopathy. *Semin Liver Dis* 1996; **16**: 321-328 [PMID: 8989817 DOI: 10.1055/s-2007-1007244]

18 **Vignatelli L**, Mattarozzi K, Zanatta C, Stracciari A. Cognitive function and Epworth Sleepiness Scale in 'minimal' hepatic encephalopathy. *Eur J Neurol* 2001; **8**: 369 [PMID: 11422439 DOI: 10.1046/j.1468-1331.2001.00238.x]

19 **Steindl PE**, Finn B, Bendok B, Rothke S, Zee PC, Blei AT. Disruption of the diurnal rhythm of plasma melatonin in cirrhosis. *Ann Intern Med* 1995; **123**: 274-277 [PMID: 7611593]

20 **Ardizzi A**, Grugni G, Saglietti G, Morabito F. [Circadian rhythm of melatonin in liver cirrhosis]. *Minerva Med* 1998; **89**: 1-4 [PMID: 9561018]

21 **Bittencourt LR**, Silva RS, Santos RF, Pires ML, Mello MT. [Excessive daytime sleepiness]. *Rev Bras Psiquiatr* 2005; **27** Suppl 1: 16-21 [PMID: 16082450 DOI: 10.1590/S1516-44462005000500004]

22 **Rechtschaffen A,** Kales A. Manual of standardized terminology, techniques, and scoring system for sleep stages of human subjects. Los Angeles: Brain Information Service/Brain Research Institute/UCLA, 1968

23 EEG arousals: scoring rules and examples: a preliminary report from the Sleep Disorders Atlas Task Force of the American Sleep Disorders Association. *Sleep* 1992; **15**: 173-184 [PMID: 11032543]

24 Recording and scoring leg movements. The Atlas Task Force. *Sleep* 1993; **16**: 748-759 [PMID: 8165390]

25 Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. The Report of an American Academy of Sleep Medicine Task Force. *Sleep* 1999; **22**: 667-689 [PMID: 10450601]

26 **Crespo J**, Cifrián J, Pinto JA, Jiménez-Gómez A, Pons-Romero F. Sleep apnea obstructive syndrome: a new complication previously undescribed in cirrhotic patients with ascites. *Am J Gastroenterol* 2003; **98**: 2815-2816 [PMID: 14687850 DOI: 10.1016/j.amjgastroenterol.2003.09.024]

27 **Mostacci B**, Ferlisi M, Baldi Antognini A, Sama C, Morelli C, Mondini S, Cirignotta F. Sleep disturbance and daytime sleepiness in patients with cirrhosis: a case control study. *Neurol Sci* 2008; **29**: 237-240 [PMID: 18810597 DOI: 10.1007/s10072-008-0973-7]

28 **Oken BS**, Salinsky MC, Elsas SM. Vigilance, alertness, or

sustained attention: physiological basis and measurement. *Clin Neurophysiol* 2006; **117**: 1885-1901 [PMID: 16581292 DOI: 10.1016/j.clinph.2006.01.017]
29 **Baldessarini RJ**, Fischer JE. Serotonin metabolism in rat brain after surgical diversion of the portal venous circulation. *Nat New Biol* 1973; **245**: 25-27 [PMID: 4516938]
30 **Lozeva-Thomas V**. Serotonin brain circuits with a focus on hepatic encephalopathy. *Metab Brain Dis* 2004; **19**: 413-420 [PMID: 15554431]
31 **Montplaisir J,** Michaud M, Lavigne GJ. Periodic limb movements in sleep. In: Chokroverty S, Hening WA, Walters AS, editors. Sleep and movement disorders. Philadelphia: Butterworth Heinemann, 2003
32 **Esteves AM**, de Mello MT, Pradella-Hallinan M, Tufik S. Effect of acute and chronic physical exercise on patients with periodic leg movements. *Med Sci Sports Exerc* 2009; **41**: 237-242 [PMID: 19092683 DOI: 10.1249/MSS.0b013e318183bb22]
33 **Nicolas A**, Lespérance P, Montplaisir J. Is excessive daytime sleepiness with periodic leg movements during sleep a specific diagnostic category? *Eur Neurol* 1998; **40**: 22-26 [PMID: 9693228 DOI: 10.1159/000007951]
34 **Saleh AM,** Mohamed H, El Bendary M, lsayad S. Sleep disorder breathing in liver cirrhosis: a cross sectional study based on child classification. *Sleep* 2008; **31:** A497
35 **Brower KJ**. Insomnia, alcoholism and relapse. *Sleep Med Rev* 2003; **7**: 523-539 [PMID: 15018094 DOI: 10.1053/smrv.2002.0248]
36 **Nunes Júnior GP**, Tufik S, Nobrega JN. Autoradiographic analysis of D1 and D2 dopaminergic receptors in rat brain after paradoxical sleep deprivation. *Brain Res Bull* 1994; **34**: 453-456 [PMID: 8082038 DOI: 10.1016/0361-9230(94)90018-3]
37 **Dzirasa K**, Ribeiro S, Costa R, Santos LM, Lin SC, Grosmark A, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA. Dopaminergic control of sleep-wake states. *J Neurosci* 2006; **26**: 10577-10589 [PMID: 17035544 DOI: 10.1523/JNEUROSCI.1767-06.2006]
38 **Weissenborn K**, Berding G, Köstler H. Altered striatal dopamine D2 receptor density and dopamine transport in a patient with hepatic encephalopathy. *Metab Brain Dis* 2000; **15**: 173-178 [PMID: 11206586 DOI: 10.1007/BF02674526]
39 **Watanabe Y**, Kato A, Sawara K, Butterworth RF, Sasaki T, Terasaki K, Sera K, Suzuki K. Selective alterations of brain dopamine D(2) receptor binding in cirrhotic patients: results of a (11)C-N-methylspiperone PET study. *Metab Brain Dis* 2008; **23**: 265-274 [PMID: 18686022 DOI: 10.1007/s11011-008-9092-7]

P- Reviewer Yang YF **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3439

World J Gastroenterol 2013 June 14; 19(22): 3439-3446
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Deep sedation during gastrointestinal endoscopy: Propofol-fentanyl and midazolam-fentanyl regimens

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Received: November 3, 2012 Revised: December 22, 2012
Accepted: January 11, 2013
Published online: June 14, 2013

Abstract

AIM: To compare deep sedation with propofol-fentanyl and midazolam-fentanyl regimens during upper gastrointestinal endoscopy.

METHODS: After obtaining approval of the research ethics committee and informed consent, 200 patients were evaluated and referred for upper gastrointestinal endoscopy. Patients were randomized to receive propofol-fentanyl or midazolam-fentanyl (n = 100/group). We assessed the level of sedation using the observer's assessment of alertness/sedation (OAA/S) score and bispectral index (BIS). We evaluated patient and physician satisfaction, as well as the recovery time and complication rates. The statistical analysis was performed using SPSS statistical software and included the Mann-Whitney test, χ^2 test, measurement of analysis of variance, and the κ statistic.

RESULTS: The times to induction of sedation, recovery, and discharge were shorter in the propofol-fentanyl group than the midazolam-fentanyl group. According to the OAA/S score, deep sedation events occurred in 25% of the propofol-fentanyl group and 11% of the midazolam-fentanyl group (P = 0.014). Additionally, deep sedation events occurred in 19% of the propofol-fentanyl group and 7% of the midazolam-fentanyl group according to the BIS scale (P = 0.039). There was good concordance between the OAA/S score and BIS for both groups (κ = 0.71 and κ = 0.63, respectively). Oxygen supplementation was required in 42% of the propofol-fentanyl group and 26% of the midazolam-fentanyl group (P = 0.025). The mean time to recovery was 28.82 and 44.13 min in the propofol-fentanyl and midazolam-fentanyl groups, respectively (P < 0.001). There were no severe complications in either group. Although patients were equally satisfied with both drug combinations, physicians were more satisfied with the propofol-fentanyl combination.

CONCLUSION: Deep sedation occurred with propofol-fentanyl and midazolam-fentanyl, but was more frequent in the former. Recovery was faster in the propofol-fentanyl group.

Key words: Endoscopy; Deep sedation; Anesthetic administration; Anesthetic dose; Adverse effects

Lera dos Santos ME, Maluf-Filho F, Chaves DM, Matuguma SE, Ide E, Luz GO, de Souza TF, Pessorrusso FCS, de Moura EGH, Sakai P. Deep sedation during gastrointestinal endoscopy: Propofol-fentanyl and midazolam-fentanyl regimens. *World J Gastroenterol* 2013; 19(22): 3439-3446 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3439.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3439

INTRODUCTION

The routine use of sedation during endoscopic procedures is increasing worldwide[1,2]. In a survey conducted in the United States in 2006, > 98% of endoscopies and colonoscopies were performed under sedation[1,3]. A similar trend has been observed in Switzerland, Germany, and Australia[2,4-7]. The combination of a benzodiazepine and opioid is reportedly used at approximately 75% of all healthcare facilities in the United States[1,3] and considered the combination of choice by most endoscopists worldwide[1,2,4-9]. As a result of its anxiolytic and sedative properties, its ability to provide anterograde amnesia, and its short half-life, midazolam is the most widely used benzodiazepine. Fentanyl is the most widely used opioid, although meperidine is still frequently used[2-4,8,9].

Propofol is a hypnotic agent that induces anesthesia almost immediately and has a short half-life. It also allows the patient to recover rapidly and be discharged. Patient and physician satisfaction is high with propofol. As a result of these properties, the use of propofol has been adopted at endoscopy centers worldwide[1-7,10]. However, its use has also been associated with deep sedation[11-14].

During endoscopy, sedation and analgesia improve the efficiency of the procedure, quality of the results, and comfort of the patient[5,8]. However, sedation is also responsible for the majority of complications related to diagnostic endoscopy[15]. During sedation and analgesia, there is a continuum of states, ranging from mild sedation to general anesthesia. In the middle of this continuum is conscious sedation, which is the target level of sedation for patients undergoing upper or lower gastrointestinal endoscopy[4,5,10,11,13,14,16-19]. A level of sedation deeper than that intended is associated with a higher rate of complications[11,15,20]. The guidelines established in 2002 by the American Society of Anesthesiologists (ASA) task force on sedation and analgesia by non-anesthesiologists, which have also been endorsed by the American Society for Gastrointestinal Endoscopy, recommend that a distinction be made between conscious sedation and deep sedation and that one professional be dedicated to the assessment and monitoring of patients during sedation[11-13].

The level of consciousness is typically assessed with the subjective clinical scale known as the observer's assessment of alertness/sedation (OAA/S) score, as validated by Chernik *et al*[21], which ranks sedation as mild, conscious, or deep. Another means of assessing the level of consciousness is the calculation of the bispectral index (BIS). Through the use of a BIS monitor, complex mathematical calculations of electroencephalography waves are transformed into numbers ranging from 0 (no brain activity) to 100 (fully conscious). This provides an objective measure of the level of consciousness. The BIS is considered a viable alternative for monitoring the level of consciousness in patients submitted to general anesthesia. Although its use in endoscopy is controversial, it has been investigated with increasing frequency, and further studies are recommended[22-27].

In the past 10 years, new sedation guidelines have been established. Many of those guidelines state that propofol can be safely administered by an endoscopist or nurse under the supervision of a physician[1,3-5,8,10,12,14,17,25,28,29]. However, because propofol has been associated with deep sedation events and complications, some have recommended that propofol be administered exclusively by anesthesiologists[5,11-13,17].

In view of these facts, we evaluated the use of propofol-fentanyl versus midazolam-fentanyl for sedation of patients undergoing upper gastrointestinal endoscopy. The primary endpoint of this study was to compare the frequency of deep sedation in each group. We also compared the two drug combinations in terms of time to induction, time to recovery, time to discharge, efficacy, and safety, as well as patient and endoscopist satisfaction.

MATERIALS AND METHODS

This was a prospective, single-blind, randomized controlled trial carried out between January 2007 and October 2010 at the Gastrointestinal Endoscopy Clinic of the Department of Gastroenterology at the University of São Paulo Medical School - Hospital das Clínicas, Brazil.

Patients

We recruited 262 patients from those scheduled to undergo upper gastrointestinal endoscopy at the Gastrointestinal Endoscopy Unit. The inclusion criteria were age > 18 years, physical status classified as ASA Ⅰ, Ⅱ or Ⅲ, and having a contact telephone number. The exclusion criteria were as follows: pregnancy; a history of allergy to the medications to be administered; a history of allergy to soy or eggs; a psychotic disorder; being under treatment with psychoactive medications; being an illicit drug user or a heavy consumer of alcohol; Child-Pugh class C cirrhosis; presence of chronic kidney disease (being on dialysis); and being submitted to endoscopy as an emergency procedure. Of the 262 patients recruited, 62 were excluded (Figure 1). The final sample comprised 200 patients. Through a drawing of sealed envelopes, patients were randomized to two groups of 100: propofol-fentanyl and midazolam-fentanyl. The endoscopists scheduled to perform the procedures had no access to the envelopes.

Drug administration

Drug infusion was performed by the nursing staff and attending endoscopist. In both groups, the objective was to

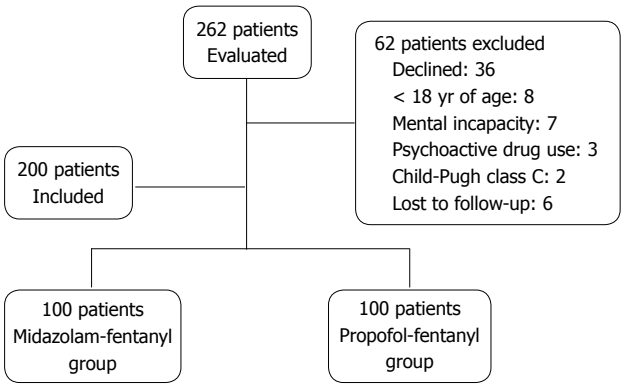

Figure 1 Flowchart of the study design.

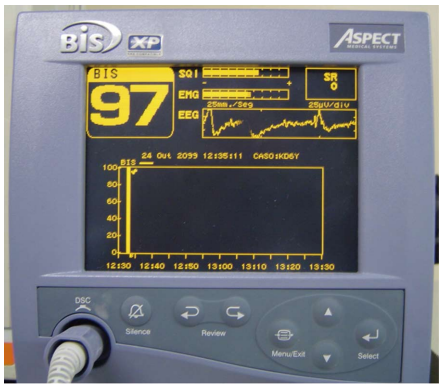

Figure 2 Bispectral index monitor.

achieve conscious sedation by using the dose calculated for that purpose (Table 1). The propofol and midazolam were administered by intravenous bolus and supplemented as necessary by the endoscopist to maintain the desired level of sedation. A single dose of fentanyl was used in both groups. The fixed maximum dose for midazolam was 10 mg or 0.1 mg/kg of body weight. If additional sedation became necessary, the endoscopist had the option of suspending the initial regimen and adding propofol.

Patient evaluation and monitoring

Prior to the procedure, a clinical history and physical examination was performed for each patient. Additionally, the anesthetic risk was assessed with the ASA classification of physical status, and the patients completed a demographic questionnaire. Continuous monitoring during the procedure included the noninvasive measurement of blood pressure, heart rate, respiratory rate (thoracic excursion measurement), and oxygen saturation (SpO_2). We defined the following evaluation time points: "baseline" (immediately before the procedure); "duodenum" (the approximate midpoint of the procedure, when the endoscopist was evaluating the duodenum or the jejunal loop in post-gastrectomy patients); and "recovery" (when the patient was awake and underwent the final evaluation).

Evaluation of the level of sedation

During the procedure, the level of sedation was evaluated in two ways. We applied the OAA/S scale[21], which is scored as 1 for deep sedation, 2-4 for conscious sedation, and 5 for mild sedation. In addition, after cleaning the skin with gauze and alcohol, we applied disposable electrodes to the forehead and connected the leads to a BIS monitor (A-2000 BIS XP; Aspect Medical Systems, Newton, MA, United States) (Figure 2). The BIS monitor output was evaluated continuously throughout the procedure and recovery period. BIS $\leqslant$ 65 indicates deep sedation, 66-85 indicates conscious sedation, and BIS > 85 indicates mild sedation. The OAA/S scale and BIS were determined simultaneously every 2 min.

An independent observer was responsible for the monitoring, which included evaluating the level of consciousness, readout of other vital signs, collection of data regarding drugs and doses used, use of benzodiazepine or opioid antagonists, and occurrence of cardiorespiratory events, such as hypoxemia (defined as SpO_2 < 90% for > 30 s after application of the jaw thrust maneuver), hypotension (defined as $\geqslant$ 20% decrease in systolic or diastolic blood pressure), and bradycardia (heart rate < 50 bpm). Hypoxemia was classified as mild if it responded to supplemental oxygen delivered at 3-4 L/min; it was classified as severe if it did not respond to supplemental oxygen and the patient required noninvasive ventilatory support (*e.g.*, bag-mask ventilation) or intubation. The same observer also reported any other adverse events that occurred secondary to sedation. The observer was blinded to the randomization.

As described by Cohen *et al*[30], we compared the two groups in terms of the time to induction (interval between the first drug bolus administration and initiation of the procedure), time to recovery (interval between removal of the endoscope and final evaluation), and time to discharge (interval between removal of the endoscope and departure from the endoscopy unit). The final evaluation began when the BIS monitor indicated at least 90. Patients were discharged only when they had achieved an OAA/S score of 5 (the maximum), a BIS > 90, and reported no pain or any other type of discomfort.

Visual analog scale and questionnaires

At discharge, patient satisfaction was assessed with a 10-point visual analog scale (1 = least satisfied and 10 = most satisfied). The patients also completed a satisfaction questionnaire before leaving the facility. At 24 h after discharge, the same observer who was responsible for the monitoring contacted the patients by telephone to administer a questionnaire that evaluated patient satisfaction with the procedure, adverse events and the resumption of domestic activities. All 200 patients completed both questionnaires. The visual analog scale was also applied to the endoscopists who performed the procedures to assess their level of satisfaction with the sedation regimen and was scored as follows: 1-3 = considerable difficulty in performing the procedure; 4-7 = minor difficulty in

Table 1 Sedation regimens

Midazolam-fentanyl	
Midazolam	
Initial dose	
ASA Ⅰ	3-5 mg
ASA Ⅱ or Ⅲ	2-3 mg
Maintenance	0.5-1.0 mg every 2-3 min, up to a maximum cumulative dose of 10 mg or 0.1 mg/kg of body weight
Fentanyl	
Single dose	
ASA Ⅰ	50 μg
ASA Ⅱ or Ⅲ	20-30 μg
Propofol-fentanyl	
Propofol	
Initial dose	
ASA Ⅰ	0.5 mg/kg
ASA Ⅱ or Ⅲ	0.25 mg/kg
Maintenance	10-20 mg bolus at 60 s intervals
Fentanyl	
Single dose	
ASA Ⅰ	50 μg
ASA Ⅱ or Ⅲ	20-30 μg

ASA: American Society of Anesthesiologists.

Table 2 Patient characteristics

Variable	Group		Total	*P* value
	Midazolam-fentanyl	Propofol-fentanyl		
Sex				
Female	66 (60.0)	71 (71.0)	137 (68.5)	0.543
Male	34 (34.0)	29 (29.0)	63 (31.5)	
ASA				
Ⅰ	63 (63.0)	55 (55.0)	118 (59.0)	0.316
Ⅱ	37 (37.0)	44 (44.0)	81 (40.5)	
Ⅲ	0 (0.0)	1 (0.5)	1 (0.5)	
Age (yr)	52.14 ± 15.01	54.40 ± 15.44		0.352
Weight (kg)	67.45 ± 11.28	70.93 ± 17.64		0.242
Height (cm)	1.62 ± 0.09	1.61 ± 0.10		0.546
BMI (kg/m^2)	25.91 ± 4.54	27.39 ± 6.59		0.251
DM proportion	4.5%	7.5%		0.276
SH proportion	16.0%	18.0%		0.640
Cardiopathy proportion	0.5%	0.0%		0.999
Other proportion	11.5%	9.0%		0.404

Data are expressed as absolute numbers (percentage) or mean ± SD. BMI: Body mass index; DM: Diabetes mellitus; SH: Systemic hypertension.

performing the procedure (patient moved at the beginning or end of the procedure); and 8-10 = no difficulty in performing the procedure.

Ethics

This work was conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The study was approved by the Hospital das Clínicas Research Ethics Committee, and all participating patients provided written informed consent.

Statistical analysis

To calculate the sample size, we estimated the proportion of patients who received deep sedation by analyzing the BIS curve. We hypothesized that this proportion would be 10% for the midazolam-fentanyl group and 25% for the propofol-fentanyl group. By adopting an α error tolerance (false-positive risk) of 5% and β error tolerance (false-negative risk) of 20%, we determined that 112 patients per group would be required to provide sufficient power to detect significant differences. It was agreed that we would perform an interim analysis involving 100 patients in each group.

The data were entered into a Microsoft Excel spreadsheet and analyzed with the assistance of the Statistics Sector of the University of São Paulo Medical School, Department of Gastroenterology. The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, United States). We applied the Mann-Whitney test to evaluate continuous variables and used the χ^2 test to evaluate categorical variables. To study the effects of the variable "group" at the various time points, we used repeated measures analyses of variance. We used the κ statistic to evaluate the degree of concordance between the OAA/S scale and BIS.

Data were collected by a researcher who was blinded to each patient's group. However, such masking was not possible when we assessed endoscopist satisfaction with the sedation regimen.

RESULTS

Patient characteristics

Most patients presented with a low anesthetic risk (ASA class Ⅰ or Ⅱ), although one patient in the propofol-fentanyl group was classified as ASA Ⅲ. There were no significant differences between the two groups regarding demographics, weight, height, body mass index, level of education, or ASA class (Table 2). All of the patients completed the procedure with adequate sedation throughout. None of the procedures were suspended or halted prematurely.

Drug doses

Patients in the midazolam-fentanyl group received midazolam and fentanyl at mean doses of 5.25 ± 1.7 mg and 43.1 ± 9.87 μg, respectively. Patients in the propofol-fentanyl group received propofol and fentanyl at mean doses of 70.3 ± 38.9 mg and 41.0 ± 10.25 μg, respectively. Sixty minutes after the end of the procedure, a patient in the midazolam-fentanyl group presented with persistent drowsiness, despite normal cardiorespiratory function, and was given 0.2 mg flumazenil.

Level of sedation

As seen in Table 3, the OAA/S classification of sedation in the midazolam-fentanyl group was mild in 1%, conscious in 88%, and deep in 11%, compared with 2%, 73%, and 25%, respectively, in the propofol-fentanyl group. There was a statistically significant difference between the two groups in terms of the OAA/S score (P = 0.014). Based on the BIS values, sedation in the midazolam-fentanyl and propofol-fentanyl groups was classified

Table 3 Level of sedation *n* (%)

Variable	Group		Total	*P* value
	Midazolam-fentanyl	Propofol-fentanyl		
OAA/S score				
1	11 (11.0)	25 (25.0)	36 (18.0)	0.014
2-4	88 (88.0)	73 (73.0)	161 (80.5)	
5	1(1.0)	2 (2.0)	3 (1.5)	
BIS				
≤ 65	7 (7.0)	19 (19.0)	26 (13.0)	0.039
66-85	75 (75.0)	67 (67.0)	142 (71.0)	
> 85	18 (18.0)	14 (14.0)	32 (16.0)	

OAA/S: Observer's assessment of alertness/sedation score; BIS: Bispectral index.

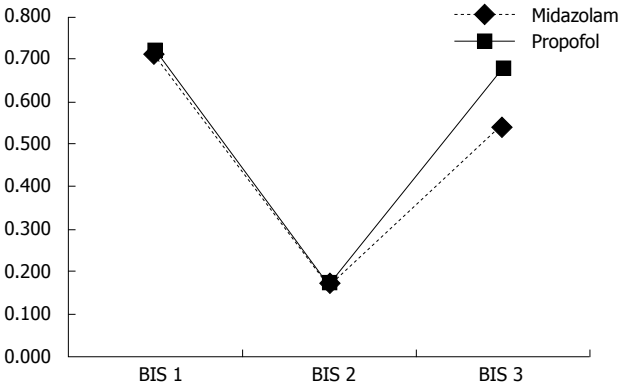

Figure 3 Bispectral values before, during, and after diagnostic upper gastrointestinal endoscopy according to group. Bispectral (BIS) 1: Before the procedure; BIS 2: During the procedure; BIS 3: After the procedure.

as mild in 18% and 14%, as conscious in 75% and 67%, and as deep in 7% and 19%, respectively ($P = 0.039$). Comparing the BIS values obtained before, during, and after the procedure, we found that there was a trend toward a return to its initial value more rapidly in the propofol-fentanyl group than in the midazolam-fentanyl group (Figure 3). The OAA/S score showed good concordance with the BIS in the midazolam-fentanyl group ($\kappa = 0.635$, $P < 0.001$), the propofol-fentanyl group ($\kappa = 0.710$, $P < 0.001$), and the sample as a whole ($\kappa = 0.696$, $P < 0.001$).

Satisfaction

The mean score on the visual analog scale of patient satisfaction was 9.84 ± 0.4 in the midazolam-fentanyl group and 9.64 ± 0.8 in the propofol-fentanyl group ($P = 0.178$). The mean score on the visual analog scale for endoscopist satisfaction was 8.90 ± 1.2 for the midazolam-fentanyl regimen and 9.30 ± 0.9 for the propofol-fentanyl regimen ($P = 0.012$). The time to induction was significantly shorter in the propofol-fentanyl group (2.63 ± 1.62 min *vs* 2.96 ± 1.5 min, $P = 0.012$). The times to recovery and discharge were also shorter in the propofol-fentanyl group ($P < 0.001$ and $P < 0.001$, respectively).

Pre- and post-discharge questionnaires

We found no statistically significant differences between the two groups in terms of the patient-reported quality of sedation or pain/discomfort related to the procedure. The proportion of patients who remembered being awake at the beginning, middle, and end of the procedure was greater in the propofol-fentanyl group than the midazolam-fentanyl group ($P < 0.001$ for all three time points). According to the results of the post-discharge questionnaire, none of the patients experienced any adverse reactions within the first 24 h after discharge. On average, the patients in the propofol-fentanyl group reported having resumed their domestic activities 60 min after discharge compared with 80 min after discharge for the midazolam-fentanyl group ($P < 0.001$).

Safety and complications

No serious complications were observed in either group. During the procedure, 42% of the propofol-fentanyl group patients developed mild transient hypoxemia (SpO_2 of 85%-90% for > 30 s after the jaw thrust maneuver), which also occurred in 26% of the midazolam-fentanyl group patients ($P = 0.025$). In all of those cases, the hypoxemia responded to supplemental oxygen delivered by nasal cannula at 3-4 L/min. There were no instances of arrhythmia. Systolic hypotension was observed in 5% of the midazolam-fentanyl group and 10% of the propofol-fentanyl group, whereas diastolic hypotension was observed in 6% of the midazolam-fentanyl group and 16% of the propofol-fentanyl group. All of the variations in arterial blood pressure, heart rate, and respiratory rate were transient and required no mechanical or pharmacological intervention. There were no cases of perforation, bleeding, or death, and none of the patients required invasive ventilatory support, or hospitalization.

DISCUSSION

Although there are abundant data in the literature and various guidelines on sedation during endoscopy[1-6,10,12,17,31], few studies have compared propofol with midazolam for conscious sedation in patients undergoing diagnostic upper gastrointestinal endoscopy, especially when using the incidence of deep sedation events as the primary outcome. In the present study, we used the BIS and OAA/S scale in an innovative manner and assessed the frequency of deep sedation events for two sedation regimens frequently used during endoscopy[11,12,16,17,32,33]. In the midazolam-fentanyl group, deep sedation, defined according to the BIS and OAA/S score, occurred in 11% and 7% of the patients, respectively, compared with 25% and 19% of those in the propofol-fentanyl group. It was clear that despite the use of doses targeting conscious sedation, deep sedation events were common in the midazolam-fentanyl group. These findings are in agreement with those of Patel *et al*[20], who evaluated the use of a benzodiazepine-opioid combination with the objective of achieving conscious sedation in patients undergoing diagnostic upper gastrointestinal endoscopy. The authors found that deep sedation, as evaluated by a modified OAA/S scale, occurred in 26% of the patients.

In the present study, the overall rates of deep sedation were 18% and 13% when assessed by the OAA/S score and BIS, respectively. However, we found that the frequency of clinically relevant complications was negligible, which is likely attributable to the relatively good overall health status of the patients. It is notable that the adverse effects arising from deep sedation were easily reversed with simple clinical maneuvers. There was a trend toward the BIS returning to its initial value more rapidly in the propofol-fentanyl group, which suggests faster recovery in those patients.

The principal complication that occurred in our study was hypoxemia, which was observed in 42% of the patients in the propofol-fentanyl group and 26% of the midazolam-fentanyl group. In all of those patients, hypoxemia responded to supplemental oxygen delivery. The close monitoring of the patients during sedation could explain the relatively high rates of transient mild hypoxemia found in both of the groups. These findings again raise the controversy regarding giving supplemental oxygen routinely during upper gastrointestinal endoscopy, which is our current practice. In addition, the small size of our sample prevented us from effectively evaluating the incidence of severe complications, which are rare in endoscopy.

In the pre-endoscopy, pre-medication period, patients typically have a BIS > 93, whereas a BIS of 60-70 is indicative of deep sedation[24]. There are some discrepancies between the data in the literature and information provided by the manufacturer in terms of the relationship between the numerical values and levels of consciousness[23,24,26,27]. Some authors define deep sedation as a BIS of 60-70 and conscious sedation as > 70[22], while others define deep sedation as BIS < 75 in the presence of an OAA/S score of 1 or 2[27]. To improve the sensitivity and specificity of our evaluations, we defined deep sedation as BIS ≤ 65 and an OAA/S score of 1 and conscious sedation as BIS > 65 and an OAA/S score > 1. Bower *et al*[24] have suggested that a BIS of 75-85 indicates conscious sedation, which is an appropriate level of sedation for endoscopic procedures. In assessing the level of consciousness of patients undergoing endoscopic procedures, those authors demonstrated a strong temporal correlation between the BIS and OAA/S score ($r = 0.59$, $P < 0.0001$). In the present study, we observed good concordance between the BIS and OAA/S score ($\kappa = 0.7$, $P < 0.001$). However, the BIS has obvious advantages over the OAA/S. The primary advantage is that the BIS is a much simpler and more continuous measure. The BIS allows objective measurements of sedation in patients undergoing endoscopic procedures[26].

The use of the BIS in monitoring the level of sedation of patients undergoing endoscopic procedures is controversial because its impact remains unclear[11,13,17,27]. In a study of patients undergoing colonoscopy, the sedation was administered by nurses under the supervision of gastroenterologists, and there was no reduction in the propofol dose used or the time to recovery[34]. Other authors have shown that BIS monitoring leads to the use of a lower mean dose of propofol in endoscopic retrograde cholangiopancreatography[23]. In a study of BIS monitoring during sedation for endoscopic submucosal dissection, the propofol dose was not reduced, although there was increased satisfaction on the part of the patients and endoscopists[25]. Nevertheless, some authors question the accuracy of BIS monitoring in detecting deep sedation[27]. There is no evidence to support the routine use of BIS monitoring in the ambulatory setting of diagnostic endoscopic procedures. In the future, it may prove beneficial for more complex therapeutic endoscopic procedures.

The present study had some limitations. The fact that physical status was classified as ASA I or Ⅱ in 99.5% of the patients might have limited the external validity of the study. However, the exclusion of patients with poor physical status allowed us to focus more closely on the relationship between propofol and deep sedation in the clinical setting most often encountered in endoscopy clinics. In fact, the inclusion of patients with more comorbidity who were undergoing endoscopic procedures of greater complexity would have shifted the discussion to the efficacy and safety of sedation in complex situations. Although that is an important topic and is currently being investigated by other authors[23,35], it was not the focus of the present study. There were also some potential biases related to the difficulty of achieving full blinding of the sedation regimen. However, double blinding was achieved for the collection of data, such as the level of sedation (OAA/S and BIS), the time to induction, recovery, and discharge, and the level of patient satisfaction. All procedures were performed consecutively, respecting the sedation regimens initially proposed and with the observer present.

In the present study, we demonstrated that the times to induction, recovery, and discharge were significantly shorter in the propofol-fentanyl group. These findings replicate the results obtained by other authors who have demonstrated that propofol allows patients to resume their work activities sooner, thereby also increasing overall productivity[3,5,9,19,30,31,33,35-38]. We found no significant difference between the two regimens in terms of patient satisfaction, although there was a difference in terms of satisfaction on the part of endoscopists. The endoscopists expressed a preference for the propofol-fentanyl combination. This finding is in keeping with the global trend toward the use of propofol sedation by gastroenterologists and endoscopists[2,3].

Our findings show that although the use of the midazolam-fentanyl regimen results in deep sedation less often than the propofol-fentanyl regimen does, the difference is not clinically relevant. In our opinion, there is little evidence to support the position that propofol should be administered only by anesthesiologists or that the use of propofol is disproportionately associated with the occurrence of unwanted deep sedation. In fact, deep sedation can also occur when the midazolam-fentanyl regimen is used. This underscores the importance of monitoring

the vital signs of patients under sedation. We could also add that both drug dosage and titration are crucial for the success of the sedation regimen.

In our opinion, patients classified as ASA I or II, if properly evaluated and monitored, can be safely subjected to diagnostic upper gastrointestinal endoscopy under sedation with the propofol-fentanyl combination at doses targeting conscious sedation. We also believe that the presence of an anesthesiologist is not mandatory in this setting. The use of this regimen can increase physician satisfaction and productivity.

COMMENTS

Background

The routine use of sedation during endoscopic procedures is increasing worldwide. The combination of a benzodiazepine (*e.g.*, midazolam) and opioid (*e.g.*, fentanyl) is reportedly used at approximately 75% of all healthcare facilities in the United States and considered the combination of choice by most endoscopists worldwide. Propofol is a hypnotic agent and its use has been adopted at endoscopy centers worldwide. However, its use has also been associated with deep sedation. In this study, authors evaluated the use of propofol-fentanyl versus midazolam-fentanyl for sedation of patients undergoing upper gastrointestinal endoscopy, comparing the frequency of deep sedation in each group. They also compared the two drug combinations in terms of time to induction, time to recovery, time to discharge, efficacy, and safety, as well as patient and endoscopist satisfaction.

Research frontiers

Nowadays, propofol is only used routinely by anesthesiologists. The research hotspots are to find a secure way to provide safety and lower complications of propofol use by non-anesthesiologists.

Innovations and breakthroughs

Although there are abundant data in the literature and various guidelines on sedation during endoscopy, few studies have compared propofol with midazolam for conscious sedation in patients undergoing diagnostic upper gastrointestinal endoscopy. In the present study, authors used the bispectral index (BIS) monitor and observer's assessment of alertness/sedation (OAA/S) scale in an innovative manner and assessed the frequency of deep sedation events for two sedation regimens frequently used during endoscopy.

Applications

The results suggest that patients classified as low anesthetic risk, if properly evaluated and monitored, can be safely subjected to diagnostic upper gastrointestinal endoscopy under sedation with the propofol-fentanyl combination at doses targeting conscious sedation. Authors also believe that the presence of an anesthesiologist is not mandatory in this setting. The use of this regimen can increase physician satisfaction and productivity.

Terminology

Deep sedation is represented by a BIS ≤ 65 and/or OAA/S scale = 1. The BIS monitors cerebral activity. BIS ≤ 65 indicates deep sedation, 66-85 indicates conscious sedation, and > 85 indicates mild sedation. OAA/S scale was developed to measure the level of alertness in subjects who are sedated. It is scored as 1 for deep sedation, 2-4 for conscious sedation, and 5 for mild sedation.

Peer review

An interesting study that assessed the differences and reliability between upper gastrointestinal endoscopies performed with midazolam-fentanyl *versus* propofol-fentanyl sedation. The main findings of the paper were that deep sedation was more frequent in the propofol-fentanyl group, however, this group had a more rapid recovery after the procedure.

REFERENCES

1 **McQuaid KR**, Laine L. A systematic review and meta-analysis of randomized, controlled trials of moderate sedation for routine endoscopic procedures. *Gastrointest Endosc* 2008; **67**: 910-923 [PMID: 18440381 DOI: 10.1016/j.gie.2007.12.046]

2 **Benson AA**, Cohen LB, Waye JD, Akhavan A, Aisenberg J. Endoscopic sedation in developing and developed countries. *Gut Liver* 2008; **2**: 105-112 [PMID: 20485619 DOI: 10.5009/gnl.2008.2.2.105]

3 **Cohen LB**, Wecsler JS, Gaetano JN, Benson AA, Miller KM, Durkalski V, Aisenberg J. Endoscopic sedation in the United States: results from a nationwide survey. *Am J Gastroenterol* 2006; **101**: 967-974 [PMID: 16573781 DOI: 10.1111/j.1572-0241.2006.00500.x]

4 **Thomson A**, Andrew G, Jones DB. Optimal sedation for gastrointestinal endoscopy: review and recommendations. *J Gastroenterol Hepatol* 2010; **25**: 469-478 [PMID: 20370725 DOI: 10.1111/j.1440-1746.2009.06174.x]

5 **Cohen LB**, Ladas SD, Vargo JJ, Paspatis GA, Bjorkman DJ, Van der Linden P, Axon AT, Axon AE, Bamias G, Despott E, Dinis-Ribeiro M, Fassoulaki A, Hofmann N, Karagiannis JA, Karamanolis D, Maurer W, O'Connor A, Paraskeva K, Schreiber F, Triantafyllou K, Viazis N, Vlachogiannakos J. Sedation in digestive endoscopy: the Athens international position statements. *Aliment Pharmacol Ther* 2010; **32**: 425-442 [PMID: 20456310 DOI: 10.1111/j.1365-2036.2010.04352.x]

6 **Heuss LT**, Peter S. Propofol use by gastroenterologists-the European experience. *Gastrointest Endosc Clin N Am* 2008; **18**: 727-38, ix [PMID: 18922411 DOI: 10.1016/j.giec.2008.06.007]

7 **Riphaus A**, Rabofski M, Wehrmann T. Endoscopic sedation and monitoring practice in Germany: results from the first nationwide survey. *Z Gastroenterol* 2010; **48**: 392-397 [PMID: 20140841 DOI: 10.1055/s-0028-1109765]

8 **Meining A**, Semmler V, Kassem AM, Sander R, Frankenberger U, Burzin M, Reichenberger J, Bajbouj M, Prinz C, Schmid RM. The effect of sedation on the quality of upper gastrointestinal endoscopy: an investigator-blinded, randomized study comparing propofol with midazolam. *Endoscopy* 2007; **39**: 345-349 [PMID: 17285514 DOI: 10.1055/s-2006-945195]

9 **Sipe BW**, Rex DK, Latinovich D, Overley C, Kinser K, Bratcher L, Kareken D. Propofol versus midazolam/meperidine for outpatient colonoscopy: administration by nurses supervised by endoscopists. *Gastrointest Endosc* 2002; **55**: 815-825 [PMID: 12024134 DOI: 10.1067/mge.2002.124636]

10 **Fanti L**, Testoni PA. Sedation and analgesia in gastrointestinal endoscopy: what's new? *World J Gastroenterol* 2010; **16**: 2451-2457 [PMID: 20503443 DOI: 10.3748/wjg.v16.i20.2451]

11 **American Society of Anesthesiologists Task Force on Sedation and Analgesia by Non-Anesthesiologists.** Practice guidelines for sedation and analgesia by non-anesthesiologists. *Anesthesiology* 2002; **96**: 1004-1017 [PMID: 11964611 DOI: 10.1097/00000542-200204000-00031]

12 **Faigel DO**, Baron TH, Goldstein JL, Hirota WK, Jacobson BC, Johanson JF, Leighton JA, Mallery JS, Peterson KA, Waring JP, Fanelli RD, Wheeler-Harbaugh J. Guidelines for the use of deep sedation and anesthesia for GI endoscopy. *Gastrointest Endosc* 2002; **56**: 613-617 [PMID: 12397263 DOI: 10.1016/S0016-5107(02)70104-1]

13 **Lichtenstein DR**, Jagannath S, Baron TH, Anderson MA, Banerjee S, Dominitz JA, Fanelli RD, Gan SI, Harrison ME, Ikenberry SO, Shen B, Stewart L, Khan K, Vargo JJ. Sedation and anesthesia in GI endoscopy. *Gastrointest Endosc* 2008; **68**: 815-826 [PMID: 18984096 DOI: 10.1016/j.gie.2008.09.029]

14 **Dumonceau JM**, Riphaus A, Aparicio JR, Beilenhoff U, Knape JT, Ortmann M, Paspatis G, Ponsioen CY, Racz I, Schreiber F, Vilmann P, Wehrmann T, Wientjes C, Walder B. European Society of Gastrointestinal Endoscopy, European Society of Gastroenterology and Endoscopy Nurses and Associates, and the European Society of Anaesthesiology Guideline: Non-anesthesiologist administration of propofol for GI endoscopy. *Endoscopy* 2010; **42**: 960-974 [PMID: 21072716 DOI: 10.1055/s-0030-1255728]

15 **Cohen LB**. Patient monitoring during gastrointestinal endoscopy: why, when, and how? *Gastrointest Endosc Clin N*

Am 2008; **18**: 651-63, vii [PMID: 18922405 DOI: 10.1016/j.giec.2008.06.015]
16 **Carvalho PHB,** Posso IP, Matuguma SE. Tratado de Endoscopia Digestiva, Diagnóstica e Terapêutica - Intestino delgado, cólon e reto. Vol. 4. Sao Paulo: Atheneu, 2008: 37-48
17 **Waring JP**, Baron TH, Hirota WK, Goldstein JL, Jacobson BC, Leighton JA, Mallery JS, Faigel DO. Guidelines for conscious sedation and monitoring during gastrointestinal endoscopy. *Gastrointest Endosc* 2003; **58**: 317-322 [PMID: 14528201 DOI: 10.1067/S0016-5107(03)00001-4]
18 **Tan G**, Irwin MG. Recent advances in using propofol by non-anesthesiologists. *F1000 Med Rep* 2010; **2**: 79 [PMID: 21170368]
19 **Rex DK**, Overley C, Kinser K, Coates M, Lee A, Goodwine BW, Strahl E, Lemler S, Sipe B, Rahmani E, Helper D. Safety of propofol administered by registered nurses with gastroenterologist supervision in 2000 endoscopic cases. *Am J Gastroenterol* 2002; **97**: 1159-1163 [PMID: 12014721 DOI: 10.1111/j.1572-0241.2002.05683.x]
20 **Patel S**, Vargo JJ, Khandwala F, Lopez R, Trolli P, Dumot JA, Conwell DL, Zuccaro G. Deep sedation occurs frequently during elective endoscopy with meperidine and midazolam. *Am J Gastroenterol* 2005; **100**: 2689-2695 [PMID: 16393221 DOI: 10.1111/j.1572-0241.2005.00320.x]
21 **Chernik DA**, Gillings D, Laine H, Hendler J, Silver JM, Davidson AB, Schwam EM, Siegel JL. Validity and reliability of the Observer's Assessment of Alertness/Sedation Scale: study with intravenous midazolam. *J Clin Psychopharmacol* 1990; **10**: 244-251 [PMID: 2286697]
22 **DeWitt JM**. Bispectral index monitoring for nurse-administered propofol sedation during upper endoscopic ultrasound: a prospective, randomized controlled trial. *Dig Dis Sci* 2008; **53**: 2739-2745 [PMID: 18274899 DOI: 10.1007/s10620-008-0198-x]
23 **Paspatis GA**, Chainaki I, Manolaraki MM, Vardas E, Theodoropoulou A, Tribonias G, Konstantinidis K, Karmiris K, Chlouverakis G. Efficacy of bispectral index monitoring as an adjunct to propofol deep sedation for ERCP: a randomized controlled trial. *Endoscopy* 2009; **41**: 1046-1051 [PMID: 19967620 DOI: 10.1055/s-0029-1215342]
24 **Bower AL**, Ripepi A, Dilger J, Boparai N, Brody FJ, Ponsky JL. Bispectral index monitoring of sedation during endoscopy. *Gastrointest Endosc* 2000; **52**: 192-196 [PMID: 10922090 DOI: 10.1067/mge.2000.107284]
25 **Imagawa A**, Fujiki S, Kawahara Y, Matsushita H, Ota S, Tomoda T, Morito Y, Sakakihara I, Fujimoto T, Taira A, Tsugeno H, Kawano S, Yagi S, Takenaka R. Satisfaction with bispectral index monitoring of propofol-mediated sedation during endoscopic submucosal dissection: a prospective, randomized study. *Endoscopy* 2008; **40**: 905-909 [PMID: 19023932 DOI: 10.1055/s-2008-1077641]
26 **Moses PL,** Vargo JJ, Mitty RD, Pleskow DK, Walker JA, Rex DK. BIS values correlate with clinical sedation scores during midazolam/narcotic or propofol sedation for endoscopy. *Gastrointest Endosc* 2004; **59:** 130 [DOI: 10.1016/S0016-5107(04)00641-8]
27 **Qadeer MA**, Vargo JJ, Patel S, Dumot JA, Lopez AR, Trolli PA, Conwell DL, Stevens T, Zuccaro G. Bispectral index monitoring of conscious sedation with the combination of meperidine and midazolam during endoscopy. *Clin Gastroenterol Hepatol* 2008; **6**: 102-108 [PMID: 18065278 DOI: 10.1016/j.cgh.2007.10.005]
28 **Dinis-Ribeiro M**, Vargo JJ. Sedation by non-anesthesiologists: are opioids and benzodiazepines outdated? *Digestion* 2010; **82**: 100-101 [PMID: 20407255 DOI: 10.1159/000287215]
29 **Chen SC**, Rex DK. Review article: registered nurse-administered propofol sedation for endoscopy. *Aliment Pharmacol Ther* 2004; **19**: 147-155 [PMID: 14723606 DOI: 10.1111/j.0269-2813.2004.01833.x]
30 **Cohen LB**, Hightower CD, Wood DA, Miller KM, Aisenberg J. Moderate level sedation during endoscopy: a prospective study using low-dose propofol, meperidine/fentanyl, and midazolam. *Gastrointest Endosc* 2004; **59**: 795-803 [PMID: 15173791 DOI: 10.1016/S0016-5107(04)00349-9]
31 **Vargo JJ**, Cohen LB, Rex DK, Kwo PY. Position statement: nonanesthesiologist administration of propofol for GI endoscopy. *Gastrointest Endosc* 2009; **70**: 1053-1059 [PMID: 19962497 DOI: 10.1016/j.gie.2009.07.020]
32 **de Azevedo MP.** Sedação e anestesia em endoscopia digestiva. *Medicina perioperatória* 2005; **81:** 709-724
33 **Conselho Federal de Medicina.** Resoluções do CFM 1.363/93, 1.409/94 e 1.670/03. [cited 2011 Aug 25]. Available from: URL: http: //www.portalmedico.org.br/resolucoes/cfm
34 **Drake LM**, Chen SC, Rex DK. Efficacy of bispectral monitoring as an adjunct to nurse-administered propofol sedation for colonoscopy: a randomized controlled trial. *Am J Gastroenterol* 2006; **101**: 2003-2007 [PMID: 16968506 DOI: 10.1111/j.1572-0241.2006.00806.x]
35 **Correia LM**, Bonilha DQ, Gomes GF, Brito JR, Nakao FS, Lenz L, Rohr MR, Ferrari AP, Libera ED. Sedation during upper GI endoscopy in cirrhotic outpatients: a randomized, controlled trial comparing propofol and fentanyl with midazolam and fentanyl. *Gastrointest Endosc* 2011; **73**: 45-51, 51.e1 [PMID: 21184869 DOI: 10.1016/j.gie.2010.09.025]
36 **Cohen LB**, Dubovsky AN, Aisenberg J, Miller KM. Propofol for endoscopic sedation: A protocol for safe and effective administration by the gastroenterologist. *Gastrointest Endosc* 2003; **58**: 725-732 [PMID: 14595310 DOI: 10.1016/S0016-5107(03)02010-8]
37 **Rex DK**. Review article: moderate sedation for endoscopy: sedation regimens for non-anaesthesiologists. *Aliment Pharmacol Ther* 2006; **24**: 163-171 [PMID: 16842446 DOI: 10.1111/j.1365-2036.2006.02986.x]
38 **Cohen LB**, Delegge MH, Aisenberg J, Brill JV, Inadomi JM, Kochman ML, Piorkowski JD. AGA Institute review of endoscopic sedation. *Gastroenterology* 2007; **133**: 675-701 [PMID: 17681185 DOI: 10.1053/j.gastro.2007.06.002]

P- Reviewers Familiari L, Galloro G, Lakatos PL
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3447

World J Gastroenterol 2013 June 14; 19(22): 3447-3452
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Endoscopic gastrojejunostomy with a natural orifice transluminal endoscopic surgery technique

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Supported by A grant from the Asan Institute for Life Sciences, Seoul, South Korea, No. 2013-201
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Received: October 30, 2012 Revised: April 2, 2013
Accepted: April 18, 2013
Published online: June 14, 2013

Abstract

AIM: To determine the technical feasibility and safety of an endoscopic gastrojejunostomy with a pure natural orifice transluminal endoscopic surgery (NOTES) technique using a T-anchoring device in a porcine survival model.

METHODS: An endoscopic gastrojejunostomy with a pure NOTES technique using a T-anchoring device was performed on 10 healthy female minipigs weighing approximately 40 kg each under general anesthesia. All procedures were performed with a transgastric approach using a 2-channel therapeutic endoscope.

RESULTS: The transgastric gastrojejunostomy was technically successful in all cases. A total of four to six stitched pairs of a T-anchoring device were used to secure the anastomosis. The median time required to enter the peritoneal cavity and pull the small bowel into the stomach was 34 min (range: 19-41 min); the median time required to suture the anastomosis was 67 min (range: 44-78 min). An obstruction of the efferent limb occurred in one case, and a rupture of the anastomosis site occurred in another case. As a result, the functional success rate was 80% (8/10). Small bowel adhesion to the stomach and liver occurred in one case, but the anastomosis was intact without leakage or obstruction.

CONCLUSION: A transgastric gastrojejunostomy with a T-anchoring device may be safe and technically feasible. A T-anchoring device may provide a simple and effective endoscopic suturing method.

Key words: Natural orifice transluminal endoscopic surgery; Endoscopy; Pigs; Aanastomosis

Core tip: Natural orifice transluminal endoscopic surgery (NOTES) have become part of the growing trend of minimally invasive surgery and have been gradually used in more diverse areas. An endoscopic gastrojejunostomy using a pure NOTES technique may be attractive because it can be a simple and less invasive method for bypassing a gastric outlet or duodenal obstruction. An endoscopic transgastric gastrojejunostomy with T-anchoring devices may be a technically feasible, useful alternative to invasive surgery. However, a great deal of care and further improvement is needed because of the risk of procedure-related complications.

Song TJ, Seo DW, Kim SH, Park DH, Lee SS, Lee SK, Kim MH. Endoscopic gastrojejunostomy with a natural orifice transluminal endoscopic surgery technique. *World J Gastroenterol* 2013; 19(22): 3447-3452 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3447.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3447

INTRODUCTION

Gastric outlet obstruction or duodenal obstruction is one of the most serious problems that frequently occur in patients with advanced malignancies of the stomach or periampullary region[1-3]. In cases in which an oral diet was impossible due to gastric outlet obstruction or duodenal obstruction, open surgery using hand-sewn techniques, staplers, or compression devices, as well as laparoscopic surgery using staplers, have been the major treatment modalities thus far[4-8]. However, these obstructions usually occur in patients with unresectable malignancies of an advanced stage, and invasive surgery may be impossible or quite burdensome for these end-stage patients[9]. Therefore, less invasive methods to address malignant obstructions may be attractive to these patients[10,11].

Various procedures using natural orifice transluminal endoscopic surgery (NOTES) techniques are part of the growing trend of minimally invasive surgery, and these NOTES techniques have been gradually used in more diverse areas[12-16]. Because NOTES techniques have proven to have many advantages in terms of being less invasive compared with existing surgical methods, they are considered to be effective treatment measures that are not burdensome for terminal-stage patients, particularly those with advanced-stage malignancies[17,18]. The first NOTES anastomosis, a cholecystogastrostomy, was reported in 2005, and subsequent studies have demonstrated that various forms of intraperitoneal surgery performed using laparoscopy could be conducted using a flexible endoscope[19,20]. Performing a gastrojejunostomy using a NOTES technique is attractive because it can be a simple and less invasive method for bypassing a gastric outlet obstruction or duodenal obstruction.

The aim of these experiments was to assess the technical feasibility and safety of an endoscopic peroral transgastric gastrojejunostomy procedure with a prototype T-anchoring device in a porcine model.

MATERIALS AND METHODS

Subjects

For this experiment, 10 minipigs, which were breeds of pig developed for medical research, weighing approximately 40 kg each were used. The anesthesia was performed by one veterinarian, and the gastrojejunostomy was performed by two endoscopists and two nurses. Permission for this study was obtained from the Animal Experiment Review Board of Asan Medical Center.

Experiment method

Pretreatment: The animals were fed a soft liquid diet beginning 48 h before the procedure; then, they abstained from food-except for only a small quantity of water-beginning 24 h before the procedure. They were anesthetized with a combination of anesthetic agents, including tiletamine hydrochloride, zolazepam hypochloride (Zoletil®, Virbac do Brasil Ltda., Brazil), and xylazine (Rompun®, Bayer Korea Co. Ltd., South Korea) before the procedures, and general anesthesia was maintained with 1.5% isoflurane (Forane®, Choongwae Pharma Co. Ltd., South Korea). Premedication was performed 30 min before anesthesia using an intramuscular injection of atropine sulfate (Bayer Korea Co. Ltd., South Korea).

Procedure: A multibending two-channel endoscope (2TQ260®, Olympus Optical Co. Ltd., Japan) was used for the procedures. The minipigs were placed in a supine position, and the endoscope was inserted into the stomach. The anterior wall of the body of the stomach was punctured with a needle knife (Micro knife®, Boston Scientific, Natick, MA, United States). After the puncture, a 0.035-inch guidewire (Jagwire®, Boston Scientific, Natick, MA, United States) was inserted into the peritoneal cavity through the needle knife, and the needle knife was then removed. A papillotome was inserted along the guidewire, and a 2-cm-long incision was made on the stomach wall. The incision was made in four directions-up, down, left, and right-while changing the direction of the papillotome. A dilating balloon with a diameter of 20 mm (CRE®, Boston Scientific, Natick, MA, United States) was inserted along the guidewire, and the puncture site was dilated twice for one minute each. After dilation, the dilating balloon was pushed inside the peritoneal cavity, together with the endoscope, while being deflated. After entry into the peritoneal cavity, the guidewire and inflating balloon were removed from the scope.

An appropriate loop of the small intestine was selected for the anastomosis. Usually, the mid-jejunum was selected using indications provided by the anatomic position relative to other structures. When the location of the anastomosis in the jejunum had been determined, grasping forceps were inserted into a channel of the endoscope, and a snare was inserted into another channel. After the snare was opened, the grasping forceps were pushed through the inside of the open snare, and the snare was slowly closed. Then, the predetermined antimesenteric site on the mid-jejunum was grasped using the grasping forceps, and the jejunum was drawn into the inside of the snare. Thereafter, the snare was closed to grasp the jejunum, which was brought into the stomach. The jejunum was grasped with care to avoid blocking the mesenteric vessel, and the air in the abdominal cavity was sufficiently aspirated before the jejunum was placed into the stomach. Because the small intestine would go back into the peritoneal cavity after being drawn into the stomach, the snare was held closed to hold the small intestine and prevent it from returning to the abdominal cavity while the grasping forcep was being removed. In the endoscopic channel (from which the grasping forceps had been removed), a needle, installed with the first T-anchoring tag, was inserted. Using the needle, the small intestine was punctured, and the first T-anchoring tag was detached through the inside of the needle. Then, the needle was removed, and the second T-anchoring tag was installed into the needle. This needle was inserted through the same channel, and the stomach wall (*i.e.*, the wall closest to the T-anchoring tag that was inserted first)

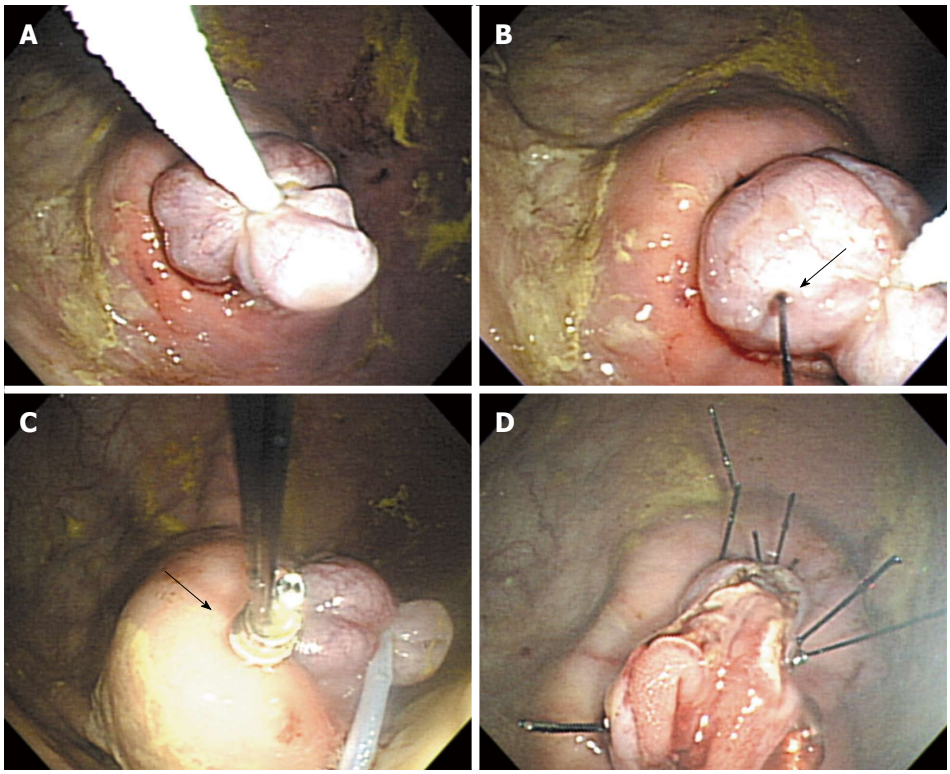

Figure 1 Technique for endoscopic gastrojejunostomy using a T-anchoring device. A: After the gastric wall incision and balloon dilatation, the small intestine was held in the stomach; B: A needle installed with the first T-anchoring tag was used to start suturing. The first T-anchoring tag was attached to the small intestine (arrow), and then, the second was attached to the gastric wall; C: A pair of sutures was locked together using a locking device (arrow). The process was repeated four to six times to anchor the small intestine to the stomach; D: Finally, an incision using a needle knife was made on the jejunum.

was punctured. The second T-anchoring tag was detached through the inside of the needle, and the needle was removed. Then, a pair of T-anchoring tags was locked using a locking device. Thereafter, a pair of scissor forceps was inserted to cut the suture thread connected to the T-anchoring tag, thereby completing the suture. These processes were repeated four to six times to fix the jejunum to the stomach. Finally, the snare was opened, and an incision was made on the jejunum using a needle knife to open the jejunal lumen to the inside of the stomach (Figure 1).

Postoperative care: Third-generation cephalosporin and analgesics were administered intravenously after the procedures, and a liquid diet was administered after 24 h. All animals survived for seven days before they were euthanized. Then, autopsies were conducted. The health conditions and abnormal reactions of the animals were monitored for seven days. After seven days, the animals were euthanized, and the anastomosis site, afferent and efferent loops, and peritoneal cavity were observed.

RESULTS

The detailed results of the endoscopic peroral transgastric gastrojejunostomy performed on the 10 animals are shown in Table 1. The transgastric gastrojejunostomy was technically successful in all cases (100%, 10/10). A total of four to six stitched pairs of T-anchoring devices were used to secure the anastomosis. The median time from when the endoscope was inserted through the mouth to puncture the stomach, enter the peritoneal cavity, find an appropriate region of the small intestine, and draw it into the stomach was 34 min (range: 19-41 min). The median time required to complete the anastomosis using the T-anchoring devices was 67 min (range: 44-78 min). During the operations, there were no adverse events, such as bleeding or internal organ injury, and the vital signs of the animals were stable. On the postmortem examination, although the afferent limb was patent, the efferent limb was obstructed in one case. A rupture of the anastomosis site occurred in another case. As a result, the functional success rate was 80% (8/10). Mild adhesion of the short segment of the small intestine to the stomach and liver occurred in one case, but the anastomosis was intact without leakage or obstruction. In all other cases, the small intestine loops of the anastomosis reached the stomach without tension or abnormal rotation.

DISCUSSION

In this study, the peroral transgastric gastrojejunostomy performed with a pure NOTES technique using a flexible endoscope and a T-anchoring device was technically feasible. Peroral transgastric gastrojejunostomy might be another potential therapeutic option for gastric outlet obstruction or duodenal obstruction in addition to surgical anastomosis. The method of using a pure NOTES

Table 1 The results of an endoscopic peroral transgastric gastrojejunostomy on 10 animals *n* (%)

Technical success	10 (100)
Functional success	8 (80)
Number of stitched pairs of the T-anchoring device	
4	4
5	4
6	2
Median time required to enter the peritoneal cavity and pull the small bowel into the stomach (min, range)	34 (19-41)
Median time required to suture the anastomosis (min, range)	67 (44-78)
Adverse events	
Rupture of anastomosis site	1
Insufficient efferent limb opening	1
Small bowel adhesion	1

technique to bypass a malignant obstruction is advantageous over surgical anastomosis because it does not require any abdominal incision; as a result, it induces less surgical stress[21]. Moreover, it can be easily performed using a flexible endoscope and accessories under a conscious sedation state without general anesthesia, which is required for the usual endoscopic procedures[22]. Therefore, this method is considered to be potentially applicable not only to the palliation of malignant obstructions but also to benign diseases in which conventional transabdominal and laparoscopic surgeries are unsuitable, such as bariatric surgery for morbidly obese patients[19].

In this study, the anastomosis was performed using a T-anchoring device. Until recently, conventional hemoclips have been used for the closure of puncture sites during NOTES procedures[23]. Although existing hemoclips can be used easily in various types of conventional endoscopic procedures, they are disadvantageous as full-thickness sutures are impossible to secure, and the sutures may fail if two ends of an opening are distant from each other[24,25]. However, T-anchoring devices are advantageous because their use enables full-thickness sutures to be secured. Moreover, even in cases in which two ends of an opening are distant from each other, T-anchoring devices secure full-thickness sutures if only a needle puncture is possible. Therefore, T-anchoring devices can be used in diverse NOTES procedures, such as the closure of various incision sites or anastomosis, and also in the management of adverse events that may occur in conventional endoscopic procedures (*e.g.*, the closure of a bowel perforation and bleeding control).

In this study, room air, which is usually used in conventional endoscopic procedures, was used for the inflation of the stomach and the peritoneal cavity. During our pure NOTES procedures, air in the stomach may have leaked into the peritoneal cavity during the anastomosis to induce abdominal distension. Although air in the peritoneal cavity was sufficiently aspirated during the process of drawing the small intestine into the stomach, the air that leaked into the peritoneal cavity during the anastomosis was not removed by the suction of the stomach. Abdominal distension occurred immediately after the anastomosis in two cases, and thus, percutaneous needle aspiration was performed after the procedures. When this procedure is performed on humans, CO_2 gas, which is easily absorbed and discharged through breathing, will be helpful in reducing this distension[26,27]. A great deal of care must be exercised to prevent pressure inside the stomach from rising too high during the procedure, and an endoscope installed with a pressure gauge at its fore-end should be developed to enable the easy measurement and constant maintenance of the pressure in the pneumoperitoneum[28,29].

In our study, complications occurred with respect to the sizes of the initial incisions on the stomach wall. The case in which there was an obstruction in the efferent loop was the first, and we believe that the obstruction in the efferent loop occurred because the stomach wall incision was not sufficiently large. Although the incision site was further enlarged using a 20-mm dilatation balloon, openings enlarged by balloon dilatation may contract again, unlike direct incisions. Therefore, the initial incisions on the stomach wall are important. In one case in which a rupture occurred in the anastomosis site, the incision was large, exceeding 2.5 cm. Our speculation is that the suture site could not tolerate the pressure in the stomach after the diet because the incision was too large. Therefore, the size of the opening should be appropriate, and a great deal of care must be taken to ensure that it is not too small or too large. In our opinion, in the case of an endoscopic gastrojejunostomy, 2.0 cm is thought to be an appropriate incision size. In the event that a large incision exceeding 2.5 cm has been made, a greater number of sutures and a longer period of fasting after the procedure are necessary. In addition, the T-anchoring tag should be improved to ensure that the suture site can tolerate sufficiently high pressure.

The present T-anchoring device is a prototype. To secure one pair of sutures using this device, a needle should be inserted through an endoscopic channel at least twice, and then, a pair of scissor forceps should be inserted to cut the thread after installing two T-anchoring tags. Given these lengthy processes, coupled with the fact that more than four pairs of sutures are necessary for the anastomosis, the overall procedure time may become too long. In addition, the threads connected to the T-anchoring tags may occasionally become tangled, causing problems during the processes of inserting and removing the needle. Therefore, improving the T-anchoring device is necessary to simplify the suturing process. Many (but not all) of the limitations of the instruments that have been used for the NOTES technique can be overcome by innovative design and engineering improvements[20].

In conclusion, a peroral transgastric gastrojejunostomy with T-anchoring devices may be a technically feasible, useful alternative to invasive surgery. However, a great deal of care is needed because of the risk of complications. The T-anchoring device is still in its early stage and needs further improvements; however, it may provide a simple and effective endoscopic suturing method.

COMMENTS

Background

Various natural orifice transluminal endoscopic surgery (NOTES) techniques have become part of the growing trend of minimally invasive surgery, and these NOTES techniques have been gradually used in more diverse areas.

Research frontiers

Performing a gastrojejunostomy using a pure NOTES technique is attractive because it can be a simple and less invasive method for bypassing a gastric outlet obstruction or duodenal obstruction.

Innovations and breakthroughs

The transgastric gastrojejunostomy was technically successful in all cases (100%, 10/10). A total of four to six stitched pairs of T-anchoring devices were used to secure the anastomosis. An obstruction of the efferent limb occurred in one case, and a rupture of the anastomosis site occurred in another case. As a result, the functional success rate was 80% (8/10). Small bowel adhesion to the stomach and liver occurred in one case, but the anastomosis was intact without leakage or obstruction in this animal.

Applications

T-anchoring devices secure full-thickness sutures if only a needle puncture is possible. Therefore, T-anchoring devices can be used in diverse NOTES procedures, such as the closure of various incision sites or anastomosis, and also in the management of adverse events that may occur in conventional endoscopic procedures.

Terminology

NOTES is a minimally invasive surgery technique that has been recently devised; the application of this technique has been gradually expanding.

Peer review

The authors reported an animal study on the transgastric gastrojejunostomy using NOTES technique. This study provides a further potential advances in NOTES, with important potential advantages for use in human in due course.

REFERENCES

1 **Alam TA**, Baines M, Parker MC. The management of gastric outlet obstruction secondary to inoperable cancer. *Surg Endosc* 2003; **17**: 320-323 [PMID: 12384765 DOI: 10.1007/s00464-001-9197-0]

2 **Mittal A**, Windsor J, Woodfield J, Casey P, Lane M. Matched study of three methods for palliation of malignant pyloroduodenal obstruction. *Br J Surg* 2004; **91**: 205-209 [PMID: 14760669 DOI: 10.1002/bjs.4396]

3 **Ly J**, O'Grady G, Mittal A, Plank L, Windsor JA. A systematic review of methods to palliate malignant gastric outlet obstruction. *Surg Endosc* 2010; **24**: 290-297 [PMID: 19551436 DOI: 10.1007/s00464-009-0577-1]

4 **Dietz UA**, Debus ES. Intestinal anastomoses prior to 1882; a legacy of ingenuity, persistence, and research form a foundation for modern gastrointestinal surgery. *World J Surg* 2005; **29**: 396-401 [PMID: 15696398 DOI: 10.1007/s00268-004-7720-x]

5 **McGuire J**, Wright IC, Leverment JN. Surgical staplers: a review. *J R Coll Surg Edinb* 1997; **42**: 1-9 [PMID: 9046134]

6 **Bergström M**, Ikeda K, Swain P, Park PO. Transgastric anastomosis by using flexible endoscopy in a porcine model (with video). *Gastrointest Endosc* 2006; **63**: 307-312 [PMID: 16427940 DOI: 10.1016/j.gie.2005.09.035]

7 **Reed DN**, Cacchione RN, Allen JW, Arlauskas V, Casey J, Larson GM, Vitale G. Laparoscopic choledochojejunostomy and gastrojejunostomy in a porcine model. *Surg Endosc* 2003; **17**: 86-88 [PMID: 12364986 DOI: 10.1007/s00464-001-8246-z]

8 **Hamad MA**, Mentges B, Buess G. Laparoscopic sutured anastomosis of the bowel. *Surg Endosc* 2003; **17**: 1840-1844 [PMID: 14959728 DOI: 10.1007/s00464-002-8618-z]

9 **Smith AC**, Dowsett JF, Russell RC, Hatfield AR, Cotton PB. Randomised trial of endoscopic stenting versus surgical bypass in malignant low bileduct obstruction. *Lancet* 1994; **344**: 1655-1660 [PMID: 7996958]

10 **Jeurnink SM**, Steyerberg EW, Hof Gv, van Eijck CH, Kuipers EJ, Siersema PD. Gastrojejunostomy versus stent placement in patients with malignant gastric outlet obstruction: a comparison in 95 patients. *J Surg Oncol* 2007; **96**: 389-396 [PMID: 17474082 DOI: 10.1002/jso.20828]

11 **Jeurnink SM**, van Eijck CH, Steyerberg EW, Kuipers EJ, Siersema PD. Stent versus gastrojejunostomy for the palliation of gastric outlet obstruction: a systematic review. *BMC Gastroenterol* 2007; **7**: 18 [PMID: 17559659 DOI: 10.1186/1471-230X-7-18]

12 **Solomon D**, Lentz R, Duffy AJ, Bell RL, Roberts KE. Female sexual function after pure transvaginal appendectomy: a cohort study. *J Gastrointest Surg* 2012; **16**: 183-16; discussion 183-16; [PMID: 21997432 DOI: 10.1007/s11605-011-1706-4]

13 **Lehmann KS**, Ritz JP, Wibmer A, Gellert K, Zornig C, Burghardt J, Büsing M, Runkel N, Kohlhaw K, Albrecht R, Kirchner TG, Arlt G, Mall JW, Butters M, Bulian DR, Bretschneider J, Holmer C, Buhr HJ. The German registry for natural orifice translumenal endoscopic surgery: report of the first 551 patients. *Ann Surg* 2010; **252**: 263-270 [PMID: 20585238 DOI: 10.1097/SLA.0b013e3181e6240f]

14 **Marks JM**, Ponsky JL, Pearl JP, McGee MF. PEG "Rescue": a practical NOTES technique. *Surg Endosc* 2007; **21**: 816-819 [PMID: 17404790 DOI: 10.1007/s00464-007-9361-2]

15 **Fischer LJ**, Jacobsen G, Wong B, Thompson K, Bosia J, Talamini M, Horgan S. NOTES laparoscopic-assisted transvaginal sleeve gastrectomy in humans--description of preliminary experience in the United States. *Surg Obes Relat Dis* 2009; **5**: 633-636 [PMID: 19656739 DOI: 10.1016/j.soard.2009.04.015]

16 **Nau P**, Anderson J, Yuh B, Muscarella P, Christopher Ellison E, Happel L, Narula VK, Melvin WS, Hazey JW. Diagnostic transgastric endoscopic peritoneoscopy: extension of the initial human trial for staging of pancreatic head masses. *Surg Endosc* 2010; **24**: 1440-1446 [PMID: 20054581 DOI: 10.1007/s00464-009-0797-4]

17 **Niu J**, Song W, Yan M, Fan W, Niu W, Liu E, Peng C, Lin P, Li P, Khan AQ. Transvaginal laparoscopically assisted endoscopic cholecystectomy: preliminary clinical results for a series of 43 cases in China. *Surg Endosc* 2011; **25**: 1281-1286 [PMID: 20927541 DOI: 10.1007/s00464-010-1360-z]

18 **Bingener J**, Gostout CJ. Update on natural orifice translumenal endoscopic surgery. *Gastroenterol Hepatol* (NY) 2012; **8**: 384-389 [PMID: 22933874]

19 **Swain P**. NOTES and anastomosis. *Gastrointest Endosc Clin N Am* 2008; **18**: 261-77; viii [PMID: 18381168 DOI: 10.1016/j.giec.2008.01.013]

20 **Park PO**, Bergström M, Ikeda K, Fritscher-Ravens A, Swain P. Experimental studies of transgastric gallbladder surgery: cholecystectomy and cholecystogastric anastomosis (videos). *Gastrointest Endosc* 2005; **61**: 601-606 [PMID: 15812420 DOI: 10.1016/S0016-5107(04)02774-9]

21 **Freeman LJ**, Rahmani EY, Al-Haddad M, Sherman S, Chiorean MV, Selzer DJ, Snyder PW, Constable PD. Comparison of pain and postoperative stress in dogs undergoing natural orifice transluminal endoscopic surgery, laparoscopic, and open oophorectomy. *Gastrointest Endosc* 2010; **72**: 373-380 [PMID: 20537637 DOI: 10.1016/j.gie.2010.01.066]

22 **Garud SS**, Willingham FF. Natural orifice transluminal endoscopic surgery. *Gastrointest Endosc* 2012; **76**: 491-495 [PMID: 22898405 DOI: 10.1016/j.gie.2012.06.025]

23 **Kalloo AN**, Singh VK, Jagannath SB, Niiyama H, Hill SL, Vaughn CA, Magee CA, Kantsevoy SV. Flexible transgastric peritoneoscopy: a novel approach to diagnostic and therapeutic interventions in the peritoneal cavity. *Gastrointest Endosc* 2004; **60**: 114-117 [PMID: 15229442]

24 **Lee SS**, Oelschlager BK, Wright AS, Soares RV, Sinan H, Montenovo MI, Hwang JH. Assessment of a simple, novel endoluminal method for gastrotomy closure in NOTES. *Surg Endosc* 2011; **25**: 3448-3452 [PMID: 21556990 DOI: 10.1007/s00464-011-1730-1]

25 **Song TJ**, Seo DW, Kim SH, Park do H, Lee SS, Lee SK, Kim MH. The Performance of Multiple Transgastric Procedures Using the Natural Orifice Transluminal Endoscopic Surgery Technique: Is Pure NOTES Satisfactory? *Gut Liver* 2012; **6**: 457-463 [PMID: 23170150 DOI: 10.5009/gnl.2012.6.4.457]

26 **von Delius S**, Sager J, Feussner H, Wilhelm D, Thies P, Huber W, Schuster T, Schneider A, Schmid RM, Meining A. Carbon dioxide versus room air for natural orifice transluminal endoscopic surgery (NOTES) and comparison with standard laparoscopic pneumoperitoneum. *Gastrointest Endosc* 2010; **72**: 161-19, 161-19 [PMID: 20381043 DOI: 10.1016/j.gie.2010.01.013]

27 **Ikechebelu JI**, Obi RA, Udigwe GO, Joe-Ikechebelu NN. Comparison of carbon dioxide and room air pneumoperitoneum for day-case diagnostic laparoscopy. *J Obstet Gynaecol* 2005; **25**: 172-173 [PMID: 15814399 DOI: 10.1080/01443610500051528]

28 **Meireles O**, Kantsevoy SV, Kalloo AN, Jagannath SB, Giday SA, Magno P, Shih SP, Hanly EJ, Ko CW, Beitler DM, Marohn MR. Comparison of intraabdominal pressures using the gastroscope and laparoscope for transgastric surgery. *Surg Endosc* 2007; **21**: 998-1001 [PMID: 17404796 DOI: 10.1007/s00464-006-9167-7]

29 **McGee MF**, Rosen MJ, Marks J, Chak A, Onders R, Faulx A, Ignagni A, Schomisch S, Ponsky J. A reliable method for monitoring intraabdominal pressure during natural orifice translumenal endoscopic surgery. *Surg Endosc* 2007; **21**: 672-676 [PMID: 17285385 DOI: 10.1007/s00464-006-9124-5]

P- Reviewers Day AS, Marinho RT, Wig JD
S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3453

World J Gastroenterol 2013 June 14; 19(22): 3453-3458
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Incidental focal colorectal ^{18}F-fluorodeoxyglucose uptake on positron emission tomography/computed tomography

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Received: December 25, 2012 Revised: March 20, 2013
Accepted: March 28, 2013
Published online: June 14, 2013

Abstract

AIM: To assess the clinical significance of incidental focal colorectal ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake on ^{18}F-FDG-positron emission tomography/computed tomography (PET/CT).

METHODS: The records of all the cases which had undergone colonoscopy after PET/CT within a two weeks interval were reviewed. Adenomas were considered advanced when they were villous, $\geq$ 10 mm in size, or had high-grade dysplasia. Colorectal cancers and advanced adenomas are collectively referred to as advanced colorectal neoplasms. Receiver-operating characteristic curve analysis was used to determine the significant predictive maximum standardized uptake value (SUVmax) cutoff value for advanced colorectal neoplasms and cancer.

RESULTS: Ninety-five colorectal lesions matched the site of incidental focal colorectal ^{18}F-FDG uptake on PET/CT and 146 did not. Colonoscopy showed advanced colorectal neoplasms corresponding to the site of ^{18}F-FDG uptake in 49 of the 95 (51.5%) lesions with incidental uptake. Of the lesions without incidental uptake, only 6 of 146 (4.1%) had advanced colorectal neoplasms on colonoscopy, indicating a statistically significant difference between the two groups (P < 0.001). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of incidental focal ^{18}F-FDG uptake in identifying advanced colorectal neoplasms were 89.1%, 75.3%, 51.6%, 95.9%, and 78.4%, respectively. In detecting only CRC, these values were 89.2%, 69.6%, 34.7%, 97.3%, and 72.6%, respectively. The significant SUVmax cutoff value for advanced colorectal neoplasms (area under the curve 0.755, P < 0.001) was 4.35, with a sensitivity, specificity, PPV, NPV, and accuracy of 75.5%, 65.2%, 69.8%, 71.4% and 70.5%, respectively. For CRC, 5.05 was the significant SUVmax cutoff value (area under the curve 0.817, P < 0.001), with a sensitivity, specificity, PPV, NPV, and accuracy of 84.8%, 71.0%, 80.9%, 89.8%, and 75.8%, respectively.

CONCLUSION: The presence of incidental focal colorectal ^{18}F-FDG uptake on PET/CT with a SUVmax $\geq$ 4.35 increases the likelihood of an advanced colorectal neoplasm.

Key words: Positron emission tomography; Adenomas; Computed tomography; Colorectal cancer; Colonoscopy

Core tip: Increased focal uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in the colon and rectum can be found incidentally during ^{18}F-FDG-positron emission tomography/computed tomography (PET/CT). The presence of incidental focal colorectal ^{18}F-FDG uptake on PET/CT indicated advanced colorectal neoplasms in more than half of the cases. Our study confirms the necessity of colonoscopy when incidental FDG uptake is detected on PET/CT to allow the early diagnosis and management of advanced colorectal neoplasms.

Cho SH, Kim SW, Kim WC, Park JM, Yoo IR, Kim SH, Oh ST. Incidental focal colorectal ^{18}F-fluorodeoxyglucose uptake on positron emission tomography/computed tomography. *World J Gastroenterol* 2013; 19(22): 3453-3458 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3453.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3453

INTRODUCTION

^{18}F-fluorodeoxyglucose (^{18}F-FDG)-positron emission tomography/computed tomography (PET/CT) is a technique used to reduce false-positive findings by combining two imaging modalities, and can distinguish physiological activity from pathology more readily than PET or CT alone. PET/CT is commonly used during the evaluation of malignant tumors, and an increased focal colorectal uptake of ^{18}F-FDG can be observed incidentally because of the capacity of PET/CT to detect the foci of tumors, which display increased glycolysis[1].

Several previous studies have shown that not only is a high incidence of unexpected gastrointestinal malignancies associated with incidental ^{18}F-FDG avidity[2], but also that the presence of these lesions can change patient management in up to 28% of cases[3]. And in symptomatic patients with proven or suspected colorectal cancer (CRC) recurrence PET detected additional sites of disease in up to 48.4% of patients[4].

However, the significance of incidental focal colorectal ^{18}F-FDG uptake observed on PET/CT has not yet been fully clarified. Only one previous prospective study has demonstrated a requirement for colonoscopy when incidental FDG uptake was found with PET/CT imaging[5]. However, those researchers could not determine the statistically significant maximum standardized uptake value (SUVmax) with which to detect advanced colorectal adenoma and cancer because of the broad overlapping SUV values among the patient groups.

As far as we know, we have evaluated the largest number of cases yet assessed to determine the value of colonoscopy when incidental FDG uptake is observed on PET/CT images. In this study, we investigated the clinical significance of incidental focal colorectal ^{18}F-FDG uptake detected on PET/CT, and determined the SUVmax for detecting advanced adenoma and cancer.

MATERIALS AND METHODS

Patients

We analyzed the records of 583 consecutive colonoscopic lesions examined over a three-year period, from those who had undergone colonoscopy after PET/CT within a two weeks interval.

The exclusion criteria were an incomplete colonoscopic examination, insufficient biopsy specimen for pathological diagnosis, diffuse colorectal ^{18}F-FDG uptake on PET/CT, and proven or suspected CRC in a previous study. A surveillance PET/CT examination performed for clinical assessment after curative CRC resection was not an exclusion criterion. Based on these criteria, 342 lesions were excluded.

A total of 241 colonoscopic lesions (88 from women and 153 from men) were eligible for the study. The median age was 62 years (range: 29-86 years). The study was approved by the Institutional Review Board of Seoul St Mary's Hospital, Catholic University of Korea College of Medicine.

PET/CT acquisition

The subjects were fasted for at least 6 h before the FDG injection. Their blood glucose levels were determined in capillary blood samples before the intravenous injection of FDG. At our institution, a cutoff blood glucose level of 8 mmol/L contraindicates FDG injection. PET/CT images were acquired 1 h after the injection of 370-570 MBq of ^{18}F-FDG. The subjects were scanned from the base of the skull to the upper thighs, with their arms raised above their heads.

The PET/CT scans (dual-section helical CT) were performed on a Biograph Duo scanner (Siemens Medical Solutions, Knoxville, TN, United Statets) with an axial spatial resolution of 6.5 mm. Neither intravenous contrast medium nor bowel preparations were used for the CT scan.

The PET/CT images were reviewed on a workstation with fusion software (Syngo, Siemens, Knoxville, TN, United Statets). An experienced nuclear medicine physician reviewed the PET/CT images. PET, CT, and fused whole-body images displayed in the axial, coronal, and sagittal planes were available for review. The PET data were also displayed in a rotating maximum-intensity projection. The intensity of the ^{18}F-FDG uptake can be analysed qualitatively and semiquantitatively by measuring the standardized uptake value, which is usually expressed as its maximum. The SUV is defined as the ratio of activity within the tissue (Bq/mL) and decay-corrected total activity injected divided by body weight (Bq/g) and it is calculated using the software provided by the workstation manufacturer. Abnormal FDG uptake, the SUVmax of the primary tumor, and distant metastases were evaluated.

PET/CT interpretation and analysis

PET studies showing focal, well-circumscribed foci of

Table 1 Baseline characteristics and pathologic diagnoses of the 241 elegible colorectal lesions *n* (%)

Variables	Total (*n* = 241)	Focal FDG uptake (+) (*n* = 95)	Focal FDG uptake (-) (*n* = 146)	*P* value
Baseline characteristics				
Age, yr[1] (range)	62 (29-86)	64 (31-86)	59.5 (29-84)	0.068
Males	153 (64)	57 (60)	96 (65)	0.366
Overweight or obesity (BMI > 25 kg/m^2)	22 (9.1)	9 (9.5)	13 (8.9)	0.367
F/U after complete CRC resection	67 (28.2)	16 (17.9)	51 (34.9)	0.006
Pathologic diagnoses				
Non specific	140 (58.1)	26 (27.5)	114 (78.1)	< 0.001
Inflammation	24 (9.9)	18 (18.9)	6 (4.1)	< 0.001
Adenoma (any size)	40 (16.6)	18 (18.9)	22 (15.1)	0.430
Advanced adenoma	18 (7.5)	16 (16.8)	2 (1.4)	< 0.001
Cancer	37 (15.4)	33 (34.7)	4 (2.7)	< 0.001
Advanced colorectal neoplasms	55 (22.9)	49/95 (51.5)	6/146 (4.1)	< 0.001

[1]Median (range). FDG: ^{18}F-Fluorodeoxyglucose; BMI: Body mass index; F/U: Follow up; CRC: Colorectal cancer.

Table 2 Positron emission tomography/computed tomograph indications of the eligible colorectal lesions

Initial cancer staging (*n* = 99)	After complete cancer resection (*n* = 87)	After cancer chemotherapy (*n* = 12)	Health screening (*n* = 31)	Others (*n* = 12)
Stomach: 33 MUO: 19 OBGY: 12 Lung: 10 Hepatobiliary: 9 Others: 16	Colorectum: 67 Stomach: 8 Others: 12	Stomach: 2 OBGY: 2 Others: 8		

MUO: Metastasis of unknown origin; OBGY: Obstetrics and gynecology.

increased abdominopelvic ^{18}F-FDG uptake, localized by PET/CT images to the colorectum, and distinguishable from the background colonic uptake, were reviewed for interpretation[6]. The incidence of incidental focally increased ^{18}F-FDG uptake in the colorectum was calculated and the intensity of the uptake was measured by calculating SUVmax from the attenuation-corrected PET data, using the software provided by the workstation manufacturer. Lesion size on PET/CT was not part of the inclusion criteria. The PET/CT findings were correlated with the various colonoscopic and histopathological results.

Colonoscopy and surgery

Two hundred forty-one colorectal pathological specimens obtained from 212 subjects who had undergone a total colonoscopy were studied. All colorectal lesions that appeared to be of neoplastic origin were biopsied and removed by polypectomy and/or surgery. Colonoscopy with a pathological examination was considered the gold standard diagnostic method.

Adenomas were considered advanced when they were villous, ⩾ 10 mm in size, or had high-grade dysplasia. Colorectal cancers and advanced adenomas are collectively referred to as advanced colorectal neoplasms. If a subject had more than one detectable colorectal lesion, then each lesion was analyzed individually.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the incidental focal ^{18}F-FDG uptake in predicting advanced colorectal neoplasms and CRC were calculated. Receiver-operating characteristic (ROC) curve analysis was used to determine the significant predictive SUVmax cutoff value for advanced colorectal neoplasms and cancer. For all tests, a P value < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS, version 16 (SPSS, Chicago, IL, United States).

RESULTS

Patient characteristics

Two hundred forty-one eligible colonoscopic lesions were obtained from 212 individual subjects. Of these, 95 lesions showed incidental focal colorectal ^{18}F-FDG uptake on PET/CT and 146 did not. The characteristics of the 241 colonoscopic lesions evaluated in this study are shown in Table 1. Of these, 153 were male and the mean age was 62 years (range: 29-86 years). The sex, age, and body mass index did not differ significantly between the ^{18}F-FDG positive and negative groups. The indications for PET/CT were as part of the initial staging of malignancies other than CRC in 99 cases, after the complete resection of malignancies other than CRC in 87 cases, and after chemotherapy for malignancies other than CRC in 12 cases. Thirty-one scanned cases were performed during health screening and 12 during screening for other purposes (Table 2).

The pathological diagnoses were 37 cancers, 24 acute or chronic inflammation, 140 non specific, and 18 advanced adenomatous lesions among a total of 40 adenomatous lesions (Table 1).

Diagnostic accuracy of focal incidental uptake on PET/CT

In those lesions showing incidental uptake on PET/CT, 49 of 95 (51.5%) had advanced colorectal neoplasms on colonoscopy corresponding to the site of ^{18}F-FDG uptake, whereas in those lesions without uptake, only 6

of 146 (4.1%) were found to have advanced colorectal neoplasms on colonoscopy. This indicates a statistically significant difference between the two groups ($P < 0.001$). Overall, the sensitivity, specificity, PPV, NPV, and accuracy of incidental focal ^{18}F-FDG uptake in predicting advanced colorectal neoplasms were 89.1%, 75.3%, 51.6%, 95.9%, and 78.4%, respectively. For detecting only CRC, the sensitivity, specificity, PPV, NPV, and accuracy of incidental focal ^{18}F-FDG uptake were 89.2%, 69.6%, 34.7%, 97.3%, and 72.6%, respectively.

SUVmax and colorectal neoplastic lesions

The statistically significant SUVmax cutoff value that allowed the discrimination of advanced colorectal neoplasms from non advanced neoplastic and non neoplastic lesions was 4.35 (area under the ROC curve 0.755, $P < 0.001$), with a sensitivity, specificity, PPV, NPV, and accuracy of 75.5%, 65.2%, 69.8%, 71.4%, and 70.5%, respectively. For CRC, 5.05 was the statistically significant SUVmax cutoff value (area under the curve 0.817, $P < 0.001$), with a sensitivity, specificity, PPV, NPV, and accuracy of 84.8%, 71.0%, 80.9%, 89.8%, and 75.8%, respectively.

DISCUSSION

PET/CT is a well-accepted technique for the diagnosis and staging of several malignancies because it provides more accurate functional and anatomical assessments than CT or other conventional imaging modalities[6]. Some researchers demonstrated that multidetector CT colonography has excellent sensitivity and specificity for lesions < 10 mm. In fact, one study reported superior sensitivity compared to conventional colonoscopy for the detection of adenomas 10 mm or larger (93.8% *vs* 85.5%)[7]. A recent study looking at the feasibility of FDG-PET/CT colonography in patients with high clinical suspicion of colorectal carcinoma showed promising results in being an "all-in-one" staging modality, but was practically challenging and still required a formal colonoscopy for histopathological confirmation[8].

Physiological ^{18}F-FDG uptake within the gastrointestinal tract, with variable intensities and localization patterns, has previously been described. Focal tracer uptake is frequently seen at the gastroesophageal junction, moderate uptake in the stomach, low-intensity uptake in the small bowel, and diffuse or focal uptake in the colon[9]. The mechanisms underlying this physiological activity are unclear. Muscular peristaltic activity, the presence of lymphoid tissue in the cecum, high concentrations of white blood cells in the bowel wall, and/or the presence within the bowel wall of cells secreting ^{18}F-FDG, especially in cases of cecum distention, have been hypothesized[10]. Increased FDG uptake can also be associated with inflammation, such as enterocolitis[11] or inflammatory bowel disease[12]. In our study, only focal or multifocal FDG findings were considered significant because diffuse uptake is considered to have a physiological origin.

Focal colorectal ^{18}F-FDG uptake indicates a high (70%-80%) probability of corresponding abnormal histopathological findings[13-15]. Despite this increase in confidence and reduction in the number of suggestive or equivocal lesions, the precise localization of increased ^{18}F-FDG foci using PET/CT cannot at present resolve the diagnostic dilemma of abnormal tracer uptake in the colorectum. For this reason, we assessed the clinical significance of incidental focal colorectal ^{18}F-FDG uptake on PET/CT and also determined a clinically useful SUVmax cutoff value for the detection of advanced adenoma and cancer. Our results are consistent with two large studies that showed that PET/CT is a sensitive tool with which to detect premalignant lesions[15,16]. In another previous study, PET/CT had a sensitivity of 74% and a specificity of 84% in the detection of colonic abnormalities[17]. However, that study was limited by its small sample size (39 patients).

Although previous studies have evaluated the etiology of incidental PET/CT findings in the colon[15,18], we believe that our study contributes to the clinician's perspective by providing a SUVmax cutoff value that discriminates advanced colorectal neoplasms from non advanced lesions.

Larger series, with 3210 and 2000 patients, identified 20 (12 villous adenomas, six carcinomas, and two tubular adenomas) and 13 abnormalities (seven adenomatous polyps and six carcinomas), respectively, with colonoscopy[5,19]. However, they failed to determine a significant SUVmax cutoff value that would be clinically useful in discriminating advanced colorectal neoplasms from malignant carcinomas. In our study, the cutoff SUVmax value was higher in the CRC group than in the advanced neoplasms group (5.05 *vs* 4.35, respectively). We showed that the intensity of FDG uptake correlated with the severity of the lesion. This is consistent with the results of previous studies, although in those, the cutoff SUVmax value that allowed advanced and non advanced neoplasms to be distinguished was not determined, whereas this was one of the main goals of our study[3,20]. In the present study, a SUVmax $\geqslant$ 4.35 increased the likelihood of advanced colorectal neoplasms and a SUVmax $\geqslant$ 5.05 increased the likelihood of CRC.

One limitation of our study was a potential selection bias because we included those cases which had undergone PET/CT for the initial staging of a non colorectal malignancy and those in whom surveillance PET/CT had been performed for clinical assessments after curative CRC resection. However, we believe that our study more accurately reflects the real-life clinical situation by including them. The prevalence of focal colonic lesions in our study is also similar to that in previous studies that have used PET to detect colonic lesions[19,21,22].

In conclusion, our study shows that advanced colorectal adenomas and malignant carcinomas should be suspected when focal ^{18}F-FDG uptake is detected by PET/CT and that this is clinically significant in most cases. Incidental focal colorectal ^{18}F-FDG uptake detected on PET/CT with a SUVmax $\geqslant$ 4.35 and a SUVmax

⩾ 5.05 increases the likelihood of advanced colorectal neoplasms and CRC, respectively.

Our study confirms the necessity of colonoscopy when incidental FDG uptake is detected on PET/CT to allow the early diagnosis and management of advanced colorectal neoplasms.

COMMENTS

Background

Positron emission tomography (PET)/computed tomography (CT) is commonly used during the evaluation of malignant tumors. Increased focal uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in the colon and rectum can be found incidentally during FDG-PET/CT.

Research frontiers

Several previous studies have shown that not only is a high incidence of unexpected gastrointestinal malignancies associated with incidental ^{18}F-FDG avidity, but also that the presence of these lesions can change patient management. However, the significance of incidental focal colorectal ^{18}F-FDG uptake observed on PET/CT has not yet been fully clarified.

Innovations and breakthroughs

The authors have evaluated the largest number of cases yet assessed to determine the value of colonoscopy when incidental FDG uptake is observed on PET/CT images. In this study, the authors investigated the clinical significance of incidental focal colorectal ^{18}F-FDG uptake detected on PET/CT, and determined the maximal standardized uptake value (SUVmax) for detecting advanced adenoma and cancer.

Applications

This study shows that advanced colorectal adenomas and malignant carcinomas should be suspected when focal ^{18}F-FDG uptake is detected by PET/CT and that this is clinically significant in most cases. This study confirms the necessity of colonoscopy when incidental FDG uptake is detected on PET/CT to allow the early diagnosis and management of advanced colorectal neoplasms.

Terminology

FDG-PET/CT display increased sites of glycolysis and by increased focal colorectal uptake of ^{18}F-FDG and is capable to detect the foci of tumors. The SUVmax of ^{18}F-FDG is a way to measure the intensity of the ^{18}F-FDG uptake during FDG-PET/CT. It is measured using the software provided by the FDG-PET/CT workstation manufacturer.

Peer review

The authors assessed the clinical significance of incidental focal colorectal ^{18}F-FDG uptake on FDG-PET/CT. The accuracy of incidental focal ^{18}F-FDG uptake in identifying advanced colorectal neoplasms and colorectal cancer (CRC) were 78.4% and 72.6%, respectively. In addition, they demonstrated higher cutoff SUVmax value in the CRC group than in the advanced neoplasms group (5.05 *vs* 4.35, respectively).

REFERENCES

1 **Bar-Shalom R**, Valdivia AY, Blaufox MD. PET imaging in oncology. *Semin Nucl Med* 2000; **30**: 150-185 [PMID: 10928381 DOI: 10.1053/snuc.2000.7439]

2 **Kostakoglu L**, Hardoff R, Mirtcheva R, Goldsmith SJ. PET-CT fusion imaging in differentiating physiologic from pathologic FDG uptake. *Radiographics* 2004; **24**: 1411-1431 [PMID: 15371617 DOI: 10.1148/rg.245035725]

3 **Shreve PD**, Anzai Y, Wahl RL. Pitfalls in oncologic diagnosis with FDG PET imaging: physiologic and benign variants. *Radiographics* 1999; **19**: 61-77; quiz 150-151 [PMID: 9925392]

4 **Scott AM**, Gunawardana DH, Kelley B, Stuckey JG, Byrne AJ, Ramshaw JE, Fulham MJ. PET changes management and improves prognostic stratification in patients with recurrent colorectal cancer: results of a multicenter prospective study. *J Nucl Med* 2008; **49**: 1451-1457 [PMID: 18703607 DOI: 10.2967/jnumed.108.051615]

5 **Peng J**, He Y, Xu J, Sheng J, Cai S, Zhang Z. Detection of incidental colorectal tumours with 18F-labelled 2-fluoro-2-deoxyglucose positron emission tomography/computed tomography scans: results of a prospective study. *Colorectal Dis* 2011; **13**: e374-e378 [PMID: 21831098 DOI: 10.1111/j.1463-1318.2011.02727.x]

6 **Riedl CC**, Akhurst T, Larson S, Stanziale SF, Tuorto S, Bhargava A, Hricak H, Klimstra D, Fong Y. 18F-FDG PET scanning correlates with tissue markers of poor prognosis and predicts mortality for patients after liver resection for colorectal metastases. *J Nucl Med* 2007; **48**: 771-775 [PMID: 17475966 DOI: 10.2967/jnumed.106.037291]

7 **Pickhardt PJ**, Choi JR, Hwang I, Butler JA, Puckett ML, Hildebrandt HA, Wong RK, Nugent PA, Mysliwiec PA, Schindler WR. Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. *N Engl J Med* 2003; **349**: 2191-2200 [PMID: 14657426 DOI: 10.1056/NEJMoa031618]

8 **Veit P**, Kühle C, Beyer T, Kuehl H, Herborn CU, Börsch G, Stergar H, Barkhausen J, Bockisch A, Antoch G. Whole body positron emission tomography/computed tomography (PET/CT) tumour staging with integrated PET/CT colonography: technical feasibility and first experiences in patients with colorectal cancer. *Gut* 2006; **55**: 68-73 [PMID: 15970580 DOI: 10.1136/gut.2005.064170]

9 **Cook GJ**, Fogelman I, Maisey MN. Normal physiological and benign pathological variants of 18-fluoro-2-deoxyglucose positron-emission tomography scanning: potential for error in interpretation. *Semin Nucl Med* 1996; **26**: 308-314 [PMID: 8916319 DOI: 10.1016/S0001-2998(96)80006-7]

10 **Delbeke D**. Oncological applications of FDG PET imaging: brain tumors, colorectal cancer, lymphoma and melanoma. *J Nucl Med* 1999; **40**: 591-603 [PMID: 10210218]

11 **Meyer MA**. Diffusely increased colonic F-18 FDG uptake in acute enterocolitis. *Clin Nucl Med* 1995; **20**: 434-435 [PMID: 7628148 DOI: 10.1097/00003072-199505000-00012]

12 **Neurath MF**, Vehling D, Schunk K, Holtmann M, Brockmann H, Helisch A, Orth T, Schreckenberger M, Galle PR, Bartenstein P. Noninvasive assessment of Crohn's disease activity: a comparison of 18F-fluorodeoxyglucose positron emission tomography, hydromagnetic resonance imaging, and granulocyte scintigraphy with labeled antibodies. *Am J Gastroenterol* 2002; **97**: 1978-1985 [PMID: 12190164 DOI: 10.1111/j.1572-0241.2002.05836.x]

13 **Agress H**, Cooper BZ. Detection of clinically unexpected malignant and premalignant tumors with whole-body FDG PET: histopathologic comparison. *Radiology* 2004; **230**: 417-422 [PMID: 14699176 DOI: 10.1148/radiol.2302021685]

14 **Israel O**, Mor M, Guralnik L, Hermoni N, Gaitini D, Bar-Shalom R, Keidar Z, Epelbaum R. Is 18F-FDG PET/CT useful for imaging and management of patients with suspected occult recurrence of cancer? *J Nucl Med* 2004; **45**: 2045-2051 [PMID: 15585480]

15 **Tatlidil R**, Jadvar H, Bading JR, Conti PS. Incidental colonic fluorodeoxyglucose uptake: correlation with colonoscopic and histopathologic findings. *Radiology* 2002; **224**: 783-787 [PMID: 12202714 DOI: 10.1148/radiol.2243011214]

16 **Chen YK**, Kao CH, Liao AC, Shen YY, Su CT. Colorectal cancer screening in asymptomatic adults: the role of FDG PET scan. *Anticancer Res* 2003; **23**: 4357-4361 [PMID: 14666651]

17 **Pandit-Taskar N**, Schöder H, Gonen M, Larson SM, Yeung HW. Clinical significance of unexplained abnormal focal FDG uptake in the abdomen during whole-body PET. *AJR Am J Roentgenol* 2004; **183**: 1143-1147 [PMID: 15385321]

18 **Yasuda S**, Fujii H, Nakahara T, Nishiumi N, Takahashi W, Ide M, Shohtsu A. 18F-FDG PET detection of colonic adenomas. *J Nucl Med* 2001; **42**: 989-992 [PMID: 11438616]

19 **Kamel EM**, Thumshirn M, Truninger K, Schiesser M, Fried M, Padberg B, Schneiter D, Stoeckli SJ, von Schulthess GK, Stumpe KD. Significance of incidental 18F-FDG accumula-

tions in the gastrointestinal tract in PET/CT: correlation with endoscopic and histopathologic results. *J Nucl Med* 2004; **45**: 1804-1810 [PMID: 15534047]

20 **Lee ST**, Tan T, Poon AM, Toh HB, Gill S, Berlangieri SU, Kraft E, Byrne AJ, Pathmaraj K, O'Keefe GJ, Tebbutt N, Scott AM. Role of low-dose, noncontrast computed tomography from integrated positron emission tomography/computed tomography in evaluating incidental 2-deoxy-2-[F-1-]fluoro-D-glucose-avid colon lesions. *Mol Imaging Biol* 2008; **10**: 48-53 [PMID: 17994266 DOI: 10.1007/s11307-007-0117-0]

21 **Israel O**, Yefremov N, Bar-Shalom R, Kagana O, Frenkel A, Keidar Z, Fischer D. PET/CT detection of unexpected gastrointestinal foci of 18F-FDG uptake: incidence, localization patterns, and clinical significance. *J Nucl Med* 2005; **46**: 758-762 [PMID: 15872347]

22 **Ishimori T**, Patel PV, Wahl RL. Detection of unexpected additional primary malignancies with PET/CT. *J Nucl Med* 2005; **46**: 752-757 [PMID: 15872346]

P- Reviewer Assadi M **S- Editor** Huang XZ
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3459

World J Gastroenterol 2013 June 14; 19(22): 3459-3465
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Perceptions about preventing hepatocellular carcinoma among patients with chronic hepatitis in Taiwan

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Received: January 31, 2013 Revised: April 2, 2013
Accepted: April 10, 2013
Published online: June 14, 2013

Abstract

AIM: To measure patient perceptions about preventing hepatocellular carcinoma (HCC) and to predict the factors that influence patient willingness to receive therapy.

METHODS: A cross-sectional descriptive study was conducted at an outpatient clinic of a medical institution in southern Taiwan. Four hundred patients with chronic hepatitis B/C were recruited as participants. Two structured questionnaires based on the health belief model were utilized in this study, including the scales of perceptions about preventing HCC and knowledge of hepatitis B/C.

RESULTS: The statistical results demonstrated that the participants' perceived susceptibility (r = -0.22, P < 0.001), benefits (r = -0.11, P = 0.028) and cues to action (r = -0.12, P = 0.014) about the prevention of HCC was significantly correlated with their age. The participants' perceptions were also associated with their educational levels, household incomes and knowledge of hepatitis. Older patients and those with a lower socioeconomic status tended to have negative perceptions and less knowledge of hepatitis. Multivariate logistic regression further indicated that the participants' age (B = -0.044, SE = 0.017, odds ratio = 0.957, P = 0.008, 95%CI: 0.926-0.989) and perceived barriers (B = -0.111, SE = 0.030, odds ratio = 0.895, P < 0.001, 95%CI: 0.845-0.949) were correlated with their willingness to receive antiviral therapy.

CONCLUSION: Healthcare professionals should provide appropriate and effective guidance to increase their patients' awareness and to decrease the perceived barriers for continuing surveillance and antiviral therapy.

Key words: Antiviral therapy; Health perception; Hepatitis B; Hepatitis C; Hepatocellular carcinoma; Health knowledge

Core tip: Chronic hepatitis B/C carriers may benefit from regular surveillance for allowing an early diagnose of hepatocellular carcinoma (HCC). In addition, raising awareness of and health perceptions about HCC, and increasing willingness to receive antiviral therapy for preventing the development of HCC are crucial in patients with chronic hepatitis B/C, particularly in rural areas.

Chen YW, Liu CC, Perng DS. Perceptions about preventing hepatocellular carcinoma among patients with chronic hepatitis in Taiwan. *World J Gastroenterol* 2013; 19(22): 3459-3465 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3459.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3459

INTRODUCTION

Chronic liver disease is the eighth most common cause of death, and hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death in Taiwan[1]. More than 80% of primary HCC is attributed to chronic infection with hepatitis B/C viruses[2,3]. To prevent further development of HCC, patients infected with hepatitis B/C should maintain a healthy lifestyle, reduce alcohol consumption, undergo regular liver function tests and abdominal sonography, and receive antiviral therapy, if necessary[4,5]. Chronic infection with hepatitis B/C is the most important cause of HCC. The early development of HCC is asymptomatic, and patients with chronic hepatitis are usually unaware of their carrier status, the symptoms and signs of HCC, and the importance of regular surveillance and treatment. During the early stage of HCC, when tumors are less than 2 cm in diameter, even expert radiologists have difficulty differentiating a cirrhotic nodule from a malignant tumor[4]. These tumors are highly heterogeneous, and HCC is nearly always fatal once the tumors cannot be eradicated by surgical or ablative approaches[4]. In addition, patients with HCC detected at the early asymptomatic stage have better survival rates than those who are diagnosed when symptomatic[6]. Therefore, increasing awareness of and health perceptions about HCC are crucial in patients with chronic hepatitis B/C, particularly in rural areas.

Perceptions about preventing HCC

Henchoz *et al*[7] defined perceived health as "an individual's evaluation of his or her own health" which can determine personal health values and influence health behaviors[8]. Furthermore, Kartal *et al*[9] stated that "self-perceived health is a subjective measure that can be calculated at an individual level. It gives an indication of how an individual feels about the condition of his/her own health." To examine patient perceptions about preventing HCC, a health belief model was utilized as a theoretical framework in the present study. The health belief model was formulated using five concepts to explain attitudes and behaviors of individuals, including perceived susceptibility, perceived severity, perceived benefits of action, perceived barriers to action, and cues to action[10,11]. The model was developed to predict compliance with preventive health recommendations[6,12] and to specify the individuals' values and beliefs about health and their influence on choices, especially for explaining screening behaviors[2,13].

Perceived susceptibility refers to a person's experience with a potentially harmful condition[11]. This concept measures whether patients with asymptomatic hepatitis perceive themselves as being at high risk and believe that they could acquire HCC. Perceived severity indicates individual concerns about the seriousness and consequences to the quality of life upon development of cancer, including the physical, mental, and social perspectives[11]. This concept can measure the impact of chronic hepatitis on a person's life and his or her belief that people infected could possibly die from liver cancer.

Perceived benefits of action refer to whether one believes the potential effectiveness of recommended health actions in preventing or reducing the risk or seriousness of a disease[11]. Perceived benefits of action can assess one's beliefs that regular screening and antiviral therapy reduce the risks of progressing to liver cirrhosis and cancer.

Perceived barriers to action are the negative aspects of anticipated health behaviors that patients adopt for prevention or early detection of hepatic cancer, such as the inconvenience of seeing a doctor, the waiting time and pain involved in giving blood samples, and the side effects from taking medications. Patients may believe that regular screening of liver function and abdominal sonography are not necessary if they do not experience any symptoms of discomfort. Moreover, if patients receive antiviral therapy, they may develop physical and psychological side effects, such as fever, headache, fatigue, nausea, vomiting, anxiety and even depression. These adverse events often make patients feel uncomfortable, resulting in early withdrawal from therapy.

Cues to action measure the perceived social and environmental influences that stimulate an individual's desire to take health-related action[11]. The concept can assess whether individuals with hepatitis have any family members, relatives or friends who are infected with hepatitis or whether the patients have ever followed the advice of healthcare professionals about regular check-ups of liver function, abdominal sonography, and antiviral therapy. Therefore, the five concepts of the health belief model were applied in the present study to explore health perceptions about preventing HCC among patients with chronic hepatitis B/C.

Knowledge of hepatitis

Most people are not aware of the routes of viral transmission of hepatitis B/C and that these viruses spread more easily than the human immunodeficiency virus[14]. Ma *et al*[15] found that populations with minimal education and low socioeconomic status are vulnerable to hepatitis infection. If high-risk groups with chronic hepatitis are not aware of the importance of regular surveillance and antiviral therapy, then their condition could gradually progress to liver cirrhosis and hepatic cancer. Wai *et al*[16] identified that those hepatitis B carriers with high knowledge were significantly younger, and more likely to have received college education in Singapore. Treloar *et al*[17] conducted a cross-sectional survey and indicated that knowledge is a precursor to decisions about treatment of hepatitis C, particularly for those patients who are less engaged with hepatitis C treatment and those with lower literacy.

The prevention of HCC in high-risk populations depends on regular serum screening, abdominal sonography and antiviral therapy. Regular screening of liver function, including assays to measure serum aspartate aminotransferase and alanine aminotransferase levels, α-fetoprotein assays and abdominal sonography are strongly recom-

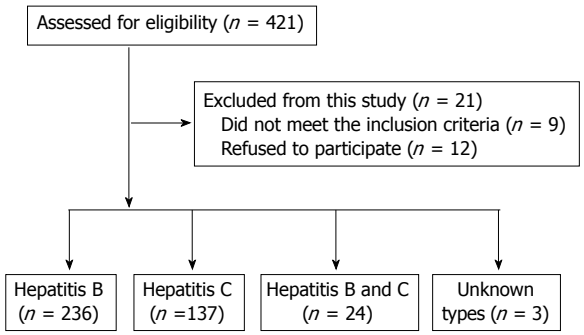

Figure 1 Sampling schema.

mended for patients with chronic hepatitis to ensure earlier detection of HCC and better survival[6,18]. Therefore, the purpose of the present study was to measure patient perceptions about the prevention of HCC, knowledge of hepatitis B/C, and frequency of hepatic check-ups, as well as to predict the factors that influence patient willingness to receive antiviral therapy.

MATERIALS AND METHODS

Participants

Four hundred patients with chronic hepatitis B/C from the outpatient clinic in a medical institution were recruited as participants. The teaching hospital is located in a rural area in southern Taiwan. The markers for hepatitis B are positive hepatitis B surface antigen (HBsAg) and positive antibody for hepatitis C (anti-HCV)[18]. The inclusion criteria for participants were (1) HBsAg (+) and anti-HBs (-), and/or (2) anti-HCV (+) in serum tests for more than 6 mo. The patients who were younger than 18 years and those who did not meet the inclusion criteria were excluded from this study. The sampling schema is shown in Figure 1.

Instruments

This study had a cross-sectional, descriptive design. Two structured questionnaires developed by Chao *et al*[19] and based on a health belief model were utilized in this investigation, namely, the scales of perceptions about preventing HCC and knowledge of hepatitis B/C. After obtaining permission from the original developers, a pilot test was conducted with 40 target adults (who did not participate in the main study) to examine whether there were any ambiguous statements in the questionnaires and to establish the reliability and validity of the instruments.

The scale of perceptions about preventing HCC consisted of five dimensions and 34 items, including the dimensions of perceived susceptibility (3 items), perceived severity (5 items), perceived benefits (3 items), perceived barriers (15 items), and cues to action (8 items). Each item was designed as a 5-point Likert scale, ranging from 1 "strongly disagree" to 5 "strongly agree." Higher scores indicated greater degrees of perceived susceptibility, severity, benefits, barriers and cues to action. Cronbach's alpha values were as follows: whole scale, 0.8; perceived susceptibility, 0.71; perceived severity, 0.78; perceived benefits, 0.68; perceived barriers, 0.84; and cues to action, 0.77[19]. The internal consistency reliability with Cronbach's alpha was 0.72 in this study. The values of Cronbach's alpha for each dimension of perception indicated the reliability of the questionnaire utilized in this study.

The scale of knowledge of hepatitis B/C had 15 items, including liver function (2 items), blood tests for hepatitis (1 item), symptoms of hepatitis (2 items), definition of hepatitis (1 item), modes of viral transmission (4 items), screening to prevent liver cancer (2 items) and infectious status of hepatitis (3 items). Participants responded to these items using the following options: 0 = "do not know" or "false" and 1 = "true." The total scores of 15 items represented a dimension of the participants' knowledge. A higher score indicated a better understanding of hepatitis. The internal consistency with the Kuder-Richardson coefficient was previously determined to be 0.71[19], whereas in the present study, the Kuder-Richardson coefficient was 0.62.

Procedure

Approval to conduct this study was obtained from the institutional review board. This study was conducted between January and December of 2010. When patients visited the outpatient hepatic clinic and met the inclusion criteria, the researchers used convenient sampling to choose the participants. Subsequently, the participants were placed in another room to complete the questionnaires without interruption. All of the questionnaires were anonymous and separated from the consent forms. Prior to administering the questionnaires, the researchers informed the patients of the purpose of this study and explained that the responses would not influence their treatment.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, United States) version 17.0 was used to analyze the data. The data analysis for this study included descriptive statistics, independent-samples *t*-tests, the chi-square test, a one-way analysis of variance (ANOVA), Pearson's correlation, and logistic regression.

RESULTS

Participants' demographics

Four hundred questionnaires were distributed and completed by 174 females (43.5%) and 226 males (56.5%). The average age of the respondents was 54 years (rang: 18-89 years). The participants' demographic information is summarized in Table 1.

Participants' hepatitis-related information

In this survey, 236 (59%) participants had hepatitis B, and 137 (34.3%) had hepatitis C. Twenty-four (6.0%) participants reported having both hepatitis B and C, and three (0.5%) did not know what types of hepatitis they had. In terms of vaccination, 298 patients (74.5%) responded

Table 1 Participants' demographics (n = 400) n (%)

Participants' demographics	Value
Gender	
Female	174 (43.5)
Male	226 (56.5)
Marital status	
Married	353 (88.3)
Single	27 (6.8)
Widowed	10 (2.5)
Divorced/separated	9 (2.3)
Educational level	
Illiterate	41 (10.3)
Elementary school	109 (27.3)
Junior high school	73 (18.3)
High school	104 (26)
College/university	62 (15.5)
Graduate school	11 (2.8)
Occupation	
Laborers	82 (20.5)
Businessmen	61 (15.3)
Farmers	36 (9.0)
Fishermen	9 (2.3)
Military personnel	2 (0.5)
Government employees	19 (4.8)
Homemakers	78 (19.5)
Others	113 (28.3)
Household income (NT dollars/mo)	
Below 10000	101 (25.3)
10000-20000	63 (15.8)
21000-30000	78 (19.5)
31000-40000	47 (11.8)
41000-50000	35 (8.8)
Above 50000	76 (19.0)

that they had never been vaccinated for hepatitis B. Thirty-eight patients (9.5%) had received the vaccine, and 64 (16.0%) did not know if they had been vaccinated.

Most of the patients (n = 292, 73.0%) reported that the virus had been found during a regular physical examination, 45 (11.3%) through the screening process for blood donation, 21 (5.3%) upon health screening in the community and 42 (10.5%) in other ways. Moreover, 332 patients (83.0%) were willing to receive antiviral therapy, 35 patients (8.8%) did not want to receive the therapy, and 33 patients (8.3%) were unaware that this antiviral therapy was available or whether they needed to receive this therapy.

Regarding the sources of hepatitis-related health information, 282 participants stated that they obtained this information from healthcare professionals, 189 from their relatives and friends, 220 from television, 173 from newspapers and magazines, and 70 from educational pamphlets or brochures. In addition, over one-half of the participants underwent regular blood screening every 3 mo (n = 247, 61.8%) and abdominal sonography every 6 mo (n = 183, 45.8%) according to the statistical results.

Health perceptions and knowledge of hepatitis

The mean scores for each dimension of the health perceptions were as follows: perceived susceptibility, 10.26 ± 2.18; perceived severity, 20.43 ± 3.53; perceived benefits, 12.07 ± 1.63; perceived barriers, 37.60 ± 7.06; and cues to action, 27.01 ± 4.73. The mean score for knowledge of hepatitis B/C in this study was 7.36 ± 1.87.

Relationships among the patients' demographics, health perceptions, knowledge, screening, and willingness to receive antiviral therapy

Pearson's correlation revealed significantly negative correlations between the participants' age and their health perceptions, except for perceived severity and barriers. Likewise, the participants' age was significantly and negatively correlated with their level of knowledge (γ = -0.20, $P < 0.001$). The statistical results also suggested that the participants' perceptions had a significantly positive correlation with their knowledge of hepatitis. In addition, the patients' gender was not significantly correlated with their perceptions (except for perceived barriers) and knowledge, as presented in Table 2.

Moreover, a one-way ANOVA indicated that there were significantly positive correlations between the participants' educational levels and their perceptions (except for perceived severity) and knowledge of hepatitis. Significant relationships were found between the patients' frequency of blood screening for HCC prevention and the perceived severity (F = 3.67, P = 0.001) as well as between the frequency of abdominal sonography and perceived barriers (F = 3.66, P = 0.001).

Furthermore, the frequency of abdominal sonography was significantly correlated with the patients' knowledge (F = 2.74, P = 0.009) and with being vaccinated (F = 13.88, $P < 0.001$). A significant correlation existed between the participants' willingness to receive antiviral therapy and perceived barriers (F = 3.55, $P < 0.001$). The researchers further divided the participants into three groups by age (18-39 years, 40-64 years, and 65-89 years old). A significantly positive correlation was found between the participants' age and their willingness to receive antiviral therapy (χ^2 = 13.97, P = 0.007), but there was no correlation between the participants' gender and their willingness to receive therapy (χ^2 = 2.35, P = 0.309).

Predictors for willingness to receive antiviral therapy

The participants' age, five dimensions of health perceptions and knowledge of hepatitis were analyzed as independent variables in the multivariate logistic regression. The results indicated that the participants' age (B = -0.044, SE = 0.017, odds ratio= 0.957, P = 0.008, 95%CI: 0.926-0.989) and perceived barriers (B = -0.111, SE = 0.030, odds ratio = 0.895, $P < 0.001$, 95%CI: 0.845-0.949) were significantly associated with their willingness to receive antiviral therapy, as presented in Table 3. Adjusting for other effects in the logistic regression model, for every one-year increase in age, the participants were 0.957 times less likely to have received antiviral therapy.

DISCUSSION

Most of the participants in this study had elementary and high school educations, and their household in-

Table 2 Relationships between the participants' demographics, health perceptions, and knowledge of hepatitis (*n* = 400)

Variables	Perceived susceptibility	Perceived severity	Perceived benefits	Perceived barriers	Cues to action	Knowledge
Age[1]	-0.22 (< 0.001)	-0.07 (0.162)	-0.11 (0.028)	0.08 (0.121)	-0.12 (0.014)	-0.20 (< 0.001)
Gender[2]	1.53 (0.135)	0.92 (0.362)	0.23 (0.819)	2.14 (0.039)	-0.04 (0.967)	-1.14 (0.254)
Marital status[3]	0.64 (0.589)	1.91 (0.127)	0.40 (0.753)	0.19 (0.905)	0.08 (0.973)	1.13 (0.336)
Education level[3]	2.96 (0.008)	1.30 (0.257)	3.95 (0.001)	6.31 (<0.001)	2.27 (0.036)	9.17 (< 0.001)
Occupation[3]	2.52 (0.015)	0.92 (0.489)	0.92 (0.490)	2.55 (0.014)	0.59 (0.767)	2.61 (0.012)
Monthly income[3]	2.27 (0.047)	0.63 (0.679)	3.91 (0.002)	3.91 (0.002)	2.87 (0.015)	6.59 (< 0.001)
Willingness to receive therapy[3]	-0.75 (0.453)	-1.15 (0.252)	-0.42 (0.675)	3.55 (< 0.001)	-1.67 (0.096)	-0.48 (0.633)
Had received a vaccine[3]	2.98 (0.052)	0.07 (0.937)	2.04 (0.131)	1.94 (0.145)	3.14 (0.044)	13.88 (< 0.001)
Frequency of blood screening[3]	1.28 (0.265)	3.67 (0.001)	0.43 (0.859)	1.87 (0.084)	1.66 (0.129)	1.44 (0.198)
Frequency of abd. sonography[3]	0.51 (0.830)	1.44 (0.188)	0.55 (0.798)	3.66 (0.001)	1.48 (0.173)	2.74 (0.009)

Data are presented as [1]*r* of Pearson's correlation/ [2]*t* of independent *t*-test/ [3]*F* of one-way analysis of variance (*P* value).

Table 3 Predictors of receiving antiviral therapy: Logistic regression (*n* = 364)

Variables	*B*	SE	OR	*P*	95%CI
Age	-0.044	0.017	0.957	0.008	0.926-0.989
Perceptions					
Susceptibility	0.011	0.096	1.011	0.912	0.837-1.220
Severity	0.061	0.053	1.063	0.255	0.957-1.180
Benefits	-0.156	0.130	0.856	0.232	0.663-1.105
Barriers	-0.111	0.030	0.895	< 0.001	0.845-0.949
Cues to action	0.059	0.042	1.060	0.160	0.977-1.150
Knowledge	-0.102	0.117	0.903	0.383	0.719-1.135

comes were between $10000 and $ 30000 NT dollars. The patients in rural areas had lower educational levels and household incomes than those in urban areas. The results revealed that the participants' education levels were significantly correlated with their health perceptions and knowledge about hepatitis. This finding is consistent with the previous study[16,20]. The participants' education levels were not associated with the perceived severity in this study, which may be because the patients themselves might not have perceived the potentially severe consequences of being hepatitis carriers and might not have been aware of the asymptomatic development of HCC.

In addition, Hsu *et al*[21] stated that educational attainment and knowledge of hepatitis B are associated with individual willingness to receive screening and vaccination. As previous studies have indicated, subjects with higher levels of knowledge were significantly more likely to receive blood screening for hepatitis B and to have received the vaccine[14,22-25]. However, the majority of the participants responded that they had never been vaccinated for hepatitis B prior to infection. Therefore, healthcare providers should educate patients with chronic hepatitis to increase their awareness and knowledge and to take action for prevention or early detection of HCC.

Likewise, household income was significantly and positively correlated with perceived susceptibility, benefits, barriers, cues to action, and knowledge. This finding revealed that patients with lower incomes perceived less susceptibility, benefits, and cues to action for preventing liver cancer, and they had less knowledge of hepatitis. These results were similar to those of a previous study[14]. Thus, it is necessary to focus on the low socioeconomic population and to increase their perceptions and knowledge to reduce health disparities about liver cancer in the community.

Moreover, the vast majority of the participants usually obtained hepatitis-related health information and knowledge from healthcare professionals, public media (such as television, newspapers and magazines), and educational brochures and pamphlets. Cacoub *et al*[26] indicated that the therapeutic education for those hepatitis patients can improve the probability of compliance with antiviral therapy. Hence, to raise perceptions and knowledge related to HCC, appropriate and effective instruction based on the individual's age and educational level is crucial, especially in rural areas.

Furthermore, the predictors for patient willingness to receive antiviral therapy were age and the perceived barriers. The participants' age was negatively associated with their health perceptions and knowledge of hepatitis. This finding indicates that elderly patients tend to have lower perceived susceptibilities, benefits, and cues to action for prevention of liver cancer; less knowledge about hepatitis; and less willingness to receive antiviral therapy. Similarly, Henchoz *et al*[7] found that the paradox between health status and health perception diverges with advancing age.

Conversely, the participants' perceived barriers had a significantly negative relationship to their willingness to receive antiviral therapy. High barriers to screening and adverse consequences from antiviral therapy such as pain, high costs, and time expenditure may discourage patients from regular screenings and cause them to withdraw early from antiviral therapy. This finding is also consistent with previous studies[2,19]. Thus, identifying the patients' inherent barriers is the first crucial step for increasing participation in preventive behaviors[2].

There were two limitations in the present study. Because the participants were restricted to an outpatient clinic of a rural medical institution, generalization beyond this population may be limited. Further research should be conducted in multiple diverse areas. In addition, this study had a cross-sectional descriptive design, and its analytic results do not indicate causality.

In conclusion, patient health perceptions about preventing HCC are related to their age, education levels, household income, and knowledge of hepatitis. Members of the high-risk population of hepatitis B/C carriers should be aware of the serious consequences of chronic active infection and should receive regular surveillance and treatment. To successfully complete a series of medical regimens, detailed explanation and communication regarding possible adverse effects are essential before starting antiviral therapy, especially in elderly individuals. In terms of the implications for clinical practice, healthcare professionals should reduce patient discomfort and inconvenience as much as possible and should strive to remove barriers to screening and treatment. Moreover, clinicians may utilize educational programs as an intervention to increase patient perception, knowledge and willingness to receive antiviral therapy.

ACKNOWLEDGMENTS

The authors sincerely appreciate the research assistants for data collection and entry and thank the participants who provided information.

COMMENTS

Background

Chronic liver disease is the eighth most common cause of death, and hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death in Taiwan. More than 80% of primary HCC is attributed to chronic infection with hepatitis B/C viruses. The early development of HCC is asymptomatic, and patients with chronic hepatitis are usually unaware of their carrier status, the symptoms and signs of HCC, and the importance of regular surveillance and treatment.

Research frontiers

The participants' educational levels were significantly correlated to their health perceptions (except for perceived severity) and knowledge about hepatitis. The elderly patients and those with lower household incomes tend to have lower perceived susceptibility, benefits, and cues to action for prevention of HCC, less knowledge about hepatitis and less willingness to receive antiviral therapy. The results indicated that the participants' age and perceived barriers were significantly associated with their willingness to receive antiviral therapy.

Innovations and breakthroughs

Chronic hepatitis B/C carriers may benefit from regular surveillance for allowing an early diagnose of HCC. In addition, raising awareness of and health perceptions about HCC, and increasing willingness to receive antiviral therapy for preventing the development of HCC are crucial in patients with chronic hepatitis B/C, particularly in rural areas.

Applications

In terms of the implications for clinical practice, healthcare professionals should reduce patient discomfort and inconvenience as much as possible and should strive to remove barriers to screening and treatment. Moreover, clinicians may utilize educational programs as an intervention to increase patient knowledge and willingness to receive antiviral therapy.

Terminology

Health perception is defined as "an individual's evaluation of his or her own health". Health perception can determine personal health values and influence health behaviors.

Peer review

The authors conducted a cross-sectional descriptive study to identify several important factors of healthy perceptions about preventing HCC in Taiwan. The participants' age and perceived barriers were correlated with their willingness to receive antiviral therapy. The study was novel and well-constructed and the results have many implications in the field of preventive medicine.

REFERENCES

1 The statistical analysis of causes of death. [cited 2012 Jun 25]. Available from: http://www.doh.gov.tw/ufile/doc/2010-statistics%20of%20cause%20of%20death.pdf
2 **Ma GX**, Fang CY, Shive SE, Toubbeh J, Tan Y, Siu P. Risk perceptions and barriers to Hepatitis B screening and vaccination among Vietnamese immigrants. *J Immigr Minor Health* 2007; **9**: 213-220 [PMID: 17265128 DOI: 10.1007/s10903-006-9028-4]
3 **Robotin MC**, George J, Supramaniam R, Sitas F, Penman AG. Preventing primary liver cancer: how well are we faring towards a national hepatitis B strategy? *Med J Aust* 2008; **188**: 363-365 [PMID: 18341462]
4 **Wilson JF**. Liver cancer on the rise. *Ann Intern Med* 2005; **142**: 1029-1032 [PMID: 15968025]
5 **Riley TR**, Smith JP. Preventive care in chronic liver disease. *J Gen Intern Med* 1999; **14**: 699-704 [PMID: 10571719]
6 **Wai CT**, Wong ML, Ng S, Cheok A, Tan MH, Chua W, Mak B, Aung MO, Lim SG. Utility of the Health Belief Model in predicting compliance of screening in patients with chronic hepatitis B. *Aliment Pharmacol Ther* 2005; **21**: 1255-1262 [PMID: 15882247 DOI: 10.1111/j.1365-2036.2005.02497.x]
7 **Henchoz K**, Cavalli S, Girardin M. Health perception and health status in advanced old age: A paradox of association. *J Aging Stud* 2008; **22**: 282-290 [DOI: 10.1016/j.jaging.2007.03.002]
8 **Brandon LJ**, Proctor L. Comparison of health perceptions and health status in African Americans and Caucasians. *J Natl Med Assoc* 2010; **102**: 590-597 [PMID: 20690322]
9 **Kartal A**, İnci FH. A cross-sectional survey of self-perceived health status and metabolic control values in patients with type 2 diabetes. *Int J Nurs Stud* 2011; **48**: 227-234 [PMID: 20678769 DOI: 10.1016/j.ijnurstu.2010.07.004]
10 **Champion VL**. Instrument development for health belief model constructs. *ANS Adv Nurs Sci* 1984; **6**: 73-85 [PMID: 6426380]
11 **Rosenstock IM.** Historical origins of the health belief model. *Health Educ Monogr* 1974; **2:** 328-335
12 **Armstrong SN**, Anderson M, Le ET, Nguyen LH. Application of the Health Belief Model to bariatric surgery. *Gastroenterol Nurs* 2009; **32**: 171-178 [PMID: 19506433 DOI: 10.1097/SGA.0b013e3181a7cf5a]
13 **Yarbrough SS**, Braden CJ. Utility of health belief model as a guide for explaining or predicting breast cancer screening behaviours. *J Adv Nurs* 2001; **33**: 677-688 [PMID: 11298205]
14 **Hislop TG**, Teh C, Low A, Li L, Tu SP, Yasui Y, Taylor VM. Hepatitis B knowledge, testing and vaccination levels in Chinese immigrants to British Columbia, Canada. *Can J Public Health* 2007; **98**: 125-129 [PMID: 17441536]
15 **Ma GX**, Shive SE, Toubbeh JI, Tan Y, Wu D. Knowledge, attitudes, and behaviors of Chinese hepatitis B screening and vaccination. *Am J Health Behav* 2008; **32**: 178-187 [PMID: 18052858 DOI: 10.5555/ajhb.2008.32.2.178]
16 **Wai CT**, Mak B, Chua W, Tan MH, Ng S, Cheok A, Wong ML, Lim SG. Misperceptions among patients with chronic hepatitis B in Singapore. *World J Gastroenterol* 2005; **11**: 5002-5005 [PMID: 16124053]
17 **Treloar C**, Hull P, Bryant J, Hopwood M, Grebely J, Lavis Y. Factors associated with hepatitis C knowledge among a sample of treatment naive people who inject drugs. *Drug Alcohol Depend* 2011; **116**: 52-56 [PMID: 21194852 DOI: 10.1016/j.drugalcdep.2010.11.018]
18 **Chen TH**, Chen CJ, Yen MF, Lu SN, Sun CA, Huang GT, Yang PM, Lee HS, Duffy SW. Ultrasound screening and risk factors for death from hepatocellular carcinoma in a high risk group in Taiwan. *Int J Cancer* 2002; **98**: 257-261 [PMID: 11857416]
19 **Chao WH**, Huang MC. Use of the health belief model in predicting screening among HBsAg positive staff in a medical center. Unpublished master's dissertation. Taiwan: National

Cheng Kung University, 2008: 134
20 **Thompson MJ**, Taylor VM, Yasui Y, Hislop TG, Jackson JC, Kuniyuki A, Teh C. Hepatitis B knowledge and practices among Chinese Canadian women in Vancouver, British Columbia. *Can J Public Health* 2003; **94**: 281-286 [PMID: 12873087]
21 **Hsu CE**, Zhang G, Yan FA, Shang N, Le T. What made a successful hepatitis B program for reducing liver cancer disparities: an examination of baseline characteristics and educational intervention, infection status, and missing responses of at-risk Asian Americans. *J Community Health* 2010; **35**: 325-335 [PMID: 20135208 DOI: 10.1007/s10900-010-9238-5]
22 **Chan OK**, Lao TT, Suen SS, Lau TK, Leung TY. Knowledge on hepatitis B infection among pregnant women in a high endemicity area. *Patient Educ Couns* 2011; **85**: 516-520 [PMID: 21167671 DOI: 10.1016/j.pec.2010.11.006]
23 **Hwang JP**, Huang CH, Yi JK. Knowledge about hepatitis B and predictors of hepatitis B vaccination among Vietnamese American college students. *J Am Coll Health* 2008; **56**: 377-382 [PMID: 18316280 DOI: 10.3200/JACH.56.44.377-382]
24 **Yamazhan T**, Durusoy R, Tasbakan MI, Tokem Y, Pullukcu H, Sipahi OR, Ulusoy S. Nursing students' immunisation status and knowledge about viral hepatitis in Turkey: a multicentre cross-sectional study. *Int Nurs Rev* 2011; **58**: 181-185 [PMID: 21554290 DOI: 10.1111/j.1466-7657.2010.00869.x]
25 **Wang WL**, Wang CJ, Tseng HF. Comparing knowledge, health beliefs, and self-efficacy toward hepatitis B prevention among university students with different hepatitis B virus infectious statuses. *J Nurs Res* 2009; **17**: 10-19 [PMID: 19352225 DOI: 10.1097/JNR.0b013e3181999ca3]
26 **Cacoub P**, Ouzan D, Melin P, Lang JP, Rotily M, Fontanges T, Varastet M, Chousterman M, Marcellin P. Patient education improves adherence to peg-interferon and ribavirin in chronic genotype 2 or 3 hepatitis C virus infection: a prospective, real-life, observational study. *World J Gastroenterol* 2008; **14**: 6195-6203 [PMID: 18985810]

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L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3466

World J Gastroenterol 2013 June 14; 19(22): 3466-3472
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Rockall score in predicting outcomes of elderly patients with acute upper gastrointestinal bleeding

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Received: March 7, 2013 Revised: March 27, 2013
Accepted: April 3, 2013
Published online: June 14, 2013

Abstract

AIM: To validate the clinical Rockall score in predicting outcomes (rebleeding, surgery and mortality) in elderly patients with acute upper gastrointestinal bleeding (AUGIB).

METHODS: A retrospective analysis was undertaken in 341 patients admitted to the emergency room and Intensive Care Unit of Xuanwu Hospital of Capital Medical University with non-variceal upper gastrointestinal bleeding. The Rockall scores were calculated, and the association between clinical Rockall scores and patient outcomes (rebleeding, surgery and mortality) was assessed. Based on the Rockall scores, patients were divided into three risk categories: low risk $\leqslant$ 3, moderate risk 3-4, high risk $\geqslant$ 4, and the percentages of rebleeding/death/surgery in each risk category were compared. The area under the receiver operating characteristic (ROC) curve was calculated to assess the validity of the Rockall system in predicting rebleeding, surgery and mortality of patients with AUGIB.

RESULTS: A positive linear correlation between clinical Rockall scores and patient outcomes in terms of rebleeding, surgery and mortality was observed (r = 0.962, 0.955 and 0.946, respectively, P = 0.001). High clinical Rockall scores > 3 were associated with adverse outcomes (rebleeding, surgery and death). There was a significant correlation between high Rockall scores and the occurrence of rebleeding, surgery and mortality in the entire patient population (χ^2 = 49.29, 23.10 and 27.64, respectively, P = 0.001). For rebleeding, the area under the ROC curve was 0.788 (95%CI: 0.726-0.849, P = 0.001); For surgery, the area under the ROC curve was 0.752 (95%CI: 0.679-0.825, P = 0.001) and for mortality, the area under the ROC curve was 0.787 (95%CI: 0.716-0.859, P = 0.001).

CONCLUSION: The Rockall score is clinically useful, rapid and accurate in predicting rebleeding, surgery and mortality outcomes in elderly patients with AUGIB.

Key words: Rockall score; Acute upper gastrointestinal bleeding; Prognosis; Elderly patients

Core tip: This study verified the advantages of the Rockall score in predicting the outcomes of the elderly patients with non-variceal upper gastrointestinal bleeding (UGIB) and assessed its clinical usefulness and prognostic value in rebleeding, surgery and mortality. The results suggest that the Rockall scoring system had satisfactory validity for the prediction of rebleeding, surgery and mortality in patients with acute non-variceal UGIB, and there was a positive linear correlation between the clinical Rockall scores and patient outcomes in terms of rebleeding, surgery and mortality.

Wang CY, Qin J, Wang J, Sun CY, Cao T, Zhu DD. Rockall score in predicting outcomes of elderly patients with acute upper gastrointestinal bleeding. *World J Gastroenterol* 2013; 19(22): 3466-3472 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3466.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3466

INTRODUCTION

Acute upper gastrointestinal bleeding (AUGIB) is common, costly, and potentially life-threatening and requires prompt assessment and aggressive medical management[1]. Despite changes in management, mortality has not significantly improved over the past 50 years. Elderly patients and those with chronic medical diseases withstand AUGIB less well than younger, fitter patients, and have a higher risk of death[2,3]. AUGIB is defined as hemorrhage that emanates proximal to the ligament of Treitz, which differentiates it from lower gastrointestinal bleeding involving the colon, and middle gastrointestinal bleeding involving the small intestine distal to the ligament of Treitz[4]. Clinically, AUGIB often causes hypodynamia, hematemesis (vomiting of blood), melena (passage of black tarry stools due to the presence of altered blood), and systemic shock typically ensues upon loss of 15% or more of the circulating blood volume. The color of the vomitus depends on its contact time with hydrochloric acid in the stomach[5,6]. If vomiting occurs early after the onset of bleeding, it appears red; with delayed vomiting, it is dark red, brown, or black. Coffee-ground emesis results from the precipitation of blood clots in the vomitus. Hematochezia (red blood per rectum) usually indicates bleeding distal to the ligament of Treitz. Occasionally, rapid bleeding from an upper gastrointestinal bleeding source may result in hematochezia[7]. The rate and extent of hemorrhage, coupled with the patient's comorbidities, determine the clinical presentation of UGIB. AUGIB is a common medical emergency, the annual incidence of hospitalization for AUGIB is 50-150 per 10000 people in China, and it has a mortality of 4%-14%[4]. Early predictors of adverse prognosis in AUGIB, include increasing age (above 60 years), an increased number of co-morbid conditions, the underlying cause of bleeding (*i.e.*, variceal), red blood cells (RBCs) in the emesis or stool, shock or hypotension on presentation, an increased number of units of blood transfused, active bleeding at the time of endoscopy, bleeding from large (> 2 cm) ulcers, onset of bleeding in the hospital, and emergency surgery[8-10]. Timely and effective assessment of the patient's condition and the degree of risk is very important, which could serve as a basis for the establishment of treatment procedures to reduce medical costs and improve prognosis[11].

One of the major challenges in managing UGIB involves the identification of patients who are at high risk of rebleeding and death; conversely, the identification of patients who are suitable for early discharge and outpatient endoscopy is also important for effective resource use[12]. Similar to other common medical conditions, risk scores have been developed to try and identify those at lower or higher risk of poor outcome[13]. An ideal risk score is one that is easy to calculate, accurate for relevant outcomes and can be measured early after presentation with AUGIB. Several clinical scoring systems have been developed to help predict outcome in patients with a view to improving patient management and promoting cost-effective use of resources. In most published scoring systems, a combination of clinical, laboratory, and endoscopic variables are weighted to produce a score that predicts the risk of mortality, recurrent hemorrhage, need for clinical intervention, or suitability for early discharge. The commonly used systems are the Rockall score, the Baylor bleeding score, the Cedars-Sinai Medical Centre Predictive Index, and the Blatchford score[14-17]. The most commonly used risk scoring system in UGIB is the Rockall score, which was described in 1996 following the analysis of data from a large English audit[12]. The score was developed to assess the risk of death following presentation with UGIB and incorporates patient age, hemodynamics, comorbidities and endoscopic findings. The clinical Rockall score, which relies on only clinical variables, is used to identify patients with AUGIB who have an adverse outcome, such as death or recurrent bleeding. The complete Rockall score, which relies on clinical and endoscopic variables, is also used to identify patients with AUGIB who died or have recurrent bleeding[18,19]. Rockall scores can be calculated both before and after endoscopy, but the post-endoscopic Rockall score provides a more accurate risk assessment. Patients at high-risk for rebleeding receive endoscopic therapy to achieve hemostasis and are subsequently treated with high-dose acid suppression to promote the formation of blood clots over the arterial defect responsible for bleeding. The aim of this study was to verify the advantages of the Rockall score in 341 patients with non-variceal upper gastrointestinal bleeding admitted to the emergency room and Intensive Care Unit (ICU) of Xuanwu Hospital, and to assess its clinical usefulness and prognostic value in rebleeding, surgery and mortality.

MATERIALS AND METHODS

Patients

This study included 341 patients with non-variceal UGIB admitted to the emergency room and ICU of Xuanwu Hospital of Capital Medical University. The median age of the patients was 72.85 ± 7.11 years (range: 60-85 years) and 181 were men and 160 were women. Patients admitted to hospital through the emergency department with a primary diagnosis of UGIB (hematemesis, melena or bloody nasogastric aspirate) were considered for inclusion, endoscopies were performed to confirm the diagnosis within 6-48 h after admission, and the characteristics of the patients are presented in Table 1. Patients were selected based on the following criteria: ⩾ 60 years of age; patients with clinically significant UGIB (*i.e.*, signs of active UGIB including hematemesis, melena or hematochezia) confirmed by gastroscopy, surgery, blood or coffee grounds detected during nasogastric lavage; patients fulfilling the low-risk criteria such as having a low risk of requiring intervention (endoscopic therapy, blood transfusion, surgery) or death if they had a Rockall score ⩽ 2 and were < 70 years old. Patients were excluded based on the following criteria: < 60 years of age; patients with a record of poor compliance, such as those

Table 1 Classification of patients with acute upper gastrointestinal bleeding

Classification of diseases	*n* = 341
Esophageal diseases	74
Esophageal carcinoma	37
Esophagitis	25
Mallory-Weiss syndrome	7
Hiatus hernia	5
Gastroduodenal disease	265
Peptic ulcer	151
Stomach cancer	59
Erosive gastritis	32
Anastomotic	12
Acute gastric mucosal lesion	11
Other	2

who did not undergo endoscopy; patients with acute variceal or obscure UGIB.

Calculation of Rockall scores

The clinical Rockall score, which was calculated without endoscopic findings for each patient, was based on points assigned for clinical variables: patient age at presentation, shock status based on initial heart rate and systolic blood pressure, and presence of comorbid disease (Table 2). The associations between Rockall scores and rebleeding rate, mortality rate and surgical rate were evaluated. Scores ranged from 0 to 9 and were divided into three risk categories: low risk $\leqslant$ 3, moderate risk 3-4, and high risk $\geqslant$ 4. We used the observed percentages of rebleeding/death/surgery in each risk category in the original patient sample of Rockall as the predicted probabilities of rebleeding/mortality for both validation samples. Calibration and discrimination were assessed as measures of validity of the scoring system. Calibration was evaluated by a χ^2 goodness of fit test, and discrimination was evaluated by calculating the area under the receiver operating characteristic (ROC) curve.

Comorbidity was based on reference standard diagnostic criteria, including cardiovascular and cerebrovascular disease, chronic obstructive pulmonary disease, chronic liver disease and cancer.

Rebleeding or bleeding recurrence was defined as a separate episode of hematemesis or melena, or nasogastric evidence of new bleeding, occurring during admission and within 24 h of initial presentation, as witnessed by hospital staff. Hematemesis was defined as the vomiting of fresh or old blood, including "coffee grounds." Melena was defined as the passage of black or tarry stools. Mortality was defined as death occurring within 30 d of hospital admission.

Successful hemostasis was defined as endoscopic hemostasis or negative occult blood in the feces, and patients were hemostatically stable when no hematemesis or melena was observed.

Rebleeding manifestations were defined by at least one of the following: recurrent hematemesis or melena, bloody or red colored vomit, or bloody stools (blood in the stool that may appear as maroon or red), or the patient had hyperactive bowel sounds; hemorrhagic peripheral circulatory failure (due to excessive blood loss and rapid bleeding) was not improved or hemodynamic status was temporarily improved after fluid infusion and blood transfusion, and the central venous pressure fluctuated and then decreased; RBC counts and hemoglobin levels continued to decline, and high reticulocyte count (increased RBC destruction such as bleeding or hemolysis) was observed; serum creatinine level increased when 24-h total volumes of fluid infusion and urinary output were normal; a relatively large amount of fresh blood was drained by nasogastric tube lavage.

Surgical treatment guidelines were as follows: conservative treatment was not sufficient and the bleeding continued, and patients suspected of having a perforated duodenal ulcer were transferred to the surgical ICU.

Statistical analysis

SPSS statistical software version 19.0 (SPSS Inc., Chicago, IL, United States) was used for data analysis and management. The sensitivity and specificity of detecting patients who needed clinical intervention, had recurrent bleeding, or died were calculated for the clinical Rockall score and the complete Rockall score with confidence interval. The Rockall scores for all patients were calculated based on their pre-endoscopic variables. The correlation between the variables was analyzed using the Pearson product-moment correlation. Categorical variables were analyzed by χ^2 tests. We assessed the validity of the scoring systems by plotting ROC curves. A two-sided *P* value of less than 0.05 was considered statistically significant.

RESULTS

Rockall scores and clinical outcomes

Of 341 patients, 63 (18.47%) patients developed recurrent bleeding, 30 (8.79%) patients died and 31 (9.09%) patients required endoscopic treatment. The Rockall scores were calculated based on the collected data (Table 3). A positive linear correlation between the clinical Rockall scores and patient outcomes in terms of rebleeding, surgery and mortality (r = 0.962, 0.955 and 0.946, respectively, P = 0.001) was observed. High clinical Rockall scores > 3 were associated with adverse outcomes (rebleeding, surgery and death).

Distribution of patients in the risk categories

The distribution of patients classified into the three risk categories (low, moderate, high), as determined by the Rockall risk scoring system, and the observed percentages of rebleeding, surgery and mortality in each risk category are shown in Table 4. The Rockall score identified 114 of 341 patients as low risk $\leqslant$ 3), 110 of 341 patients as moderate risk (3-4) and 117 of 341 patients as high risk $\geqslant$ 4). There were significant correlations between high Rockall scores and the occurrence of rebleeding, surgery and mortality in the entire patient population (χ^2 = 49.29, 23.10 and 27.64, respectively, P = 0.001).

Table 2 Rockall scores in patients with upper gastrointestinal bleeding

Variable	Scores			
	0	1	2	3
Age (yr)	< 60	60-79	⩾ 80	
Shock	No shock; SBP ⩾ 100 mmHg; pulse < 100 bpm	SBP ⩾ 100 mmHg; Pulse ⩾ 100 bpm	SBP < 100 mmHg; Pulse ⩾ 100 bpm	
Comorbidity	No major		CHF, IHD, major morbidity	Renal failure, liver failure, metastatic cancer
Diagnosis	Mallory-Weiss syndrome	All other diagnoses	GI malignancy	
Evidence of bleeding	None		Blood, adherent clot, spurting vessel	

CHF: Chronic hearth failure; IHD: Ischaemic heart disease; SBP: Systolic blood pressure; GI: Gastrointestinal.

Table 3 Relationship between clinical Rockall scores and patient outcomes

Variables	Rockall score							
	1	2	3	4	5	6	7	⩾ 8
Number	65	49	44	66	51	31	20	15
Rebleeding	2	3	4	9	14	11	10	10
Mortality	0	1	2	4	7	8	4	4
Surgery	0	1	3	5	9	6	4	3

Table 4 Percentages of rebleeding/death/surgery in each risk category *n* (%)

Category	Cases	Outcome		
		Rebleeding	Surgery	Mortality
Low-risk	114	5 (4.38)	1 (0.87)	1 (0.87)
Moderate-risk	110	13 (11.81)	8 (7.27)	6 (5.45)
High-risk	117	45 (38.46)	22 (18.80)	23 (19.65)

Predictive value of the Rockall score for rebleeding, surgery and mortality in patients with upper gastrointestinal bleeding

The discriminative ability of the Rockall score for the prediction of rebleeding and mortality were compared. The Rockall score was found to have good predictive value for rebleeding (area under the ROC curve was 0.788, 95%CI: 0.726-0.849, $P = 0.001$), surgery (area under the ROC curve was 0.752, 95%CI: 0.679-0.825, $P = 0.001$) and mortality (area under the ROC curve was 0.787, 95%CI: 0.716-0.859, $P = 0.001$).

DISCUSSION

Acute non-variceal UGIB remains a common and challenging emergency for gastroenterologists and general physicians[20]. The annual incidence is 50-150 per 100000 of the population, and although there have been significant improvements in endoscopic and supportive therapies, the overall mortality stubbornly remains around 10% (4%-14%), and may even reach 27% in hospitalized patients with serious co-morbidity[21]. AUGIB results in considerable patient morbidity and significant medical costs. Elderly patients (aged over 80 years) now account for around 25% of all AUGIB and 33% of AUGIB occurring in hospitalized patients, and therefore tend to account for much of the poor outcome associated with this condition[1]. Many risk factors are associated with bleeding, and these must be addressed. Pharmacists, physicians, and dentists should record patients' medical history and analgesic requirements. The initial evaluation of patients with AUGIB involves recognition of a range of symptoms depending on the source, rate, and volume of blood loss[2]. Medical comorbidities and the use of antiplatelet medications can complicate the severity and management of bleeding, especially in the elderly. Symptoms of AUGIB include anemia, hematemesis (vomiting bright red blood or "coffee ground" material), and melena[5,6]. Other symptoms may include epigastric pain, dyspnea, and syncope (due to volume depletion). Bleeding may be obscure when the gastrointestinal blood loss is of unknown origin[7]. Certain prognostic factors in patients who present with AUGIB can increase the incidence of complications, including morbidity and mortality[22]. The patient should be admitted to the ICU if one or more of the following prognostic factors are present: age greater than 60 years; shock; comorbidities (*e.g.*, cardiac, renal and hepatic diseases); current bleeding; low systolic blood pressure; need for more than 6 units of blood; and endoscopy showing major signs of bleeding.

Several clinical scoring systems, *e.g.*, the Rockall score, the Blatchford score and Acute Physiology and Chronic Health Evaluation Ⅱ score, have been developed to direct appropriate patient management and predict mortality as well as rebleeding. These systems weigh a combination of clinical, laboratory and endoscopic variables to produce a score that predicts the risk of mortality, recurrent hemorrhage, the need for clinical intervention or suitability for early discharge[23-25]. Factors commonly associated with poor outcome in patients with AUGIB may be related to presentation and co-morbidities, or to the behavior of the ulcer. Before endoscopy is performed, use of the Rockall risk scoring system is recommended. This assessment tool, which predicts the patient's outcome and estimates rebleeding risk, is the most widely

used scoring system and has been validated by several studies. The patient's age, systolic blood pressure, pulse rate and the presence of comorbidities are used for scoring. Patients with a score of 0 should be considered for non-admission or early discharge with outpatient follow-up; if the score is above 0, there is a significant risk of mortality, and endoscopy is recommended for a full assessment of bleeding risk[14-16].

Rockall included 4185 cases of AUGIB from 74 hospitals in the United Kingdom over a four-month period in 1993. Their scoring system was based on multivariate analysis of information from history, examination, blood tests, and endoscopic investigation. The complete Rockall score makes use of both clinical and endoscopic criteria to predict the risks of rebleeding and death; the scale ranges from 0 to 11 points, with higher scores indicating higher risk. In the present study, we used Rockall's risk scoring system to classify patients and found that high clinical Rockall scores > 3 were associated with adverse outcomes (rebleeding, surgery and death), and the results obtained were widely corroborated in clinical practice. The complete Rockall score has been validated as a clinically useful score in predicting outcomes (rebleeding, mortality) of patients with acute non-variceal UGIB[26,27]. As the original study included only 180 of 4185 patients with esophagus-stomach fundus variceal hemorrhage, some investigators argued that the Rockall score might not be ideal or accurate in predicting rebleeding and mortality in patients with esophagus-stomach fundus variceal hemorrhage.

In the present study, we found that 63 (18.47%) patients developed recurrent bleeding, 30 (8.79%) patients died and 31 (9.09%) patients required endoscopic treatment. These results were consistent with earlier research[28]. A positive linear correlation between the clinical Rockall scores and patient outcomes in terms of rebleeding, surgery and mortality (r = 0.962, 0.955 and 0.946, respectively, P = 0.001) was observed. High clinical Rockall scores > 3 were associated with adverse outcomes (rebleeding, surgery and death). Our results validated the clinical Rockall score in predicting patient outcome (*i.e.*, rebleeding, surgery and mortality) after acute non-variceal UGIB, which will help identify low-risk patients for delayed, elective or outpatient endoscopy, whereas those at high risk could have urgent endoscopy and a higher level of hospital care[29-33].

Recurrence of bleeding is one of the most important factors affecting prognosis, and early prediction and treatment of rebleeding would improve the outcome in patients with acute non-variceal UGIB, as rebleeding is associated with high mortality[34-36]. The commonly used scoring systems are the Rockall score, the Baylor bleeding score, the Cedars-Sinai Medical Centre Predictive Index, and the Blatchford score[37]. The Cedars-Sinai Medical Centre Predictive Index was less accurate than the Rockall score in predicting patient outcome (*i.e.*, rebleeding, surgery and mortality), the Baylor bleeding score is commonly used in predicting the likelihood of rebleeding after endoscopic hemostasis of peptic ulcers, while the complete Rockall score has been found to have good predictive value for mortality and in-hospital rebleeding. In this study, we showed that a low clinical Rockall risk score in patients with AUGIB without endoscopy was not associated with adverse outcomes (rebleeding or mortality), whereas a high clinical risk score was associated with adverse outcomes. A positive linear correlation between the clinical Rockall scores and patient outcomes in terms of rebleeding, surgery and mortality (r = 0.962, 0.955 and 0.946, respectively, P = 0.001) was observed. The discriminative ability of the Rockall score for the prediction of rebleeding and mortality was compared. For rebleeding, the area under the ROC curve was 0.788 (95%CI: 0.726-0.849. P = 0.001). For mortality, the area under the ROC curve was 0.787 (95%CI: 0.716-0.859. P = 0.001). Our results were consistent with those of other studies and suggested that the Rockall score had good predictive value for mortality and in-hospital rebleeding, and was validated as a clinically useful scoring system for stratifying patients into high-risk and low-risk categories for mortality and in-hospital rebleeding[38,39]. However, other reports have suggested that the Rockall score showed inadequate sensitivity and poor specificity for outcome prediction in terms of rebleeding and mortality, thus, further clinical research is needed to confirm our observations[40].

In conclusion, our results suggest that the Rockall risk scoring system had satisfactory validity for the prediction of rebleeding, surgery and mortality in patients with acute non-variceal UGIB, and a positive linear correlation between the clinical Rockall scores and patient outcomes in terms of rebleeding, surgery and mortality was observed. The problems associated with AUGIB are challenging for patients and physicians, and a combination of clinical and laboratory assessments (including the Cedars-Sinai Medical Centre Predictive Index and Baylor bleeding score) should be performed to comprehensively assess and correctly diagnose various conditions in patients in order to develop appropriate treatment programs and improve the prognosis of patients.

COMMENTS

Background

Acute upper gastrointestinal bleeding (AUGIB) is common, costly, and potentially life-threatening and requires prompt assessment and aggressive medical management. The most commonly used risk scoring system in UGIB is the Rockall score, which was described in 1996 following the analysis of data from a large English audit.

Research frontiers

Rockall scores can be calculated both before and after endoscopy, but the post-endoscopic Rockall score provides a more accurate risk assessment. Patients at high-risk for rebleeding receive endoscopic therapy to achieve hemostasis and are subsequently treated with high-dose acid suppression to promote the formation of blood clots over the arterial defect responsible for bleeding.

Innovations and breakthroughs

Several clinical scoring systems have been developed to help predict outcome in patients with a view to improving patient management and promoting cost-effective use of resources. In most published scoring systems, a combination of clinical, laboratory, and endoscopic variables are weighted to produce a score that predicts the risk of mortality, recurrent hemorrhage, need for clinical intervention, or suitability for early discharge. The commonly used systems are the Rockall score, the Baylor bleeding score, the Cedars-Sinai Medical Centre

Predictive Index, and the Blatchford score. This study verified the advantages of the Rockall score in predicting the outcomes of the elderly patients with non-variceal UGIB and assessed its clinical usefulness and prognostic value in rebleeding, surgery and mortality.

Applications

The authors found that the Rockall score is clinically useful, rapid and accurate in predicting rebleeding, surgery and mortality outcomes in elderly patients with acute upper gastrointestinal bleeding.

Peer review

This manuscript is very interesting. The authors intented to validate the clinical Rockall score in predicting outcomes (rebleeding, surgery and mortality) in elderly patients with acute upper gastrointestinal bleeding. The study is well designed and the data well support the conclusion. Rockall score plays an important role in predicting the outcomes of the elderly patients with non-variceal upper gastrointestinal bleeding. This study will be informative for the readers.

REFERENCES

1 **van Leerdam ME**. Epidemiology of acute upper gastrointestinal bleeding. *Best Pract Res Clin Gastroenterol* 2008; **22**: 209-224 [PMID: 18346679 DOI: 10.1016/j.bpg.2007.10.011]

2 **Charatcharoenwitthaya P**, Pausawasdi N, Laosanguaneak N, Bubthamala J, Tanwandee T, Leelakusolvong S. Characteristics and outcomes of acute upper gastrointestinal bleeding after therapeutic endoscopy in the elderly. *World J Gastroenterol* 2011; **17**: 3724-3732 [PMID: 21990954 DOI: 10.3748/wjg.v17.i32.3724]

3 **Hearnshaw SA**, Logan RF, Lowe D, Travis SP, Murphy MF, Palmer KR. Acute upper gastrointestinal bleeding in the UK: patient characteristics, diagnoses and outcomes in the 2007 UK audit. *Gut* 2011; **60**: 1327-1335 [PMID: 21490373 DOI: 10.1136/gut.2010.228437]

4 **Vreeburg EM**, Snel P, de Bruijne JW, Bartelsman JF, Rauws EA, Tytgat GN. Acute upper gastrointestinal bleeding in the Amsterdam area: incidence, diagnosis, and clinical outcome. *Am J Gastroenterol* 1997; **92**: 236-243 [PMID: 9040198]

5 **Rivkin K**, Lyakhovetskiy A. Treatment of nonvariceal upper gastrointestinal bleeding. *Am J Health Syst Pharm* 2005; **62**: 1159-1170 [PMID: 15914876]

6 **Wilcox CM**, Clark WS. Causes and outcome of upper and lower gastrointestinal bleeding: the Grady Hospital experience. *South Med J* 1999; **92**: 44-50 [PMID: 9932826 DOI: 10.1097/00007611-199901000-00008]

7 **Laine L**, Peterson WL. Bleeding peptic ulcer. *N Engl J Med* 1994; **331**: 717-727 [PMID: 8058080 DOI: 10.1056/NEJM199409153311107]

8 **Mondardini A**, Barletti C, Rocca G, Garripoli A, Sambataro A, Perotto C, Repici A, Ferrari A. Non-variceal upper gastrointestinal bleeding and Forrest's classification: diagnostic agreement between endoscopists from the same area. *Endoscopy* 1998; **30**: 508-512 [PMID: 9746157 DOI: 10.1055/s-2007-1001335]

9 **Himal HS**, Watson WW, Jones CW, Miller L, Maclean LD. The management of upper gastrointestinal hemorrhage: a multiparametric computer analysis. *Ann Surg* 1974; **179**: 489-493 [PMID: 4544547 DOI: 10.1097/00000658-197404000-00019]

10 **Sung JJ**, Tsoi KK, Ma TK, Yung MY, Lau JY, Chiu PW. Causes of mortality in patients with peptic ulcer bleeding: a prospective cohort study of 10,428 cases. *Am J Gastroenterol* 2010; **105**: 84-89 [PMID: 19755976 DOI: 10.1038/ajg.2009.507]

11 **Fleischer D**. Etiology and prevalence of severe persistent upper gastrointestinal bleeding. *Gastroenterology* 1983; **84**: 538-543 [PMID: 6600435]

12 **Rockall TA**, Logan RF, Devlin HB, Northfield TC. Influencing the practice and outcome in acute upper gastrointestinal haemorrhage. Steering Committee of the National Audit of Acute Upper Gastrointestinal Haemorrhage. *Gut* 1997; **41**: 606-611 [PMID: 9414965]

13 **Vreeburg EM**, Terwee CB, Snel P, Rauws EA, Bartelsman JF, Meulen JH, Tytgat GN. Validation of the Rockall risk scoring system in upper gastrointestinal bleeding. *Gut* 1999; **44**: 331-335 [PMID: 10026316 DOI: 10.1136/gut.44.3.331]

14 **Blatchford O**, Davidson LA, Murray WR, Blatchford M, Pell J. Acute upper gastrointestinal haemorrhage in west of Scotland: case ascertainment study. *BMJ* 1997; **315**: 510-514 [PMID: 9329304 DOI: 10.1136/bmj.315.7107.510]

15 **Enns RA**, Gagnon YM, Barkun AN, Armstrong D, Gregor JC, Fedorak RN. Validation of the Rockall scoring system for outcomes from non-variceal upper gastrointestinal bleeding in a Canadian setting. *World J Gastroenterol* 2006; **12**: 7779-7785 [PMID: 17203520]

16 **Saeed ZA**, Ramirez FC, Hepps KS, Cole RA, Graham DY. Prospective validation of the Baylor bleeding score for predicting the likelihood of rebleeding after endoscopic hemostasis of peptic ulcers. *Gastrointest Endosc* 1995; **41**: 561-565 [PMID: 7672549]

17 **Ali H**, Lang E, Barkan A. Emergency department risk stratification in upper gastrointestinal bleeding. *CJEM* 2012; **14**: 45-49 [PMID: 22417958]

18 **Longstreth GF**, Feitelberg SP. Successful outpatient management of acute upper gastrointestinal hemorrhage: use of practice guidelines in a large patient series. *Gastrointest Endosc* 1998; **47**: 219-222 [PMID: 9540873]

19 **Lahiff C**, Shields W, Cretu I, Mahmud N, McKiernan S, Norris S, Silke B, Reynolds JV, O'Toole D. Upper gastrointestinal bleeding: predictors of risk in a mixed patient group including variceal and nonvariceal haemorrhage. *Eur J Gastroenterol Hepatol* 2012; **24**: 149-154 [PMID: 22113209 DOI: 10.1097/MEG.0b013e32834e37d6]

20 **Wee E**. Management of nonvariceal upper gastrointestinal bleeding. *J Postgrad Med* 2011; **57**: 161-167 [PMID: 21654147 DOI: 10.4103/0022-3859.81868]

21 **Weng SC**, Shu KH, Tarng DC, Tang YJ, Cheng CH, Chen CH, Yu TM, Chuang YW, Huang ST, Sheu WH, Wu MJ. In-hospital mortality risk estimation in patients with acute nonvariceal upper gastrointestinal bleeding undergoing hemodialysis: a retrospective cohort study. *Ren Fail* 2013; **35**: 243-248 [PMID: 23336331]

22 **Chiu PW**, Sung JJ. Acute nonvariceal upper gastrointestinal bleeding. *Curr Opin Gastroenterol* 2010; **26**: 425-428 [PMID: 20703110]

23 **Kim BJ**, Park MK, Kim SJ, Kim ER, Min BH, Son HJ, Rhee PL, Kim JJ, Rhee JC, Lee JH. Comparison of scoring systems for the prediction of outcomes in patients with nonvariceal upper gastrointestinal bleeding: a prospective study. *Dig Dis Sci* 2009; **54**: 2523-2529 [PMID: 19104934 DOI: 10.1007/s10620-008-0654-7]

24 **Atkinson RJ**, Hurlstone DP. Usefulness of prognostic indices in upper gastrointestinal bleeding. *Best Pract Res Clin Gastroenterol* 2008; **22**: 233-242 [PMID: 18346681 DOI: 10.1016/j.bpg.2007.11.004]

25 **Tham TC**, James C, Kelly M. Predicting outcome of acute non-variceal upper gastrointestinal haemorrhage without endoscopy using the clinical Rockall Score. *Postgrad Med J* 2006; **82**: 757-759 [PMID: 17099097]

26 **Bessa X**, O'Callaghan E, Ballesté B, Nieto M, Seoane A, Panadès A, Vazquez DJ, Andreu M, Bory F. Applicability of the Rockall score in patients undergoing endoscopic therapy for upper gastrointestinal bleeding. *Dig Liver Dis* 2006; **38**: 12-17 [PMID: 16314150 DOI: 10.1016/j.dld.2005.05.012]

27 **Zhang J**, Zhang JY, Ding SG, Wang Y, Zhou LY. [Clinical value of endoscopic hemostasis in acute nonvariceal upper gastrointestinal bleeding]. *Beijing Daxue Xuebao* 2012; **44**: 582-587 [PMID: 22898852]

28 **Chen IC**, Hung MS, Chiu TF, Chen JC, Hsiao CT. Risk scoring systems to predict need for clinical intervention for patients with nonvariceal upper gastrointestinal tract bleeding. *Am J Emerg Med* 2007; **25**: 774-779 [PMID: 17870480 DOI:

10.1016/j.ajem.2006.12.024]
29 **Morales Uribe CH**, Sierra Sierra S, Hernández Hernández AM, Arango Durango AF, López GA. Upper gastrointestinal bleeding: risk factors for mortality in two urban centres in Latin America. *Rev Esp Enferm Dig* 2011; **103**: 20-24 [PMID: 21341933 DOI: 10.4321/S1130-01082011000100004]
30 **Espinoza Ríos J**, Huerta-Mercado Tenorio J, Huerta-Mercado Tenorio J, Lindo Ricce M, García Encinas C, Rios Matteucci S, Vila Gutierrez S, Pinto Valdivia J, De Los Rios Senmache R, Piscoya Rivera A, Bussalleu Rivera A. [Prospective validation of the Rockall Scoring System in patients with upper gastrointestinal bleeding in Cayetano Heredia Hospital Lima- Peru]. *Rev Gastroenterol Peru* 2009; **29**: 111-117 [PMID: 19609325]
31 **Soncini M**, Triossi O, Leo P, Magni G, Bertelè AM, Grasso T, Ferraris L, Caruso S, Spadaccini A, Brambilla G, Verta M, Muratori R, Attinà A, Grasso G. Management of patients with nonvariceal upper gastrointestinal hemorrhage before and after the adoption of the Rockall score, in the Italian Gastroenterology Units. *Eur J Gastroenterol Hepatol* 2007; **19**: 543-547 [PMID: 17556899 DOI: 10.1097/MEG.0b013e3281532b89]
32 **Youn YH**, Park YJ, Kim JH, Jeon TJ, Cho JH, Park H. Weekend and nighttime effect on the prognosis of peptic ulcer bleeding. *World J Gastroenterol* 2012; **18**: 3578-3584 [PMID: 22826623 DOI: 10.3748/wjg.v18.i27.3578]
33 **Musa SA**, Brecker SJ, Rahman TM, Kang JY. Upper gastrointestinal haemorrhage in the acute cardiac care setting: antiplatelets and endoscopy. *Scott Med J* 2012; **57**: 88-91 [PMID: 22555229 DOI: 10.1258/smj.2012.012006]
34 **Levin DA**, Watermeyer GA, Deetleefs E, Metz DC, Thomson SR. The efficacy of endoscopic therapy in bleeding peptic ulcer patients. *S Afr Med J* 2012; **102**: 290-293 [PMID: 22554334]
35 **Fattahi E**, Somi MH, Moosapour MR, Fouladi RF. Independent predictors of in-hospital re-bleeding, need of operation and mortality in acute upper gastrointestinal bleeding. *Pak J Biol Sci* 2011; **14**: 849-853 [PMID: 22590836 DOI: 10.3923/pjbs.2011.849.853]
36 **Wilkins T**, Khan N, Nabh A, Schade RR. Diagnosis and management of upper gastrointestinal bleeding. *Am Fam Physician* 2012; **85**: 469-476 [PMID: 22534226]
37 **Camellini L**, Merighi A, Pagnini C, Azzolini F, Guazzetti S, Scarcelli A, Manenti F, Rigo GP. Comparison of three different risk scoring systems in non-variceal upper gastrointestinal bleeding. *Dig Liver Dis* 2004; **36**: 271-277 [PMID: 15115340 DOI: 10.1016/j.dld.2003.10.017]
38 **Sanders DS**, Carter MJ, Goodchap RJ, Cross SS, Gleeson DC, Lobo AJ. Prospective validation of the Rockall risk scoring system for upper GI hemorrhage in subgroups of patients with varices and peptic ulcers. *Am J Gastroenterol* 2002; **97**: 630-635 [PMID: 11922558 DOI: 10.1111/j.1572-0241.2002.05541.x]
39 **Sarwar S**, Dilshad A, Khan AA, Alam A, Butt AK, Tariq S, Ahmad I. Predictive value of Rockall score for rebleeding and mortality in patients with variceal bleeding. *J Coll Physicians Surg Pak* 2007; **17**: 253-256 [PMID: 17553319]
40 **Farooq FT**, Lee MH, Das A, Dixit R, Wong RC. Clinical triage decision vs risk scores in predicting the need for endotherapy in upper gastrointestinal bleeding. *Am J Emerg Med* 2012; **30**: 129-134 [PMID: 21185674 DOI: 10.1016/j.ajem.2010.11.007]

P- Reviewers Chung SCS, Shapira S **S- Editor** Wang JL
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3473

World J Gastroenterol 2013 June 14; 19(22): 3473-3480
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Combination treatment with comprehensive cryoablation and immunotherapy in metastatic hepatocellular cancer

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Received: December 21, 2012 Revised: March 21, 2013
Accepted: May 9, 2013
Published online: June 14, 2013

Abstract

AIM: To retrospectively assess the effect of comprehensive cryosurgery (ablation of intra- and extra-hepatic tumors) plus dendritic cell-cytokine-induced killer cell immunotherapy in metastatic hepatocellular cancer.

METHODS: We divided 45 patients into cryo-immunotherapy (21 patients), cryotherapy (n = 12), immunotherapy (n = 5) and untreated (n = 7) groups. Overall survival (OS) after diagnosis of metastatic hepatocellular cancer was assessed after an 8-year follow-up.

RESULTS: Median OS was higher following cryo-immunotherapy (32 mo) or cryotherapy (17.5 mo; $P < 0.05$) than in the untreated group (3 mo) and was higher in the cryo-immunotherapy group than in the cryotherapy group ($P < 0.05$). In the cryo-immunotherapy group, median OS was higher after multiple treatments (36.5 mo) than after a single treatment (21 mo; $P < 0.05$).

CONCLUSION: Cryotherapy and, especially, cryo-immunotherapy significantly increased OS in metastatic hepatocellular cancer patients. Multiple cryo-immunotherapy was associated with a better prognosis than single cryo-immunotherapy.

Key words: Cryoablation; Dendritic cell-cytokine-induced killer cell; Immunotherapy; Metastatic hepatocellular cancer; Survival time

Core tip: Fourty-five patients of metastatic hepatocellular cancer were divided into cryo-immunotherapy, cryotherapy, immunotherapy and untreated groups. Median overall survival (OS) was higher following cryo-immunotherapy (32 mo) or cryotherapy (17.5 mo) than in the untreated group (3 mo); In the cryo-immunotherapy group, median OS was higher after multiple treatments (36.5 mo) than after a single treatment (21 mo). In a word, cryotherapy and, especially, cryo-immunotherapy significantly increased OS in metastatic hepatocellular cancer patients. Multiple cryo-immunotherapy was associated with a better prognosis than single cryo-immunotherapy.

Niu LZ, Li JL, Zeng JY, Mu F, Liao MT, Yao F, Li L, Liu CY, Chen JB, Zuo JS, Xu KC. Combination treatment with comprehensive cryoablation and immunotherapy in metastatic hepatocellular cancer. *World J Gastroenterol* 2013; 19(22): 3473-3480 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3473.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3473

INTRODUCTION

Hepatocellular carcinoma (HCC), which is the fifth most common cancer worldwide, is usually discovered late and has a poor prognosis[1]. In about 80% of patients, HCC is associated with chronic liver disease (*i.e.*, hepatitis and cirrhosis), with major implications for the prognosis and therapeutic options[2]. Many patients are unsuitable for tumor resection because of factors such as poor hepatic reserve (cirrhosis), multicentric tumors or extrahepatic disease[3,4]. Until recently, no systemic chemotherapy has significantly increased survival in patients with advanced HCC[5,6]. External beam radiation has had a limited role in the treatment of HCC because of radiation toxicity to the adjacent normal liver[7,8]. Percutaneous ablation is currently considered the best therapeutic modality for patients with early stage HCC who are not candidates for surgery; it principally involves percutaneous ethanol injection, radiofrequency ablation, microwave ablation, laser ablation or cryoablation[9].

Because cryoablation forms an ice ball that can be visualized by many imaging methods, it has been an attractive option for reasons of safety. Technically, cryoablation of tumors in multiple organs (*i.e.,* liver, lung, kidney, breast, pancreas and prostate) has been proved to be safe and effective[10]. A long term study of medium to large tumors (more than 5 cm in diameter) treated with cryoablation and/or transarterial chemoembolization (TACE) showed a 5 year survival rate of 23% and local progression rate of 24%[11,12]. To our knowledge, there are currently no reports on the long term effects of simultaneous cryoablation of intra- and extra-hepatic tumors in metastatic HCC patients.

Another potential advantage of the in situ freezing of malignant disease is the cryo-immunologic response[13], which is an antitumor immune response triggered by the natural absorption of malignant tissue[14]. Immunotherapy mediated by autologous dendritic cells (DCs) is a promising treatment option for long lasting control of unresectable HCC[15-17]. Increased knowledge regarding vaccination with DCs co-cultured with cytokine-induced killer (CIK) cells has led to improved clinical treatment strategies[18]. Whether slow release of tumor antigen after cryoablation can improve the effect of immunotherapy remains unknown.

Here, we retrospectively compared the effects of comprehensive cryosurgery (simultaneous cryoablation of intra- and extrahepatic tumors and of liver tumors of diameter greater than 5 cm, with TACE performed once or twice before cryoablation to reduce the tumor to 5 cm) and/or DC-CIK immunotherapy in patients with untreated metastatic HCC. To measure the survival time of patients with metastatic cancer, overall survival (OS) after diagnosis of metastatic disease was the main evaluation index.

MATERIALS AND METHODS

Patient selection

Between January, 2004 and October, 2011, 45 HCC patients with metastatic HCC met our inclusion criteria and were enrolled in this study. Surgery and chemotherapy were deemed unsuitable in any of the following situations: multifocal disease, unresectable HCC, patient refused to undergo surgery and chemotherapy or was seeking further treatment after failure of chemotherapy, severe complications (*i.e.,* hypertension and ascites) and advanced age. Ideal patients for comprehensive cryoablation are those with: Karnofsky performance status (KPS) score $\geqslant$ 70; platelet count $\geqslant 80 \times 10^9$/L; white blood cell count $\geqslant 3 \times 10^9$/L; neutrophil count $\geqslant 2 \times 10^9$/L; hemoglobin $\geqslant$ 90 g/L; prothrombin time international normalized ratio $\geqslant$ 1.5; hepatic tumor not obviously invading the gallbladder, diaphragm or large vessels; absence of level 3 hypertension, severe coronary disease, myelosuppression, respiratory disease and acute or chronic infection; and adequate hepatic function (bilirubin $<$ 30 μmol/L, aminotransferase $<$ 60 U/L and Child-Pugh score A or B) and renal function (serum creatinine $<$ 130 μmol/L, serum urea $<$ 10 mmol/L).

The diagnosis of HCC was confirmed by liver pathology in 41 patients; in the remaining cases, HCC was diagnosed by classical imaging methods, including computed tomography (CT) or magnetic resonance imaging, or by biochemical markers such as increased alpha-fetoprotein. Twenty-four patients had only one mass in the liver, of 3.8-15 cm in diameter with an average of 6.5 cm. Twenty-one patients had two to four masses of 4.5-13 cm in diameter. There were a total of 71 masses in 45 patients. All except two cases had cirrhosis. Using the Child-Pugh score to assess the severity of cirrhosis, 25 patients were class A and 18 were class B. All patients received their final treatment in our hospital within an 8 year follow-up period.

TACE

The preferred treatment for 25 patients with a hepatic tumor of long diameter $\geqslant$ 5 cm was TACE[19,20], which was performed after cross-sectional imaging as previously described[21]. A French vascular sheath was placed into the femoral artery and a 0.035 inch diameter Mickaelson catheter was advanced into the celiac and superior mesenteric arteries. Contrast was injected into the arteries during rapid-sequence radiographic imaging. Arterial branches supplying the tumor were then located and the venous phase was examined carefully for patency of the portal veins. A 0.018 inch diameter Tracker catheter was advanced through the Mickaelson catheter to the arterial branches supplying the tumor. A mixture of doxorubicin (50 mg), mitomycin (10 mg) and lipiodol (4-15 mL) was injected into the arterial branches until hemostasis was achieved. If the tumor showed no shrinkage 2 wk after the procedure, a second TACE was performed.

Cryoablation procedure

Comprehensive cryosurgery was performed on 33 patients, with complete cryoablation of obvious intra- and extrahepatic masses. Each procedure comprised two freeze/thaw cycles accomplished using an argon gas-

based cryosurgical unit (Endocare, Irvine, CA, United States). Cryoprobes (3, 5 or 8 mm) were inserted into the center of the tumor mass under ultrasonographic guidance and two freeze/thaw cycles were performed, each reaching a temperature of -180 ℃ at the tip of the probe. The duration of freezing was dependent on the achievement of an ice ball, visible as a hypoechoic region on ultrasonography. Generally, the maximal freezing time was 15 min, followed by thawing for 5 min; this cycle was then repeated. A margin of at least 1 cm of normal hepatic tissue was frozen circumferentially around the tumor. For masses larger than 5 cm in diameter, two or three cryoprobes were placed within the center and periphery of the tumor, to ensure freezing of the entire mass. The tracts formed were sealed with fibrin glue immediately after removal of the cryoprobes to ensure hemostasis.

Immunotherapy

Twenty-six patients opted for immunotherapy (adoptive transfer of DC-CIK cells performed four times). DC-CIK cells were generated according to previously published protocols[22,23]; 70 mL peripheral blood was drawn before cryosurgery and the treatment was given 3-5 d after cryosurgery. Using Ficoll-Hypaque density centrifugation, we harvested peripheral blood mononuclear cells (PBMCs) from peripheral blood samples (80 mL) collected from the 48 patients 2 d before cryosurgery.

For DC culture, PBMCs were resuspended in DC medium [X-VIVO 15 (Lonza, Basel, Switzerland), 25 ng/mL interleukin (IL)-4 (Peprotech, Rocky Hill, NJ, United States) and 30 ng/mL granulocyte macrophage colony stimulating factor (GM-CSF; Peprotech)], at a concentration of 1×10^6 to 2×10^6/mL. The cells were then allowed to adhere in two plastic flasks (T75; Corning Costa, Cambridge, MA, United States), each containing 50 mL DC medium and approximately 10^8 cells. After overnight culture at 37 ℃ with 5% CO_2, the suspended cells were transferred to two fresh flasks. The cells sticking to the initial two flasks were continuously cultured in DC medium and a small amount of fresh medium was added daily to the cultures.

For culture of CIK cells, PBMCs were suspended in CIK medium [X-VIVO 15 (Lonza), 1000 U/mL IL-2 (Peprotech), 2.5 μg/mL monoclonal antibody to CD3 (OKT-3; Jansen-Kyowa, Tokyo, Japan), 25 μg/mL phytohemagglutinin (Peprotech) and 1000 U/mL interferon gamma (Peprotech)]. The CIK cells were allowed to grow and then continuously passaged. At approximately 7 d of culture, the CIK cells were passaged to fourteen T225 flasks. Cells adhering to the flasks were removed with a cell spatula, centrifuged and resuspended in DC-CIK medium [X-VIVO 15 (Lonza), 400 U/mL IL-2 and 0.5 μg/mL monoclonal antibody to CD3]. All DCs were distributed evenly in the 14 T225 flasks containing CIK cells (approximately 10^8 DCs per flask). After co-culture for 24-48 h, almost 1 wk after cryosurgery, the DC-CIKs were harvested and suspended in 100 mL saline for intravenous injection (cells were collected on four consecutive days; 6×10^9 to 10×10^9 cells were collected on each day). The final cell products were assessed for viability by the dye-exclusion test and checked twice for possible contamination by bacteria, fungi and endotoxins. All cell preparation processes were performed by the same technician and assessed by another technician.

Seven patients refused to undergo cryo- or immunotherapy owing to its cost or their health or age. These patients received no treatment and left the hospital.

Ethics

The study protocol received ethical approval from the Regional Ethics Committee of Guangzhou Fuda Cancer Hospital and conformed to the provisions of the World Medical Association's Declaration of Helsinki in 1995 (as revised in Tokyo in 2004). Written informed consent was obtained from each participant.

Statistical analysis

Complications were recorded and classified in accordance with the Common Terminology Criteria of Adverse Events v4.0. Local tumor control and OS were also evaluated. Radiographic local tumor control was assessed using image-guided tumor ablation criteria[24]. Thoracic and/or abdominal ultrasonography was performed both 1 d and 1 wk after the minimally invasive treatment of primary and metastatic tumors. Follow-up dynamic CT was performed at 1 mo and then at 3-4 mo intervals. The revised Response Evaluation Criteria in Solid Tumors v1.1 were used to assess the response of the thoracic and abdominal tumors[25]. Three diagnostic radiologists reviewed CT scans for every case to determine whether progression or recurrence had occurred. Diagnoses were made independently, although the radiologists discussed cases over which they disagreed. Using the Dunnett test, we compared the OS of patients who had received cryo- and/or immunotherapy with that of untreated patients. The Kaplan-Meier test with log-rank analysis was used for comparison of OS between two groups. Significant differences were indicated by $P < 0.05$ or $P < 0.01$. All analyses were conducted using GraphPad software (San Diego, CA, United States).

RESULTS

Clinical data

Twenty-eight men and five women underwent comprehensive cryoablation and/or TACE. Their ages ranged from 29 to 79 years, with a mean age of 53 years. Twenty-eight patients had histories of hepatitis B infection and two had hepatitis C infection. Fifteen patients were from China and 18 were from Southeast Asia. Of these patients, 18 had initially been treated with surgery and 13 with systemic chemotherapy in other centers; a total of 22 patients came to our hospital for further treatment 1-7 mo after metastases were found and 11 patients came to our hospital for first treatment. Bone metastases (17 lesions) were found in 11 patients, lung metastases (21 lesions) in 15 and multiple organ metastases (18 lesions) in seven. Moderate/severe abdominal pain, evaluated as 5-10

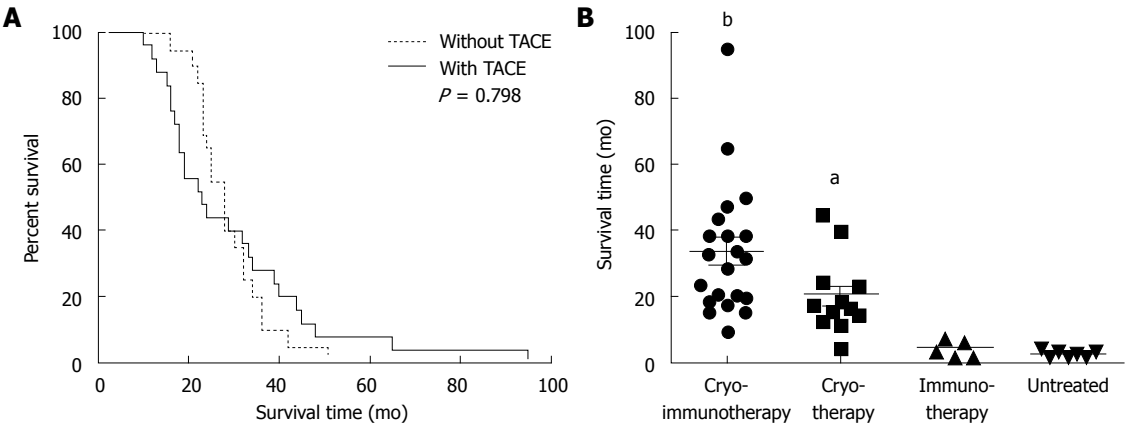

Figure 1 Correlation of overall survival with type of treatment. All 45 patients with metastatic hepatocellular carcinoma died before June, 2012. There were 21 patients in the cryo-immunotherapy group, 12 in the cryotherapy group, five in the immunotherapy group and seven in the untreated group. The overall follow-up period was 8 years. A: Overall survival (OS) of patients who underwent comprehensive cryosurgery with or without transarterial chemoembolization (TACE). Thirty-three patients were enrolled; based on the long diameter of their hepatic tumors (≥ 5 cm), 25 underwent TACE before hepatic cryoablation. Kaplan-Meier test with long-rank analysis; B: OS in the cryo-immunotherapy, cryotherapy and/or immunotherapy groups *vs* that in the untreated group using the Dunnett test. Horizontal lines represent the average and standard deviation. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* untreated group.

on a visual analog scale (VAS) (17 patients), and mild/moderate ascites (15 patients) were common complaints. For metastasis or recurrence of HCC after treatment, 16 patients received multiple treatments (10 in the cryo-immunotherapy group and 6 in the cryotherapy group); 17 patients refused to continue treatment (11 in the cryo-immunotherapy group and 6 in the cryotherapy group).

The untreated group (those who refused cryoablation, TACE and immunotherapy for reasons of treatment concept, age or economic ability) comprised 12 patients (47-77 years of age, median age 63 years; 8 male, 4 female). All of these patients had histories of hepatitis B or C infection. Five patients were from China and seven were from Southeast Asia. Of these patients, eight had initially been treated with surgery or systemic chemotherapy in other centers; a total of seven patients came to our hospital for further treatment 1-6 mo after metastases were found and five patients came to our hospital for first treatment. Bone metastases (5 lesions) were found in three patients, lung metastases (12 lesions) in seven and multiple organ metastases (6 lesions) in two. These patients had complaints similar to those of the comprehensive treatment group.

Perioperative outcomes

Percutaneous cryoablation of primary and metastatic HCC was successful in every case. No severe complications, such as liver cracking and failure or acute renal failure with myoglobinuria, were discovered post-cryoablation. After the first comprehensive cryosurgery in 33 patients, many slight side effects of cryoablation were observed but recovered with or without symptomatic treatment. Slightly hepatorrhagia was found in six patients (18%) but all healed within 5 d, after injection of a hemostatic agent. Liver capsular cracking was found in one patient (3%) who recovered after blood transfusion. Transient thrombocytopenia occurred in seven patients (21%) within 1 wk after cryoablation; two received platelet transfusions. Two patients (6%) had tumor in the right lobe and developed asymptomatic right-sided pleural effusions close to the dome of the diaphragm; these disappeared spontaneously within 2-3 wk. Two patients (6%) developed liver abscess at the previous cryoablation site 2 and 4 d respectively following cryoablation, but recovered after antibiotic and drainage treatment. Four patients were found to have slight fever (body temperature less than 39 ℃). No obvious side effects associated with TACE were found during the perioperative stage. In the first 2 wk after comprehensive cryosurgery, the VAS pain score decreased to 0-3 in 13 patients (76%) who had suffered pretreatment abdominal pain, with consumption of analgesics decreased by 50% and KPS score increased by ≥ 20.

Influence of treatment method and frequency on OS

In our therapeutic protocol, large hepatic tumors (long diameter ≥ 5 cm) were treated by TACE first and considerably reduced in size before cryoablation. Whether patient life span is significantly affected by liver tumor size and additional TACE treatment remains to be determined. Of the 33 patients who received comprehensive cryosurgery, the median OS of those who underwent TACE first was 29 mo; those who received cryoablation directly had a median OS of 26 mo. There was no difference in the OS of these two groups according to the log-rank test ($P = 0.798$, Kaplan-Meier test with log-rank analysis; Figure 1A). Thus, a large hepatic tumor successfully shrunk by TACE can be treated as a small tumor, with no difference in the results of cryoablation.

To the date of the last follow-up, the median OS of all patients was 18 mo (25% percentile, 6 mo; 75% percentile, 33.5 mo). Median OS in the cryo-immunotherapy, cryotherapy, immunotherapy and untreated groups was 32, 17.5, 4 and 3 mo, respectively. OS was significantly higher in the cryo-immunotherapy ($P < 0.01$) and cryotherapy ($P < 0.05$) groups than in the untreated group (by

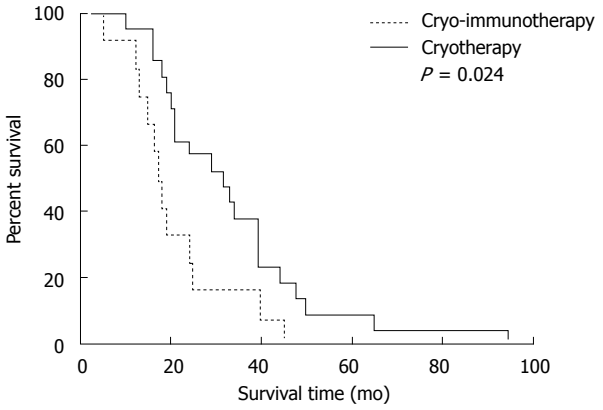

Figure 2 Overall survival of patients who underwent comprehensive cryosurgery with or without immunotherapy. The Kaplan-Meier test with log-rank analysis was used to compare the overall survival of 21 patients in the cryo-immunotherapy group with that of 12 patients in the cryotherapy group.

the Dunnett test, with the untreated group as the control group; Figure 1B).

Comparing the two groups in which there were obvious therapeutic effects, OS was higher in the cryo-immunotherapy group than in the cryotherapy group ($P = 0.024$, Kaplan-Meier test with log-rank analysis; Figure 2).

Repeated cryo- and immunotherapy for tumor progression and/or recurrence was performed in 10 patients in the cryo-immunotherapy group (twice in five patients, thrice in four patients and four times in one patient) and 6 patients in the cryotherapy group (twice in four patients and thrice in two patients); the remaining patients refused repeat treatments. Due to the shorter survival time, all patients in the immunotherapy group received one treatment. In the cryo-immunotherapy group, the median OS of the patients who underwent repeated treatment (36.5 mo) was higher than that of those who underwent a single treatment (21 mo; $P = 0.039$, Kaplan-Meier test with log-rank analysis; Figure 3A). In the cryotherapy group, the median OS after repeated treatment was 21.5 mo, whereas that after a single treatment was 14 mo ($P = 0.035$, Kaplan-Meier test with log-rank analysis; Figure 3B).

DISCUSSION

In this study, we retrospectively reviewed our hospital's database to evaluate the survival time of patients with metastatic HCC. These patients had received various therapies in different medical centers before the metastases were found, and our treatment program directly determined their survival time in the metastatic stage. Increasing numbers of patients are undergoing cryoablation of their primary tumor and metastases, termed comprehensive cryoablation. With skilled operators and strict patient selection, this combined technology can be effective in preventing the occurrence of severe complications (*i.e.,* liver cracking and failure, acute renal failure with myoglobinuria), reducing the probability of side effects (*i.e.,* hepatorrhagia, liver capsular cracking, thrombocytopenia and liver abscess) and provide guarantee for the success of cryotherapy. Theoretically, most of the side effects can be further reduced, with the exception of thrombocytopenia. Development of systemic thrombocytopenia after cryosurgery is associated with excessive platelet trapping and destruction within the cryolesion[26]. This symptom is difficult to avoid simply through improved care, but can heal spontaneously or with platelet supplements.

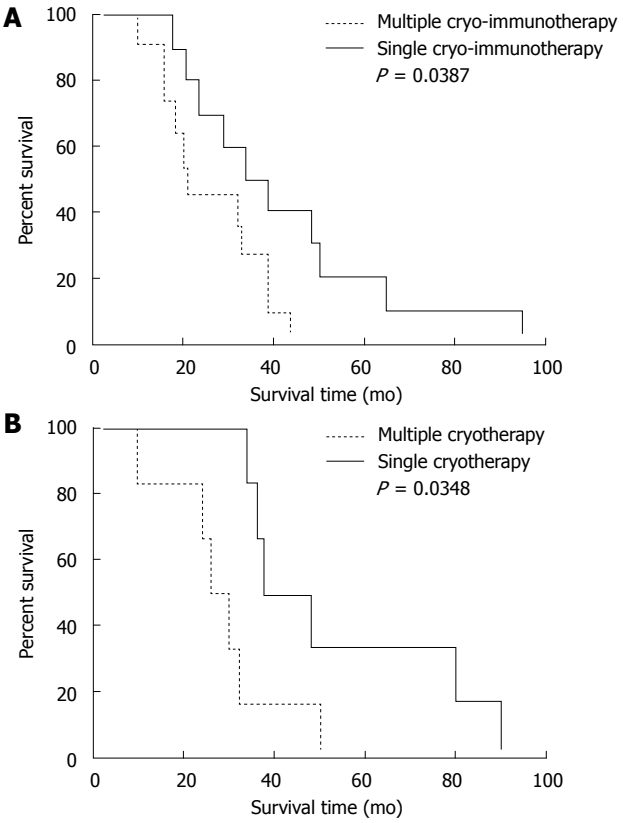

Figure 3 Correlation of overall survival with number of cryo- and/or immunotherapy procedures, using the Kaplan-Meier test with long-rank analysis. A: Comparison of overall survival (OS) between 10 patients who underwent repeated treatments and 11 patients who underwent a single treatment in the cryo-immunotherapy group; B: Comparison of OS between six patients who underwent repeated cryoablation and six who underwent a single cryoablation in the cryotherapy group.

It is increasingly clear that immunotherapy can be useful in cancer therapy, but there are also obstacles that need to be overcome. Due to their organ-like structural environment, these tumors are able to escape immune surveillance[27], and immunotherapy for HCC must therefore be combined with additional therapy to disrupt this structure. Adoptive transfer of CIK cells along with DCs has been shown to be efficacious when the tumor burden is relatively low or when used as an adjuvant therapy rather than as a treatment for bulky tumors[18], indicating the importance of cytoreductive cryoablation before immunotherapy. DCs have been the subject of much research in the last decade and are widely used in immunotherapy protocols. These bone marrow-derived cells have been identified as the most potent immune-stimulatory cells known and are specialized for the initiation and shaping of immune responses. Activated DCs after cryoablation

are potent stimulators of both $CD4^+$ and $CD8^+$ T cells, as supported by evidence from experimental[28] and human[29,30] studies. DCs are often pulsed with synthetic peptides derived from known tumor antigens[31], tumor cell lysates[32], apoptotic tumor cells[33] or RNA derived from tumor antigens[34] and transfected with whole tumor cell DNA[35] or RNA[36]. Moreover, DCs have been fused with tumor cells to induce antigen-specific, polyclonal cytotoxic T lymphocyte responses[37].

On account of continued antigen release after cryoablation[14], *in vitro* activation of DCs was omitted and the DCs were stimulated *in vivo* in the present study. We found that combined cryo- and immunotherapy extended the median OS of metastatic HCC patients from 3 to 32 mo (Figure 1B). Desirable results were achieved, and OS was longer in the cryo-immunotherapy group than in the cryotherapy group, demonstrating the synergistic effect of these two therapies (Figure 2). Owing to procedural costs, age or health, some of our patients underwent cryo-immunotherapy only once. We found that, compared with a single treatment, multiple cytoreductive cryoablation combined with immunotherapy was therapeutically valuable (Figure 3A) and prolonged survival time. Continued cryotherapy delayed disease progression, maintained function of multiple organs and improved quality of life and KPS scores, thereby achieving a better effect than single cryotherapy (Figure 3B).

In studies of the sequential use of TACE and percutaneous cryosurgery for unresectable HCC, pre-cryosurgical TACE was shown to increase the efficacy of cryoablation and decrease its adverse effects in patients with large HCCs (> 5 cm in diameter)[19]. It is well known that the presence of large HCCs often predicts rapid loss of liver function and a poor prognosis, and reducing their size before treatment is more effective than direct treatment of a large tumor. Data are available from two studies on the possible effect of TACE on immune stimulation[38,39], which may further increase the therapeutic effect of combination therapy. Depending on whether single or multiple TACE is performed, a large HCC can first be reduced to 5 cm in diameter and then completely ablated by the combined application of multiple cryoprobes[19]. Consistent with the 2009 report of Shibata *et al*[40], treatment of larger tumors with sequential TACE and cryoablation can achieve significantly better effects than TACE or cryoablation alone. The findings of these authors and our own results indicate that not the frequency of TACE but the shrinkage of large HCCs contributed to the increase in median OS of about 30 mo, and differences due to HCC diameter can be eliminated by additional TACE procedures (median OS was 29 and 26 mo, respectively; $P = 0.798$; Figure 1A).

In conclusion, we combined a minimally invasive procedure (percutaneous cryoablation of primary and metastatic tumors) with a common immunotherapy method (DC-CIK) to treat metastatic HCC. This new strategy extended the median OS from 3 to 32 mo. Better outcomes are expected as more patients undergo cryo-immunotherapy.

COMMENTS

Background

Hepatocellular carcinoma (HCC), which is the fifth most common cancer worldwide, is usually discovered late and has a poor prognosis. In about 80% of patients, HCC is associated with chronic liver disease (*i.e.,* hepatitis and cirrhosis), with major implications for the prognosis and therapeutic options. Many patients are unsuitable for tumor resection because of factors such as poor hepatic reserve (cirrhosis), multicentric tumors or extrahepatic disease. Until recently, no systemic chemotherapy has significantly increased survival in patients with advanced HCC. External beam radiation has had a limited role in the treatment of HCC because of radiation toxicity to the adjacent normal liver

Research frontiers

The effects of comprehensive cryosurgery (simultaneous cryoablation of intra- and extrahepatic tumors and of liver tumors of diameter greater than 5 cm, with transarterial chemoembolization (TACE) performed once or twice before cryoablation to reduce the tumor to 5 cm) and/or dendritic cells - cytokine-induced killer (DC-CIK) immunotherapy in patients with untreated metastatic HCC.

Innovations and breakthroughs

Cryotherapy and, especially, cryo-immunotherapy significantly increased overall survival in metastatic hepatocellular cancer patients. Multiple cryo-immunotherapy was associated with a better prognosis than single cryo-immunotherapy.

Applications

For metastatic HCC, comprehensive cryotherapy and cryo-immunotherapy can help patients improve symptoms, reduce pain and prolong life.

Terminology

Comprehensive cryotherapy: simultaneous cryoablation of intra- and extrahepatic tumors and of liver tumors of diameter greater than 5 cm, with TACE performed once or twice before cryoablation to reduce the tumor to 5 cm; cryo-immunotherapy: Immunotherapy is performed shortly after comprehensive cryosurgery, breakong products of tumor may continually stimulate immune cells to clean up the systemic metastases lesions.

Peer review

In this study, authors combined a minimally invasive procedure (percutaneous cryoablation of primary and metastatic tumors) with a common immunotherapy method (DC-CIK) to treat metastatic HCC. This new strategy extended the median overall surviva from 3 mo to 32 mo. Better outcomes are expected as more patients undergo cryo-immunotherapy.

REFERENCES

1 **Said A**, Wells J. Management of hepatocellular carcinoma. *Minerva Med* 2009; **100**: 51-68 [PMID: 19277004]
2 **Turner PM**, Turner TJ. Validation of the crisis triage rating scale for psychiatric emergencies. *Can J Psychiatry* 1991; **36**: 651-654 [PMID: 1773400 DOI: 10.1136/gut.51.4.459]
3 **Hodgson HJ**. Primary hepatocellular carcinoma. *Br J Hosp Med* 1983; **29**: 240, 246, 250 passim [PMID: 6191818]
4 **Dusheiko GM**, Hobbs KE, Dick R, Burroughs AK. Treatment of small hepatocellular carcinomas. *Lancet* 1992; **340**: 285-288 [PMID: 1353202 DOI: 10.1016/0140-6736(92)92367-O]
5 **Burroughs A**, Hochhauser D, Meyer T. Systemic treatment and liver transplantation for hepatocellular carcinoma: two ends of the therapeutic spectrum. *Lancet Oncol* 2004; **5**: 409-418 [PMID: 15231247 DOI: 10.1016/S1470-2045(04)01508-6]
6 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]
7 **Davis CR**. Interventional radiological treatment of hepatocellular carcinoma. *Cancer Control* 2010; **17**: 87-99 [PMID: 20404792]
8 **Fuss M**, Salter BJ, Herman TS, Thomas CR. External beam radiation therapy for hepatocellular carcinoma: potential of

intensity-modulated and image-guided radiation therapy. *Gastroenterology* 2004; **127**: S206-S217 [PMID: 15508086 DOI: 10.1053/j.gastro.2004.09.035]
9 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
10 **Chen JB**, Li JL, He LH, Liu WQ, Yao F, Zeng JY, Zhang Y, Xu KQ, Niu LZ, Zuo JS, Xu KC. Radical treatment of stage IV pancreatic cancer by the combination of cryosurgery and iodine-125 seed implantation. *World J Gastroenterol* 2012; **18**: 7056-7062 [PMID: 23323008 DOI: 10.3748/wjg.v18.i47.7056]
11 **Orlacchio A**, Bazzocchi G, Pastorelli D, Bolacchi F, Angelico M, Almerighi C, Masala S, Simonetti G. Percutaneous cryoablation of small hepatocellular carcinoma with US guidance and CT monitoring: initial experience. *Cardiovasc Intervent Radiol* 2008; **31**: 587-594 [PMID: 18236104 DOI: 10.1007/s00270-008-9293-9]
12 **Shimizu T**, Sakuhara Y, Abo D, Hasegawa Y, Kodama Y, Endo H, Shirato H, Miyasaka K. Outcome of MR-guided percutaneous cryoablation for hepatocellular carcinoma. *J Hepatobiliary Pancreat Surg* 2009; **16**: 816-823 [PMID: 19466377 DOI: 10.1007/s00534-009-0124-4]
13 **Sabel MS**. Cryo-immunology: a review of the literature and proposed mechanisms for stimulatory versus suppressive immune responses. *Cryobiology* 2009; **58**: 1-11 [PMID: 19007768 DOI: 10.1016/j.cryobiol.2008.10.126]
14 **Rovere-Querini P**, Manfredi AA. Tumor destruction and in situ delivery of antigen presenting cells promote antineoplastic immune responses: implications for the immunotherapy of pancreatic cancer. *JOP* 2004; **5**: 308-314 [PMID: 15254366]
15 **Palmer DH**, Midgley RS, Mirza N, Torr EE, Ahmed F, Steele JC, Steven NM, Kerr DJ, Young LS, Adams DH. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009; **49**: 124-132 [PMID: 18980227 DOI: 10.1002/hep.22626]
16 **Lee WC**, Wang HC, Hung CF, Huang PF, Lia CR, Chen MF. Vaccination of advanced hepatocellular carcinoma patients with tumor lysate-pulsed dendritic cells: a clinical trial. *J Immunother* 2005; **28**: 496-504 [PMID: 16113606 DOI: 10.1097/01.cji.0000171291.72039.e2]
17 **Iwashita Y**, Tahara K, Goto S, Sasaki A, Kai S, Seike M, Chen CL, Kawano K, Kitano S. A phase I study of autologous dendritic cell-based immunotherapy for patients with unresectable primary liver cancer. *Cancer Immunol Immunother* 2003; **52**: 155-161 [PMID: 12649744]
18 **Thanendrarajan S**, Nowak M, Abken H, Schmidt-Wolf IG. Combining cytokine-induced killer cells with vaccination in cancer immunotherapy: more than one plus one? *Leuk Res* 2011; **35**: 1136-1142 [PMID: 21652069 DOI: 10.1016/j.leukres.2011.05.005]
19 **Fazio MJ**, Olsen DR, Uitto JJ. Skin aging: lessons from cutis laxa and elastoderma. *Cutis* 1989; **43**: 437-444 [PMID: 2721242 DOI: 10.3748/wjg.15.3664]
20 **Azizi A**, Naguib NN, Mbalisike E, Farshid P, Emami AH, Vogl TJ. Liver metastases of pancreatic cancer: role of repetitive transarterial chemoembolization (TACE) on tumor response and survival. *Pancreas* 2011; **40**: 1271-1275 [PMID: 21975434 DOI: 10.1097/MPA.0b013e318220e5b9]
21 **Liaw YF**, Lin DY. Transcatheter hepatic arterial embolization in the treatment of hepatocellular carcinoma. *Hepatogastroenterology* 1990; **37**: 484-488 [PMID: 2174827]
22 **Li H**, Wang C, Yu J, Cao S, Wei F, Zhang W, Han Y, Ren XB. Dendritic cell-activated cytokine-induced killer cells enhance the anti-tumor effect of chemotherapy on non-small cell lung cancer in patients after surgery. *Cytotherapy* 2009; **11**: 1076-1083 [PMID: 19929470 DOI: 10.3109/14653240903121252]
23 **Nakamura M**, Wada J, Suzuki H, Tanaka M, Katano M, Morisaki T. Long-term outcome of immunotherapy for patients with refractory pancreatic cancer. *Anticancer Res* 2009; **29**: 831-836 [PMID: 19414316]
24 **Goldberg SN**, Grassi CJ, Cardella JF, Charboneau JW, Dodd GD, Dupuy DE, Gervais D, Gillams AR, Kane RA, Lee FT, Livraghi T, McGahan J, Phillips DA, Rhim H, Silverman SG. Image-guided tumor ablation: standardization of terminology and reporting criteria. *J Vasc Interv Radiol* 2005; **16**: 765-778 [PMID: 15947040 DOI: 10.1097/01.RVI.0000170858.46668.65]
25 **Eisenhauer EA**, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**: 228-247 [PMID: 19097774 DOI: 10.1016/j.ejca.2008.10.026]
26 **Pistorius GA**, Alexander C, Krisch CM, Feifel G, Schilling MK, Menger MD. Local platelet trapping as the cause of thrombocytopenia after hepatic cryotherapy. *World J Surg* 2005; **29**: 657-660; discussion 661 [PMID: 15827856 DOI: 10.1007/s00268-005-7543-4]
27 **Plate J**. Clinical trials of vaccines for immunotherapy in pancreatic cancer. *Expert Rev Vaccines* 2011; **10**: 825-836 [PMID: 21692703 DOI: 10.1586/erv.11.77]
28 **Walker AR**, Walker BF, Vorster HH. Functional significance of mild-to-moderate malnutrition. *Am J Clin Nutr* 1990; **52**: 178-179 [PMID: 2360548 DOI: 10.1038/sj.bjc.6603341]
29 **Schueller G**, Stift A, Friedl J, Dubsky P, Bachleitner-Hofmann T, Benkoe T, Jakesz R, Gnant M. Hyperthermia improves cellular immune response to human hepatocellular carcinoma subsequent to co-culture with tumor lysate pulsed dendritic cells. *Int J Oncol* 2003; **22**: 1397-1402 [PMID: 12739010]
30 **Ali MY**, Grimm CF, Ritter M, Mohr L, Allgaier HP, Weth R, Bocher WO, Endrulat K, Blum HE, Geissler M. Activation of dendritic cells by local ablation of hepatocellular carcinoma. *J Hepatol* 2005; **43**: 817-822 [PMID: 16087270 DOI: 10.1016/j.jhep.2005.04.016]
31 **Nestle FO**, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat Med* 1998; **4**: 328-332 [PMID: 9500607 DOI: 10.1038/nm0398-328]
32 **Mackensen A**, Herbst B, Chen JL, Köhler G, Noppen C, Herr W, Spagnoli GC, Cerundolo V, Lindemann A. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. *Int J Cancer* 2000; **86**: 385-392 [PMID: 10760827]
33 **Palucka AK**, Ueno H, Connolly J, Kerneis-Norvell F, Blanck JP, Johnston DA, Fay J, Banchereau J. Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. *J Immunother* 2006; **29**: 545-557 [PMID: 16971810 DOI: 10.1097/01.cji.0000211309.90621.8b]
34 **Nair SK**, Boczkowski D, Morse M, Cumming RI, Lyerly HK, Gilboa E. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. *Nat Biotechnol* 1998; **16**: 364-369 [PMID: 9555728 DOI: 10.1038/nbt0498-364]
35 **Zhu CZ**, Norris JW. Seizures after stroke. *Arch Neurol* 1991; **48**: 18-19 [PMID: 1986720 DOI: 10.1016/S0264-410X(99)00271-6]
36 **Gilboa E**, Vieweg J. Cancer immunotherapy with mRNA-transfected dendritic cells. *Immunol Rev* 2004; **199**: 251-263 [PMID: 15233739 DOI: 10.1111/j.0105-2896.2004.00139.x]
37 **Gong J**, Chen D, Kashiwaba M, Kufe D. Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. *Nat Med* 1997; **3**: 558-561 [PMID: 9142127 DOI: 10.1038/nm0597-558]

38 **Akizuki S**, Magara T, Tanaka T. [Diminution of the number of gamma delta T lymphocytes in hepatocellular carcinoma patients treated with transcatheter arterial embolization]. *Nihon Rinsho Meneki Gakkai Kaishi* 1998; **21**: 108-117 [PMID: 9754013 DOI: 10.2177/jsci.21.108]

39 **Ayaru L**, Pereira SP, Alisa A, Pathan AA, Williams R, Davidson B, Burroughs AK, Meyer T, Behboudi S. Unmasking of alpha-fetoprotein-specific CD4(+) T cell responses in hepatocellular carcinoma patients undergoing embolization. *J Immunol* 2007; **178**: 1914-1922 [PMID: 17237442]

40 **Shibata T**, Isoda H, Hirokawa Y, Arizono S, Shimada K, Togashi K. Small hepatocellular carcinoma: is radiofrequency ablation combined with transcatheter arterial chemoembolization more effective than radiofrequency ablation alone for treatment? *Radiology* 2009; **252**: 905-913 [PMID: 19567647 DOI: 10.1148/radiol.2523081676]

P- Reviewers Gokhan K, Mario K **S- Editor** Zhai HH
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3481

World J Gastroenterol 2013 June 14; 19(22): 3481-3486
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Efficacy of combined therapy in patients with hepatitis B virus-related decompensated cirrhosis

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Author contributions: Lv GC, Yao JM performed the majority of experiments; Sheng JF and Zheng L provided analytical tools and were involved in revising the manuscript; Li LJ and Chen Y designed the study; Yang YD wrote the manuscript.

Supported by The China National Science and Technology Major Project, No. 2010ZB063; Health Department of Zhejiang Province, China, No. 2010KYA067

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Received: November 17, 2012 Revised: March 23, 2013
Accepted: March 28, 2013
Published online: June 14, 2013

Abstract

AIM: To investigate the efficacy and safety of combined *de novo* lamivudine (LAM) and adefovir dipivoxil (ADV) therapy in hepatitis B virus (HBV)-related decompensated liver cirrhosis patients.

METHODS: One hundred and forty patients with HBV-related decompensated cirrhosis were recruited, 70 patients were treated with combined LAM and ADV *de novo* therapy, and the other 70 patients were treated with LAM alone as controls. The follow-up period was 144 wk. All patients with LAM resistance were shifted to ADV.

RESULTS: The percentage of HBV-related decompensated cirrhosis patients with undetectable HBV DNA in *de novo* combination group was 51.6% (33/64), 84.2% (48/57), and 92.3% (49/53) by weeks 48, 96, and 144, respectively. In monotherapy group, HBV DNA negativity rate was 46.1% (30/65), 56.1% (32/57), and 39.2% (20/51) by weeks 48, 96 and 144, respectively. There was a significant difference between the two groups by weeks 96 and 144 (P = 0.012 and 0.001). The hepatitis B e antigen seroconversion rate was 28.1% (9/32), 40.0% (12/30), and 53.6% (15/28) in the combination group by weeks 48, 96 and 144, respectively, and 24.2% (8/33), 31.0% (9/29), and 37.0% (10/27) by weeks 48, 96 and 144, respectively, in monotherapy group. A total of 68.6% (44/64), 84.2% (48/57), and 92.5% (49/53) patients achieved alanine aminotransferase (ALT) normalization by weeks 48, 96 and 144, respectively in the combination group. In monotherpy group, the ALT normalization rate was 64.6% (42/65) by week 48, 73.7% (42/57) by week 96, and 80.4% (41/51) by week 144. No patients in the combination group exhibited detectable resistance for at least 144 wk. The cumulative resistance rate in monotherapy group at weeks 48, 96, and 144 was 20.0%, 36.8%, and 56.9%. Both combination group and monotherapy group demonstrated an improvement in Child-Turcotte Pugh and Model for End-Stage Liver Disease scores at weeks 48, 96, and 144. All patients tolerated both combination and monotherapy. The ceratinine levels and glomerular filtration rate remained normal in all patients during the follow-up period.

CONCLUSION: In HBV-related decompensated liver cirrhosis patients, the combined *de novo* LAM and ADV therapy is more efficacious and safer compared to LAM alone.

Key words: Liver cirrhosis; Lamivudine; Adefovir dipivoxil; Efficacy; Alanine transaminase

Core tip: Present treatment guidelines advocate oral nucleos(t)ide analogues in decompensated chronic hepatitis B (CHB) patients. Studies with lamivudine

(LAM) have demonstrated decreased mortality and improved liver function in CHB decompensated patients. However, LAM resistance mutations emerging during monotherapy can negate therapeutic benefit. Adefovir dipivoxil had no cross resistance with LAM. Consistent with outcomes in patients with LAM-resistance, no patient in *de novo* combination therapy group showed detectable resistance up to 144 wk in this study. The *de novo* combination therapy markedly improved liver function, reduced Child-Turcotte Pugh and Model for End-Stage Liver Disease scores in hepatitis B virus-related decompensated cirrhosis patients.

Lv GC, Yao JM, Yang YD, Zheng L, Sheng JF, Chen Y, Li LJ. Efficacy of combined therapy in patients with hepatitis B virus-related decompensated cirrhosis. *World J Gastroenterol* 2013; 19(22): 3481-3486 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3481.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3481

INTRODUCTION

It is estimated that over 350 million people worldwide are chronically infected with hepatitis B virus (HBV). The majority of these individuals reside in the Asia-Pacific region[1]. Chronic hepatitis B (CHB) infection is the principal cause of liver cirrhosis and hepatocellular carcinoma (HCC). In cirrhotic patients, the 5-year probability of decompensation is 15%-20%, and the risk increases as the HBV DNA level increases[2]. The 5-year survival rate in decompensated cirrhosis patients is 14%-35%, compared to 84% for those with compensated cirrhosis[3].

Previous studies have shown that high serum HBV DNA is a major risk factor for disease progression to cirrhosis or HCC. Lamivudine (LAM) is the first nucleoside analog widely prescribed for CHB patients due to its antiviral efficacy and safety profile. LAM is found effective for patients with HBV-related decompensated liver cirrhosis[4,5]. However, LAM is associated with a high risk of drug resistance and virological breakthrough[6]. Adefovir dipivoxil (ADV) exhibits specificity, low drug resistance, and no cross resistance with other nucleoside analogs, which has been strongly considered as a rescue therapeutic agent to combat resistance[7,8]. In China, as the first approved drug for CHB patients, LAM has been widely prescribed for its clinical efficacy and low cost. Clinical trials have demonstrated the superiority of combined LAM and ADV therapy compared to ADV monotherapy in LAM resistant patients[9,10]. In this study, LAM and ADV are utilized as *de novo* combination treatment. To date, no study has been performed to systematically evaluate the efficacy and safety of *de novo* combined therapy in patients with HBV-related decompensated liver cirrhosis.

In this study, we aimed to compare the efficacy and safety of *de novo* combination therapy with monotherapy in patients with HBV-related decompensated liver cirrhosis.

MATERIALS AND METHODS

Study population

From January 2007 to December 2008, 140 consecutive nucleoside analogs treatment-naïve patients with HBV-related decompensated cirrhosis were enrolled in this study in the First Affiliated Hospital of Zhejiang University.

Diagnostic criteria: The diagnosis of decompensated liver cirrhosis was based on clinical, laboratory, previous histological, ultrasonographic and radiological signs of cirrhosis with Child-Turcotte-Pugh (CTP) score. The inclusion criteria for this study were as follows: aged 18-65 years, with HBV DNA $\geqslant 10^3$ copies/mL, a CTP score of 7-12 (inclusive), calculated serum creatinine clearance $\geqslant$ 50 mL/min, hemoglobin $\geqslant$ 75 g/L, total white blood cell $\geqslant 2.5\times10^9$/L, platelet count $\geqslant 30 \times 10^9$/L, α-fetoprotein $\leqslant$ 20 ng/mL, and no evidence of HCC.

Exclusion criteria: Patients were excluded for resistance to LAM, co-infection with hepatitis C virus, hepatitis D virus, hepatitis E virus or human immunodeficiency virus, and autoimmune hepatitis, alcoholic cirrhosis, hepatorenal syndrome, grade 3 or 4 hepatic encephalopathy, or spontaneous bacterial peritonitis, and severe heart, renal, brain diseases.

Study design

Among 140 patients with HBV-related decompensated cirrhosis, 70 patients were treated with *de novo* combination therapy of 100 mg/d LAM and 10 mg/d ADV; the other 70 patients were treated with 100 mg/d LAM alone as controls. The duration of the treatment was 144 wk. All patients who exhibited LAM resistance were administered ADV.

All patients were followed up every 3-6 mo with examinations of liver and renal function, prothrombin time (PT), international normalized ratio (INR), serum HBV DNA, hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B e antigen (HBeAg), hepatitis B e antibody (anti-HBe), and hepatitis B core antibody (anti-HBc), as well as ultrasonographic or computerized tomography examination. Routine biochemical and hematological tests, and clinical examination were performed at all clinical visits.

Biochemical and virological analysis

Biochemistry, hematology, PT, INR, and urinalysis were analyzed immediately. Serum hepatitis B viral markers, including HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were detected using commercially available enzyme immunoassays (Abbott Laboratories, Chicago, IL, United States). Serum HBV DNA was measured by polymerase chain reaction with a linear range between 1×10^3 and 5×10^8 copies/mL (Shanghai ZJ Bio-Tech Co., Ltd., China). LAM and ADV associated mutations were assessed by direct sequencing.

Ethics

Informed consent for inclusion in the study was obtained

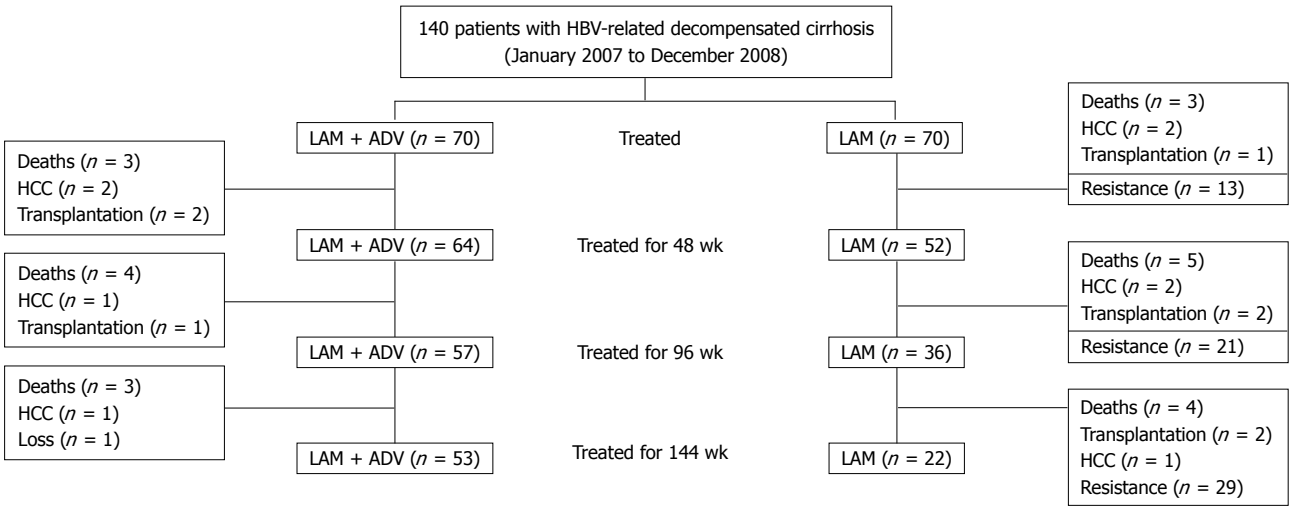

Figure 1 Flow chart of patients disposition in *de novo* lamivudine and adefovir dipivoxil combination group and lamivudine monotherapy group. HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; ADV: Adefovir dipivoxil; LAM: Lamivudine.

Table 1 Baseline characteristics of patients with hepatitis B virus-related decompensated cirrhosis (mean ± SD)

Characteristics	*De novo* combination group (*n* = 70)	Lamivudine group (*n* = 70)	*P* value
Age (yr)	46.8 ± 10.3	47.1 ± 10.9	0.91
Male/female	41/29	40/30	0.89
ALT (IU/L)	134.6 ± 101.3	132.4 ± 120.8	0.78
TBIL (μmol/L)	38.5 ± 12.1	37.8 ± 11.9	0.84
Albumin (g/dL)	3.2 ± 0.7	3.3 ± 0.6 0.34	
Prothrombin time (s)	17.1 ± 4.5	17.6 ± 3.1	0.61
HBV DNA (log10 copies/mL)	6.87 ± 1.21	6.94 ± 1.15	0.73
HBeAg positive	34 (48.6)	33 (47.1)	0.94
Platelet count (× 10^9/L)	78.9 ± 24.2	76.7 ± 32.3	0.67
Creatinine (μmol/L)	89.7 ± 12.3	88.6 ± 13.1	0.45
GFR (mL/min)	115.2 ± 34.5	113.5 ± 22.9	0.68
CTP score	8.9 ± 2.1	8.8 ± 1.7	0.83
CTP class			
A	6 (8.6)	7 (10)	0.65
B	48 (68.6)	49 (70)	0.77
C	16 (22.9)	14 (20)	0.59
MELD score	12.4 ± 3.7	11.9 ± 2.5	0.75

ALT: Alanine aminotransferase; TBIL: Total bilirubin; GFR: Glomerular filtration rate; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; CTP: Child-Turcotte-Pugh score; MELD: Model for End-Stage Liver Disease.

from all patients before recruitment. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Zhejiang University.

Statistical analysis

Statistical analysis was conducted using SPSS (version 16.0, IL, United States). Continuous variables were expressed as mean ± SD. Continuous variables were examined using the Students's *t* test or Mann-Whitney *U* test. Categorical variables were compared by χ^2 test. A *P* value < 0.05 was considered statistically significant.

RESULTS

Patients

A total of 140 patients were recruited: 70 received LAM and ADV d*e novo* combination therapy and 70 received LAM monotherapy. Baseline characteristics were comparable between the two groups and are shown in Table 1. In *de novo* combination therapy group, 37 (52.9%) patients exhibited ascites, 9 (12.8%) exhibited episodes of hepatic encephalopathy, and 15 (21.4%) exhibited variceal bleeding. In LAM monotherapy group, 36 (51.4%) patients presented ascites, 8 (11.4%) presented episodes of hepatic encephalopathy, and 16 (22.9%) exhibited variceal bleeding. No patent in either group discontinued antiviral therapy during the study period. The characteristics of the patients during the 144 wk are shown in Figure 1.

Virological, serological, and biochemical responses

The percentage of HBV related decompensated cirrhosis patients with undetectable HBV DNA in the *de novo* combination group was 51.6% (33/64), 84.2% (48/57) and 92.3% (49/53) by weeks 48, 96, and 144, respectively. In the monotherapy group, the rate of HBV DNA negativity was 46.1% (30/65), 56.1% (32/57) and 39.2% (20/51) by weeks 48, 96, and 144, respectively. A significant difference was observed between the two groups by weeks 96 and 144 ($P = 0.012$ and 0.001).

Patients who were HBeAg-positive at baseline, 34/70 (48.6%) in *de novo* combination group and 33/70 (47.1%) in monotherapy group, exhibited HBeAg seroconversion: 28.1% (9/32) *vs* 24.2% (8/33) by week 48 ($P = 0.372$), 40.0% (12/30) *vs* 31.0% (9/29) by week 96 ($P = 0.021$), and 53.6% (15/28) *vs* 37.0% (10/27) by week 144 ($P = 0.014$), respectively.

Of the 70 decompensated cirrhosis patients receiving *de novo* combination therapy, 68.6% (44/64), 84.2% (48/57) and 92.5% (49/53) of the patients achieved alanine aminotransferase (ALT) normalization by weeks 48, 96 and 144, respectively. In monotherpy group, the ALT

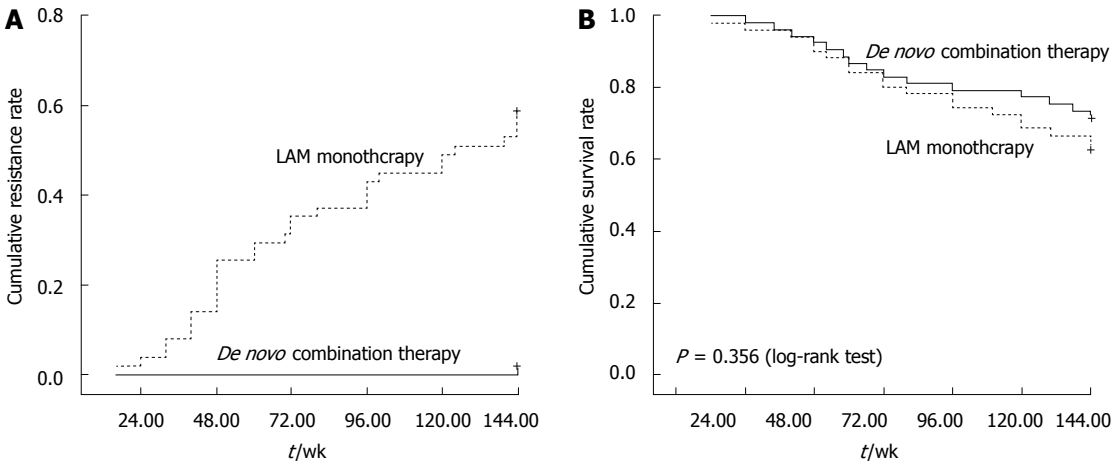

Figure 2 Kaplan-Meier analysis of cumulative resistance (A) and survival (B) rate in patients with hepatitis B virus-related decompensated cirrhosis on *de novo* lamivudine and adefovir dipivoxil combination therapy and lamivudine monotherapy. A: No resistance occurred in *de novo* combination group up to 144 wk. The cumulative resistance rate in lamivudine monotherapy group at weeks 48, 96 and 144 was 20%, 36.8% and 56.9%, respectively; B: The cumulative survival rate in *de novo* combination group was 91.4%, 81.6% and 75.7% at weeks 48, 96 and 144, respectively. The cumulative survival rate in lamivudine monotherapy group was 90.6%, 81.6% and 72.8%, respectively.

Table 2 Comparison of change in hepatic and renal function between two treatment groups (mean ± SD)

Characteristics	*De novo* combination group				Lamivudine monotherapy group			
	Baseline	48 wk	96 wk	144 wk	Baseline	48 wk	96 wk	144 wk
Hepatic function								
Albumin (g/dL)	3.2 ± 0.7	3.5 ± 0.4	3.8 ± 0.1	4.1 ± 0.3[a]	3.3 ± 0.6	3.4 ± 0.3	3.6 ± 0.8	3.8 ± 0.3[c]
TBIL (μmol/L)	38.5 ± 12.1	24.3 ± 11.4	20.4 ± 10.8	16.1 ± 6.7[a]	37.8 ± 11.9	26.5 ± 12.3	23.2 ± 10.5	18.1 ± 12.3
PT (s)	17.1 ± 4.5	14.5 ± 5.3	13.3 ± 9.1[a]	11.4 ± 5.8[a]	17.6 ± 3.1	15.4 ± 3.5	14.5 ± 6.9	13.4 ± 5.8[c]
CTP score	8.9 ± 2.1	7.0 ± 1.2	6.3 ± 2.7[a]	5.7 ± 1.9[a]	8.8 ± 1.7	7.6 ± 2.4	6.6 ± 1.9	6.1 ± 3.8
MELD score	12.4 ± 3.7	10.0 ± 6.5	9.6 ± 5.3	8.8 ± 2.7[a]	11.9 ± 2.5	10.8 ± 3.7	8.9.7 ± 4.8	8.9 ± 4.3
Renal function								
BUN (mmol/L)	6.7 ± 0.7	6.8 ± 1.4	7.1 ± 1.1	6.9 ± 0.3	6.8 ± 0.6	7.4 ± 1.3	7.2 ± 0.8	7.0 ± 1.1
Cr (μmol/L)	96.1 ± 12.3	98.3 ± 8.9	97.8 ± 10.7	99.7 ± 9.6	95.6 ± 12.1	97.8 ± 7.9	98.6 ± 12.7	99.5 ± 9.7
GFR (mL/min)	115.2 ± 34.5	112.8 ± 56.3	103.4 ± 76.1	109.1 ± 21.5	113.5 ± 22.9	107.6 ± 13.4	112.4 ± 45.5	109.4 ± 13.4

[a]$P < 0.05$ *vs* baseline combination group; [c]$P < 0.05$ *vs* lamivudine monotherapy group. TBIL: Total bilirubin; PT: Prothrombin time; CTP: Child-Turcotte-Pugh score, MELD: Model for End-Stage Liver Disease; BUN: Blood urine nitrogen; Cr: Creatinine; GFR: Glomerular filtration rate.

normalization rate was 64.6% (42/65) by week 48, 73.7% (42/57) by week 96, and 80.4% (41/51) by week 144, respectively. Compared to *de novo* combination therapy group, ALT normalization rates were lower in monotherapy group by weeks 96 and 144 ($P = 0.026$ and 0.037).

Drug resistance

No patient in the *de novo* combination group exhibited detectable resistance within 144 wk. The cumulative resistance rate in monotherapy group at weeks 48, 96 and 144 was 20%, 36.8%, and 56.9%, respectively as shown in Figure 2A, significantly higher compared to *de novo* combination group.

Change in hepatic function

Through week 144, both *de novo* combination group and monotherapy group achieved improvement in hepatic function, as evaluated by change from baseline in serum albumin, total bilirubin, prothrombin time; the degree of improvement in *de novo* combination group for albumin and prothrombin at week 144 was superior to monotherapy group, as shown in Table 2.

Both *de novo* combination and monotherapy groups demonstrated an improvement in CTP and Model for End-Stage Liver Disease (MELD) scores at weeks 48, 96 and 144. The mean change from baseline in CTP scores was -1.9, -2.6, and -3.2 at weeks 48, 96 and 144, respectively, for *de novo* combination group, and -1.7, -2.2 and -2.7 at weeks 48, 96, and 144, respectively, for monotherapy group. As a result, 45 (84.9%) of cirrhosis patients in *de novo* combination therapy group achieved CTP class A (score 5 or 6) after 144 wk of treatment, whereas in monotherapy group 68.6% (35/51) of patients achieved CTP class A after 144 wk.

The mean change from baseline in MELD scores was -2.4, -3.2, and -4.0 at weeks 48, 96, and 144, respectively, for *de novo* combination group, and -1.8, -2.3, and -3.0 at weeks 48, 96 and 144, respectively, for monotherapy group as shown in Table 2.

The 70 patients with decompensated cirrhosis in *de novo* combination therapy group exhibited a cumulative HCC incidence of 3.1% at week 48, 5.3% at week 96,

and 7.5% at week 144, respectively; four patients developed HCC during the follow-up period. In monotherapy group, the cumulative incidence of HCC was 3.1%, 7.0% and 9.8% at weeks 48, 96 and 144, respectively; five patients developed HCC during the follow-up period. In addition, the cumulative mortality or orthotopic liver transplantation rate was 8.6%, 18.4% and 24.3% at weeks 48, 96 and 144, respectively in *de novo* combination group, and 9.4%, 18.4% and 27.2% at weeks 48, 96 and 144, respectively, in the monotherapy group, as shown in Figure 2B.

Side effects

All patients in this study tolerated both the *de novo* combination therapy and monotherapy. No patient in either group discontinued the treatment during the follow-up period. In the *de novo* combination group, the blood urine nitrogen (BUN) of three patients increased to 8.6, 9.1 and 9.7 mmol/L respectively, without a concomitant increase in creatinine levels. BUN level of these patients were normalized during the follow-up period. The creatinine levels and glomerular filtration rate remained normal in all patients during the follow-up period, as shown in Table 2.

DISCUSSION

A consensus on the benefit of antiviral therapy for HBV-related cirrhosis has been achieved. Liaw *et al*[11] reported that continuous treatment with LAM delays clinical progression in patients with CHB and advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and risk of HCC. However, LAM exhibits a high incidence of resistance mutations compared to other nucleos(t)ide analogs. The current CHB treatment guidelines advocate initial combination therapy or agents with a high genetic barrier for patients with a high risk of developing drug resistance and potentially life-threatening associated diseases, such as HBV-related liver cirrhosis[12-14]. A combination with other drugs that do not share cross resistance may promote or exhibit synergistic antiviral effects; most importantly, it may exhibit the potential to prevent resistance. Ghany *et al*[15] recently reported that extended combined therapy with LAM and ADV was associated with a high rate of long-term virological and biochemical response; none of the 22 combination therapy treated CHB patients developed resistance for up to 192 wk. Fan *et al*[16] demonstrated that *de novo* combination therapy with LAM and ADV was superior to add-on combination therapy in terms of CTP score, virus inhibition, and renal function. In this study, for patients with HBV-related decompensated liver cirrhosis, *de novo* combination therapy resulted in a superior virological response than monotherapy at weeks 96 and 144. The cumulative tyrosinemethionine-aspartic acid-aspartic acid resistance rate was higher in monotherapy group by weeks 48, 96 and 144, whereas none of the *de novo* combination therapy treated patients developed resistance during the follow-up period.

Higher HBeAg seroconversion rates and ALT normalization rates were also observed in *de novo* combination group compared to LAM monotherapy group at weeks 96 and 144. It is well known that HBeAg seroconversion is accompanied by biochemical and histological regression of liver diseases. Previous studies have indicated that combination therapy is efficacious in preventing drug resistance, but does not improve virological and serological response[17]. Our results and other studies suggest that *de novo* combination therapy can not only prevent drug resistance, but also improve virological and serological response[15,16]. Further elucidation concerning the mechanisms underlying the higher negative rates of HBV DNA and higher rates of HBeAg seroconversion by *de novo* combination therapy is warranted. In general, patients with CHB-related liver cirrhosis exhibit lower levels of HBV DNA and HBeAg compared with patients with CHB. Alternatively, during the course of HBV-related liver cirrhosis, the antiviral immune response is vigorously activated. In concordance with the findings of previous studies, the results that we present in this study suggest that *de novo* combination therapy is potentially suitable as an initial treatment for HBV-related decompensated liver cirrhosis in order to reduce resistance.

Previous clinical studies have demonstrated the positive effects of LAM therapy on the functional improvement in patients with HBV-related decompensated liver cirrhosis[4,5,18]. However, these benefits were offset due to drug-resistance. The CTP and MELD scores, both of which reflect components of liver function, including serum albumin, total bilirubin, and prothrombin time, were markedly improved during *de novo* combination therapy. These results clearly indicate that due to inhibition of HBV replication, *de novo* combination treatment potentially prevents clinical progression to liver failure, reduces complication risk, and delays or avoids the need for liver transplantation.

Renal impairment is a known risk of severe liver disease and is considered a potential side effect of *de novo* combination therapy. In this study, both *de novo* combination and monotherapy were well-tolerated, with no case of renal failure attributable to combination therapy.

This study has several limitations. It was not randomized or blinded, as this would be difficult for patients with CHB-related decompensated liver cirrhosis. This may contribute to the discord in results among previous studies.

In conclusion, this study demonstrated that *de novo* combination therapy is superior to monotherpy in suppressing HBV DNA and achieving ALT normalization and HBeAg seroconversion by weeks 96 and 144. Both treatments improved liver function as measured by reduction of CTP and MELD scores. In HBV-related decompensated liver cirrhosis patients, *de novo* combination therapy has also been proven safe. Our findings strongly support *de novo* combination therapy as a viable and effective therapeutic strategy in patients with HBV-related decompensated liver cirrhosis.

COMMENTS

Background

The mortality rate of hepatitis B virus (HBV)-related decompensated cirrhosis is very high. Recommended treatment options are nucleos(t)ide analogues. There are few reports regarding the issue of treatment for these patients with *de novo* combination therapy or monotherapy.

Research frontiers

In China, tenofovir is not available yet and entecavir is expensive for most patients. Combination therapy with lamivudine (LAM) and adefovir dipivoxil (ADV) is better than ADV in LAM-resistance chronic hepatitis B (CHB) patients. But in patients with HBV-related decompensated live cirrhosis, it remains unclear whether *de novo* combination therapy is better than monotherapy. In this study, the authors demonstrate that LAM and ADV *de novo* combination therapy is more effective than LAM monotherapy for patients with HBV-related decompensated liver cirrhosis.

Innovations and breakthroughs

Many clinical studies showed that the combined LAM and ADV therapy is more effective than ADV monotherapy after LAM-resistance. However, the efficacy of *de novo* LAM and ADV combination therapy in patients with HBV-related decompensated liver cirrhosis is unclear. This is the first study to report that *de novo* LAM and ADV is more effective than LAM monotherapy, especially the combination therapy can reduce the drug resistance.

Applications

By understanding that LAM and ADV *de novo* combination therapy is more effective than LAM monotherapy in patients with HBV-related decompensated liver cirrhosis, this study may represent a future strategy for therapeutic intervention in CHB patients with HBV-related decompensated liver cirrhosis.

Terminology

De novo combination therapy means combination with two or more drugs form the beginning of the treatment. The diagnosis of decompensated liver cirrhosis was based on clinical, laboratory, previous histological, ultrasonographic and radiological signs of cirrhosis with Child-Turcotte-Pugh (CTP) score. The CTP score is a system to assess the disease stage for decompensated cirrhotic patients.

Peer review

This is a good clinical study in which the authors compared the effect of *de novo* LAM and ADV combination therapy with LAM monotherapy. The authors concluded that LAM and ADV should be combined at the beginning for treatment of the patients with HBV-related decompensated liver cirrhosis.

REFERENCES

1 **Ganem D**, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med* 2004; **350**: 1118-1129 [PMID: 15014185 DOI: 10.1056/NEJMra031087]

2 **McMahon BJ**. Epidemiology and natural history of hepatitis B. *Semin Liver Dis* 2005; **25** Suppl 1: 3-8 [PMID: 16103976 DOI: 10.1055/s-2005-915644]

3 **Huber R**, Orzeszyna M, Pokorny N, Kravitz EA. Biogenic amines and aggression: experimental approaches in crustaceans. *Brain Behav Evol* 1997; **50** Suppl 1: 60-68 [PMID: 9217993 DOI: 10.1016/S0140-6736(09)60207-5]

4 **Yao FY**, Bass NM. Lamivudine treatment in patients with severely decompensated cirrhosis due to replicating hepatitis B infection. *J Hepatol* 2000; **33**: 301-307 [PMID: 10952248 DOI: 10.1016/S0168-8278(00)80371-2]

5 **Fontana RJ**, Hann HW, Perrillo RP, Vierling JM, Wright T, Rakela J, Anschuetz G, Davis R, Gardner SD, Brown NA. Determinants of early mortality in patients with decompensated chronic hepatitis B treated with antiviral therapy. *Gastroenterology* 2002; **123**: 719-727 [PMID: 12198698 DOI: 10.1053/gast.2002.35352]

6 **Liaw YF**, Leung NW, Chang TT, Guan R, Tai DI, Ng KY, Chien RN, Dent J, Roman L, Edmundson S, Lai CL. Effects of extended lamivudine therapy in Asian patients with chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *Gastroenterology* 2000; **119**: 172-180 [PMID: 10889166 DOI: 10.1053/gast.2000.8559]

7 **Yang H**, Westland CE, Delaney WE, Heathcote EJ, Ho V, Fry J, Brosgart C, Gibbs CS, Miller MD, Xiong S. Resistance surveillance in chronic hepatitis B patients treated with adefovir dipivoxil for up to 60 weeks. *Hepatology* 2002; **36**: 464-473 [PMID: 12143057 DOI: 10.1053/jhep.2002.34740]

8 **Yeon JE**, Yoo W, Hong SP, Chang YJ, Yu SK, Kim JH, Seo YS, Chung HJ, Moon MS, Kim SO, Byun KS, Lee CH. Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. *Gut* 2006; **55**: 1488-1495 [PMID: 16461777 DOI: 10.1136/gut.2005.077099]

9 **Kim HJ**, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, Kim BI. Rescue therapy for lamivudine-resistant chronic hepatitis B: comparison between entecavir 1.0 mg monotherapy, adefovir monotherapy and adefovir add-on lamivudine combination therapy. *J Gastroenterol Hepatol* 2010; **25**: 1374-1380 [PMID: 20659226 DOI: 10.1111/j.1440-1746.2010.06381.x]

10 **Vassiliadis TG**, Giouleme O, Koumerkeridis G, Koumaras H, Tziomalos K, Patsiaoura K, Grammatikos N, Mpoumponaris A, Gkisakis D, Theodoropoulos K, Panderi A, Katsinelos P, Eugenidis N. Adefovir plus lamivudine are more effective than adefovir alone in lamivudine-resistant HBeAg-chronic hepatitis B patients: a 4-year study. *J Gastroenterol Hepatol* 2010; **25**: 54-60 [PMID: 19780875 DOI: 10.1111/j.1440-1746.2009.05952.x]

11 **Liaw YF**, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, Dixon JS, Gray DF, Sabbat J. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; **351**: 1521-1531 [PMID: 15470215 DOI: 10.1056/NEJMoa033364]

12 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283 [PMID: 19669255 DOI: 10.1007/s12072-008-9080-3]

13 **Lok AS**, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009; **50**: 661-662 [PMID: 19714720 DOI: 10.1002/hep.23190]

14 **European Association For The Study Of The Liver.** EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242 [PMID: 19054588 DOI: 10.1016/j.jhep.2008.10.001]

15 **Ghany MG,** Feld JJ, Zhao X, Heller T, Doo E, Rotman Y, Nagabhyru P, Koh C, Kleiner DE, Wright EC, Liang TJ, Hoofnagle JH. Randomised clinical trial: the benefit of combination therapy with adefovir and lamivudine for chronic hepatitis B. *Aliment Pharmacol Ther* 2012 Mar 26 [PMID: 22449251 DOI: 10.1111/j.1365-2036.2012.05059.x]

16 **Fan XH**, Geng JZ, Wang LF, Zheng YY, Lu HY, Li J, Xu XY. De novo combination therapy with lamivudine and adefovir dipivoxil in chronic hepatitis B patients. *World J Gastroenterol* 2011; **17**: 4804-4809 [PMID: 22147982 DOI: 10.3748/wjg.v17.i43.4804]

17 **Lau GK**, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695 [PMID: 15987917 DOI: 10.1056/NEJMoa043470]

18 **Villeneuve JP**, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, Leduc R, Peltekian K, Wong F, Margulies M, Heathcote EJ. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. *Hepatology* 2000; **31**: 207-210 [PMID: 10613747]

P- Reviewer Bruha R **S- Editor** Huang XZ
L- Editor Ma JY **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3487

World J Gastroenterol 2013 June 14; 19(22): 3487-3493
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Role of nesfatin-1 in a rat model of visceral hypersensitivity

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Supported by Natural Science Foundation of China, No. 81070308/H0308
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Received: January 13, 2013 Revised: May 15, 2013
Accepted: May 22, 2013
Published online: June 14, 2013

Abstract

AIM: To explore the role of nesfatin-1 on irritable bowel syndrome (IBS)-like visceral hypersensitivity.

METHODS: The animal model of IBS-like visceral hypersensitivity was induced by intracolonic infusion of 0.5% acetic acid (AA) in saline once daily from postnatal days 8-21. Experiments were performed when rats became adults. The visceral sensitivity of rats was evaluated by abdominal withdrawal reflex (AWR) and electromyographic (EMG) activity of the external oblique muscle to graded colorectal distension. The content of nesfatin-1 in serum was determined using enzyme-linked immunosorbent assay. After implantation of an intracerebroventricular (ICV) cannula and two electrodes into the external oblique muscle, model rats were randomly divided into four groups. Animals then received ICV injection of 8 μg of anti-nesfatin-1/nucleobindin-2 (NUCB2), 50 μg of α-helical corticotropin releasing factor (CRF) 9-41 (non-selective CRF receptor antagonist), 50 μg of NBI-27914 (selective CRF1 receptor antagonist) or 5 μL of vehicle. After 1 h of ICV administration, visceral sensitivity of each group was measured again, and comparisons between groups were made.

RESULTS: Rats treated with AA showed higher mean AWR scores and EMG activity at all distension pressures compared with controls ($P < 0.05$). On histopathologic examination, no evidence of inflammation or abnormalities in structure were noted in the colon of either control or AA-treated groups. Myeloperoxidase values were not significantly different between the two groups. The level of nesfatin-1 in serum was significantly higher in the AA-treated group than in the control group (5.34 ± 0.37 ng/mL *vs* 4.81 ± 0.42 ng/mL, $P < 0.01$). Compared with rats injected with vehicle, rats which received ICV anti-nesfatin-1/NUCB2, α-helical CRF9-41 or NBI-27914 showed decreased mean AWR scores and EMG activity at all distension pressures ($P < 0.05$).

CONCLUSION: Nesfatin-1 may be associated with IBS-like visceral hypersensitivity, which may be implicated in brain CRF/CRF1 signaling pathways.

Key words: Irritable bowel syndrome; Nesfatin-1; Visceral hypersensitivity; Corticotropin releasing factor; Intracerebroventricular injection

Core tip: This is a well conducted experimental study on the possible effect of nesfatin-1 in visceral hypersensitivity. Currently no reports have been published concerning the role of nesfatin-1 in irritable bowel syndrome (IBS). In a well-established visceral hypersensitivity animal model, we found an elevated nesfatin-1 level in the serum, and there was a reduction in evoked abdominal electromyography and abdominal withdrawal reflex scores after treatment with nesfatin-1 antibody, a non-selective corticotropin releasing factor (CRF) receptor antagonist, or a selective CRF1

receptor antagonist. These results suggest that nesfatin-1 may be associated with visceral hypersensitivity in model rats and implicated in brain CRF/CRF1 signaling pathways, which contribute to the visceral hypersensitivity of IBS.

Jia FY, Li XL, Li TN, Wu J, Xie BY, Lin L. Role of nesfatin-1 in a rat model of visceral hypersensitivity. *World J Gastroenterol* 2013; 19(22): 3487-3493 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3487.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3487

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by abdominal pain and alterations in bowel habits. The pathogenic mechanism of IBS remains incompletely understood. Visceral hypersensitivity is common in IBS patients and probably plays a major role in development of symptoms[1]. Nesfatin-1 is a newly discovered anorexigenic peptide derived from the precursor peptide nucleobindin-2 (NUCB2)[2]. Putative post-translational processing of NUCB2 by the enzyme pro-hormone convertase-1/3 results in nesfatin-1, nesfatin-2 and nesfatin-3[2]. Intracerebroventricular (ICV) injection of nesfatin-1 decreased dark phase food intake in freely fed rats, whereas injection of an antibody specific to nesfatin-1 potently stimulated appetite[2]. The hypothalamic selective corticotropin releasing factor-2 (CRF2) signaling system was also shown to be involved in the underlying mechanisms of nesfatin-1 induced reduction of dark phase food intake[3]. Nesfatin-1 is distributed in stress-related brain areas, such as the supraoptic nucleus, hypothalamic paraventricular nucleus (PVN), nucleus of the solitary tract, locus coeruleus and raphe pallidus nucleus[4,5]. Furthermore, nesfatin-1 is colocalized with CRF in the PVN[5]. The hormone CRF is the hallmark initiator of the stress response[6]. It exerts its biological actions by interacting with CRF1 and CRF2 receptors[7]. It has been well established that the brain CRF/CRF1 signaling system modulates pain responses although the exact sites mediating this modulation remain unidentified[8,9]. These observations suggest that nesfatin-1 may be involved in the autonomic regulation of visceral sensation. Given that visceral hypersensitivity is the major pathophysiology of IBS, nesfatin-1 may be an important contributor to the symptoms of IBS. Currently no reports have been published concerning the role of nesfatin-1 in IBS. The purpose of the present study, therefore, was to investigate the effect of nesfatin-1 on visceral sensitivity in IBS and the possible underlying mechanisms of this action. This was achieved by establishing a rat model of visceral hypersensitivity associated with IBS.

MATERIALS AND METHODS

Animals

Experiments were performed on male Sprague-Dawley rats bought from the Experimental Animal Center of Nanjing Medical University (China). Rats were housed with *ad libitum* food and water in standard rodent cages at 22 ± 2 ℃ in a 12-h light-dark controlled room. All animal procedures strictly adhered to the guidelines of the Institution Council of Animal Care and were approved by the Ethics Committee of Nanjing Medical University.

Induction of chronic visceral hyperalgesia

Pups received an infusion of 0.3 mL of 0.5% acetic acid (AA) solution in saline into the colon 2 cm from the anus once daily on postnatal days 8-21[10]. Controls received an equal volume of saline. Experiments were conducted in these rats between 6 and 9 wk of age.

Behavioral testing

We used a grading system based on the abdominal withdrawal reflex (AWR), as well as a measure of the electromyographic (EMG) activity of the external oblique muscle to evaluate visceral hypersensitivity 6 wk after treatment, by grading the response of rats to colorectal distention (CRD). Briefly, under mild sedation with ether, a flexible balloon (5 cm) constructed from a surgical glove finger attached to tygon tubing was inserted (8 cm) into the descending colon and rectum via the anus and held in place by taping the tubing to the tail. Rats were placed in small lucite cubicles (20 cm × 6 cm × 8 cm) and allowed to adapt for 30 min. CRD was performed by rapidly inflating the balloon to a constant pressure using a sphygmomanometer connected to a pressure transducer. The balloon was inflated to various pressures (20, 40, 60 and 80 mmHg) for 20 s followed by a 2-min rest. Behavioral responses to CRD were measured by visual observation of the AWR by a blinded observer. The assignment of an AWR score was as follows: 1 = normal behavior without response; 2 = contraction of abdominal muscles; 3 = lifting of abdominal wall; and 4 = body arching and lifting of pelvic structures[11].

To obtain EMG measurements of visceromotor responses, two electrodes were implanted, under anesthesia with pentobarbital sodium (100 mg/kg, intraperitoneally), into the external oblique muscle and externalized behind the head. Rats were allowed 1 wk recovery from surgery. CRD was performed as described previously with 20 s of distention followed by 2-min rest between distentions of 20, 40, 60 and 80 mmHg. Wires were connected to a Bio Amp, which was connected to a power lab (AD Instruments, Australia) used as an EMG acquisition system with Chart 7 software. The area under the curve during the 20-s distention for the EMG signal was calculated by subtracting the area under the curve for the 20 s before distention[12].

Evaluation of the colon for inflammation/damage

After behavioral testing, 4 cm of colonic tissue proximal to the anus was removed and rinsed briefly with saline. The proximal half of each colon was placed in 4% paraformaldehyde, embedded in paraffin, cut into 4 μm sections, and used for histologic examination. The distal

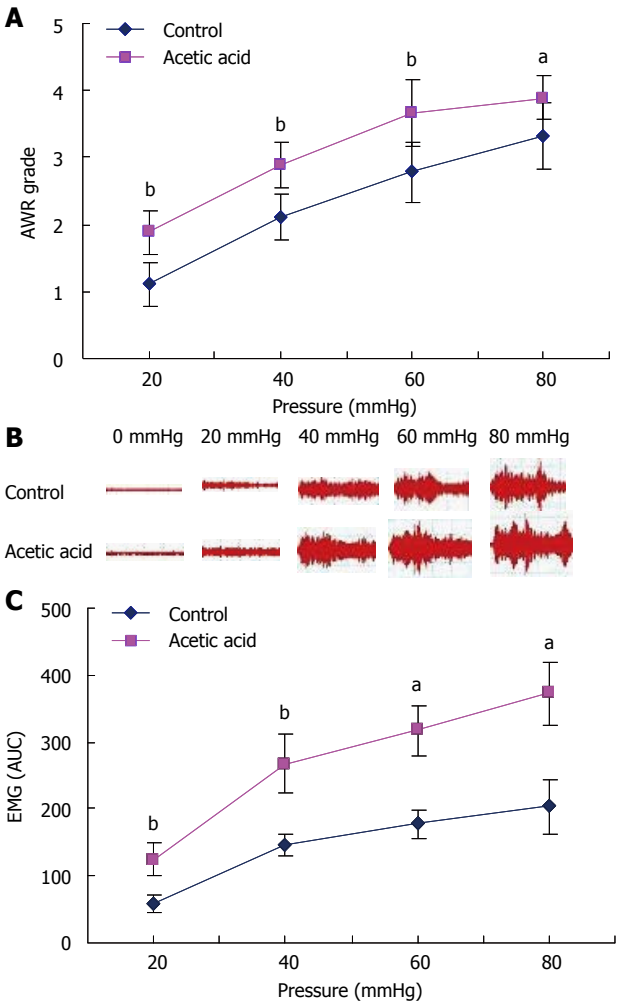

Figure 1 Evaluation of visceral sensitivity in neonatal acetic acid-treated rats when adults. A: Abdominal withdrawal reflex (AWR) scores were used as an index in response to distension pressure. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group, $n = 9$; B: Typical electromyographic (EMG) activity in the external oblique muscle in response to graded colorectal distension; C: Area under the curve (AUC) of EMG activity in the external oblique muscle in response to graded colorectal distension. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group, $n = 9$.

half was snap-frozen and stored at -80 ℃ until use. Myeloperoxidase (MPO) activity was determined later by an enzyme-linked immunosorbent assay (ELISA) kit (RB, Minneapolis, MN, United States).

Blood sampling and processing

Under mild sedation with ether, approximately 1 mL blood was taken from the orbital canthus vein plexus. The blood was centrifuged, then the serum was separated and stored at -80 ℃ until assayed. Serum nesfatin-1 levels were measured using the ELISA kit (RD, CA, United States).

ICV cannula and external oblique muscle electrode implantation

Model rats were selected and anaesthetized with pentobarbital sodium (100 mg/kg *ip*). A chronic guide cannula was implanted into the right lateral ventricle of the brain following coordinates from Bregma: anteroposterior, -0.8 mm; lateral, -1.5 mm; dorsoventral, -3.5 mm[13]. Two stainless steel screws were fixed to the skull, and then the cannula was secured with dental cement. Finally, two electrodes were implanted in the external oblique muscle as described above. After surgery, rats were housed individually and allowed to recuperate for 1 wk.

ICV injection

After ICV cannula and external oblique muscle electrode implantation, model rats were randomly divided into 4 groups and administered, by ICV injection, 8 μg of anti-nesfatin-1/NUCB2[2] (Bioss, Beijing, China), 50 μg of α-helical CRF9-41[14] (Tocris, Minneapolis, MN, United States), 50 μg of NBI-27914[13] (Tocris, Minneapolis, MN, United States) or 5 μL of vehicle[2]. After 1 h, the visceral sensitivity of each group to CRD was measured and compared between groups.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, United States). All data are expressed as mean ± SE. For AWR behavioral grades, a Friedman analysis of variance (ANOVA) was used. Median AWR scores at each distention pressure were compared between treatment groups using the Mann-Whitney *U* rank sum test. EMG data were analyzed by two-way repeated-measures ANOVA. Other data were analyzed by the Student *t* test or one-way ANOVA where appropriate. *P* values < 0.05 were considered statistically significant.

RESULTS

Neonatal AA treatment produced persistent visceral hypersensitivity

Visceral sensitivity to CRD was determined at 6 wk of age. Rats treated with AA exhibited higher mean AWR scores at all distension pressures tested than controls (Figure 1A, $P < 0.05$). In a separate experiment, EMG activity, measured in response to graded CRD, was significantly higher in neonatal AA-treated rats than in controls (Figure 1B and C, $P < 0.05$). Taken together, these data showed that rats treated with AA between postnatal days 8 and 21 were more sensitive to CRD than controls, suggesting that neonatal AA treatment produced persistent visceral hypersensitivity when animals became adults.

Evaluation of adult colons for inflammation

To determine whether persistent visceral hypersensitivity achieved in adults of neonatal AA-treated rats was due to the development of chronic colitis in adults, hematoxylin and eosin-stained sections and MPO activity of the colons of adult rats were examined for histopathologic signs of inflammation. On examination, no significant inflammation or abnormalities in structure were noted in either saline- or AA-treated groups and no inflammatory infiltrates were observed (Figure 2). Likewise, there was

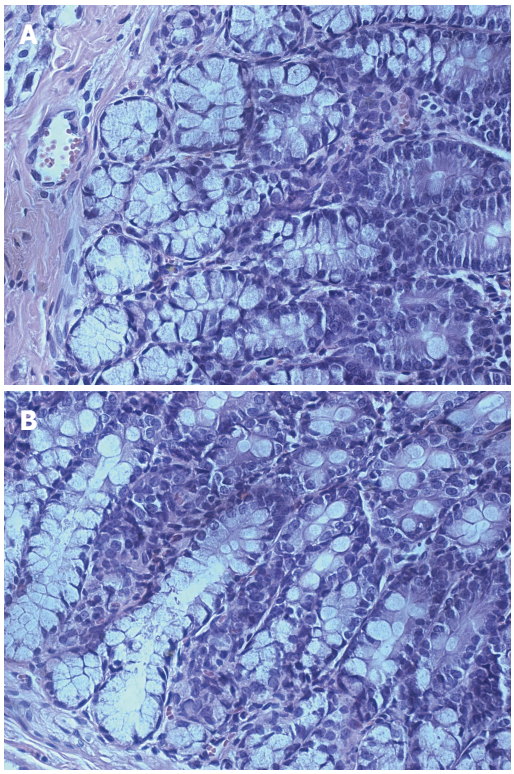

Figure 2 Photomicrographs of hematoxylin and eosin-stained sections from colons of control adult rats (A) and neonatal acetic acid-treated adult rats (B). No significant inflammation or abnormalities in structure were observed in neonatal acetic acid-treated rats.

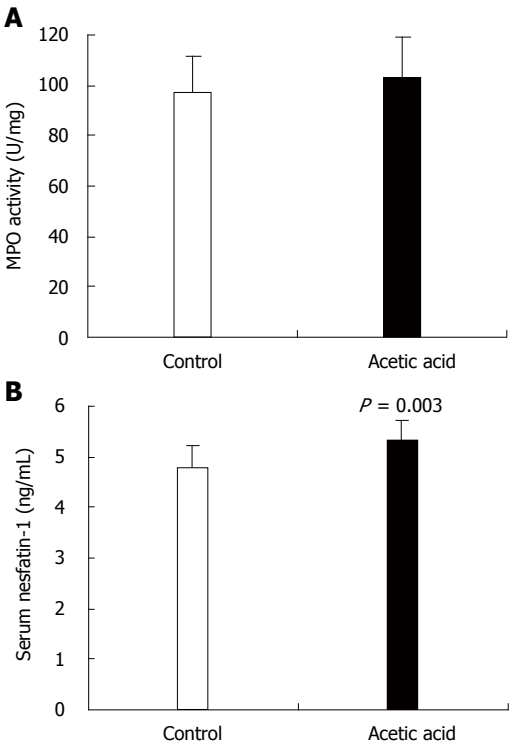

Figure 3 Myeloperoxidase activity in rat colon (A) and nesfatin-1 levels in rat serum (B). A: There was no difference in myeloperoxidase (MPO) activity in neonatal acetic acid-treated rats compared with control rats; B: The average serum nesfatin-1 level was statistically higher in neonatal acetic acid-treated rats than in controls (n = 12).

no significant difference in the level of MPO between control and neonatal AA-treated rats (Figure 3A, $P > 0.05$). Thus, these data showed that inflammation/abnormalities were absent in our model, ruling out their involvement in persistent hypersensitivity. Therefore, we have a rat model of persistent visceral hypersensitivity caused by neonatal exposure to a mild acid in the absence of ongoing inflammation.

Serum nesfatin-1 levels in rats

The mean serum nesfatin-1 level in the neonatal AA-treated group (5.34 ± 0.37 ng/mL) was significantly higher than in the control rats (4.81 ± 0.42 ng/mL; Figure 3B, $P = 0.003$).

Effect of ICV administration of antibody on visceral sensitivity

To determine the effect of nesfatin-1 on visceral sensitivity, adult model rats were given ICV injection of anti-nesfatin-1/NUCB2 or vehicle, and visceral sensitivity was measured 1 h later. The AWR scores of nesfatin-1 antibody-treated rats were significantly lower than those of vehicle-treated rats at 20, 40, 60 and 80 mmHg (Figure 4A). Similarly, in model rats, anti-nesfatin-1/NUCB2 treatment caused a significant decline in the EMG activity to graded CRD when compared with vehicle injection. To examine whether brain CRF/CRF1 signaling pathways were involved in visceral hypersensitivity in model rats, animals were injected intracerebroventricularly with non-selective CRF receptor antagonists (α-helical CRF9-41), selective CRF1 receptor antagonist (NBI-27914) or vehicle 1 h before graded CRD. In comparison with model rats receiving a vehicle injection, model rats that received α-helical CRF9-41 and NBI-27914 showed decreased mean AWR scores and EMG activity at all distension pressures (Figure 4B).

DISCUSSION

IBS is one of the most common functional gastrointestinal disorders worldwide. The mechanism of this disease is not clear, but an important role for visceral hypersensitivity in the development of symptoms compatible with IBS has become evident. It has been confirmed that a lower pain threshold to colonic distention was observed in patients with IBS compared with healthy subjects[1], and visceral hypersensitivity is a biological marker for IBS[15].

Visceral hypersensitivity may be associated with intestinal irritation (pain or inflammation) in the neonatal period. Nociceptive neuronal circuits are formed during the embryonic and postnatal period when painful stimuli are normally absent or limited. Pain and inflammation during this critical period, particularly before the maturation of the descending inhibitory systems, can lead to prolonged structural and functional alterations in pain

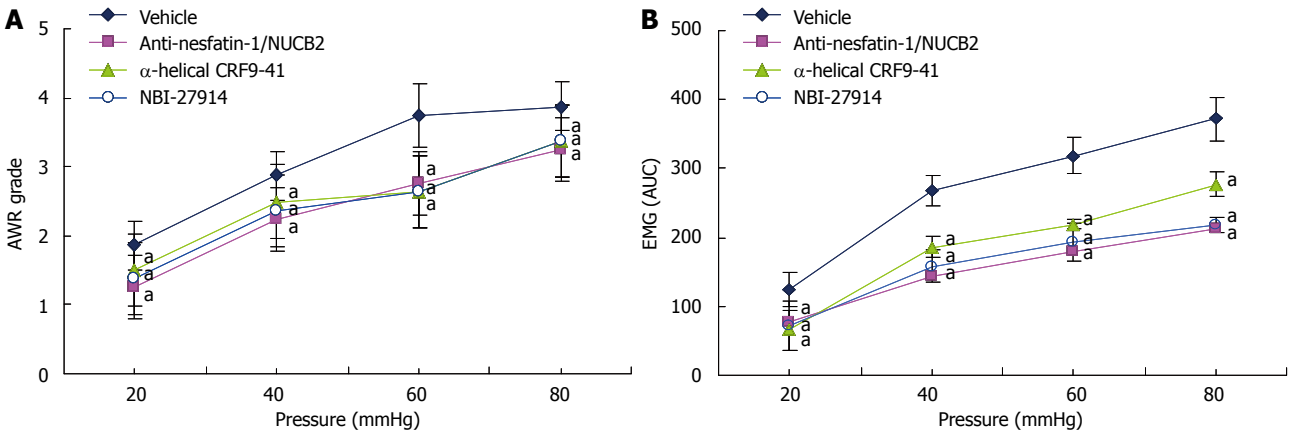

Figure 4 Effect of anti-nesfatin-1/nucleobindin-2, α-helical corticotropin releasing factor 9-41 and NBI-27914 treatment on visceral sensitivity in model rats. A: Abdominal withdrawal reflex (AWR) scores were used as an index in response to distension pressure. Model rats receiving intracerebroventricular injection of anti-nesfatin-1/nucleobindin-2 (NUCB2), α-helical corticotropin releasing factor (CRF) 9-41 and NBI-27914 showed decreased mean AWR scores compared with model rats receiving vehicle injection, at 20, 40, 60 and 80 mmHg, $^aP < 0.05$ *vs* vehicle group, $n = 8$; B: Electromyographic (EMG) activity in the external oblique muscle in response to graded colorectal distension. Compared with vehicle administration, EMG activity in model rats administered intracerebroventricularly with anti-nesfatin-1/NUCB2, α-helical CRF9-41 or NBI-27914 was significantly decreased at 20, 40, 60 and 80 mmHg compared with model rats receiving vehicle injection, $^aP < 0.05$ *vs* vehicle group, $n = 8$.

pathways that can last into adult life. This visceral hypersensitivity is associated with central neural sensitization, as well as central sensitization[16].

In this study, a rat model of visceral hypersensitivity associated with IBS was established by intracolonic instillation of dilute AA between P8 and P21. In our model, dilute AA treatment of pups had no effect on the growth of rats. As adults, these rats showed no identifiable peripheral pathology, which was in line with the characteristics of IBS. These rats exhibited higher mean AWR scores and EMG activity at all distension pressures compared with controls. These findings suggest that neonatal AA treatment induced long-lasting visceral hypersensitivity without significant inflammation in the colon, which was in agreement with previous findings[17]. Therefore, this model can better reflect the chronic hyperesthetic state of IBS and is applicable to the study of visceral hypersensitivity.

Nesfatin-1 is a recently discovered 82-amino-acid satiety peptide and has a predicted molecular mass of 9.7 kDa. Nesfatin-1, injected intracerebroventricularly or peripherally in rats, reduced food intake in a dose-dependent manner[2,18]. As yet, however, the nesfatin-1 receptor has not been identified. Shimizu *et al*[18] reported that NUCB2 can be potentially processed into an active derivative, nesfatin-1. However, this mature peptide has not been detected in protein extracts from rat brain[2]. Likewise, the western blots of all tissues and cells studied here failed to show a band at 9.7 kDa[19,20]. It has been confirmed that nesfatin-1 exists in the blood of rodents and humans using a sandwich-type ELISA, but normal values have not yet been established for nesfatin-1[21]. Nesfatin-1 can cross the blood-brain barrier without saturation[22,23], therefore the measurement of nesfatin-1 in the blood may partly reflect its level in the brain. The present study showed that the average serum nesfatin-1 level was significantly higher in neonatal AA-treated rats than in control rats, suggesting nesfatin-1 may be associated with a visceral hypersensitivity state in model rats.

Furthermore, we found that model rats receiving ICV injection of anti-nesfatin-1/NUCB2 showed decreased mean AWR scores and EMG activity at 20, 40, 60 and 80 mmHg compared with model rats receiving vehicle injection. Results suggest that ICV injection of nesfatin-1 antibody may neutralize endogenous nesfatin-1 and therefore dramatically attenuate visceral hypersensitivity in model rats.

Taken together, these results strongly suggest that nesfatin-1 may be involved in visceral hypersensitivity in model rats. To date, the evidence for the role of a brain CRF/CRF1 signaling system in the modulation of visceral sensitivity in rodents has been based on the study of visceral hypersensitivity in acute models of stress[24]. In this model, our results also showed that ICV administration of α-helical CRF9-41 and NBI-27914 caused a significant decrease in mean AWR scores and EMG activity at 20, 40, 60 and 80 mmHg in comparison with ICV administration of vehicle, which was consistent with the previous studies[25,26]. These data demonstrate that brain CRF/CRF1 signaling pathways may be involved in visceral hypersensitivity in the present study.

Previous studies have shown that nesfatin-1 was co-localized with CRF in the PVN[5]. ICV administration of nesfatin-1 increased the incidence of c-Fos expression in CRF neurons, and nesfatin-1 increased cytosolic Ca^{2+} concentration in the CRF-immunoreactive neurons isolated from the PVN[27]. Taken together with our present study, these results suggested that nesfatin-1 may be implicated in brain CRF/CRF1 signaling pathways, which then contribute to visceral hypersensitivity in model rats.

In conclusion, nesfatin-1 may be associated with the visceral hypersensitivity state of IBS, and this may be mediated at least in part by brain CRF/CRF1 signaling pathways.

COMMENTS

Background

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder in clinical practice, but the pathophysiology of IBS has not been completely elucidated. Visceral hypersensitivity is common in IBS patients and has recently been considered as a biological marker for IBS. Nesfatin-1 is a recently discovered 82-amino-acid satiety peptide. Nesfatin-1 is co-localized with corticotropin releasing factor (CRF) in the hypothalamic paraventricular nucleus (PVN). Furthermore, intracerebroventricular administration of nesfatin-1 induced c-Fos expression in CRF neurons, and nesfatin-1 increased cytosolic Ca^{2+} concentrations in single CRF neurons in the PVN. It is now well established that the brain CRF/CRF1 signaling system modulates pain responses. These observations suggest that nesfatin-1 may be involved in the autonomic regulation of visceral sensation.

Research frontiers

Visceral hypersensitivity is a topic of intense research in gastrointestinal disorders. The research hotspot in terms of the visceral hypersensitivity mechanism is the signaling system and the exact sites mediating modulation.

Innovations and breakthroughs

The authors found that the average serum nesfatin-1 level was significantly higher in neonatal acetic acid-treated rats than in control rats, suggesting nesfatin-1 may be associated with a visceral hypersensitivity state in IBS-like model rats. Currently, no reports have been published discussing the role of nesfatin-1 in IBS. The purpose of the present study, therefore, was to investigate the effect of nesfatin-1 on visceral sensitivity in IBS and the possible underlying mechanisms of this action.

Applications

The study results suggest that nesfatin-1 may be associated with the visceral hypersensitivity state of IBS, and this may be mediated, at least in part, by brain CRF/CRF1 signaling pathways. This may provide new targets for the treatment of IBS.

Peer review

The authors investigated serum nesfatin-1 in a rat model of visceral hypersensitivity associated with IBS to explore the role of nesfatin-1 in the pathogenesis of IBS-like visceral hypersensitivity. The study found that nesfatin-1 may be associated with visceral hypersensitivity in model rats and may be implicated in brain CRF/CRF1 signaling pathways. Overall, the study has been well designed and the results are of great scientific significance.

REFERENCES

1 **Kanazawa M**, Hongo M, Fukudo S. Visceral hypersensitivity in irritable bowel syndrome. *J Gastroenterol Hepatol* 2011; **26 Suppl 3**: 119-121 [PMID: 21443723 DOI: 10.1111/j.1440-1746.2011.06640.x]

2 **Oh-I S**, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature* 2006; **443**: 709-712 [PMID: 17036007]

3 **Stengel A**, Goebel M, Wang L, Rivier J, Kobelt P, Mönnikes H, Lambrecht NW, Taché Y. Central nesfatin-1 reduces dark-phase food intake and gastric emptying in rats: differential role of corticotropin-releasing factor2 receptor. *Endocrinology* 2009; **150**: 4911-4919 [PMID: 19797401 DOI: 10.1210/en.2009-0578]

4 **Goebel M**, Stengel A, Wang L, Taché Y. Restraint stress activates nesfatin-1-immunoreactive brain nuclei in rats. *Brain Res* 2009; **1300**: 114-124 [PMID: 19733157 DOI: 10.1016/j.brainres.2009.08.082]

5 **Brailoiu GC**, Dun SL, Brailoiu E, Inan S, Yang J, Chang JK, Dun NJ. Nesfatin-1: distribution and interaction with a G protein-coupled receptor in the rat brain. *Endocrinology* 2007; **148**: 5088-5094 [PMID: 17627999]

6 **Bale TL**, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol* 2004; **44**: 525-557 [PMID: 14744257]

7 **Stengel A**, Taché Y. Neuroendocrine control of the gut during stress: corticotropin-releasing factor signaling pathways in the spotlight. *Annu Rev Physiol* 2009; **71**: 219-239 [PMID: 18928406 DOI: 10.1146/annurev.physiol.010908.163221]

8 **Lariviere WR**, Melzack R. The role of corticotropin-releasing factor in pain and analgesia. *Pain* 2000; **84**: 1-12 [PMID: 10601667]

9 **Mousa SA**, Bopaiah CP, Richter JF, Yamdeu RS, Schäfer M. Inhibition of inflammatory pain by CRF at peripheral, spinal and supraspinal sites: involvement of areas coexpressing CRF receptors and opioid peptides. *Neuropsychopharmacology* 2007; **32**: 2530-2542 [PMID: 17375137]

10 **Qian AH**, Liu XQ, Yao WY, Wang HY, Sun J, Zhou L, Yuan YZ. Voltage-gated potassium channels in IB4-positive colonic sensory neurons mediate visceral hypersensitivity in the rat. *Am J Gastroenterol* 2009; **104**: 2014-2027 [PMID: 19491827 DOI: 10.1038/ajg.2009.227]

11 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285 [PMID: 11054385]

12 **Traub RJ**, Tang B, Ji Y, Pandya S, Yfantis H, Sun Y. A rat model of chronic postinflammatory visceral pain induced by deoxycholic acid. *Gastroenterology* 2008; **135**: 2075-2083 [PMID: 19000677 DOI: 10.1053/j.gastro.2008.08.051]

13 **Martínez V**, Taché Y. Role of CRF receptor 1 in central CRF-induced stimulation of colonic propulsion in rats. *Brain Res* 2001; **893**: 29-35 [PMID: 11222989]

14 **Bonaz B**, Taché Y. Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor. *Brain Res* 1994; **641**: 21-28 [PMID: 8019847]

15 **Mayer EA**, Naliboff BD, Chang L, Coutinho SV. V. Stress and irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G519-G524 [PMID: 11254476]

16 **Lin C**, Al-Chaer ED. Long-term sensitization of primary afferents in adult rats exposed to neonatal colon pain. *Brain Res* 2003; **971**: 73-82 [PMID: 12691839]

17 **Bouin M**, Plourde V, Boivin M, Riberdy M, Lupien F, Laganière M, Verrier P, Poitras P. Rectal distention testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. *Gastroenterology* 2002; **122**: 1771-1777 [PMID: 12055583]

18 **Shimizu H**, Oh-I S, Hashimoto K, Nakata M, Yamamoto S, Yoshida N, Eguchi H, Kato I, Inoue K, Satoh T, Okada S, Yamada M, Yada T, Mori M. Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin-independent mechanism. *Endocrinology* 2009; **150**: 662-671 [PMID: 19176321 DOI: 10.1210/en.2008-0598]

19 **Stengel A**, Goebel M, Yakubov I, Wang L, Witcher D, Coskun T, Taché Y, Sachs G, Lambrecht NW. Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa. *Endocrinology* 2009; **150**: 232-238 [PMID: 18818289 DOI: 10.1210/en.2008-0747]

20 **Gonzalez R**, Tiwari A, Unniappan S. Pancreatic beta cells colocalize insulin and pronesfatin immunoreactivity in rodents. *Biochem Biophys Res Commun* 2009; **381**: 643-648 [PMID: 19248766 DOI: 10.1016/j.bbrc.2009.02.104]

21 **Li QC**, Wang HY, Chen X, Guan HZ, Jiang ZY. Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans. *Regul Pept* 2010; **159**: 72-77 [PMID: 19896982 DOI: 10.1016/j.regpep.2009.11.003]

22 **Pan W**, Hsuchou H, Kastin AJ. Nesfatin-1 crosses the blood-brain barrier without saturation. *Peptides* 2007; **28**: 2223-2228 [PMID: 17950952]

23 **Price TO**, Samson WK, Niehoff ML, Banks WA. Permeability of the blood-brain barrier to a novel satiety molecule nesfatin-1. *Peptides* 2007; **28**: 2372-2381 [PMID: 18006117]

24 **Martinez V**, Taché Y. CRF1 receptors as a therapeutic target for irritable bowel syndrome. *Curr Pharm Des* 2006; **12**: 4071-4088 [PMID: 17100612]
25 **Taché Y**, Martinez V, Wang L, Million M. CRF1 receptor signaling pathways are involved in stress-related alterations of colonic function and viscerosensitivity: implications for irritable bowel syndrome. *Br J Pharmacol* 2004; **141**: 1321-1330 [PMID: 15100165]
26 **Sagami Y**, Shimada Y, Tayama J, Nomura T, Satake M, Endo Y, Shoji T, Karahashi K, Hongo M, Fukudo S. Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. *Gut* 2004; **53**: 958-964 [PMID: 15194643]
27 **Yoshida N**, Maejima Y, Sedbazar U, Ando A, Kurita H, Damdindorj B, Takano E, Gantulga D, Iwasaki Y, Kurashina T, Onaka T, Dezaki K, Nakata M, Mori M, Yada T. Stressor-responsive central nesfatin-1 activates corticotropin-releasing hormone, noradrenaline and serotonin neurons and evokes hypothalamic-pituitary-adrenal axis. *Aging* (Albany NY) 2010; **2**: 775-784 [PMID: 20966530]

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L- Editor Cant MR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3494

World J Gastroenterol 2013 June 14; 19(22): 3494-3501
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Psychometrics of chronic liver disease questionnaire in Chinese chronic hepatitis B patients

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Author contributions: All authors participated in the design of the study; Zhou KN, Zhang M, Ji ZH and Zhang XM collected data; Zhou KN, Zhang M and Zhuang GH were involved in data analysis and interpretation; Zhou KN and Zhuang GH drafted the manuscript; all authors have read and approved the final manuscript.
Supported by The National TS Major Project of China, No. 2008ZX10002-001 and No. 2012ZX10002001
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Received: January 08, 2013 Revised: March 25, 2013
Accepted: March 28, 2013
Published online: June 14, 2013

Abstract

AIM: To evaluate psychometrics of the Chinese (mainland) chronic liver disease questionnaire (CLDQ) in patients with chronic hepatitis B (CHB).

METHODS: A cross-sectional sample of 460 Chinese patients with CHB was selected from the Outpatient Department of the Eighth Hospital of Xi'an, including CHB (CHB without cirrhosis) (n = 323) and CHB-related cirrhosis (n = 137). The psychometrics includes reliability, validity and sensitivity. Internal consistency reliability was measured using Cronbach's α. Convergent and discriminant validity was evaluated by item-scale correlation. Factorial validity was explored by principal component analysis with varimax rotation. Sensitivity was assessed using Cohen's effect size (ES), and independent sample t test between CHB and CHB-related cirrhosis groups and between alanine aminotransferase (ALT) normal and abnormal groups after stratifying the disease (CHB and CHB-related cirrhosis).

RESULTS: Internal consistency reliability of the CLDQ was 0.83 (range: 0.65-0.90). Most of the hypothesized item-scale correlations were 0.40 or over, and all of such hypothesized correlations were higher than the alternative ones, indicating satisfactory convergent and discriminant validity. Six factors were extracted after varimax rotation from the 29 items of CLDQ. The eligible Cohen's ES with statistically significant independent sample t test was found in the overall CLDQ and abdominal, systematic, activity scales (CHB *vs* CHB-related cirrhosis), and in the overall CLDQ and abdominal scale in the stratification of patients with CHB (ALT normal *vs* abnormal).

CONCLUSION: The CLDQ has acceptable reliability, validity and sensitivity in Chinese (mainland) patients with CHB.

Key words: Chronic hepatitis B; Chronic liver disease questionnaire; Reliability; Validity; Sensitivity

Core tip: Chronic hepatitis B (CHB) is a common chronic liver disease in mainland China, and its adverse prognosis might impair the patients' health-related quality of life (HRQoL). The chronic liver disease questionnaire (CLDQ) is the first liver specific HRQoL instrument, however, few studies have examined psychometrics of the Chinese (mainland) CLDQ in CHB patients. This study tested psychometrics of the Chinese (mainland) CLDQ in patients with CHB (including CHB without cirrhosis and CHB-related cirrhosis). The findings will help find suitable disease-specific questionnaire in the management of CHB in mainland China and provide evidence for expanding the use of the CLDQ.

Zhou KN, Zhang M, Wu Q, Ji ZH, Zhang XM, Zhuang GH. Psychometrics of chronic liver disease questionnaire in Chinese chronic hepatitis B patients. *World J Gastroenterol* 2013; 19(22): 3494-3501 Available from: URL: http://www.wjgnet.

com/1007-9327/full/v19/i22/3494.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3494

INTRODUCTION

Chronic hepatitis B (CHB), caused by persistent infection with hepatitis B virus (HBV), is a common chronic liver disease in mainland China. According to the latest national hepatitis B seroepidemiological survey[1], the estimated current HBV carriers in mainland China run up to 93 million, including 20-30 million patients with CHB[2]. CHB patients suffer recurrent symptoms in the long disease natural history and are at a high risk of developing fatal complications of cirrhosis and hepatocellular carcinoma[3,4]. Therefore, CHB may result in a heavy disease burden not only in premature death but also in health impairment[5-8].

Health-related quality of life (HRQoL) is defined as how the individual rates his/her life in terms of physical, psychological and social aspects[9]. In comparison with clinical parameters, HRQoL is a more holistic assessment of health status considering the individual's functional health and well-being, especially in chronic disease in which mortality is not an immediate concern[3]. Due to the complex natural history and phases of CHB[10], it is particularly important to use HRQoL as the primary endpoint for evaluating health and treatment effects in patients with such disease.

Generally, HRQoL can be measured by generic and disease-specific instruments. Generic instruments are used to compare the HRQoL between groups of patients, but disease-specific questionnaires distinguish the impairment of a specific illness and are more sensitive to the change[11,12]. The 36-item short-form health survey version 2 (SF-36v2) performs well as a generic instrument in patients with CHB[13]. However, not every scale or summary component of the SF-36v2 has the required or eligible sensitivity[14]. Therefore, a disease-specific HRQoL instrument is recommended as a complement for clinical studies in CHB.

The chronic liver disease questionnaire (CLDQ) is the first liver specific instrument developed by Younossi *et al*[15]. It has been translated into different languages for cross-cultural adaptation[16-23] and proved as a valid tool for HRQoL measurement and evaluation in patients with chronic liver disease[24,25]. The Chinese (Hong Kong, China) CLDQ has been validated in patients with CHB infection[14]. However, a dearth of study assessed psychometrics of the Chinese (mainland) CLDQ in mainland Chinese patients with CHB[26].

The purpose of the study was to evaluate psychometrics including reliability, validity and sensitivity of the Chinese (mainland) CLDQ in CHB patients. The findings of this study will help find suitable disease-specific questionnaire in the management of CHB in mainland China and add to the body of evidence for expanding the use of the CLDQ.

MATERIALS AND METHODS

Patients and data collection

Participants were CHB outpatients from the Eighth Hospital of Xi'an, which is the only local infectious disease hospital in Xi'an, Shaanxi Province, China. Inclusion criteria were aged 18 years or over, Chinese-speaking, with diagnosis of CHB (CHB without cirrhosis) or CHB-related cirrhosis. The diagnosis was made following the standards in the Guideline of Prevention and Treatment for Chronic Hepatitis B (2005 version) issued by the Chinese Society of Hepatology and the Chinese Society of Infectious Diseases[27]. If the patients had other chronic diseases (including hypertension, diabetes, chronic obstructive pneumonia disease, cardiovascular disease, mental illness, arthritis, tuberculosis, or gallstone), non-hepatitis B related liver disease, malignancies of liver or other organs, liver transplantation, cognitive disorders, or co-infected with human immunodeficiency virus or other types of hepatitis virus (*e.g.*, hepatitis A virus, hepatitis C virus , hepatitis D virus or hepatitis E virus), or refused to give written informed consent, they would be excluded.

Data were collected from April to June 2010. An individual face-to-face interview was administered by the trained interviewers in a quiet and well-lit room. The patients answered questions of sociodemographics and the Chinese (mainland) CLDQ. In addition to this, a free alanine aminotransferase (ALT) test was provided for the patients immediately after the questionnaire survey. The test was conducted in the laboratory of the Eighth Hospital of Xi'an.

Chinese (mainland) CLDQ

The Chinese (mainland) CLDQ was provided by the developers of the original CLDQ[15]. It consists of 29 items measuring six scales on abdominal symptoms (AB), fatigue (FA), systemic symptoms (SY), activity (AC), emotional function (EM) and worry (WO). Each item is rated on a 7-point Likert scale (1 = all of the time to 7 = none of the time). The six scale scores are calculated by the summated averages of corresponding endorsed item scores. The total score is calculated by the mean of all scale scores. Each scale and the total score ranged from 1 to 7, with a higher score indicating better HRQoL.

Reliability and floor/ceiling effects

Cronbach's α coefficient was used to assess the internal consistency reliability, with the value greater than 0.70 representing acceptable reliability[28]. Floor and ceiling effects were calculated as the number and percentage of CHB patients at the lowest and highest possible scores for each scale and the overall CLDQ. It should be less than 20% regarding both the floor and ceiling effect to ensure that the scales are capturing the full range of potential responses in the population and that changes over time can be detected[29].

Table 1 Social-demographic characteristics *n* (%)

Items	Total (*n* = 460)	CHB (*n* = 323)	CHB-related cirrhosis (*n* = 137)
Age (yr)			
18-35	240 (52.2)	216 (90.0)	24 (10.0)
36-50	152 (33.0)	87 (57.2)	65 (42.8)
51-65	64 (13.9)	20 (31.3)	44 (68.8)
66-76	4 (0.9)	0 (0.0)	4 (100.0)
mean ± SD	35.75 ± 12.82	31.53 ± 10.94	45.71 ± 11.36
Gender			
Male	305 (66.3)	212 (69.5)	93 (30.5)
Female	155 (33.7)	111 (71.6)	44 (28.4)
Marital status			
Single	125 (27.2)	119 (95.2)	6 (4.8)
Married	328 (71.3)	200 (61.0)	128 (39.0)
Others	7 (1.5)	4 (57.1)	3 (42.9)
Education level			
No schooling and primary	63 (13.7)	31 (49.2)	32 (50.8)
Secondary	311 (67.6)	217 (69.8)	94 (30.2)
Tertiary	86 (18.7)	75 (87.2)	11 (12.8)
Occupation			
Peasants	153 (33.3)	73 (47.7)	80 (52.3)
Non-peasants	307 (66.7)	250 (81.4)	57 (18.6)
Annual per capita household incomes (RMB)			
< 5000	236 (51.3)	147 (62.3)	89 (37.7)
5000-9999	111 (24.1)	88 (79.3)	23 (20.7)
⩾ 10000	113 (24.6)	88 (77.9)	25 (22.1)
Received ALT test			
Yes	375 (81.5)	311 (82.9)	64 (17.1)
No	85 (18.5)	12 (14.1)	73 (85.9)

CHB: Chronic hepatitis B; ALT: Alanine aminotransferase.

Validity

The hypothesized item-scale correlation (γ coefficient) ⩾ 0.40 was considered as satisfactory convergent validity[30]. Discriminant validity was supported whenever the hypothesized item-scale correlation was significantly higher than the correlation of the item with other scales[31]. Factorial validity of the CLDQ was explored using the principal component analysis with varimax rotation. The predictive items with factor loading coefficient (FLC) ⩾ 0.45[32] and the extracted factors with an eigenvalue ⩾ 1.0[33] were considered to be relevant.

Sensitivity

Sensitivity was evaluated by Cohen's effect size (ES), and independent sample *t* test between CHB and CHB-related cirrhosis groups and between ALT normal and abnormal groups after stratifying the disease (CHB and CHB-related cirrhosis). The ES value was calculated as the difference between group mean scores divided by overall standard deviation. According to Cohen, the ES of 0.2-0.5 is small, of 0.5-0.8 moderate, and those of 0.8 or above large[31]. Besides, multiple linear regression analysis was used to further prove sensitivity of the CLDQ under the influence of ALT (normal *vs* abnormal). The six scales and overall CLDQ scores were dependent variables, respectively, while the controlled independent variables were age, gender, marital status, education level, occupation, annual per capita household incomes and the disease (CHB and CHB-related cirrhosis).

Table 2 Internal consistency reliability, floor and ceiling effects of the chronic liver disease questionnaire (*n* = 460) *n* (%)

Items	Cronbach's α	Floor and ceiling effects[1]	
		Floor effects	Ceiling effects
Abdominal symptoms	0.80	1 (0.2)	50 (10.9)
Fatigue	0.82	1 (0.2)	1 (0.2)
Systemic symptoms	0.65	2 (0.4)	16 (3.5)
Activity	0.66	1 (0.2)	36 (7.8)
Emotional function	0.90	1 (0.2)	5 (1.1)
Worry	0.85	1 (0.2)	17 (3.7)
Overall	0.83	1 (0.2)	1 (0.2)

[1]Floor and ceiling effect: The number and percentage of the chronic hepatitis B patients at the lowest and highest possible scores.

Ethics statement

The study protocol was reviewed and approved by the Human Research Ethics Committee of Xi'an Jiaotong University. The written informed consent was obtained from each recruited patient before the questionnaire survey.

Statistical analysis

A database was built using the software EpiData 3.1 and the data were double-entered by two different persons to capture data entry errors. All analyses were performed with SPSS version 13.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Sociodemographics

A total of 460 patients were recruited. In the face-to-face interview, they understood the questions of the CLDQ well, and finished the questionnaire completely. The patients aged 35.75 ± 12.82 years (range: 18-76 years), with 305 (66.3%) males and 155 (33.7%) females. Three hundred and twenty-eight (71.3%) patients were married. Education levels of the patients were no schooling and primary (*n* = 63, 13.7%), secondary (*n* = 311, 67.6%) and tertiary (*n* = 86, 18.7%); 153 (33.3%) were peasants and the others were non-peasants. Annual per capita household incomes (RMB) of the patients were < 5000 (*n* = 236, 51.3%), 5000-9999 (*n* = 111, 24.1%) or ⩾ 10000 (*n* = 113, 24.6%). With respect to the disease, the CHB and CHB-related cirrhosis patients accounted for 70.2% (*n* = 323) and 29.8% (*n* = 137) respectively. Three hundred and seventy five (81.5%) patients received ALT test immediately after the questionnaire survey, 311 (82.9%) with CHB and 64 (17.1%) with CHB-related cirrhosis. The detailed information of patients with CHB or CHB-related cirrhosis is shown in Table 1.

Reliability and floor/ceiling effects

Cronbach's α of the total CLDQ was 0.83, with the

Table 3 Convergent validity and discriminant validity of the chronic liver disease questionnaire (n = 460)

Items	Item-scale correlation (Spearman γ)					
	AB	FA	SY	AC	EM	WO
Abdominal symptoms						
1. Abdominal bloating	0.82[1]	0.40	0.42	0.38	0.33	0.22
5. Abdominal pain	0.79[1]	0.41	0.56	0.39	0.35	0.30
17. Abdominal discomfort	0.91[1]	0.52	0.57	0.47	0.46	0.33
Fatigue						
2. Tiredness or fatigue	0.46	0.78[1]	0.48	0.42	0.53	0.37
4. Feel sleepy during the day	0.23	0.61[1]	0.30	0.27	0.30	0.23
8. Decreased strength	0.45	0.79[1]	0.50	0.64	0.52	0.41
11. Decreased energy	0.46	0.79[1]	0.50	0.62	0.65	0.42
13. Drowsiness	0.37	0.76[1]	0.43	0.42	0.50	0.31
Systemic symptoms						
3. Bodily pain	0.61	0.47	0.71[1]	0.40	0.46	0.33
6. Shortness of breath	0.46	0.50	0.66[1]	0.49	0.42	0.30
21. Muscle cramps	0.35	0.31	0.59[1]	0.36	0.28	0.21
23. Dry mouth	0.32	0.35	0.64[1]	0.26	0.38	0.31
27. Itching	0.35	0.31	0.65[1]	0.29	0.28	0.23
Activity						
7. Not able to eat as much as you would like	0.37	0.49	0.35	0.78[1]	0.41	0.28
9. Trouble in lifting or carrying heavy objects	0.41	0.54	0.49	0.84[1]	0.38	0.28
14. Bothered by a limitation of the diet	0.45	0.53	0.43	0.73[1]	0.44	0.28
Emotional function						
10. Anxiety	0.36	0.57	0.46	0.43	0.81[1]	0.53
12. Unhappiness	0.34	0.50	0.36	0.39	0.80[1]	0.44
15. Irritability	0.33	0.53	0.42	0.41	0.78[1]	0.43
16. Difficulty in sleeping at night	0.30	0.44	0.39	0.32	0.70[1]	0.33
19. Mood swings	0.36	0.51	0.42	0.35	0.77[1]	0.54
20. Difficulty in falling asleep at night	0.33	0.38	0.40	0.35	0.67[1]	0.35
24. Depression	0.39	0.58	0.48	0.43	0.80[1]	0.58
26. Problems with concentration	0.36	0.54	0.43	0.36	0.73[1]	0.48
Worry						
18. Worries about the impact of the liver disease	0.19	0.32	0.30	0.24	0.47	0.78[1]
22. Worries that symptoms will develop into major problem	0.33	0.45	0.38	0.31	0.53	0.89[1]
25. Worries that the condition is getting worse	0.33	0.41	0.38	0.31	0.53	0.88[1]
28. Worries about never feeling any better	0.31	0.39	0.34	0.30	0.50	0.83[1]
29. Availability of a liver for transplant	0.19	0.15	0.24	0.18	0.22	0.35[2]

Convergent validity: [1]The hypothesized item-scale correlations ⩾ 0.40; [2]correlations < 0.40. Discriminant validity: The hypothesized item-scale correlations are higher than the alternative ones. AB: Abdominal symptoms; FA: Fatigue; SY: Systemic symptoms; AC: Activity; EM: Emotional function; WO: Worry.

range from 0.65 to 0.90 in the six scales. Both floor and ceiling effect percentages of the six scales and total score of the CLDQ were less than 20% (Table 2).

Validity

The hypothesized item-scale correlation of the six scales (range) were AB (0.79-0.91), FA (0.61-0.79), SY (0.59-0.71), AC (0.73-0.84), EM (0.67-0.81) and WO (0.35-0.89), all were higher than the range of the corresponding items with other scales, indicating better convergent and discriminant validity (Table 3).

Six factors were extracted from the 29 items of CLDQ, which explained 61.69% of the total variance. Factor loadings of the 29 items were not entirely consistent with the six scales. The items of EM scale loaded on factor 1, except for "difficulty in sleeping at night" (item 16) and "difficulty in falling asleep at night" (item 20), which loaded on factor 6. Factor 2 covered WO scale. The items of FA and AC scales loaded on factor 3, except for "tiredness or fatigue" (item 2), "feel sleepy during the day" (item 4) and "drowsiness" (item 13) loaded on factor 5. Both of AB scale and item 3 (bodily pain) loaded on factor 4 (Table 4).

Sensitivity

Regarding the between-group comparison in CHB and CHB-related cirrhosis patients, the eligible ES was found in AC (0.71), SY (0.52), AB (0.33) scales and the overall CLDQ (0.37). After controlling the influences of the disease (CHB and CHB-related cirrhosis), the ES in AB (0.30) scale and the overall CLDQ (0.23) were satisfactory by comparison between the normal and abnormal ALT groups in the stratification of patients with CHB. Meanwhile, the corresponding between-group independent sample t test was statistically significant ($P < 0.05$) (Tables 5 and 6). Other eligible ES was also found in patients with normal or abnormal ALT after stratification, including SY (0.22) scale (CHB), and AB (0.27), FA (0.23), AC (0.23) scales and the overall CLDQ (0.21) (CHB-related cirrhosis). However, the corresponding between-group t test was not statistically significant ($P > 0.05$) (Table 6).

Based on controlling the influences of sociodemo-

Table 4 Factor analysis of the chronic liver disease questionnaire (n = 460)

Items	F 1 (EM)	F 2 (WO)	F 3 (AC + FA)	F 4 (AB + SY)	F 5 (FA)	F 6 (sleep)
Abdominal symptoms						
1. Abdominal bloating				0.68		
5. Abdominal pain				0.82		
17. Abdominal discomfort				0.75		
Fatigue						
2. Tiredness or fatigue					0.52	
4. Feel sleepy during the day					0.75	
8. Decreased strength			0.66			
11. Decreased energy			0.63			
13. Drowsiness					0.72	
Systemic symptoms						
3. Bodily pain				0.68		
6. Shortness of breath				(0.32)		
21. Muscle cramps				(0.35)		
23. Dry mouth				(0.32)		
27. Itching				(0.28)		
Activity						
7. Not able to eat as much as you would like			0.76			
9. Trouble in lifting or carrying heavy objects			0.64			
14. Bothered by a limitation of the diet			0.65			
Emotional function						
10. Anxiety	0.73					
12. Unhappiness	0.78					
15. Irritability	0.77					
16. Difficulty in sleeping at night						0.79
19. Mood swings	0.66					
20. Difficulty in falling asleep at night						0.75
24. Depression	0.65					
26. Problems with concentration	0.55					
Worry						
18. Worries about the impact of the liver disease		0.69				
22. Worries that symptoms will develop into major problem		0.82				
25. Worries that the condition is getting worse		0.81				
28. Worries about never feeling any better		0.75				
29. Availability of a liver for transplantation		0.51				
Eigenvalue	36.14%	7.94%	5.44%	4.57%	4.01%	3.59%
Cumulative	36.14%	44.08%	49.52%	54.09%	58.10%	61.69%

Kaiser-Meyer-Olkin measure of sampling adequacy: 0.929. Bartlett's test of sphericity: $P < 0.001$. The items with factor (F) loading coefficient less than 0.45 are written in bracket. EM: Emotional function; WO: Worry; AC: Activity; FA: Fatigue; AB: Abdominal symptoms; SY: Systemic symptoms.

Table 5 Sensitivity of the chronic liver disease questionnaire: scores (mean ± SD) and effect size

Scales	Total (n = 460)	CHB (n = 323)	CHB-related cirrhosis (n = 137)	ES
AB	5.48 ± 1.11	5.59 ± 1.07	5.22 ± 1.16	0.33[1,b]
FA	4.76 ± 1.11	4.82 ± 1.08	4.61 ± 1.17	0.19
SY	5.41 ± 0.86	5.54 ± 0.82	5.09 ± 0.87	0.52[1,b]
AC	5.45 ± 1.13	5.69 ± 1.03	4.89 ± 1.15	0.71[1,b]
EM	4.85 ± 1.10	4.87 ± 1.13	4.80 ± 1.01	0.06
WO	5.06 ± 1.19	5.03 ± 1.23	5.11 ± 1.08	-0.07
Overall	5.17 ± 0.83	5.26 ± 0.83	4.95 ± 0.78	0.37[1,b]

[1]The effect size (ES) ≥ 0.20. Effect size is calculated as the difference between chronic hepatitis B (CHB) and CHB-related cirrhosis groups mean score divided by the overall standard deviation. Significant difference between CHB and CHB-related cirrhosis groups by independent sample t test: [b]$P < 0.01$. AB: Abdominal symptoms; FA: Fatigue; SY: Systemic symptoms; AC: Activity; EM: Emotional function; WO: Worry.

graphics and the disease (CHB and CHB-related cirrhosis), multiple linear regression analysis detected differences (unstandardized coefficient, 95%CI) in AB scale [-0.39, (-0.63, -0.15), P = 0.001], SY scale [-0.23, (-0.41, -0.06), P = 0.009] and the overall CLDQ [-0.22, (-0.40, -0.04), P = 0.018] with statistical significance under the influence of ALT (normal *vs* abnormal) (Table 7).

DISCUSSION

The CLDQ is a non-generic, disease-specific instrument for assessing HRQoL in patients with chronic liver disease. We used the Chinese (mainland) CLDQ in patients with CHB and proved that this instrument has acceptable reliability, validity and sensitivity.

Internal consistency reliability of AB, FA, EM, WO scales and the overall CLDQ were satisfactory, with Cronbach's α coefficient greater than 0.70. It was consistent with the reports from similar studies using the original and other different language versions of CLDQ[15,16,19,34]. However, Cronbach's α of SY (0.65) and AC (0.66) scales were less than the recommended value of 0.70. This is probably due to heterogeneous manifestations of system-

Table 6 Sensitivity of the chronic liver disease questionnaire in different alanine aminotransferase results after stratifying the disease

Scales	CHB with ALT test (n = 311)				CHB-related cirrhosis with ALT test (n = 64)			
	Total score	Normal (n = 170)	Abnormal (n = 141)	ES	Total score	Normal (n = 29)	Abnormal (n = 35)	ES
Abdominal symptoms	5.59 ± 1.08	5.73 ± 1.04	5.41 ± 1.11	0.30[1,b]	5.34 ± 1.16	5.51 ± 1.17	5.20 ± 1.14	0.27[1]
Fatigue	4.81 ± 1.08	4.88 ± 1.09	4.72 ± 1.08	0.15	4.61 ± 1.13	4.75 ± 1.21	4.49 ± 1.06	0.23[1]
Systemic symptoms	5.54 ± 0.83	5.62 ± 0.82	5.44 ± 0.84	0.22[1]	5.17 ± 0.83	5.24 ± 0.83	5.11 ± 0.84	0.16
Activity	5.70 ± 1.03	5.76 ± 1.00	5.62 ± 1.06	0.14	4.94 ± 1.21	5.09 ± 1.30	4.81 ± 1.14	0.23[1]
Emotional function	4.85 ± 1.13	4.94 ± 1.16	4.75 ± 1.09	0.17	4.77 ± 1.06	4.85 ± 1.14	4.71 ± 1.00	0.13
Worry	5.02 ± 1.23	5.10 ± 1.23	4.93 ± 1.23	0.14	4.98 ± 1.20	4.94 ± 1.31	5.01 ± 1.12	-0.06
Overall	5.25 ± 0.84	5.34 ± 0.83	5.15 ± 0.84	0.23[1,a]	4.97 ± 0.81	5.06 ± 0.88	4.89 ± 0.74	0.21[1]

[1]The effect size (ES) ⩾ 0.20. Effect size was calculated as the difference between alanine aminotransferase (ALT) normal and abnormal group mean scores divided by standard deviation of the total score. Significant difference by independent sample t-test between ALT normal and abnormal groups: [a]$P < 0.05$, [b]$P < 0.01$.

Table 7 Multiple linear regression analysis (n = 460)

Dependent variable	B	SE	t	P	95%CI
Abdominal symptoms	-0.39	0.12	-3.22	0.001	-0.63, -0.15
Systemic symptoms	-0.23	0.09	-2.63	0.009	-0.41, -0.06
Overall CLDQ	-0.22	0.09	-2.37	0.018	-0.40, -0.04

Independent variable: alanine aminotransferase (normal *vs* abnormal). The other controlled independent variables were age, gender, marital status, education level, occupation, annual per capita household incomes and the disease [chronic hepatitis B (CHB) and CHB-related cirrhosis]. CLDQ: Chronic liver disease questionnaire.

atic symptoms severity and activity limitations in patients with CHB or CHB-related cirrhosis and, consequently, influences the corresponding scoring. Percentages of the floor and ceiling effects regarding the six scales and total score of the CLDQ were less than 20%, indicating that this instrument can capture the full range of potential responses and detect small changes in CHB patients during treatment process.

The convergent and discriminant validity of the CLDQ was satisfactory, proving that the items of each scale can reflect the major characteristics consistently. Lam *et al*[14] and Mahmoudi *et al*[35] reported similar results in their studies. However, "availability of a liver for transplant" (item 29) in the present study did not better correlate to WO scale (γ = 0.35), possibly due to few patients confronted with the problem of liver transplantation. Therefore, this item was less correlated to WO scale than the other four items within the same dimension.

The factor structure of the Chinese (mainland) CLDQ conform to the six scale structure of the original US version, with a new scale of "sleep" (factor 6) covering the item "difficulty in sleeping at night" (item 16) and "difficulty in falling asleep at night" (item 20) being the only major difference. Such new factor was also found in the Spanish, Italian and German population[19,20,34]. It was reasonable to believe that sleeping habits vary among cultures (napping habits and bedtimes), so it may not be surprising that these items clustered differently. For patients with CHB or CHB-related cirrhosis, they may have sleep difficulties due to various reasons other than emotional problems, including pain or other disease-related factors. Except for the two items of "sleeping difficulty", all other items of the EM scale loaded on factor 1 consistently.

The items of WO, AC and AB scales loaded on factor 2, 3 and 4. However, AC and AB scales also combined with other items. The finding showed that "decreased strength" (item 8) and "decreased energy" (item 11) of FA scale loaded on factor 3 (AC scale), whereas "bodily pain" (item 3) of SY scale loaded on factor 4 (AB scale), which demonstrated that decreased strength and energy highly correlate to activity[14], and bodily pain may be better related to AB, especially abdominal pain in CHB patients. "Tiredness or fatigue" (item 2), "feel sleepy during the day" (item 4) and "drowsiness" (item 13) were found loading on factor 5 as measuring fatigue symptoms. However, the FLC of "shortness of breath" (item 6) (0.32), "muscle cramps" (item 21) (0.35), "dry mouth" (item 23) (0.32) and "itching" (item 27) (0.28) were less than the recommended value of 0.45. It was probably because that most of the patients were in CHB stage and did not have distinct clinical manifestations as the items reflected.

By comparison between CHB and CHB-related cirrhosis groups, the CLDQ was sensitive in detecting differences in the overall and AB, SY and AC scale scores. It suggests that, based on measuring the physical and psychological health, the CLDQ could further detect the difference of hepatitis-related physical symptoms in CHB patients with or without cirrhosis. After controlling for influences of the disease (CHB and CHB-related cirrhosis), the CLDQ was very good in measuring differences in the overall and AB and SY scale scores between normal and abnormal ALT groups in CHB patients without cirrhosis. Multiple linear regression analysis also confirmed this result through further controlling other confounding factors such as sociodemographics.

However, due to close scores, FA, EM and WO scales did not show significant difference in CHB patients with or without considering ALT test results and controlling for influences of the disease (CHB and CHB-related cirrhosis). Specifically, the difference of between-group comparison in AC scale changed from significant to not significant after stratification, indicating the confounding role of the disease (CHB and CHB-related cirrhosis).

Unlike such finding, Lam *et al*[14] used the Chinese (Hong Kong, China) CLDQ in patients with CHB infection and reported significant differences in FA, AC, EM and WO scales between uncomplicated and complicated CHB patients. Such discrepancy might be the result that CHB-related complications have impact on the patients' health regardless of the disease (CHB and CHB-related cirrhosis) and, subsequently, influence the scoring of the corresponding scales. Therefore, the sensitivity of FA, AC, EM and WO scales of the CLDQ in patients with CHB or CHB-related cirrhosis need further examination.

In patients with CHB-related cirrhosis, the ES values of AB (0.27), FA (0.23), AC (0.23) scales and the overall CLDQ (0.21) were greater than 0.20, confirming the major impact of ALT on the hepatitis-related physical health. Other scales including SY (0.16), EM (0.13) and WO (-0.06) did not have the required ES value, indicating the similar conditions of hepatitis-related symptoms and mental health under the influence of ALT (normal *vs* abnormal) in cirrhotic patients. However, no statistical significance of independent sample *t* test was found in any scales and the overall CLDQ in the present study. The probable explanation for this might be the small sample size (n = 64). Different from such result, Lam *et al*[13] found significant differences in SY scale and the overall CLDQ between impaired liver function and cirrhosis groups in CHB patients. Accordingly, more attention should be paid to proving the sensitivity of the CLDQ in CHB-related cirrhosis patients under the influence of normal and abnormal ALT.

There were some limitations in the present study. First, the Chinese (mainland) CLDQ was administered using a face-to-face interview, the performance of the instrument by self-completion will need to be confirmed by future work. Second, the responsiveness of the Chinese (mainland) CLDQ in detecting changes with disease progression or ALT status will also need to be determined. Third, this study was conducted in the single-site of the Eighth Hospital of Xi'an. Therefore, it was limited to generalize the results to all of Chinese mainland CHB patients.

The Chinese (mainland) CLDQ has been proved as a valid tool for assessing HRQoL in patients with CHB. Both reliability and validity were demonstrated to be strongly satisfactory, and better sensitivity was confirmed in detecting the difference of AB, SY and AC scales and the overall CLDQ, especially the differences in AB scale and the overall CLDQ under the influence of ALT status. The Chinese (mainland) CLDQ can be a suitable disease-specific questionnaire for evaluating the change of HRQoL and treatment effects of CHB in clinical practice. Future work in a larger cohort of patients is needed to further prove the responsiveness of the Chinese (mainland) CLDQ.

COMMENTS

Background

Chronic hepatitis B (CHB) is a common chronic liver disease in mainland China. The patients are at high risk of developing fatal complications of cirrhosis and hepatocellular carcinoma, which consequently lead to the impairments of the patients' health-related quality of life (HRQoL).

Research frontiers

HRQoL should be used as the primary endpoint in the evaluation of treatment effectiveness for patients with CHB, since it is a more holistic assessment of health status considering the individual's functional health and well-being.

Innovations and breakthroughs

The chronic liver disease questionnaire (CLDQ) has been translated into many different languages for cross-cultural adaptation and proved as a valid tool for HRQoL measurement and evaluation in patients with chronic liver disease. However, a dearth of study assessed psychometrics of the Chinese (mainland) CLDQ in mainland Chinese patients with CHB. This study determined that the Chinese (mainland) CLDQ has acceptable reliability, validity and sensitivity in patients with CHB. It can be applied to mainland Chinese CHB patients to evaluate their HRQoL.

Applications

The Chinese (mainland) CLDQ can be used as a suitable disease-specific questionnaire for evaluating the change of HRQoL and treatment effects of CHB in clinical practice. The CLDQ can be used as a cross-cultural HRQoL measure in international studies that include mainland Chinese.

Terminology

Reliability concerns the random variability associated with measurements. Validity refers to the extent to which a test measures what it is intended to measure. Sensitivity is the ability of measurements to detect differences between patients or groups of patients.

Peer review

The authors tested psychometrics of the Chinese (mainland) CLDQ and proved that this questionnaire was reliable, valid and sensitive for Chinese mainland patients with CHB. The study was well done and used appropriate methodology to validate and test the questionnaire.

REFERENCES

1 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Wang F, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Epidemiological serosurvey of hepatitis B in China--declining HBV prevalence due to hepatitis B vaccination. *Vaccine* 2009; **27**: 6550-6557 [PMID: 19729084 DOI: 10.1016/j.vaccine.2009.08.048]

2 **Lu FM**, Li T, Liu S, Zhuang H. Epidemiology and prevention of hepatitis B virus infection in China. *J Viral Hepat* 2010; **17** Suppl 1: 4-9 [PMID: 20586928 DOI: 10.1111/j.1365-2893.2010.01266.x]

3 **Ong SC**, Mak B, Aung MO, Li SC, Lim SG. Health-related quality of life in chronic hepatitis B patients. *Hepatology* 2008; **47**: 1108-1117 [PMID: 18318043 DOI: 10.1002/hep.22138]

4 **Lai CL**, Yuen MF. Chronic hepatitis B--new goals, new treatment. *N Engl J Med* 2008; **359**: 2488-2491 [PMID: 19052131 DOI: 10.1056/NEJMe0808185]

5 **Dan AA**, Kallman JB, Srivastava R, Younoszai Z, Kim A, Younossi ZM. Impact of chronic liver disease and cirrhosis on health utilities using SF-6D and the health utility index. *Liver Transpl* 2008; **14**: 321-326 [PMID: 18306356 DOI: 10.1002/lt.21376]

6 **Sobhonslidsuk A**, Silpakit C, Kongsakon R, Satitpornkul P, Sripetch C, Khanthavit A. Factors influencing health-related quality of life in chronic liver disease. *World J Gastroenterol* 2006; **12**: 7786-7791 [PMID: 17203521]

7 **Younossi ZM**, Boparai N, Price LL, Kiwi ML, McCormick M, Guyatt G. Health-related quality of life in chronic liver disease: the impact of type and severity of disease. *Am J Gastroenterol* 2001; **96**: 2199-2205 [PMID: 11467653 DOI: 10.1111/j.1572-0241.2001.03537.x]

8 **Kondo Y**, Yoshida H, Tateishi R, Shiina S, Mine N, Yamashiki N, Sato S, Kato N, Kanai F, Yanase M, Yoshida H, Akamatsu M, Teratani T, Kawabe T, Omata M. Health-related quality of life of chronic liver disease patients with

and without hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 197-203 [PMID: 17295871 DOI: 10.1111/j.1440-1746.2006.04456.x]
9 **Guyatt GH**, Feeny DH, Patrick DL. Measuring health-related quality of life. *Ann Intern Med* 1993; **118**: 622-629 [PMID: 8452328]
10 **Fattovich G**. Natural history and prognosis of hepatitis B. *Semin Liver Dis* 2003; **23**: 47-58 [PMID: 12616450 DOI: 10.1055/s-2003-37590]
11 **Osoba D.** Guidelines for measuring health-related quality of life in clinical trials. In: Staquet MJ, Hays RD, Fayers PM, editors. Quality of Life Assessment in Clinical Trials. New York: Oxford University Press, 1999
12 **Younossi ZM**, Guyatt G. Quality-of-life assessments and chronic liver disease. *Am J Gastroenterol* 1998; **93**: 1037-1041 [PMID: 9672326 DOI: 10.1111/j.1572-0241.1998.00325.x]
13 **Lam ET**, Lam CL, Lai CL, Yuen MF, Fong DY, So TM. Health-related quality of life of Southern Chinese with chronic hepatitis B infection. *Health Qual Life Outcomes* 2009; **7**: 52 [PMID: 19500349 DOI: 10.1186/1477-7525-7-52]
14 **Lam ET**, Lam CL, Lai CL, Yuen MF, Fong DY. Psychometrics of the chronic liver disease questionnaire for Southern Chinese patients with chronic hepatitis B virus infection. *World J Gastroenterol* 2009; **15**: 3288-3297 [PMID: 19598306 DOI: 10.3748/wjg.15.3288]
15 **Younossi ZM**, Guyatt G, Kiwi M, Boparai N, King D. Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease. *Gut* 1999; **45**: 295-300 [PMID: 10403745 DOI: 10.1136/gut.45.2.295]
16 **Sobhonslidsuk A**, Silpakit C, Kongsakon R, Satitpornkul P, Sripetch C. Chronic liver disease questionnaire: translation and validation in Thais. *World J Gastroenterol* 2004; **10**: 1954-1957 [PMID: 15222044]
17 **Sumskiene J**, Sumskas L, Petrauskas D, Kupcinskas L. Disease-specific health-related quality of life and its determinants in liver cirrhosis patients in Lithuania. *World J Gastroenterol* 2006; **12**: 7792-7797 [PMID: 17203522]
18 **Häuser W**, Schnur M, Steder-Neukamm U, Muthny FA, Grandt D. Validation of the German version of the Chronic Liver Disease Questionnaire. *Eur J Gastroenterol Hepatol* 2004; **16**: 599-606 [PMID: 15167163 DOI: 10.1097/00042737-200406000-00014]
19 **Ferrer M**, Córdoba J, Garin O, Olivé G, Flavià M, Vargas V, Esteban R, Alonso J. Validity of the Spanish version of the Chronic Liver Disease Questionnaire (CLDQ) as a standard outcome for quality of life assessment. *Liver Transpl* 2006; **12**: 95-104 [PMID: 16382456 DOI: 10.1002/lt.20551]
20 **Rucci P**, Taliani G, Cirrincione L, Alberti A, Bartolozzi D, Caporaso N, Colombo M, Coppola R, Chiaramonte M, Craxi A, De Sio I, Floreani AR, Gaeta GB, Persico M, Secchi G, Versace I, Mele A. Validity and reliability of the Italian version of the Chronic Liver Disease Questionnaire (CLDQ-I) for the assessment of health-related quality of life. *Dig Liver Dis* 2005; **37**: 850-860 [PMID: 16221576 DOI: 10.1016/j.dld.2005.02.014]
21 **Kollia Z**, Patelarou E, Vivilaki V, Kollia E, Kefou F, Elefsiniotis I, Dourakis SP, Brokalaki H. Translation and validation of the Greek chronic liver disease questionnaire. *World J Gastroenterol* 2010; **16**: 5838-5844 [PMID: 21155005 DOI: 10.3748/wjg.v16.i46.5838]
22 **Ray I**, Dutta D, Basu P, De BK. Quality of life assessment of patients with chronic liver disease in eastern India using a Bengali translation chronic liver disease questionnaire. *Indian J Gastroenterol* 2010; **29**: 187-195 [PMID: 20740340 DOI: 10.1007/s12664-010-0036-x]
23 **Benito de Vale M**, Josefsson A, Lindkvist B, Kalaitzakis E. Validation of the Swedish version of the chronic liver disease questionnaire. *Scand J Gastroenterol* 2012; **47**: 614-615 [PMID: 22364527 DOI: 10.3109/00365521.2012.661763]
24 **Younossi ZM**, Kiwi ML, Boparai N, Price LL, Guyatt G. Cholestatic liver diseases and health-related quality of life. *Am J Gastroenterol* 2000; **95**: 497-502 [PMID: 10685757 DOI: 10.1111/j.1572-0241.2000.01774.x]
25 **Martin LM**, Younossi ZM. Health-related quality of life (HRQL) in chronic liver disease. *Dig Liver Dis* 2005; **37**: 819-820 [PMID: 15935747 DOI: 10.1016/j.dld.2005.04.022]
26 **Wu CH,** Deng QW, Ji XS, Yan LM. Preliminary use of the CLDQ in chronic hepatitis B patients. *Zhongguo Linchuang Xinli Xue Zazhi* 2003; **11:** 60-62
27 **Chinese Society of Hepatology and Chinese Society of Infectious Diseases, Chinese Medical Association.** [The guideline of prevention and treatment for chronic hepatitis B (2010 version)]. *Zhonghua Ganzangbing Zazhi* 2011; **19:** 13-24 [PMID: 21272453 DOI: 10.3760/cma.j.issn.1007-3418.2011.01.007]
28 **Nunnally J,** Bernstein I. Psychometric Theory. New York: McGraw-Hill, 1994
29 **Lim LL**, Seubsman SA, Sleigh A. Thai SF-36 health survey: tests of data quality, scaling assumptions, reliability and validity in healthy men and women. *Health Qual Life Outcomes* 2008; **6**: 52 [PMID: 18634552 DOI: 10.1186/1477-7525-6-52]
30 **Ware JE**, Gandek B. Methods for testing data quality, scaling assumptions, and reliability: the IQOLA Project approach. International Quality of Life Assessment. *J Clin Epidemiol* 1998; **51**: 945-952 [PMID: 9817111 DOI: 10.1016/S0895-4356(98)00085-7]
31 **Fayers PM,** Machin D. Quality of life: The assessment, analysis and interpretation of patient-reported outcomes. 2nd ed. New York: John Wiley-Sons, 2007
32 **Jolliffe IT.** Principal components analysis. New York: Springer, 1986
33 **Gómez-Besteiro MI**, Santiago-Pérez MI, Alonso-Hernández A, Valdés-Cañedo F, Rebollo-Alvarez P. Validity and reliability of the SF-36 questionnaire in patients on the waiting list for a kidney transplant and transplant patients. *Am J Nephrol* 2004; **24**: 346-351 [PMID: 15205553 DOI: 10.1159/000079053]
34 **Schulz KH**, Kroencke S, Ewers H, Schulz H, Younossi ZM. The factorial structure of the Chronic Liver Disease Questionnaire (CLDQ). *Qual Life Res* 2008; **17**: 575-584 [PMID: 18389385 DOI: 10.1007/s11136-008-9332-7]
35 **Mahmoudi H**, Jafari P, Alizadeh-Naini M, Gholami S, Malek-Hosseini SA, Ghaffaripour S. Validity and reliability of Persian version of Chronic Liver Disease Questionnaire (CLDQ). *Qual Life Res* 2012; **21**: 1479-1485 [PMID: 22081217 DOI: 10.1007/s11136-011-0059-5]

P- Reviewer Tiniakos DG **S- Editor** Huang XZ
L- Editor Ma JY **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3502

World J Gastroenterol 2013 June 14; 19(22): 3502-3504
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Nodular regenerative hyperplasia related portal hypertension in a patient with hypogammaglobulinaemia

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Received: November 20, 2012 Revised: January 30, 2013
Accepted: February 5, 2013
Published online: June 14, 2013

Abstract

Nodular regenerative hyperplasia (NRH) of liver is a relatively rare liver disorder, but a frequent cause of noncirrhotic portal hypertension. We present a lady with common variable immune deficiency who presented with upper gastrointestinal bleeding and deranged liver function tests but preserved synthetic function. Upper gastrointestinal endoscope showed bleeding gastric varices and non-bleeding oesophageal varices. Although her oesophageal varices were eradicated by repeated endoscopic band ligation, the gastric varices failed to resolve after repeated endoscopic histocryl injection and she eventually needed transjugular intrahepatic portosystemic shunt placement. Liver biopsy showed NRH. We review the association of hypogammaglobinaemia and NRH and discuss the appropriate management of portal hypertension in NRH.

Key words: Nodular regenerative hyperplasia; Liver; Portal hypertension; Hypogammaglobilinaemia; Gastro-oesophageal varices

Core tip: Nodular regenerative hyperplasia (NRH) is still an evolving concept. Although a rarely identified liver disorder, it is a frequent cause of noncirrhotic portal hypertension. Liver involvement in primary hypogammaglobulinemia mainly consists of NRH leading to chronic cholestasis and portal hypertension. Optimal management of gastric variceal bleed remains unclear. Histoacryl injection is the endoscopic method of choice for gastric variceal bleed but one should keep a lower threshold for transjugular intrahepatic portosystemic shunt procedure for recurrent gastric variceal bleed.

Lal BK, Stanley A. Nodular regenerative hyperplasia related portal hypertension in a patient with hypogammaglobulinaemia. *World J Gastroenterol* 2013; 19(22): 3502-3504 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3502.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3502

INTRODUCTION

Nodular regenerative hyperplasia (NRH) is the main cause of non-cirrhotic portal hypertension in the western world being responsible for 14%-27% of these cases[1-3]. It is characterized by diffuse regenerative hepatocytic nodules without fibrosis. Common variable immune disorder (CVID) is a heterogeneous group of primary immunodeficiency conditions involving formation of antibody production and various cellular immune system defects. NRH is the main liver pathology found in patients with CVID with abnormal liver function test (LFT). It is associated with intrasinusoidal T-cell infiltration, portal vein endothelitis, autoimmune disease and peripheral lymphocytic abnormalities which suggest an autoimmune mechanism.

CASE REPORT

A 50-year-old lady presented with upper gastrointestinal bleeding. She had a history of bronchiectasis and idiopathic thrombocytopenic purpura and had previously

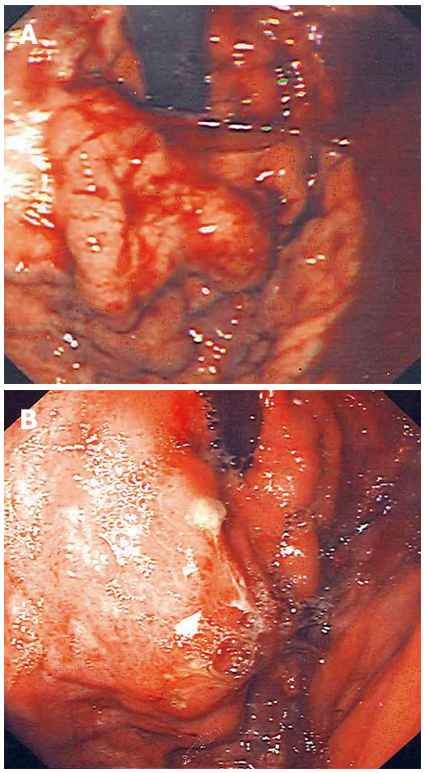

Figure 1 Gastric varices. A: Gastric varices pre glue injection; B: Gastric varices post glue.

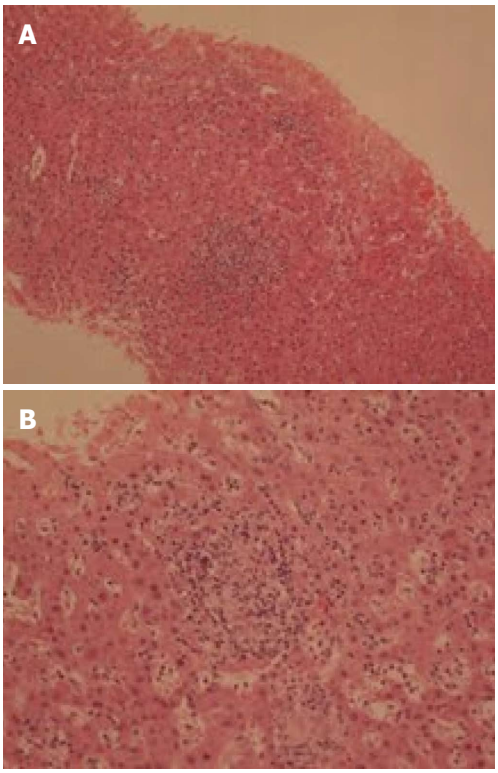

Figure 2 Nodule formation and granuloma on liver biopsy. A: Nodule; B: Granuloma.

been diagnosed with CVID. She was taking regular intravenous immunoglobulin infusion.

She was haemodynamically stable on presentation with pulse 90 per minute and blood pressure 131/65 mmHg. She had further episodes of haematemesis after admission and emergency endoscopy revealed large gastric fundal varices with evidence of active bleeding (Figure 1) and non-bleeding oesophageal varices. Haemostasis was achieved by applying glue (Histoacryl) to the gastric varices and her esophageal varices were banded. She was given five days of antibiotics and started on oral propranolol for prevention of variceal rebleeding.

She had mildly elevated liver enzymes (bilirubin 22 mmol/L, aspartate aminotransferase 55 U/L, alanine aminotransferase 35 U/L, gamma-glutamyl transpeptidase 45 U/L) with preserved synthetic function (albumin 41 g/L and prothrombin time 16 s). Ultrasonography of the abdomen revealed a coarse liver echo texture and normal flow in all hepatic veins. There was some bidirectional flow noted in the portal vein suggestive of portal hypertension but no ascites was seen.

To investigate her mildly elevated liver enzymes and portal hypertension percutaneous liver biopsy was undertaken. This showed features suggestive of NRH (Figures 2 and 3). Inflammatory cell infiltrate with granulomas were seen with a reactive appearance and immunophenotype suggestive that the NRH was related to CVID.

Subsequently, her oesophageal varices were eradicated by repeated band ligation but her gastric varices failed to resolve despite of repeated histoacryl injection (Figure 1).

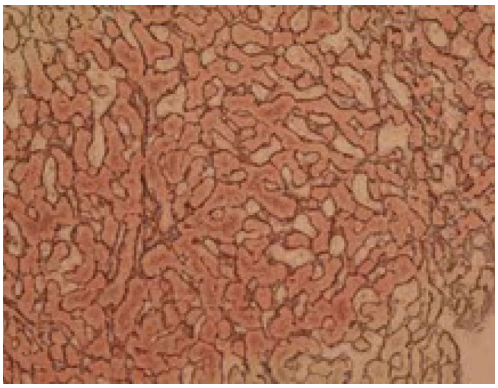

Figure 3 Thick liver cell plates on reticulin, but no fibrosis on liver biopsy.

Eventually, she had transjugular intrahepatic portosystemic shunt (TIPS) procedure with a covered graft which was placed after 6 mo of initial presentation resulting into satisfactory reduction in the portosystemic gradient with reversal of flow in the varices. She had complete resolution of her gastric varices post procedure and has remained on 6-mo TIPS checks by portography since.

She remains under regular follow up jointly in the liver, immunology, respiratory and haematology clinics. After nine years follow-up she remains well with normal LFTs.

DISCUSSION

NRH is usually associated with malignant, prothrom-

botic or rheumatologic conditions. Although liver disease and abnormal LFTs are found in approximately 10% of CVID patients[4,5] liver lesions associated with primary hypogammaglobulinaemia has been poorly described[6]. Two recent studies have identified NRH as the main histological correlate in patients with hypogammaglobulinemia[2-6]. Subsequent portal hypertension was frequently observed in only one patient cohort[6]. There has also been a case report which shows the association of hypogammaglobulinemia and major gastrointestinal bleeding from gastric varices as a result of cirrhosis of unknown cause (on biopsy)[7].

Management of patients with NRH is aimed at the treatment of the underlying systemic disorder and any complications related to the portal hypertension. A fundamental concept is that the synthetic function of the liver is generally preserved in NRH, despite the potential for development of significant portal hypertension. Liver transplantation is therefore rarely needed for NRH[8].

The immediate approach to variceal bleeding and ascites in patient with NRH does not differ from that of any other patients with the same condition. In the management of gastric varices or intractable or recurrent oesophageal variceal bleeding, TIPS should be considered[9]. Gastric variceal bleeding can be particularly challenging to the clinician. Histoacryl injection is the endoscopic method of choice for gastric variceal bleeding with immediate haemostasis figures of over 90% reported[9,10]. TIPS is used at a lower threshold for gastric compared with oesophageal variceal bleeding with uncontrolled studies demonstrating initial haemostasis obtained in over 90%, and rebleeding rates of 15%-30%[11]. As hepatic encephalopathy is rare in NRH because of preserved hepatic synthetic function, Porto systemic shunt surgery or TIPS is more suitable to treat and prevent refractory gastric variceal bleed in patients with NRH[11]. Balloon occluded retrograde transvenous obliteration is a technique for patients with gastric varices and gastrorenal shunts, although it is rarely used outside Asia[12]. Non-cardioselective beta-blockers are an alternative to TIPS for secondary prophylaxis, although the evidence is limited[11].

In conclusion, NRH is still an evolving concept. Although a rarely identified liver disorder, it is a frequent cause of noncirrhotic portal hypertension. Liver involvement in primary hypogmmaglobulinaemia mainly consists of NRH leading to chronic cholestasis and portal hypertension. Optimal management of gastric variceal bleed remains unclear. Histoacryl injection is the endoscopic method of choice for gastric variceal bleed but one should keep a lower threshold for TIPPS procedure for recurrent gastric variceal bleed.

REFERENCES

1 **Mahamid J**, Miselevich I, Attias D, Laor R, Zuckerman E, Shaoul R. Nodular regenerative hyperplasia associated with idiopathic thrombocytopenic purpura in a young girl: a case report and review of the literature. *J Pediatr Gastroenterol Nutr* 2005; **41**: 251-255 [PMID: 16056109]

2 **Nakanuma Y**. Nodular regenerative hyperplasia of the liver: retrospective survey in autopsy series. *J Clin Gastroenterol* 1990; **12**: 460-465 [PMID: 1975817 DOI: 10.1097/00004836-199008000-00023]

3 **Nakanuma Y**, Hoso M, Sasaki M, Terada T, Katayanagi K, Nonomura A, Kurumaya H, Harada A, Obata H. Histopathology of the liver in non-cirrhotic portal hypertension of unknown aetiology. *Histopathology* 1996; **28**: 195-204 [PMID: 8729037 DOI: 10.1046/j.1365-2559.1996.d01-412.x]

4 **Cunningham-Rundles C**, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin Immunol* 1999; **92**: 34-48 [PMID: 10413651 DOI: 10.1006/clim.1999.4725]

5 **Ward C**, Lucas M, Piris J, Collier J, Chapel H. Abnormal liver function in common variable immunodeficiency disorders due to nodular regenerative hyperplasia. *Clin Exp Immunol* 2008; **153**: 331-337 [PMID: 18647320 DOI: 10.1111/j.1365-2249.2008.03711.x]

6 **Malamut G**, Ziol M, Suarez F, Beaugrand M, Viallard JF, Lascaux AS, Verkarre V, Bechade D, Poynard T, Hermine O, Cellier C. Nodular regenerative hyperplasia: the main liver disease in patients with primary hypogammaglobulinemia and hepatic abnormalities. *J Hepatol* 2008; **48**: 74-82 [PMID: 17998147 DOI: 10.1016/j.jhep.2007.08.011]

7 **Rigaud S**, Lopez-Granados E, Sibéril S, Gloire G, Lambert N, Lenoir C, Synaeve C, Stacey M, Fugger L, Stephan JL, Fischer A, Picard C, Durandy A, Chapel H, Latour S. Human X-linked variable immunodeficiency caused by a hypomorphic mutation in XIAP in association with a rare polymorphism in CD40LG. *Blood* 2011; **118**: 252-261 [PMID: 21543760 DOI: 10.1182/blood-2011-01-328849]

8 **Elariny HA**, Mizrahi SS, Hayes DH, Boudreaux JP, Hussey JL, Farr GH. Nodular regenerative hyperplasia: a controversial indication for orthotopic liver transplantation. *Transpl Int* 1994; **7**: 309-313 [PMID: 7916934]

9 **de Franchis R**. Revising consensus in portal hypertension: report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2010; **53**: 762-768 [PMID: 20638742 DOI: 10.1016/j.jhep.2010.06.004]

10 **Rajoriya N**, Forrest EH, Gray J, Stuart RC, Carter RC, McKay CJ, Gaya DR, Morris AJ, Stanley AJ. Long-term follow-up of endoscopic Histoacryl glue injection for the management of gastric variceal bleeding. *QJM* 2011; **104**: 41-47 [PMID: 20871126 DOI: 10.1093/qjmed/hcq161]

11 **Tripathi D**, Ferguson JW, Therapondos G, Plevris JN, Hayes PC. Review article: recent advances in the management of bleeding gastric varices. *Aliment Pharmacol Ther* 2006; **24**: 1-17 [PMID: 16803599 DOI: 10.1111/j.1365-2036.2006.02965.x]

12 **Fukuda T**, Hirota S, Sugimura K. Long-term results of balloon-occluded retrograde transvenous obliteration for the treatment of gastric varices and hepatic encephalopathy. *J Vasc Interv Radiol* 2001; **12**: 327-336 [PMID: 11287510]

P- Reviewer Bayrahtar Y **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3505

World J Gastroenterol 2013 June 14; 19(22): 3505-3507
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Mini-loop ligation of a bleeding duodenal Dieulafoy's lesion

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Received: January 15, 2013 Revised: March 4, 2013
Accepted: March 15, 2013
Published online: June 14, 2013

Abstract

Two percent of gastrointestinal hemorrhages are caused by Dieulafoy's lesions, which are located in duodenum in only 15% of cases. There are no recommendations regarding the prime endoscopic treatment technique for this condition. A 61-year-old woman presented with melena without signs of hemodynamic instability. During an urgent upper endoscopy, blood oozing from the normal mucosa of the duodenum was seen and this was classified as a Dieulafoy's lesion. A mini-loop was opened at the rim of a transparent ligation chamber, at the end of the endoscope, and after aspiration of the lesion, closed and detached. Complete hemostasis was achieved without early or postponed complications. In every day clinical practice, mini-loop ligation is rarely used because of possible complications, such as site ulceration, organ perforation, re-bleeding and possible inexperience of the operator. To the best of our knowledge this is the first case of successful treatment of bleeding duodenal Dieulafoy's lesion by mini-loop ligation.

Key words: Dieulafoy's lesion; Duodenum; Endoscopy; Mini-loop; Hemostasis

Core tip: This is a case of 61-year-old woman who presented with upper gastrointestinal hemorrhage caused by duodenal Dieulafoy's lesion that presented with blood oozing from the normal mucosa on upper endoscopy. Complete hemostasis was achieved using a mini-loop ligation without a mucosal lesion, such as ulceration, on two month follow-up endoscopy. Thus, mini-loop ligation is an effective, easy to use and safe method for the treatment of Dieulafoy's lesion. However, case reports with longer follow-up are needed for a definitive statement because of the substantial risk of re-bleeding from a residual aberrant artery.

Gomerčić Palčić M, Ljubičić N. Mini-loop ligation of a bleeding duodenal Dieulafoy's lesion. *World J Gastroenterol* 2013; 19(22): 3505-3507 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3505.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3505

INTRODUCTION

Dieulafoy's lesion (DL), a condition first reported in 1884, and named in 1898, refers to dilated submucosal arterial malformation (width of 1-3 mm) that protrudes through overlying epithelium erosion accompanied with normal surrounding mucosa[1]. It can be found in any segment of the gastrointestinal (GI) tract with a preference for the lesser curvature of the stomach, 6 cm distally from the esophagogastric junction[2]. The incidence of DL is unknown because it is usually an asymptomatic condition that is often undiagnosed; however, its most common complication, GI hemorrhage, accounts for 2%-3% of all GI hemorrhage. A combination of abundant arterial bleeding with an inapproachable site of the lesion implies urgent and reliable treatment; the available endoscopic hemostatic methods are thermal, mechanical and injection[3,4]. To the best of our knowledge mini-loop ligation has never been reported as a treatment option in a case of DL.

CASE REPORT

A 61-year-old woman was admitted to our emergency unit because of melenic stool without abdominal pain, nausea or vomiting. Her previous history indicated that she did not have any serious health problems, just arterial hypertension and hyperlipoproteinemia, which were adequately regulated with chronic therapy. There was no history of alcohol abuse or excess intake of non-steroidal anti-inflammatory drugs (NSAIDs). She was anemic, but showed no signs of hemodynamic instability. Although a nasogastric tube aspirate was clear after insertion, a rectal examination showed evidence of melena. Laboratory tests revealed low levels of erythrocytes 2.8×10^9/L and hemoglobin 79 g/L with normal coagulation parameters. After signing an informed consent, the patient underwent an urgent upper endoscopy (GIF Q160, Olympus Optical Co., Japan). Blood oozing from a pin-point defect with normal surrounding mucosa of the duodenum was visualized and that was suggestive of DL (Figure 1A). A detachable nylon ring was opened at the rim of a transparent ligation chamber attached to the tip of the endoscope (Figure 1B). The lesion was aspirated into the chamber and subsequently the mini-loop was closed and detached, achieving complete hemostasis (Figure 1C). A blood transfusion was administered later, together with pantoprazole in the infusion over a 48 h period. A repeated endoscopy showed that the mini-loop was located at the site of the lesion with no visible sign of acute hemorrhage. The patient was discharged from the hospital on the fourth day with a satisfactory complete blood count (erythrocytes 3.9×10^9/L and hemoglobin 98 g/L). On follow up examination two months later, no pathology of a duodenal mucosa was observed, such as ulceration on the site, and values of her complete blood count were normal.

DISCUSSION

DL of the duodenum is an uncommon cause of upper GI bleeding, currently experiencing an increase of its frequency because operators' increased awareness of this condition. The duodenum is the second commonest site of DLs, accounting for 15% of patients, second only to the lesser curvature of the stomach, which accounts for around 70%[3,5]. Although the incidence of DL is unknown, it accounts for 2%-3% of all GI hemorrhages. Clinical presentations of this condition are extremely wide-ranging: from symptom free, and therefore, frequently undiagnosed cases, to signs of severe and often fatal GI hemorrhage. These lesions usually appear among the elderly, predominantly in men, and are associated with multiple co-morbidities such as chronic renal and heart failure. Other risk factors include the usage of NSAIDs, and antiplatelet and anticoagulant therapy. The patient we treated was an elderly woman with a history of arterial hypertension and hyperlipoproteinemia.

Upper endoscopy, the gold standard, is diagnostic in 90% of the cases; other available diagnostic techniques are angiography and endoscopic ultrasound (EUS). Selective catheter-directed embolization, especially in patients at high risk for surgery, is usually a salvage method in cases of unsuccessful endoscopic treatment, particularly if the lesion cannot be visualized because of excessive hemorrhage[6]. EUS is used to help detect the site of the submucosal vessel, especially in cases of spouting bleeding and to confirm ablation of a DL after therapy.

The endoscopic appearance of a DL is typically arterial spurting or oozing streaming from a minimal defect of the normal surrounding mucosa, or, less commonly, a protruding vessel without signs of active bleeding or with an adherent clot. In this case, blood oozing out of the normal mucosa was visualized without the possibility to distinguishing the underlying vessel. Once the diagnosis is established, several endoscopic techniques can be used, depending on the site of the lesion, severity of symptoms and previous operator experience. Available endoscopic procedures are electrocoagulation, heat probe coagulation, argon plasma coagulation, local epinephrine injection, sclerotherapy, banding and hemoclip. There is no consensus as to which is the most appropriate method. Based on the available data, endoscopic mechanical haemostatic methods seem to be superior to thermal or injection treatment methods[7,8]. However, the most commonly used methods are injection therapy and mechanical endoscopic therapy with hemoclips. In our case, a mechanical hemostatic method was used; the bleeding vessel with the surrounding mucosa was aspirated into the transparent ligation chamber and a preloaded nylon ring, called a mini-loop, was closed and detached with consequent complete hemostasis. A search of the published literature using PubMed with the phrase "Dieulafoy's lesion" resulted in 211 articles, of which the first was dated to early 1978 reporting two cases of DL of the jejunum[9]. First report of a duodenal DL was published in 1993[10] and to the best of our knowledge, there have been no reports of mini-loop ligation as a choice of endoscopic treatment for DL. Despite the fact that the first choice therapy for the bleeding duodenal DLs among most endoscopists includes injection of epinephrine and electrocoagulation[11], mechanical techniques are used more frequently and endoscopic band ligation (EBL) has been demonstrated to be an effective treatment for bleeding esophageal, stomach, duodenal and rectal DLs[12,13]. Chung *et al*[14] and Yanar *et al*[15] demonstrated higher efficacy of mechanical hemostatic therapy in initial hemostasis and lowering re-bleeding rate with hemoclip and band ligation compared to injection therapy in patients with DL. On the other hand, EBL has been shown to be as effective as injection therapy with epinephrine, with or without thermal therapy, with a significantly longer length of hospitalization among patients treated with injection therapy or combined therapy with epinephrine and thermal therapy[16]. In a study that compared two mechanical methods, EBL and endoscopic hemoclip placement (EHP), no difference was detected in efficacy or safety in the management of bleeding gastric DLs and only one patient from each group experienced re-bleeding[17]. A larger study (66 patients) showed a lower recurrent bleeding rate in patients treated with EBL compared with EHP (3.1% *vs* 14.7%)[18]. A combined therapy, such as

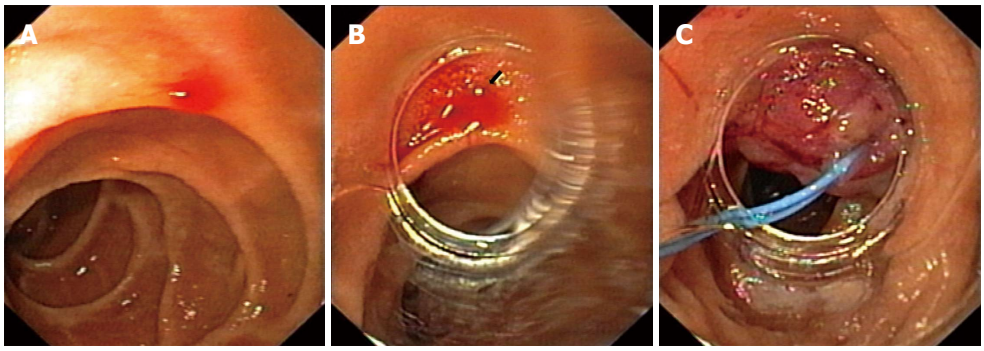

Figure 1 Sixty-one-year-old woman was admitted to our emergency unit because of melenic stool without abdominal pain, nausea or vomiting. A: Bleeding duodenal Dieulafoy's lesion; B: Visible bleeding Dieulafoy's lesion through the rim of a transparent ligation chamber attached to the tip of the endoscope; C: The mini-loop is closed and detached, achieving complete hemostasis.

EBL with EUS, promises a new era in diagnostic efficacy in terms of establishing accurate diagnosis, guiding EBL and confirming success of the treatment[19].

Therapeutic endoscopy is the first line of treatment of DL. However, the choice of technique is not specified. In our case, endoscopic mini-loop ligation proved to be an effective, easy to use and safe method for the treatment of DL. Mini-loop ligation may become a method of choice in resolving bleeding DL; however, case reports with longer follow-up periods are needed for a definitive statement because of the substantial risk of re-bleeding from a residual aberrant artery.

REFERENCES

1 **Dieulafoy G.** Exulceratio simplex: Leçons 1-3. In: Dieulafoy G. Clinique medicale de l'Hotel Dieu de Paris. Paris: Masson et Cie, 1898: 1-38

2 **Ibrarullah M**, Wagholikar GD. Dieulafoy's lesion of duodenum: a case report. *BMC Gastroenterol* 2003; **3**: 2 [PMID: 12581456 DOI: 10.1186/1471-230X-3-2]

3 **Baxter M**, Aly EH. Dieulafoy's lesion: current trends in diagnosis and management. *Ann R Coll Surg Engl* 2010; **92**: 548-554 [PMID: 20883603 DOI: 10.1308/003588410X12699663905311]

4 **Ljubicic N**. Efficacy of endoscopic clipping and long-term follow-up of bleeding Dieulafoy's lesions in the upper gastrointestinal tract. *Hepatogastroenterology* 2006; **53**: 224-227 [PMID: 16608029]

5 **Schmulewitz N**, Baillie J. Dieulafoy lesions: a review of 6 years of experience at a tertiary referral center. *Am J Gastroenterol* 2001; **96**: 1688-1694 [PMID: 11419815 DOI: 10.1111/j.1572-0241.2001.03922.x]

6 **Loffroy R**, Guiu B. Role of transcatheter arterial embolization for massive bleeding from gastroduodenal ulcers. *World J Gastroenterol* 2009; **15**: 5889-5897 [PMID: 20014452 DOI: 10.3748/wjg.15.5889]

7 **Matsui S**, Kamisako T, Kudo M, Inoue R. Endoscopic band ligation for control of nonvariceal upper GI hemorrhage: comparison with bipolar electrocoagulation. *Gastrointest Endosc* 2002; **55**: 214-218 [PMID: 11818925 DOI: 10.1067/mge.2002.121337]

8 **Ljubici**ć **N**. Endoscopic detachable mini-loop ligation for treatment of gastroduodenal angiodysplasia: case study of 11 patients with long-term follow-up. *Gastrointest Endosc* 2004; **59**: 420-423 [PMID: 14997147 DOI: 10.1016/S0016-5107]

9 **Matuchansky C**, Babin P, Abadie JC, Payen J, Gasquet C, Barbier J. Jejunal bleeding from a solitary large submucosal artery. Report of two cases. *Gastroenterology* 1978; **75**: 110-113 [PMID: 401085]

10 **Pollack R**, Lipsky H, Goldberg RI. Duodenal Dieulafoy's lesion. *Gastrointest Endosc* 1993; **39**: 820-822 [PMID: 8293911]

11 **Goldenberg SP**, DeLuca VA, Marignani P. Endoscopic treatment of Dieulafoy's lesion of the duodenum. *Am J Gastroenterol* 1990; **85**: 452-454 [PMID: 2248637]

12 **Soetikno RM**, Piper J, Montes H, Ukomadu C, Carr-Locke DL. Use of endoscopic band ligation to treat a Dieulafoy's lesion of the esophagus. *Endoscopy* 2000; **32**: S15 [PMID: 10774980]

13 **Yoshikumi Y**, Mashima H, Suzuki J, Yamaji Y, Okamoto M, Ogura K, Kawabe T, Omata M. A case of rectal Dieulafoy's ulcer and successful endoscopic band ligation. *Can J Gastroenterol* 2006; **20**: 287-290 [PMID: 16609760]

14 **Chung IK**, Kim EJ, Lee MS, Kim HS, Park SH, Lee MH, Kim SJ, Cho MS. Bleeding Dieulafoy's lesions and the choice of endoscopic method: comparing the hemostatic efficacy of mechanical and injection methods. *Gastrointest Endosc* 2000; **52**: 721-724 [PMID: 11115902 DOI: 10.1067/mge.2000.108040]

15 **Yanar H**, Dolay K, Ertekin C, Taviloglu K, Ozcinar B, Guloglu R, Barbaros U. An infrequent cause of upper gastrointestinal tract bleeding: "Dieulafoy's lesion". *Hepatogastroenterology* 2007; **54**: 1013-1017 [PMID: 17629028]

16 **Mumtaz R**, Shaukat M, Ramirez FC. Outcomes of endoscopic treatment of gastroduodenal Dieulafoy's lesion with rubber band ligation and thermal/injection therapy. *J Clin Gastroenterol* 2003; **36**: 310-314 [PMID: 12642736 DOI: 10.1097/00004836-200304000-00006]

17 **Park CH**, Joo YE, Kim HS, Choi SK, Rew JS, Kim SJ. A prospective, randomized trial of endoscopic band ligation versus endoscopic hemoclip placement for bleeding gastric Dieulafoy's lesions. *Endoscopy* 2004; **36**: 677-681 [PMID: 15280971 DOI: 10.1055/s-2004-825661]

18 **Ahn DW**, Lee SH, Park YS, Shin CM, Hwang JH, Kim JW, Jeong SH, Kim N, Lee DH. Hemostatic efficacy and clinical outcome of endoscopic treatment of Dieulafoy's lesions: comparison of endoscopic hemoclip placement and endoscopic band ligation. *Gastrointest Endosc* 2012; **75**: 32-38 [PMID: 22100302 DOI: 10.1016/j.gie.2011.08.038]

19 **Folvik G**, Nesje LB, Berstad A, Odegaard S. Endosonography-guided endoscopic band ligation of Dieulafoy's malformation: a case report. *Endoscopy* 2001; **33**: 636-638 [PMID: 11473339 DOI: 10.1055/s-2001-15322]

P- Reviewers Chamberlain SM, Kouraklis G, Loffroy R, Wilcox CM
S- Editor Gou SX **L- Editor** Stewart G **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3508

World J Gastroenterol 2013 June 14; 19(22): 3508-3511
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Colonic mucormycosis presented with ischemic colitis in a liver transplant recipient

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Received: January 17, 2013 Revised: April 3, 2013
Accepted: April 28, 2013
Published online: June 14, 2013

Abstract

Mucormycosis is an uncommon opportunistic fungal infection with high mortality in liver transplant recipients. Mucormycosis of the gastrointestinal tract can manifest with features similar to ischemic colitis. Typically signs and symptoms of non-gangrenous ischemic colitis resolve spontaneously within 24-48 h. On the other hand, the clinical course of the mucormycosis is commonly fulminant. We encountered a case of invasive fungal colitis presenting with abdominal pain and hematochezia in a liver transplant recipient. Endoscopic examination showed multiple shallow ulcerations and edema with mucosal friabilities on the sigmoid and distal descending colon, which was consistent with ischemic colitis. However, the histological examination obtained from endoscopic biopsies showed fungal hyphae with surrounding inflammatory cells and mucosal necrosis. The patient was successfully managed with antifungal agent without surgical treatment. Thus, early diagnosis and treatment is essential for improving the prognosis of invasive fungal infection after liver transplantation.

Key words: Mucormycosis; Liver transplantation; Colon; Ischemia

Do GW, Jung SW, Jun JB, Seo JH, Nah YW. Colonic mucormycosis presented with ischemic colitis in a liver transplant recipient. *World J Gastroenterol* 2013; 19(22): 3508-3511 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3508.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3508

INTRODUCTION

Mucormycosis is a rare but highly invasive opportunistic fungal infection in liver transplant recipients. Clinical manifestations can be rhino-sino-orbital, rhinocerebral, pulmonary, gastrointestinal, genitourinary, cutaneous and disseminated[1]. Mucormycosis of the gastrointestinal tract manifests with features similar to ischemic colitis[2]. *Mucor*, one of fungi that cause mucormycosis, has a special affinity for blood vessels[3], which may explain the clinical presentation of colonic infection as an ischemic colitis pattern. Ischemic colitis is associated with inadequate blood flow in the colon leading to colonic inflammation, one of the most common vascular disorders of the intestinal tract. Most cases of ischemic colitis are transient and resolve spontaneously without complication[4]. However, a deteriorating course of ischemic colitis should warrant further investigation. Herein we present a case of colonic mucormycosis presenting with ischemic colitis in a liver transplant recipient with diabetes mellitus who had been on immunosuppressive agents.

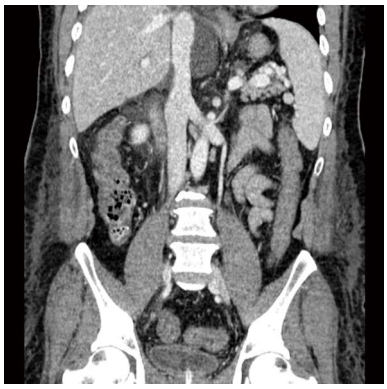

Figure 1 Abdominopelvic computed tomography scan. Mild to moderate symmetrical wall thickening with decreased mural attenuation was observed from the hepatic flexure to distal sigmoid colon.

CASE REPORT

A 42-year-old man with chronic hepatitis B underwent deceased donor liver transplantation (DDLT) due to hepatocellular carcinoma in a background of liver cirrhosis. He suffered from diabetes mellitus for 26 mo and was treated with insulin. He had an extremely high blood glucose level after he stopped insulin therapy in the last few months on his own initiative. His general condition was good after transplantation and there were no immediate complications such as acute rejection or infection. Postoperative immunosuppressive agents with methylprednisolone, mycophenolate mofetil, and tacrolimus were used. On the fifth day after DDLT, the patient complained of abdominal pain and small amount of hematochezia. On physical examination, his vital signs were normal except slightly elevated pulse rate (101 beats/min). His abdomen was soft but diffusely tender without peritoneal irritation signs. Laboratory analysis revealed a leukocyte count of 27800/mm^3 with 92% segment form, hemoglobin of 12.1 g/dL, a platelet count of 65000/mm^3, and C-reactive protein of 3.48 mg/dL. A computed tomography scan of the abdomen revealed mild to moderate symmetrical wall thickening with decreased mural attenuation was observed from the hepatic flexure to distal sigmoid colon (Figure 1). Because of suspected ischemic colitis, the patient was treated with bowel rest, intravenous fluid replacement, and antibiotics. However, the patient's symptoms continued to deteriorate. On the seventh day after DDLT, the decision was made to perform an endoscopic evaluation to elucidate the exact cause of abdominal pain and rectal bleeding. The sigmoidoscopic examination revealed multiple shallow ulcerations, edema with mucosal friabilities, and loss of the normal vascular appearance on the sigmoid and distal descending colon (Figure 2A). Microscopic examination showed several elongated fungal hyphae that stained light blue with haematoxylin and eosin (Figure 3A), were periodic acid Schiff positive and stained dark brown to black with the Grocott-Gomori methenamine silver method (Figure 3B). The fungal hyphae consisted of non-septated filaments, irregularly wide (6-9 μm diameter) hyphae with frequent right angle branching. These morphological features suggested infection by *Mucoraceae*. Based on the clinical findings and the morphological features of the fungal organisms, the diagnosis of mucormycosis was made. The patient was initially treated with intravenous high dose (1 mg/kg per day) amphotericin B, later replaced with liposomal amphotericin B due to azotemia. A follow-up sigmoidoscopy showed significant reduction in mucosal edema and inflammation compared to previous endoscopic findings (Figure 2B). The patient's symptoms disappeared and he made a good recovery.

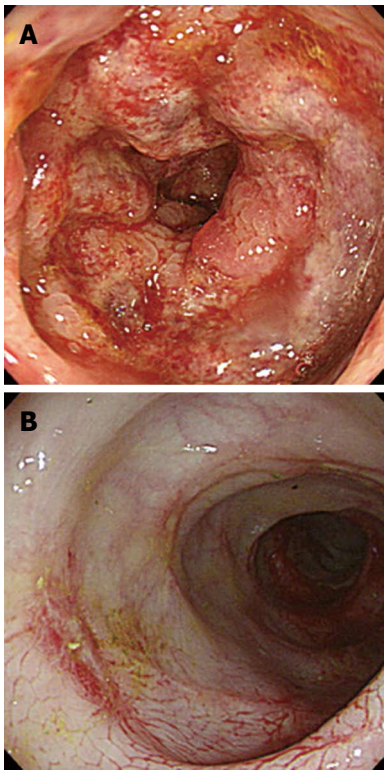

Figure 2 Sigmoidoscopic findings. A: The mucosa shows multiple shallow ulcerations, edema with friability of the mucosa, and loss of the normal vascular appearance; B: Two weeks after anti-fungal therapy, the mucosal edema and ulcerative lesions are markedly improved and mucosal scar changes were seen on the sigmoid colon.

DISCUSSION

Mucormycosis is an uncommon fungal infection caused by fungi of the class *Zygomycetes*, order *Mucorales* and *Entomorphthorales*[5]. The following risk factors are associated with mucormycosis: neutropenia, history of diabetes mellitus, hyperglycemia, acidosis, preexisting renal impairment, deferoxamine therapy, immunosuppressive agents and steroid use[1]. Our patient was diagnosed with diabetes mellitus before this diagnosis and received postoperative immunosuppressive agents after liver transplantation. Mucormycosis manifests a variety of different syndromes in humans, particularly in solid organ transplant recipients[1,6]. Although unusual, mucormycosis of the gastrointestinal tract may occur as a result of ingesting fungal spores. A review of the 116 solid organ

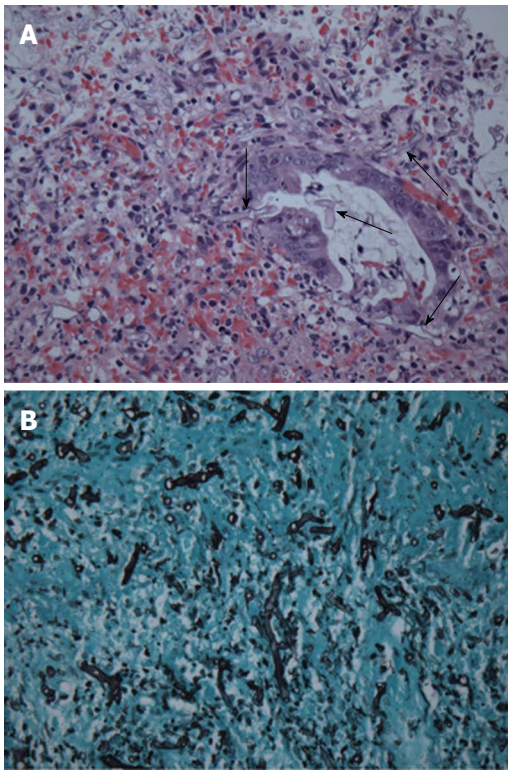

Figure 3 Histologic findings. A: Microscopic examination of the biopsy specimen obtained by endoscopy shows several fungal hyphae (arrows) (hematoxylin and eosin stain, magnification × 400); B: Right-angle branching, non-septate hyphae of *Mucoraceae* are shown (Grocott's methenamine silver stain, magnification × 400).

transplant recipients with mucormycosis found that the site of involvement of gastrointestinal mucormycosis (n = 13, 11.2%), included stomach in 9 (69.2%), colon 1 (7.6%), esophagus 1 (7.6%) and liver 1 (7.6%)[1]. Nonspecific abdominal pain and distention associated with nausea and vomiting are the most common symptoms. Fever and gastrointestinal bleeding may also occur[7]. In our case, the patient complained of abdominal pain and hematochezia. We performed a sigmoidoscopy to determine the exact cause of those symptoms. One previous study showed that endoscopic examination is a valuable diagnostic tool to establish an accurate diagnosis of transplant recipients with lower gastrointestinal complaints[8]. These symptoms occur typically in ischemic colitis[2]. In a review of 85 patients, endoscopic appearance of transient ischemic colitis included edematous and fragile mucosa, segmental erythema, scattered erosion, longitudinal ulcerations, petechial hemorrhages interspersed with pale areas, and purple hemorrhagic nodules[9]. The endoscopic findings for our patient resembled ischemic colitis. Successful current treatment for mucormycosis involves a combined approach; rapidity of diagnosis, the control of the underlying predisposing illness, appropriate surgical debridement of infected tissue and prompt initiation of antifungal therapy[5]. Until recently, only the polyene class of drugs, including amphotericin B deoxycholate and its lipid derivatives, demonstrated activity against the agents of mucormycosis. The lipid formulations of amphotericin are significantly less nephrotoxic than amphotericin B deoxycholate and can be safely administered at higher doses for a longer period of time[7]. Surgery is essential in decreasing the fungal burden by removing infarcted tissues, where amphotericin B cannot distribute. In a recent study, a patient who was precluded from extensive surgical intervention did not respond to amphotericin B but was treated successfully with a combination of posaconazole and liposomal amphotericin B without surgical treatment[10]. Mucormycosis is generally a severe infection with an overall survival rate of patients of approximately 50%[7]. Appropriate treatment must be instituted early if the clinical suspicion is high, even before the results of culture and/or histopathological studies have been obtained, and currently, the administration of systemic amphotericin B and/or surgical debridement of the lesions are considered standard therapy[7].

In this case, the patient with diabetes mellitus had received immunosuppressive agents after liver transplantation and presumptive diagnosis of ischemic colitis was initially suspected. The patient was treated with supportive care including bowel rest, intravenous fluid replacement, and antibiotics. However, the patient's symptoms showed a deteriorating condition. Non-improving ischemic colitis warrants further inquiry into causes in an immunosuppressed patient, especially liver transplant recipients with risk factors of mucormycosis. He was finally diagnosed by endoscopic biopsies and successfully treated with strict control of blood sugar level and antifungal agent. Thus, early diagnosis and treatment are essential for improving the prognosis of invasive fungal infection such as colonic mucormycosis after liver transplantation.

REFERENCES

1 **Almyroudis NG**, Sutton DA, Linden P, Rinaldi MG, Fung J, Kusne S. Zygomycosis in solid organ transplant recipients in a tertiary transplant center and review of the literature. *Am J Transplant* 2006; **6**: 2365-2374 [PMID: 16925570 DOI: 10.1111/j.1600-6143.2006.01496.x]

2 **Hosseini M**, Lee J. Gastrointestinal mucormycosis mimicking ischemic colitis in a patient with systemic lupus erythematosus. *Am J Gastroenterol* 1998; **93**: 1360-1362 [PMID: 9707066 DOI: 10.1111/j.1572-0241.1998.00417.x]

3 **Zhan HX**, Lv Y, Zhang Y, Liu C, Wang B, Jiang YY, Liu XM. Hepatic and renal artery rupture due to Aspergillus and Mucor mixed infection after combined liver and kidney transplantation: a case report. *Transplant Proc* 2008; **40**: 1771-1773 [PMID: 18589192 DOI: 10.1016/j.transproceed.2007.10.013]

4 **Green BT**, Tendler DA. Ischemic colitis: a clinical review. *South Med J* 2005; **98**: 217-222 [PMID: 15759953 DOI: 10.1097/01.SMJ.0000145399.35851.10]

5 **Severo CB**, Guazzelli LS, Severo LC. Chapter 7: zygomycosis. *J Bras Pneumol* 2010; **36**: 134-141 [PMID: 20209316 DOI: 10.1590/S1806-37132010000100018]

6 **Singh N**, Aguado JM, Bonatti H, Forrest G, Gupta KL, Safdar N, John GT, Pursell KJ, Muñoz P, Patel R, Fortun J, Martin-Davila P, Philippe B, Philit F, Tabah A, Terzi N, Chatelet V, Kusne S, Clark N, Blumberg E, Julia MB, Humar A, Houston S, Lass-Flörl C, Johnson L, Dubberke ER, Barron MA, Lortholary O. Zygomycosis in solid organ transplant recipients: a prospective, matched case-control study to assess risks for

disease and outcome. *J Infect Dis* 2009; **200**: 1002-1011 [PMID: 19659439 DOI: 10.1086/605445]
7 **Spellberg B**, Edwards J, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. *Clin Microbiol Rev* 2005; **18**: 556-569 [PMID: 16020690 DOI: 10.1128/CMR.18.3.556-569.2005]
8 **Korkmaz M**, Gür G, Yilmaz U, Karakayali H, Boyacioğlu S, Haberal M. Colonoscopy is a useful diagnostic tool for transplant recipients with lower abdominal symptoms. *Transplant Proc* 2004; **36**: 190-192 [PMID: 15013343 DOI: 10.1016/j.transproceed.2003.11.065]
9 **Zou X**, Cao J, Yao Y, Liu W, Chen L. Endoscopic findings and clinicopathologic characteristics of ischemic colitis: a report of 85 cases. *Dig Dis Sci* 2009; **54**: 2009-2015 [PMID: 19089615 DOI: 10.1007/s10620-008-0579-1]
10 **Mezhir JJ**, Mullane KM, Zarling J, Satoskar R, Pai RK, Roggin KK. Successful nonoperative management of gastrointestinal mucormycosis: novel therapy for invasive disease. *Surg Infect* (Larchmt) 2009; **10**: 447-451 [PMID: 19485785 DOI: 10.1089/sur.2008.049]

P- Reviewers Nielsen OH, Rodrigo L **S- Editor** Wen LL
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3512

World J Gastroenterol 2013 June 14; 19(22): 3512-3516
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Transarterial embolization of metastatic mediastinal hepatocellular carcinoma

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Received: January 12, 2013 Revised: April 14, 2013
Accepted: April 18, 2013
Published online: June 14, 2013

Abstract

This paper introduces an innovative treatment for extrahepatic metastasis of hepatocellular carcinoma. A 71-year-old patient had a stable liver condition following treatment for hepatocellular carcinoma, but later developed symptomatic mediastinal metastasis. This rapidly growing mediastinal mass induced symptoms including cough and hoarseness. Serial sessions of transarterial embolization (TAE) successfully controlled this mediastinal mass with limited side effects. The patient's survival time since the initial diagnosis of the mediastinal hepatocellular carcinoma was 32 mo, significantly longer than the 12 mo mean survival period of patients with similar diagnoses: metastatic hepatocellular carcinoma and a liver condition with a Child-Pugh class A score. Currently, oral sorafenib is the treatment of choice for metastatic hepatocellular carcinoma. Recent studies indicate that locoregional treatment of extrahepatic metastasis of hepatocellular carcinomas might also significantly improve the prognosis in patients with their primary hepatic lesions under control. Many effective locoregional therapies for extrahepatic metastasis, including radiation and surgical resection, may provide palliative effects for hepatocellular carcinoma-associated mediastinal metastasis. This case report demonstrates that TAE of metastatic mediastinal hepatocellular carcinoma provided this patient with tumor control and increased survival time. This finding is important as it can potentially provide an alternative treatment option for patients with similar symptoms and diagnoses.

Key words: Embolization; Hepatocellular carcinoma; Lymphatic metastasis; Endosonography; Mediastinal neoplasm

Core tip: This report presents a patient with metastatic hepatocellular carcinoma localized to his mediastinum. The metastatic tumor grew rapidly, with α-fetoprotein (AFP) elevation rates correlating with the size of the tumor and tumor growth. We used several sessions of transarterial embolization to control this metastatic tumor. After every successful intervention, the tumor had been stabilized and AFP levels were decreased significantly, resulting in a survival time of the patient that is much longer when compared to the mean survival times of others with similar diagnoses. Transarterial embolization could be an alternative treatment choice for patients with mediastinal metastasis of hepatocellular carcinoma.

Chen CC, Yeh HZ, Chang CS, Ko CW, Lien HC, Wu CY, Hung SW. Transarterial embolization of metastatic mediastinal hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(22): 3512-3516 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3512.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3512

INTRODUCTION

Liver cancer in men is the fifth most frequently diagnosed cancer and the second most frequent cause of cancer death worldwide. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death[1]. Although advances in treatment of hepatocellular carcinoma (HCC) have resulted in prolonged survival of HCC patients, the incidence of extrahepatic metastasis associated with HCC has also risen[2]. Patients with extrahepatic spread have a poor prognosis[3]. Current managements for these patients are oral sorafenib and possibly transarterial chemoembolization (TACE) for the hepatic tumors if the patient has enough remaining liver function[4]. There are few studies examining the efficacy of locoregional management for extrahepatic metastasis. Furthermore, there are even less reports of lymph node-directed therapy in distant lymph node metastasis[5]. In this report, a 71-year-old patient with symptomatic isolated progression of mediastinal lymphadenopathy of HCC was treated with transarterial embolization (TAE). The symptoms were controlled and survival time was longer than expected.

CASE REPORT

A 71-year-old man visited the Taichung Veterans General Hospital for the management of his HCC. His HCC tumor was 3 cm, localized to the right lobe, and diagnosed by elevated α-fetoprotein (AFP) levels and hypervascular appearance on abdominal computer tomography (CT). The patient was a hepatitis B carrier with a class A Child-Pugh score and had early stage HCC as assessed by the Barcelona Clinic Liver Cancer staging classification (stage A). However, this patient did not want to receive curative surgery due to old age and personal preference.

He received TACE treatment twice during the first year following his HCC diagnosis. His serum AFP level decreased from 1171 to 14.92 ng/mL following this TACE therapy. One year later, his serum AFP level increased again, but an abdominal CT scan and angiography of the liver did not reveal definite hepatic hypervascular tumors. Two years after the diagnosis of HCC, he started to experience hoarseness due to left vocal cord paralysis. A chest CT scan revealed a 5 cm tumor over the aortopulmonary window of the mediastinum (Figure 1). Metastatic HCC was identified pathologically with endosonography-guided fine needle aspiration. After discussions with the surgeon, radiation oncologist and interventional radiologist, he refused surgery and radiation.

Transarterial embolization of mediastinal tumor was adopted as the treatment modality. Although available now, molecular targeting agents for HCC were not yet a standard care option for patients at that time. The TAE procedure was performed as follows: the first branch of the right superior bronchial artery was identified as the main artery supplying the left hilar metastatic lesion during angiography. Lipiodol was administrated *via* this artery as the embolizer, and a chest CT scan verified the metastatic lymph nodes were mostly occupied by lipiodol (Figure 2). His AFP level decreased from 17089 to 1061 ng/mL and this TAE session was considered successful. There were no adverse effects like cough, chest pain, fever or dyspnea. Thereafter, he received another four sessions of TAE *via* several supplying arteries for viable or recurrent components. AFP levels decreased following each successful TAE, but these levels would rebound quickly due to recurrence of the tumor. During the 16-mo follow-up period, his AFP level was approximately 1000-4000 ng/mL. In addition, he began to have night coughs. On the sixth session of TAE, embolization was performed *via* a small branch of the left branch of internal mammary artery identified by high-resolution chest CT (Figure 3). AFP levels decreased significantly to 16.64 ng/mL and this session was considered successful and the patient remained in stable condition for seven months with no TAE-related complications. However, several months later, the patient began to experience progressive night cough and elevation of AFP levels. A chest CT scan showed progression of mediastinal tumors (Figure 4). A seventh TAE for recurrent mediastinal lymphadenopathy was performed for controlling of tumor. However, his lung condition deteriorated progressively eventually causing him to expire due to pneumonia-related respiratory failure. The patient's survival time since the initial diagnosis of mediastinal metastasis was 32 mo. During the follow up period, his hepatic tumor remained well embolized and his liver function remained stable without evidence of decompensation.

DISCUSSION

The natural course of HCC is unique. Unlike cancers of the colon, breast, lung, and others, whose causes of death are typically due to systemic processes with development of disseminated diseases, HCCs usually present as local lesions and subsequent development of systemic diseases are relatively uncommon[6]. The causes of death from HCC may not only be due to progressive cancer, but may also be due to liver failure.

The prognosis of patients with extrahepatic metastases is usually very poor. The one year and three year survival rates of patients who underwent surgical resection of extrahepatic metastases of HCC was 24% and 7%, respectively. For patients who did not undergo resection, these rates dropped to 8% and 0%, respectively[7]. Poor prognostic factors of extrahepatic metastasis of HCC are poor performance status, a high Child-Pugh score, a large number and size of intrahepatic lesions, the presence of macroscopic vascular invasion, a symptomatic extrahepatic metastasis, and high AFP levels[8]. In this report, the patient exhibited two of these prognostic factors: high AFP level and symptomatic extrahepatic metastasis. He survived for 32 mo following the initial diagnosis of extrahepatic metastasis despite these poor prognostic factors and his decision not to have surgery. The mean survival time for HCC patients with a Child-Pugh class A score and who had developed extrahepatic

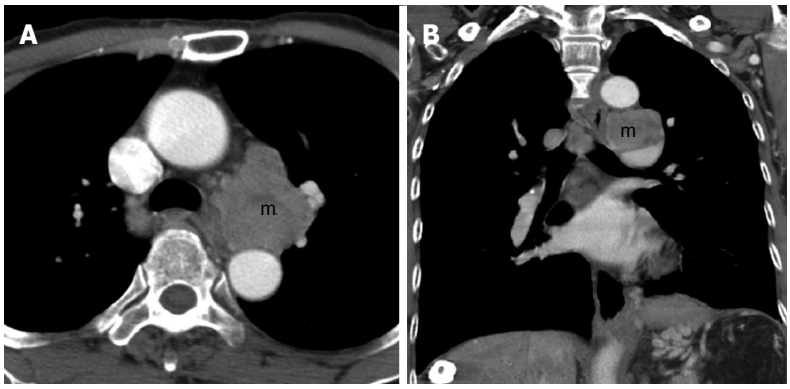

Figure 1 A chest computer tomography scan of the aortopulmonary window of the mediastinum revealed a 5 cm metastatic lymphadenopathy (m). A: Transverse view; B: Sagittal view.

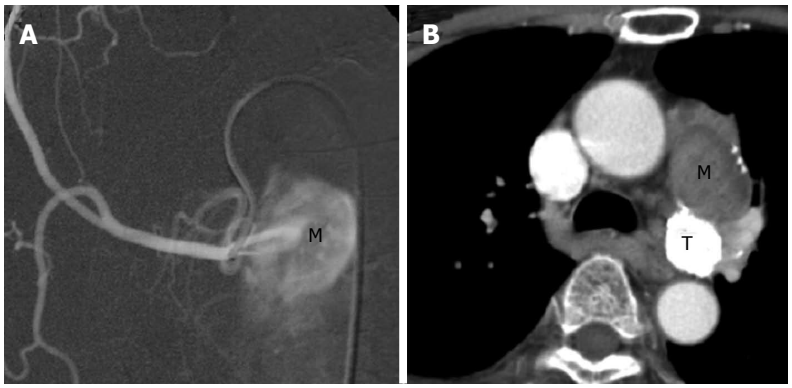

Figure 2 First session of transarterial embolization for mediastinal tumor. A: An angiogram of the right superior bronchial arterial branch showed a metastatic hepatocellular carcinoma (M) in the left hilum supplying by the first branch of right superior bronchial artery. Embolization was performed by lipiodol only; B: Chest computer tomography (CT) scan: Lipiodol retention in posterior portion of the mass (T) over aortopulmonary window region and decreased in size, but the anterior portion of the mass (M) over aortopulmonary window region increased in size as compared with previous CT.

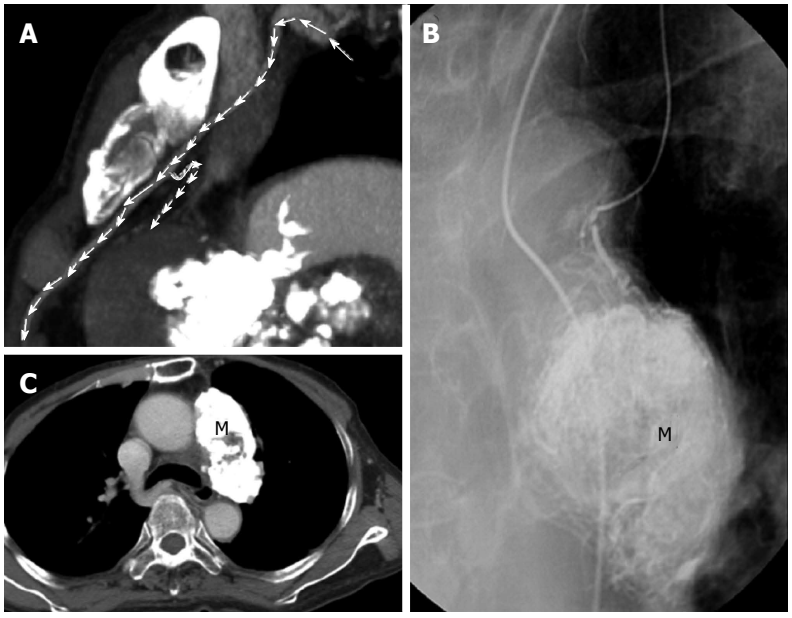

Figure 3 Successful control of mediastinal metastasis *via* selection of the supplying vessel. A: Multi-detector row computed tomography showed a supplying artery from small branch of left internal mammary artery to residual tumor (arrows); B: Tumor supplying artery of left internal thoracic (mammary) artery was selected *via* a 3 Fr microcatheter. Transarterial embolization for tumor (M) was done by using 6 mL of lipiodol; C: Follow-up chest computed tomography showed the metastatic tumor (M) in left anterior mediastinum was well embolized.

metastasis was recently reported to be 12.0 mo[6]. Therefore, this patient far exceeded the expected survival time for someone with his diagnosis.

Current management of metastatic HCC is TACE for primary tumor control and possible chemotherapy, radiation or molecular targeting with sorafenib for extrahepatic lesions[4]. Some evidence suggests that treatment of extra-hepatic metastasis of HCC can significantly improve the prognosis of patients whose hepatic lesions are under control[7,8].

Previous studies indicate that surgery for HCC-associated pulmonary[9], adrenal[10] and local lymph node metastasis[11] may be beneficial when added to these traditional treatments. Many effective locoregional therapies for extrahepatic metastasis have also been reported, including irradiation for bone[12], lymph node[13], brain[14], and adrenal[15] metastases, and percutaneous ablation for adrenal[16] and bone metastases[17]. Further, TAE has been used as a therapy for adrenal metastasis[18] while this is the first report of TAE for mediastinal metastasis.

The most common sites of lymph node metastasis of HCC are locoregional lymph nodes including paraaortic,

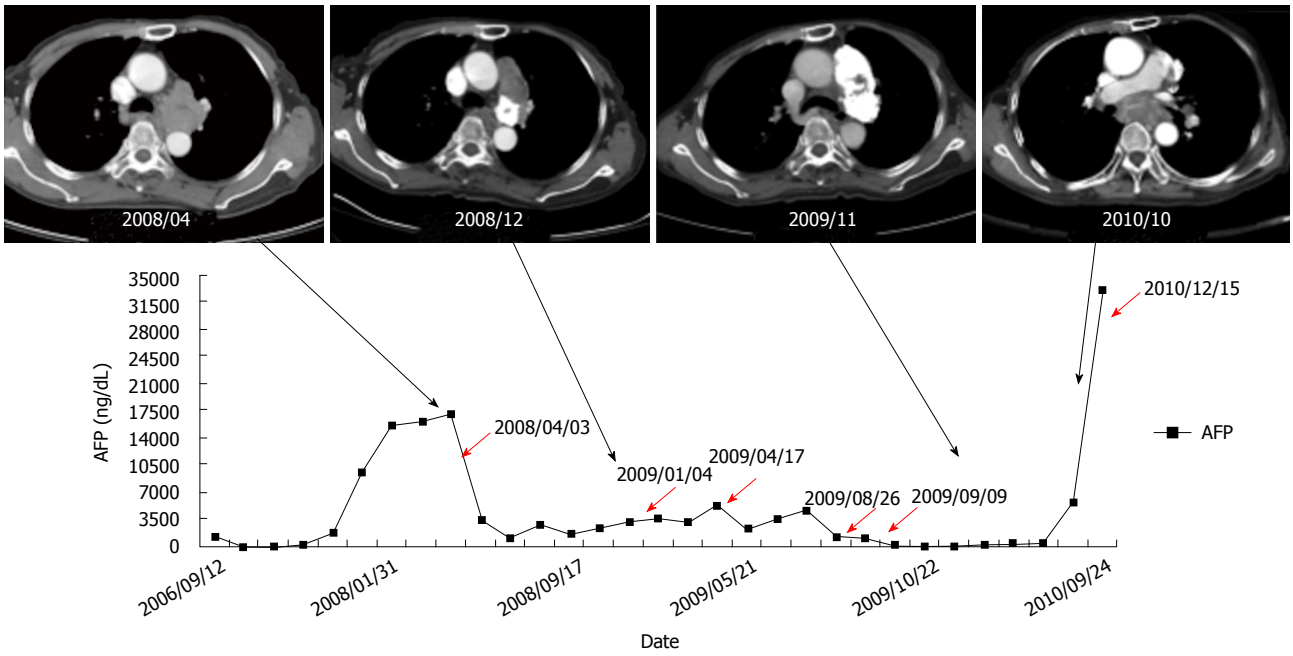

Figure 4 Alpha-fetoprotein levels in time course of treatment. Every session of transarterial embolization is shown with a red arrow. The computed tomography (CT) images represented the progression of mediastinal tumor and treatment response of transarterial embolization. The CT image on the far right was taken about three months before death of the patient. The tumor compressed the left bronchus, which may explain the cause of the patient's pneumonia.

portohepatic, periceliac and peripancreatic nodes. The most common site of HCC-associated distant metastatic lymphadenopathy is the mediastinal lymph nodes[19]. Because lymph node metastasis or dissemination itself is not the usual cause of death[5], there are relatively few studies examining the management of metastatic lymph nodes. Lymph node metastases that cause agonizing pain, or rarely life-threatening conditions like pericardial or esophageal invasion[20], may be indications for locoregional treatment such as radiotherapy or surgical resection.

Excellent radiotherapy responsiveness was reported in patients whose intrahepatic HCC was concurrently under control[13]. However, radiation at the mediastinum is associated with cardiovascular toxicity, which may present with acute pericarditis, restrict cardiomyopathy, ischemic heart disease, congestive heart failure and valvular heart disease[21]. Moreover, radiation therapy at the mediastinum is also associated with a 5%-10% risk of symptomatic radiation pneumonitis[22]. Subtle lung function impairment induced by radiation injury may further reduce the capacity of a patient to deal with future cardiopulmonary stress. Surgical resection may be an option if the patient has good liver function and isolated lymph node metastasis[23]. However, the existence of possible occult metastasis and the variable degree of hepatic decompensation in HCC patients greatly reduce the benefits of surgery.

Currently, the mainstay treatment for metastatic HCC is sorafenib[4], while options including heavy ion or proton radiotherapy also remain. TAE for mediastinal metastatic HCC may be an alternative option for certain patients with localized metastatic lymph nodes. Furthermore, TAE does not cost more than the other treatment modalities. In this report, the patient did not present significant side effects during several sessions of TAE and had satisfactory control of his tumor. However, several challenges associated with performing TAE in the mediastinum remain including: the considerable challenge of locating the supplying vessels, the close proximity to many critical arteries in this part of the body such as the spinal cord artery, and the risk of reflux of embolizer to other vital areas. Above all, the key challenge to providing a safe and successful TAE is selection of the supplying artery. Thus, it remains unclear whether this technique can be widely used.

In conclusion, this report showed that the use of TAE for treatment of a metastatic mediastinal HCC provided temporal tumor control and increased survival time. In the right environment, TAE should be an alternative option for well-selected patients with mediastinal metastasis of HCC.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
2 **Kanda M**, Tateishi R, Yoshida H, Sato T, Masuzaki R, Ohki T, Imamura J, Goto T, Yoshida H, Hamamura K, Obi S, Kanai F, Shiina S, Omata M. Extrahepatic metastasis of hepatocellular carcinoma: incidence and risk factors. *Liver Int* 2008; **28**: 1256-1263 [PMID: 18710423 DOI: 10.1111/j.1478-3231.2008.01864.x]
3 **Natsuizaka M**, Omura T, Akaike T, Kuwata Y, Yamazaki K, Sato T, Karino Y, Toyota J, Suga T, Asaka M. Clinical features of hepatocellular carcinoma with extrahepatic metastases. *J Gastroenterol Hepatol* 2005; **20**: 1781-1787 [PMID: 16246200 DOI: 10.1111/j.1440-1746.2005.03919.x]
4 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011;

365: 1118-1127 [PMID: 21992124 DOI: 10.1056/NEJMra1001683]
5 **Uka K**, Aikata H, Takaki S, Shirakawa H, Jeong SC, Yamashina K, Hiramatsu A, Kodama H, Takahashi S, Chayama K. Clinical features and prognosis of patients with extrahepatic metastases from hepatocellular carcinoma. *World J Gastroenterol* 2007; **13**: 414-420 [PMID: 17230611]
6 **Senthilnathan S**, Memon K, Lewandowski RJ, Kulik L, Mulcahy MF, Riaz A, Miller FH, Yaghmai V, Nikolaidis P, Wang E, Baker T, Abecassis M, Benson AB, Omary RA, Salem R. Extrahepatic metastases occur in a minority of hepatocellular carcinoma patients treated with locoregional therapies: analyzing patterns of progression in 285 patients. *Hepatology* 2012; **55**: 1432-1442 [PMID: 22109811 DOI: 10.1002/hep.24812]
7 **Chan KM**, Yu MC, Wu TJ, Lee CF, Chen TC, Lee WC, Chen MF. Efficacy of surgical resection in management of isolated extrahepatic metastases of hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 5481-5488 [PMID: 19916180 DOI: 10.3748/wjg.15.5481]
8 **Uchino K**, Tateishi R, Shiina S, Kanda M, Masuzaki R, Kondo Y, Goto T, Omata M, Yoshida H, Koike K. Hepatocellular carcinoma with extrahepatic metastasis: clinical features and prognostic factors. *Cancer* 2011; **117**: 4475-4483 [PMID: 21437884 DOI: 10.1002/cncr.25960]
9 **Lam CM**, Lo CM, Yuen WK, Liu CL, Fan ST. Prolonged survival in selected patients following surgical resection for pulmonary metastasis from hepatocellular carcinoma. *Br J Surg* 1998; **85**: 1198-1200 [PMID: 9752858 DOI: 10.1046/j.1365-2168.1998.00846.x]
10 **Park JS**, Yoon DS, Kim KS, Choi JS, Lee WJ, Chi HS, Kim BR. What is the best treatment modality for adrenal metastasis from hepatocellular carcinoma? *J Surg Oncol* 2007; **96**: 32-36 [PMID: 17345596 DOI: 10.1002/jso.20773]
11 **Une Y**, Misawa K, Shimamura T, Ogasawara K, Masuko Y, Sato N, Nakajima Y, Uchino J. Treatment of lymph node recurrence in patients with hepatocellular carcinoma. *Surg Today* 1994; **24**: 606-609 [PMID: 7949768 DOI: 10.1007/BF01833724]
12 **Kaizu T**, Karasawa K, Tanaka Y, Matuda T, Kurosaki H, Tanaka S, Kumazaki T. Radiotherapy for osseous metastases from hepatocellular carcinoma: a retrospective study of 57 patients. *Am J Gastroenterol* 1998; **93**: 2167-2171 [PMID: 9820391 DOI: 10.1111/j.1572-0241.1998.00614.x]
13 **Park YJ**, Lim do H, Paik SW, Koh KC, Lee JH, Choi MS, Yoo BC, Nam HR, Oh DR, Park W, Ahn YC, Huh SJ. Radiation therapy for abdominal lymph node metastasis from hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 1099-1106 [PMID: 17160521 DOI: 10.1007/s00535-006-1895-x]
14 **Chang L**, Chen YL, Kao MC. Intracranial metastasis of hepatocellular carcinoma: review of 45 cases. *Surg Neurol* 2004; **62**: 172-177 [PMID: 15261518 DOI: 10.1016/j.surneu.2003.10.002]
15 **Zeng ZC**, Tang ZY, Fan J, Zhou J, Qin LX, Ye SL, Sun HC, Wang BL, Zhang JY, Yu Y, Cheng JM, Wang XL, Guo W. Radiation therapy for adrenal gland metastases from hepatocellular carcinoma. *Jpn J Clin Oncol* 2005; **35**: 61-67 [PMID: 15709088 DOI: 10.1093/jjco/hyi020]
16 **Kashima M**, Yamakado K, Takaki H, Kaminou T, Tanigawa N, Nakatsuka A, Takeda K. Radiofrequency ablation for the treatment of bone metastases from hepatocellular carcinoma. *AJR Am J Roentgenol* 2010; **194**: 536-541 [PMID: 20093621 DOI: 10.2214/AJR.09.2975]
17 **Yamakado K**, Anai H, Takaki H, Sakaguchi H, Tanaka T, Kichikawa K, Takeda K. Adrenal metastasis from hepatocellular carcinoma: radiofrequency ablation combined with adrenal arterial chemoembolization in six patients. *AJR Am J Roentgenol* 2009; **192**: W300-W305 [PMID: 19457793 DOI: 10.2214/AJR.08.1752]
18 **Kuehl H**, Stattaus J, Forsting M, Antoch G. Transhepatic CT-guided radiofrequency ablation of adrenal metastases from hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2008; **31**: 1210-1214 [PMID: 18584241 DOI: 10.1007/s00270-008-9377-6]
19 **Katyal S**, Oliver JH, Peterson MS, Ferris JV, Carr BS, Baron RL. Extrahepatic metastases of hepatocellular carcinoma. *Radiology* 2000; **216**: 698-703 [PMID: 10966697]
20 **Huang CC**, Ng WW, Chiang JH, Chen FH, Huang CH, Wang YJ, Chang FY, Lee SD. Hepatocellular carcinoma with mediastinal and pericardial invasion: report of two cases. *Zhonghua Yixue Zazhi* (Taipei) 1999; **62**: 891-895 [PMID: 10634004]
21 **Gagliardi G**, Constine LS, Moiseenko V, Correa C, Pierce LJ, Allen AM, Marks LB. Radiation dose-volume effects in the heart. *Int J Radiat Oncol Biol Phys* 2010; **76**: S77-S85 [PMID: 20171522 DOI: 10.1016/j.ijrobp.2009.04.093]
22 **Marks LB**, Bentzen SM, Deasy JO, Kong FM, Bradley JD, Vogelius IS, El Naqa I, Hubbs JL, Lebesque JV, Timmerman RD, Martel MK, Jackson A. Radiation dose-volume effects in the lung. *Int J Radiat Oncol Biol Phys* 2010; **76**: S70-S76 [PMID: 20171521 DOI: 10.1016/j.ijrobp.2009.06.091]
23 **Chua TC**, Morris DL. Exploring the role of resection of extrahepatic metastases from hepatocellular carcinoma. *Surg Oncol* 2012; **21**: 95-101 [PMID: 21397495 DOI: 10.1016/j.suronc.2011.01.005]

P- Reviewers Enjoji M, Ferraioli G, Ramia JM, Tsuchiya A
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World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3517

World J Gastroenterol 2013 June 14; 19(22): 3517-3519
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

A long adult intussusception secondary to transverse colon cancer

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Author contributions: Xu XQ and Li BL designed the report; Xu XQ, Hong T, Liu W and He XD were attending doctors for the patient; Xu XQ, Li BL and Zheng CJ performed surgical operation; Xu XQ and Hong T organized the report; Xu XQ wrote paper.

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Received: February 28, 2013 Revised: April 2, 2013
Accepted: April 10, 2013
Published online: June 14, 2013

Abstract

The occurrence of adult intussusception arising from colorectal cancer is quite rare. We present the case of a 76-year-old man with sudden abdominal pain and vomiting. Clinical symptoms included severe abdominal distension and tenderness. Computed tomography scan of the abdomen revealed left-sided colocolic intussusception with a lead point. The patient underwent a left hemicolectomy with right transverse colostomy. Pathologic evaluation revealed moderately differentiated adenocarcinoma invading the muscularis propria; the regional lymph nodes were negative for cancer cells. The postoperative course was uneventful.

Key words: Adult intussusception; Colon cancer; Surgery; Hemicolectomy

Core tip: Intussusception is a common cause of bowel obstruction in pediatric patients, but it is rare in adults and it is often difficult to diagnose. We present the case of a 76-year-old man with sudden abdominal pain and vomiting. The patient underwent a left hemicolectomy with right transverse colostomy. This article reports the complete diagnosis and management of the patient.

Xu XQ, Hong T, Liu W, Zheng CJ, He XD, Li BL. A long adult intussusception secondary to transverse colon cancer. *World J Gastroenterol* 2013; 19(22): 3517-3519 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3517.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3517

INTRODUCTION

Intussusception is most often encountered in infants and children, and only 5% of cases occur in adults. It accounts for about 1% of all cases of adult bowel obstruction. Adult intussusception of the colon is rare and related to malignant lesions[1]. We describe a case of adult intussusception of the transverse colon caused by a malignant tumor in an elderly man preoperatively diagnosed by X-ray, barium enema and computed tomography (CT) scan.

CASE REPORT

A 76-year-old male with no significant medical history was admitted as a surgical emergency with sudden abdominal pain and vomiting and 2 episodes of bright red bloody stool. Physical examination revealed severe abdominal distension and tenderness.

The abdominal X-ray showed distension of the ascending and right half transverse colon (Figure 1). A barium enema showed the meniscus sign in the contrast material-filled distal sigmoid (Figure 2). CT showed a giant mass at the transverse colon. CT also showed "bowel within bowel" consistent with colocolic component of the intussusception with distension of the ascending

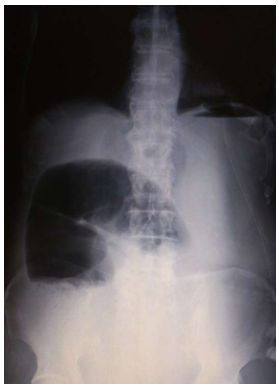

Figure 1 Abdominal X-ray showing distension of the ascending and right half transverse colon.

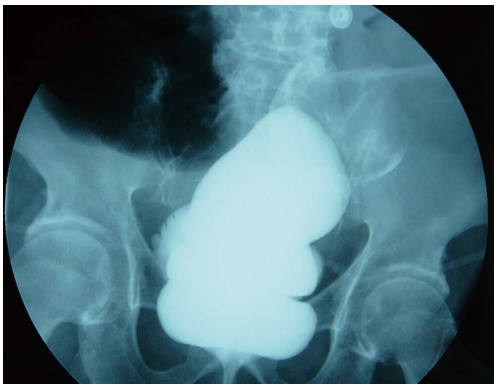

Figure 2 Barium enema examination showing the meniscus sign in the contrast material-filled distal sigmoid.

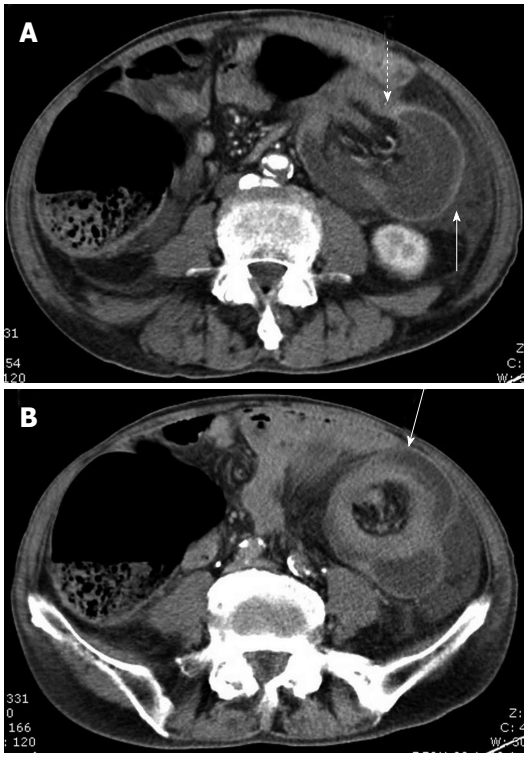

Figure 3 Abdominal computed tomography showing colocolic component of the intussusception (A, dashed arrow), thick-walled descending colon invaginated by transverse colon with mesenteric fat and vessels (A, solid arrow), and the typical "target sign" of colon within colon (B, solid arrow).

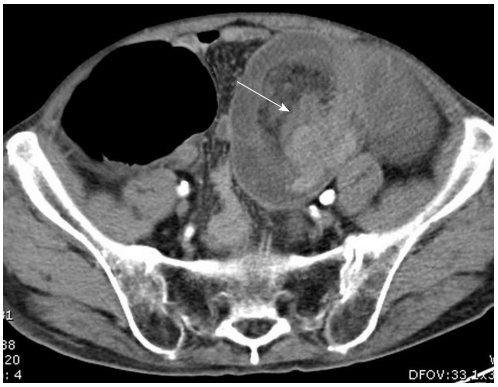

Figure 4 Abdominal computed tomography showing enlargement of the descending colon wall with the intussusception (arrow), suggesting the existence of a tumor in the head.

colon and invaginated left transverse colon into the descending colon with mesenteric fat and vessels (Figure 3).

The diagnosis of colocolic intussusception at the transverse colon was made. The patient was admitted and underwent emergency laparotomy, and radiologic findings were confirmed during the operation. A giant mass was palpable through the splenic flexure of colon which extended till the sigmoid colon. The mass comprised an intussusception of both the left part of the transverse colon and the great omentum (Figure 4). An extended left hemicolectomy with right transverse colostomy was performed. The patient had an uneventful recovery and was discharged 10 d after the surgery.

Gross examination of the resected specimen revealed a 25-cm colocolic intussusception which contained a 3.0 cm × 3.0 cm × 2.5 cm protuberant tumor originating from the splenic flexure of colon and the whole great omentum in the descending colon (Figure 5). The final pathological report showed moderately differentiated adenocarcinoma invading the muscularis propria; the regional lymph nodes were negative for cancer cells.

DISCUSSION

Colo-colonic intussusception is rare in adults (< 5%), but it is the most common cause of intestinal obstruction in infants aged 6-18 mo[1]. The characteristic pediatric presentation triad of abdominal pain, palpable abdominal mass and bloody stool is rarely seen in adult cases. Most patients present with subacute (24.4%) or chronic (51.2%) symptoms of abdominal pain, nausea, vomiting and constipation; this is the main reason why preoperative diagnosis is difficult[2]. The best preoperative diagnosis method of intussusception is CT scan. Mostly, adult intussusception is related to bowel pathology and 38%-45% cases occur in the colon, while 52%-55% occur in the small intestine[3]. It has been reported that 33%-77% of adult colonic intussusception cases are associated with malignant lesions. Adult intus-

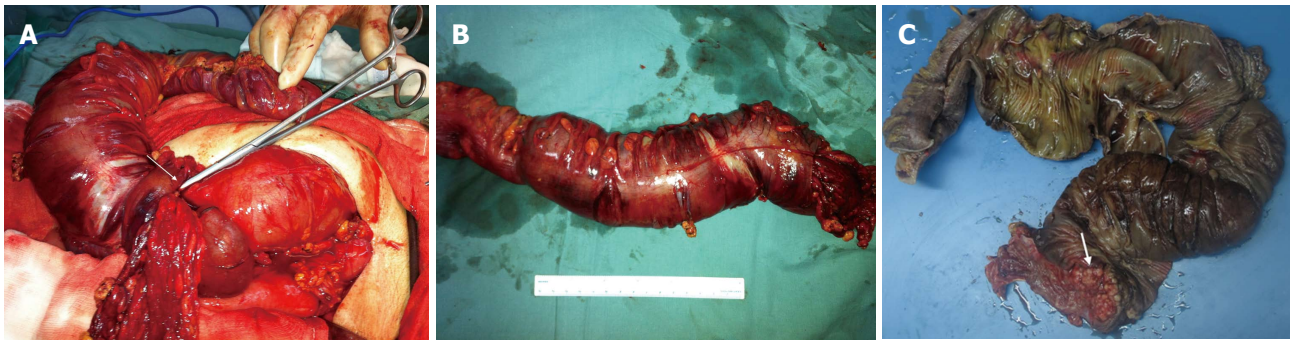

Figure 5 Gross examination of the resected specimen reveals a 25-cm colocolic intussusception. A: Perioperative view after mobilization of the left colon with forceps inside the intussusception invaginated transverse colon with great omentum; B: Operative specimen showing about 25 cm overlapped colon in the intussusceptions; C: The specimen contained a 3.0 cm × 3.0 cm × 2.5 cm protuberant tumor, thought to be the lead point of the intussusception (arrow).

susception of the colon mostly occurs in the flexible regions such as the sigmoid and transverse colon and the cecum[4]. For the management of adult intussusception, most authors think that laparotomy is mandatory due to the high incidence of underlying malignancy in colonic intussusceptions and the inability to differentiate non-operatively benign from malignant causes in enteric intussusceptions[5].

In this case, we report a relatively complete process for the diagnosis of adult intussusceptions with detailed medical pictures. Adult intussusception must be considered in the differential diagnosis of patients with abdominal pain and vomiting. The work-up must include X-ray, ultrasound and CT scan of the abdomen, and even a barium enema. Emergent surgical interventions are required once the diagnosis of intussusception is made, due to the high risk of malignancy.

REFERENCES

1 **Azar T**, Berger DL. Adult intussusception. *Ann Surg* 1997; **226**: 134-138 [PMID: 9296505 DOI: 10.1097/00000658-199708000-00003]

2 **Wang N**, Cui XY, Liu Y, Long J, Xu YH, Guo RX, Guo KJ. Adult intussusception: a retrospective review of 41 cases. *World J Gastroenterol* 2009; **15**: 3303-3308 [PMID: 19598308 DOI: 10.3748/wjg.15.3303]

3 **Schuind F**, Van Gansbeke D, Ansay J. Intussusception in adults--report of 3 cases. *Acta Chir Belg* 1985; **85**: 55-60 [PMID: 3984633]

4 **Lorenzi M**, Iroatulam AJ, Vernillo R, Banducci T, Mancini S, Tiribocchi A, Ferrari FS, Mancini S. Adult colonic intussusception caused by malignant tumor of the transverse colon. *Am Surg* 1999; **65**: 11-14 [PMID: 9915523]

5 **Dan JM**, Agarwal S, Burke P, Mahoney EJ. Adult intussusception secondary to colorectal cancer in a young man: a case report. *J Emerg Med* 2012; **43**: 983-986 [PMID: 21262558 DOI: 10.1016/j.jemermed.2010.11.030]

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L- Editor Logan S **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3520

World J Gastroenterol 2013 June 14; 19(22): 3520-3523
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Hemolymphangioma: A rare differential diagnosis of cystic-solid or cystic tumors of the pancreas

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Author contributions: Chao M designed the study; Dong F acquired and analyzed the data and drafted the manuscript; Zheng Y, Wu JJ, and Jin K analyzed the clinical and imaging data; Fu YB analyzed and interpreted the pathological data; all authors participated in the editing and have read and approved the final manuscript.
Supported by The Public Technology Research and Social Development Project of Science and Technology Department of Zhejiang Province, China, Grant No.2010C33142
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Received: January 18, 2013 Revised: March 2, 2013
Accepted: April 9, 2013
Published online: June 14, 2013

Abstract

We report a case of pancreatic hemolymphangioma. Hemolymphangioma is a malformation of both lymphatic vessels and blood vessels. The incidence of this disease in the pancreas is extremely rare. To the best of our knowledge, only seven cases have been reported worldwide (PubMed). A 39-year-old woman with a one-day history of abdominal pain was admitted to our hospital. There was no obvious precipitating factor. The preoperative examination, including ultrasonography and computed tomography, showed a cystic-solid tumor in the pancreas, and it was considered to be a mucinous cystadenoma or cystadenocarcinoma. Pancreatic body-tail resection combined with splenectomy was performed. After the operation, the tumor was pathologically demonstrated to be a pancreatic hemolymphangioma. Although pancreatic hemolymphangioma is rare, we believe that it should be considered in the differential diagnosis of cystic-solid tumors of the pancreas, particularly when there is no sufficient evidence for diagnosing cystadenoma, cystadenocarcinoma or some other relatively common disease of the pancreas.

Key words: Pancreatic neoplasm; Hemolymphangioma; Differential diagnosis; Computed tomography; Ultrasonography

Core tip: This article reports an extremely rare case of pancreatic hemolymphangioma, which is a cystic-solid tumor. The imaging findings are different from those reported in the literature. Although it is rare and the definitive diagnosis depends on histological evidence, it should be considered preoperatively in the differential diagnosis of cystic-solid or cystic tumors of the pancreas, particularly when there is no sufficient evidence for diagnosing cystadenoma, cystadenocarcinoma or some other relatively common disease of the pancreas.

Dong F, Zheng Y, Wu JJ, Fu YB, Jin K, Chao M. Hemolymphangioma: A rare differential diagnosis of cystic-solid or cystic tumors of the pancreas. *World J Gastroenterol* 2013; 19(22): 3520-3523 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3520.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3520

INTRODUCTION

Hemolymphangioma is a malformation of both lymphatic vessels and blood vessels. The incidence of pancreatic

hemolymphangioma is extremely rare. To the best of our knowledge, only seven cases have been reported worldwide (PubMed)[1-7]. Macroscopically, it is a multilocular cystic mass with endothelium covering the connective septa[2,3]. The tumor typically presents as a mass with heterogeneous enhancement on computed tomography (CT)[1,6,7]. Here, we report a case of pancreatic hemolymphangioma with different imaging findings.

CASE REPORT

A 39-year-old woman was admitted to our hospital with a complaint of abdominal pain for one day. There was no obvious precipitating factor, no weight loss and no family history of cancer. A physical examination showed mild tenderness in the left hypochondrium. Laboratory tests, including serum tumor markers, were normal.

The ultrasonography and CT findings of the tumor were as follows: (1) Location: the tumor was located at the body and tail of the pancreas; (2) Shape: it was irregular, approximately 10 cm × 7 cm in size, and had a clear border (Figures 1 and 2); (3) Composition: it was a cysticsolid tumor. The cystic area, with a mean CT attenuation value of 30 Hounsfield units (HU), comprised the main part. There was a cord-like septum in the center of the cystic area (Figure 2A, arrow). The solid part was located at the back of the tumor, with a mean CT attenuation value of 68 HU (Figure 2E, arrow); (4) Enhancement: it showed almost no change on contrast-enhanced CT images (Figure 2B-D, Figure 2F-H) compared with pre-contrast images, as assessed with the CT attenuation value; and (5) Neighborhood: the adjacent organs were compressed, and no enlarged lymph nodes were found. Based on these findings, mucinous cystadenoma or cystadenocarcinoma was initially considered.

The patient underwent an exploratory laparotomy. During the operation, the mass was identified as a large dark red mass located at the body and tail of the pancreas, slightly adhering to the gastric wall, posterior peritoneum and left kidney, with bloody fluid inside. Pancreatic body-tail resection combined with splenectomy was performed. Microscopically, some dysplastic lymphatic vessels and blood vessels were observed in the tumor (Figure 3). The pathological diagnosis was hemolymphangioma. Her postoperative course was uneventful. She is currently enjoying a normal life without signs of recurrence.

DISCUSSION

Hemolymphangioma is a congenital malformation of the vascular system. The formation of this tumor may be explained by obstruction of the venolymphatic communication between dysembryoplastic vascular tissue and the systemic circulation[1-3]. Only seven cases have been reported previously[1-7]. Most of these cases were women (6 of 7), and most of the tumors were located at the head of the pancreas (6 of 7). A patient with pancreatic hemolymphangioma can be asymptomatic for a long time. Abdominal pain and awareness of an abdominal mass are the most common symptoms[7].

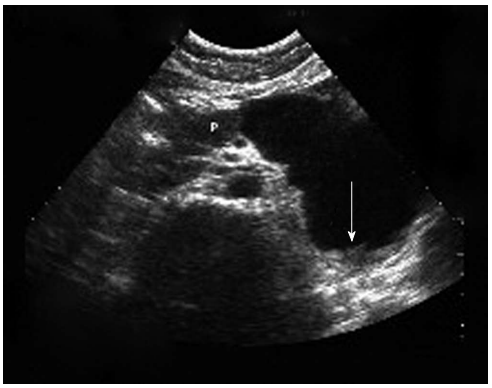

Figure 1 Ultrasonography. The tumor is located at the body and tail of the pancreas and is irregularly shaped. The cystic part has a low-intensity echo level, and the solid part (arrow) displays an isointense echo.

The imaging findings of pancreatic hemolymphangioma typically show a mass with heterogeneous enhancement[1,6,7]. In our case, the tumor showed different imaging findings. It presented as a cystic-solid tumor (a single large cyst and a small solid part), with no enhancement in the three-phase contrast scan. We believe that the cystic part might be due to the rupture and fusion of the vascular cavity and that the solid part represented the residual and compressed vascular tissue. The lack of enhancement in the three-phase contrast scan may be related to the relatively small number of blood vessels in the tumor and slow-moving blood flow in the dysplastic blood vessels.

The differential imaging diagnosis for this case includes other cystic-solid or cystic lesions of the pancreas. A mucinous cystadenoma typically shows a single large cyst with a smooth contour, fine septa, even wall, and potentially, small nodules. A thick wall and large nodules might suggest the diagnosis of cystadenocarcinoma. Both mucinous cystadenomas and cystadenocarcinomas can present septa, walls, and nodule enhancement. A solid pseudopapillary tumor of the pancreas is typically found in young women presenting a cystic-solid mass with enhancement of the solid part. A pancreatic pseudocyst typically shows a unilocular lesion with a history of pancreatitis. However, the definitive diagnosis of this tumor was made using histological evidence[1,3].

An operation is required to treat this tumor. Two operative methods are available: tumor enucleation and partial pancreatectomy[2]. Because the diagnosis is made postoperatively, pancreatoduodenectomy is typically used for the suspicion of malignancy or invasion[1,6].

Although rare, pancreatic hemolymphangioma should be considered in the differential diagnosis when a cystic-solid tumor of the pancreas is found, particularly when there is no sufficient evidence for diagnosing cystadenoma, cystadenocarcinoma or some other relatively common disease.

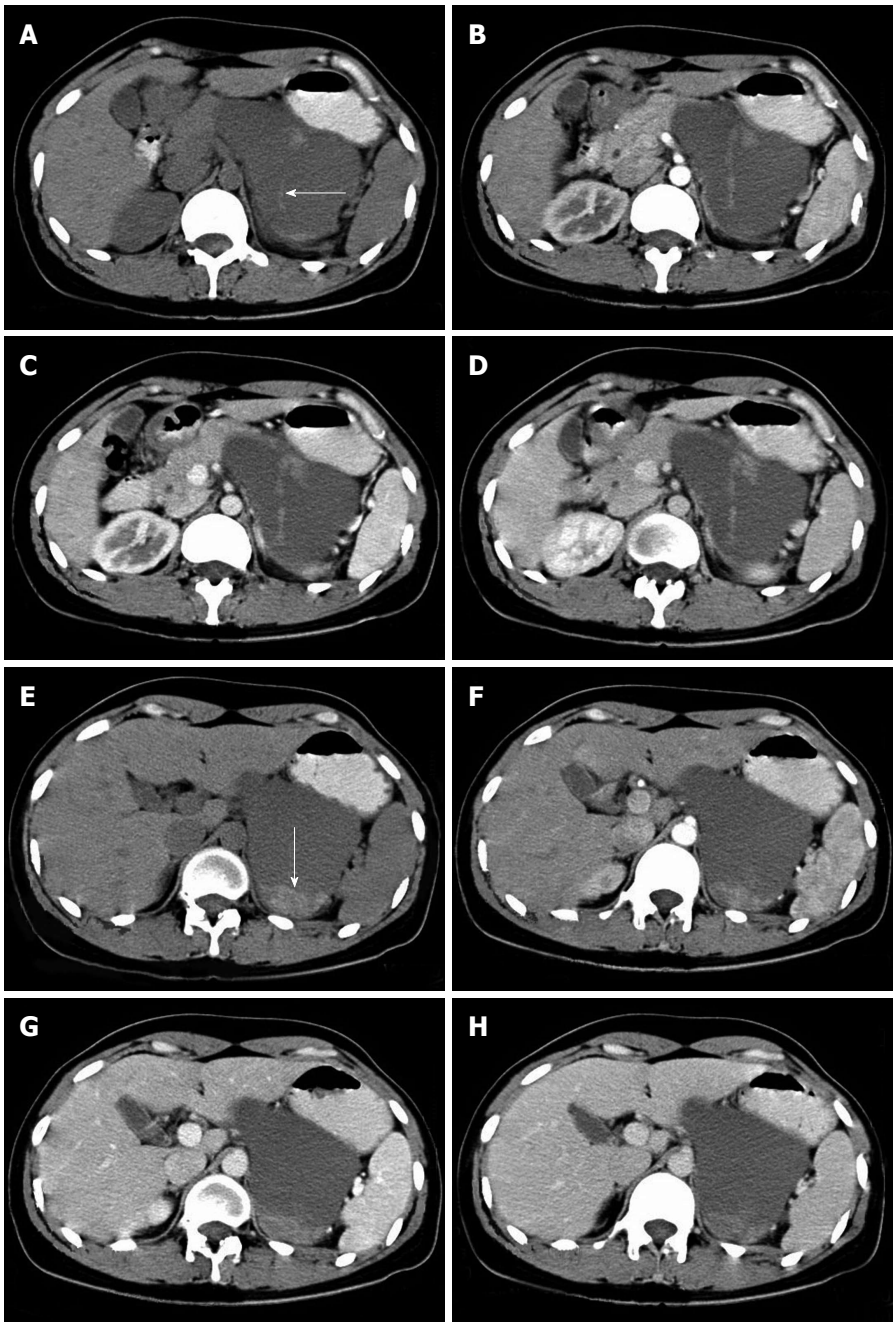

Figure 2 Computed tomography images. A-D: There is a septum (arrow) in the center of the cystic part. Neither the cystic part nor the septum shows enhancement in the arterial phase (20 s after contrast injection) (B), pancreatic phase (45 s after contrast injection) (C), or portal phase (80 s after contrast injection) (D) compared with the pre-enhanced phase (A); E-H: The solid part (arrow) shows no enhancement in the arterial phase (20 s after contrast injection) (F), pancreatic phase (45 s after contrast injection) (G), or portal phase (80 s after contrast injection) (H) compared with the pre-enhanced phase (E).

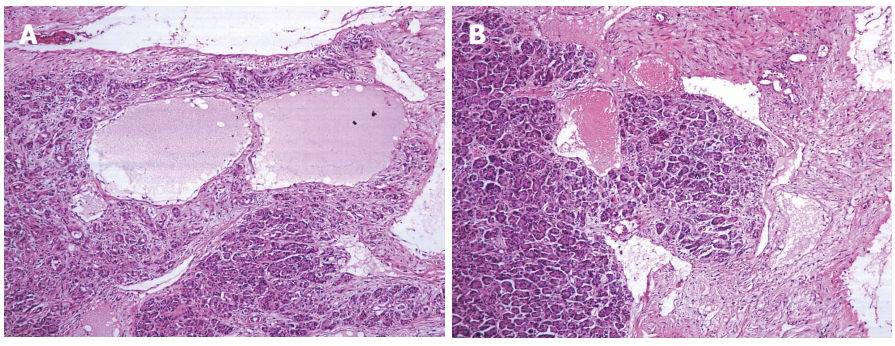

Figure 3 Pathological sections. Some dysplastic lymphatic vessels (A) and blood vessels (B) can be observed in the tumor. Hematoxylin and eosin stain, × 100.

ACKNOWLEDGMENTS

We thank Min-Ming Zhang, a responsible Professor and the Director of our department, who provided us with support during the work.

REFERENCES

1 **Balderramo DC**, Di Tada C, de Ditter AB, Mondino JC. Hemolymphangioma of the pancreas: case report and review of the literature. *Pancreas* 2003; **27**: 197-199 [PMID: 12883270 DOI: 10.1097/00006676-200308000-00014]
2 **Montete P**, Marmuse JP, Claude R, Charleux H. [Hemolymphangioma of the pancreas]. *J Chir* (Paris) 1985; **122**: 659-663 [PMID: 4086523]
3 **Banchini E**, Bonati L, Villani LG. [A case of hemolymphangioma of the pancreas]. *Minerva Chir* 1987; **42**: 807-813 [PMID: 3614745]
4 **Couinaud,** Jouan, Prot, Chalut, Schneiter. [Hemolymphangioma of the head of the pancreas]. *Mem Acad Chir* (Paris) 1966; **92**: 152-155 [PMID: 5905830]
5 **Couinaud C**. [A rare tumor of the head of the pancreas. (Hemolymphangioma weighing 1,500 kg.)]. *Presse Med* 1967; **75**: 1955-1956 [PMID: 6059596]
6 **Toyoki Y**, Hakamada K, Narumi S, Nara M, Kudoh D, Ishido K, Sasaki M. A case of invasive hemolymphangioma of the pancreas. *World J Gastroenterol* 2008; **14**: 2932-2934 [PMID: 18473426 DOI: 10.3748/wjg.14.2932]
7 **Sun LF**, Ye HL, Zhou QY, Ding KF, Qiu PL, Deng YC, Zhang SZ, Zheng S. A giant hemolymphangioma of the pancreas in a 20-year-old girl: a report of one case and review of the literature. *World J Surg Oncol* 2009; **7**: 31 [PMID: 19296838]

P- Reviewers Vila JJ, Zhang XP **S- Editor** Wen LL
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3524

World J Gastroenterol 2013 June 14; 19(22): 3524-3527
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Hepatoid adenocarcinoma of the extrahepatic duct

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Supported by The Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China, No. 2009-1001
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Received: January 10, 2013 Revised: April 29, 2013
Accepted: May 22, 2013
Published online: June 14, 2013

Abstract

Hepatoid carcinoma is a unique type of extrahepatic tumor associated with hepatic differentiation and displays the morphological and functional features of hepatocellular carcinoma. Hepatoid carcinoma of the extrahepatic duct has rarely been reported in the literature. We report a 62-year-old man who presented with epigastric discomfort, xanthochromia, dull pain of the right shoulder, nausea and pruitus. Microscopic examination of the extrahepatic duct indicated that the tumor was primarily composed of "hepatoid cells", which were characterized by an eosinophilic cytoplasm, enlarged nucleus and prominent nucleoli. The cells were arranged in nests or proliferated in a trabecular pattern. Immunohistochemistry indicated that the tumor cells were positive for hepatocyte paraffin 1 and cytokeratins 8 and 18. Based on these findings, this case was diagnosed as hepatoid carcinoma of the extrahepatic duct.

Key words: Extrahepatic bile ducts; Hepatocellular; Adenocarcinoma; Immunohistochemistry; Surgical pathology; Differential diagnosis

Core tip: Hepatoid adenocarcinoma (HAC) is a rare type of extrahepatic adenocarcinoma that resembles hepatocellular carcinoma. Although the stomach is the most common location for this tumor, the lung, pancreas, esophagus, papilla of Vater, colon, urinary bladder, renal pelvis, ovaries, uterus and cervix have also been reported as primary locations. To the best of our knowledge, HAC arising primarily from the extrahepatic duct has not previously been reported in the literature. This report presents a rare case of HAC of the hepatic duct and performs a differential diagnosis based on immunochemical results, detailed clinical history and endoscopic findings.

Wang Y, Liu YY, Han GP. Hepatoid adenocarcinoma of the extrahepatic duct. *World J Gastroenterol* 2013; 19(22): 3524-3527 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3524.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3524

INTRODUCTION

Hepatoid adenocarcinoma (HAC) of the stomach was first described by Ishikura *et al* in 1985. Carcinomas with hepatoid differentiation have since been described in a variety of locations, including the lung, kidney, female reproductive tract, pancreas, gallbladder and stomach (the most prevalent site)[1-6]. An adenocarcinoma of the papilla of Vater showing hepatoid differentiation has previously been reported[2]. This tumor was proposed to be a specific type of carcinoma of the Vater. HAC is characterized by hepatic differentiation, which is determined based on both morphological and functional features[7].

To the best of our knowledge, cases of HAC of the extrahepatic duct have not previously been reported. Herein, we present a rare case of HAC of the hepatic duct, which may be proposed as a new site of this carcinoma.

CASE REPORT

A 62-year-old man presented with epigastric discomfort, xanthochromia, dull pain of the right shoulder, nausea and pruritus. A physical examination identified edema in both lower extremities and his feet. Epigastric tenderness to deep palpation was present but rebound tenderness was undetected. Muscular tension was negative. The liver and spleen were unreachable under the ribs. The hepatojugular reflux was negative. The patient denied any history of infection. Caput medusae, spider angioma and palmar erythema were not ovserved. Bowle sounds were detected twice per minute. Hepatitis B surface antigen (HbsAg) and hepatitis C-antibody were negative. Cholecystectomy, laparoscopic common bile duct exploration and cholangio-enterostomy were performed.

Abdominal ultrasonography revealed hepatic adipose infiltration, intrahepatic cholangiectasis, and choledochectasia. The above description included the possibility of upper hepatic duct obstruction. Magnetic resonance cholangiopancreatography (MRCP) demonstrated that the branches of the intrahepatic bile duct, the ductus hepaticus sinister and the ductus hepaticus dexter flowed normally. Intrahepatic cholangiectasis and aclasis of the ductus hepaticus sinister and the ductus hepaticus dexter were observed (Figure 1). The common hepatic duct, gall bladder and upper segment of the common bile duct could not be visualized. The imaging suggested obstructive jaundice of the upper segment of the bile duct. The obstruction was observed in the proximity of the hepatic portal area, and a lesion occupying the hepatic portal area was considered.

During the exploratory laparotomy, a small degree of abdominal dropsy and intrahepatic cholestasis were observed. The spleen was normal and the gallbladder wall was thickened. Foci of severe inflammation adhered to surrounding tissues were detected and the wall of the common bile duct was thickened and the lumen dilated (diameter of 3 cm). A solid space-occupying lump could be felt within the ampulla of the common bile duct. Therefore, cholecystectomy, laparoscopic exploration of the common bile duct and a cholangioenterostomy were performed. Intraoperative consultation reported a small number of malignant cells in the necrotic tissues, however, the origin of the cells could not be confirmed. A repeated search for the origin of the cells failed to identify a primary lesion in the liver, gastrointestinal tract or pancreas. A choledochojejunostomy was subsequently performed.

A gross examination revealed a purple and red solid mass in the hepatic duct corresponding to the occupying lesion observed by MRCP, measuring 1.5 × 1.2 × 1.0 cm^3. In addition, one tubular mass in the common hepatic duct (1 cm in diameter and 0.2 cm in depth), one tubular mass in the ductus hepaticus sinister (1.0 × 0.6 × 0.2 cm^3), one gray and yellow solid mass in the ductus hepaticus dexter (1.2 × 1.2 × 0.5 cm^3), a mass of fragmented red and purple contents in the hepatic duct (3.5 × 3.0 × 0.4 cm^3), one gallbladder containing bile (4 cm in diameter and 9 cm in length), and two lymph nodes attached to the mesentery were also observed.

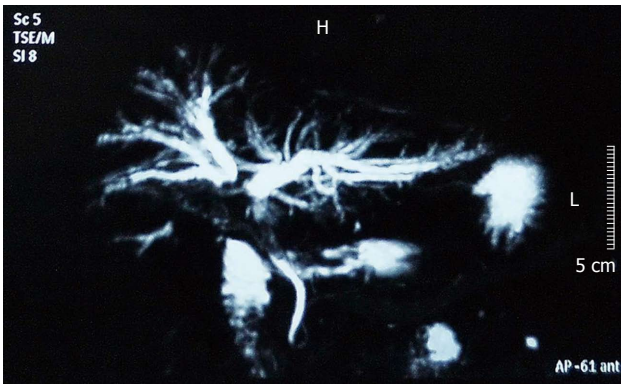

Figure 1 Magnetic resonance cholangiopancreatography. Magnetic resonance cholangiopancreatography revealed an obstruction in the proximity of the hepatic portal area and the absence of hepatic nodules within the liver lobes. L: Left; H: Height.

Microscopically, the tumor was primarily composed of "hepatoid cells", which were characterized by an eosinophilic cytoplasm, enlarged nucleus, and prominent nucleoli. The tumor cells were arranged in nests or proliferated in a trabecular pattern. The tumor consisted of marginal areas of dysplastic glands and well-differentiated intestinal-type adenocarcinomatous tissue, which formed the bulk of the tumor (Figure 2A-C). None of the lymph nodes dissected at surgery showed tumor metastasis. Immunohistochemistry indicated that the tumor cells were positive for hepatocyte paraffin 1 (HepPar-1), cytokeratins 8 and 18 (CK8/18), polyclonal carcinoembryonic antigen (pCEA) and S-100 (Figure 2D-G). Approximately 15% of the tumor cells were positive for Ki-67 (Figure 2H).

Based on the above findings, the case was diagnosed as HAC of the hepatic duct was made.

DISCUSSION

HAC a rare variant of extrahepatic adenocarcinoma, consists of foci of both adenomatous and hepatocellular differentiation that behave morphologically and functionally similar to hepatocellular carcinoma (HCC)[8,9]. As previously mentioned, the primary characteristics of HAC are histopathological features that suggest hepatoid differentiation resembling HCC. The tumor is generally composed of large or polygonal cells with abundant eosinophilic cytoplasm and proliferates in a solid or trabecular pattern, although medullary proliferation is occasionally observed[10]. The hepatoid nature of the cells can be proven based on the detection of bile production[1]. Primary gastric HAC has been the most frequently analyzed type of HAC. Glandular and hepatocyte differentiation both have been obserned and the explanation for this phenomenon is "enteroblastic differentiation". Because the stomach and liver are derived from the primordial foregut, prosoplasis that occurs during the maturation of the cells may induce the formation described above.

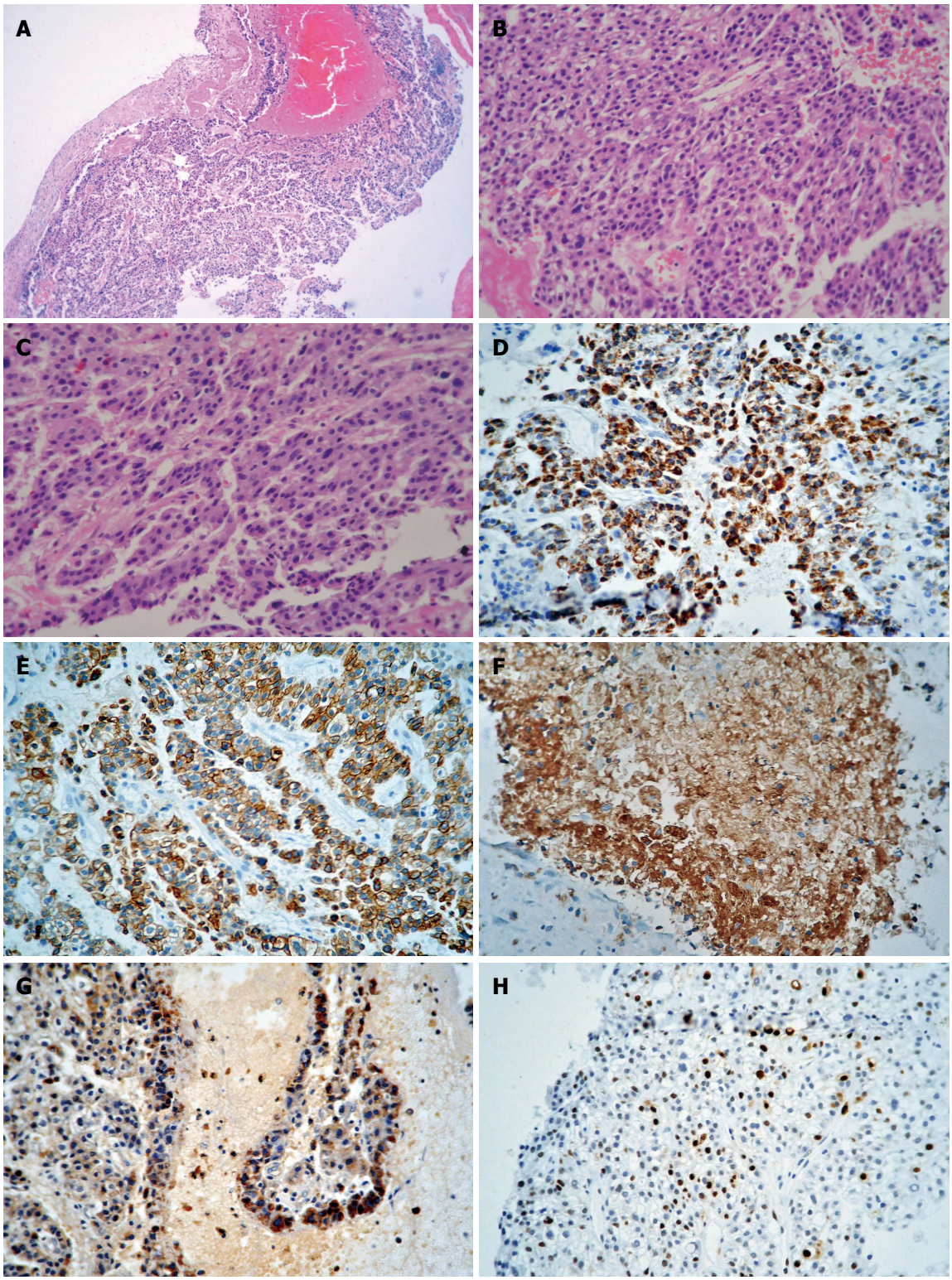

Figure 2 Histological and immunohistochemical features. A: Low-power microphotograph showing hepatoid areas (hematoxylin and eosin; original magnification, × 100); B, C: Hepatoid adenocarcinoma composed of cells with a clear cytoplasm arranged in a nested or trabecular pattern (hematoxylin and eosin; original magnification, × 400); D-H: Intracytoplasmic expression of hepatocyte paraffin 1, cytokeratin 8/18, polyclonal carcinoembryonic antigen and S-100 as well as Ki-67-positive nuclei (original magnification, × 400).

Clear cell carcinomas of the gallbladder with or without hepatoid differentiation are often associated with high serum α-fetoprotein (AFP) levels[10], however, not all HACs are associated with AFP overproduction[11]. In the present case, the preoperative serum AFP level was 6.84 μg/L, and immunohistochemistry suggested that AFP protein was not expressed. However, focal positivity for HepPar-1 suggested the presence of hepatoid differentiation, and pCEA positivity indicated canalicular differentiation and hepatocellular origin. CK8/18 ex-

pression in this case resembled HCC and could also be considered to be as a marker of hepatocellular origin.

HCC generally arises in cirrhotic livers and with hepatitis virus infection, however, the preoperative examination revealed that this patient was negative for HBsAg. In the present case, the rough region of the ductus hepaticus sinister was observed by the operator during laparoscopic exploration of the common bile duct. Combined with the clinical findings, the extrahepatic ductal origin of the HAC was verified by the clinical presentation, its morphological similarity to HCC, the immunohistochemical identification of liver-synthesized proteins, and bile production[2].

The differential diagnosis of HAC and HCC with invasion into the hepatic duct is primarily dependent upon tumor location. No primary lesion was identified in the liver, gastrointestinal tract or pancreas during surgery. In addition, MRCP and computed tomography angiography (not shown) did not reveal lumps in the liver. The literature has shown that HAC is generally found in elderly males, and poor outcomes are observed. The pathological findings in immunohistochemical analyses may also aid in the differential diagnosis of HAC from HCC: an evaluation using a panel of immunohistochemical markers (*e.g.*, cytokeratin 19, palate, lung and nasal epithelium clone, HepPar-1 and carcinoembryonic antigen), combined with detailed clinical history and endoscopic findings is essential for a definitive diagnosis.

REFERENCES

1 **Gakiopoulou H**, Givalos N, Liapis G, Agrogiannis G, Patsouris E, Delladetsima I. Hepatoid adenocarcinoma of the gallbladder. *Dig Dis Sci* 2007; **52**: 3358-3362 [PMID: 17510803 DOI: 10.1007/s10620-007-9807-3]

2 **Gardiner GW**, Lajoie G, Keith R. Hepatoid adenocarcinoma of the papilla of Vater. *Histopathology* 1992; **20**: 541-544 [PMID: 1318863 DOI: 10.1111/j.1365-2559.1992.tb01044.x]

3 **Kwon JE**, Kim SH, Cho NH. No ancillary finding is valid to distinguish a primary ovarian hepatoid carcinoma from metastatic hepatocellular carcinoma. *Int J Gynecol Cancer* 2006; **16**: 1691-1694 [PMID: 16884387 DOI: 10.1111/j.1525-1438.2006.00646.x]

4 **Takeuchi K**, Kitazawa S, Hamanishi S, Inagaki M, Murata K. A case of alpha-fetoprotein-producing adenocarcinoma of the endometrium with a hepatoid component as a potential source for alpha-fetoprotein in a postmenopausal woman. *Int J Gynecol Cancer* 2006; **16**: 1442-1445 [PMID: 16803544 DOI: 10.1111/j.1525-1438.2006.00613.x]

5 **Thamboo TP**, Wee A. Hep Par 1 expression in carcinoma of the cervix: implications for diagnosis and prognosis. *J Clin Pathol* 2004; **57**: 48-53 [PMID: 14693835 DOI: 10.1136/jcp.57.1.48]

6 **Kitamura H**, Ikeda K, Honda T, Ogino T, Nakase H, Chiba T. Diffuse hepatoid adenocarcinoma in the peritoneal cavity. *Intern Med* 2006; **45**: 1087-1091 [PMID: 17077571 DOI: 10.2169/internalmedicine.45.1760]

7 **Supriatna Y**, Kishimoto T, Uno T, Nagai Y, Ishikura H. Evidence for hepatocellular differentiation in alpha-fetoprotein-negative gastric adenocarcinoma with hepatoid morphology: a study with in situ hybridisation for albumin mRNA. *Pathology* 2005; **37**: 211-215 [PMID: 16175893]

8 **Sakamoto K**, Monobe Y, Kouno M, Moriya T, Sasano H. Hepatoid adenocarcinoma of the gallbladder: Case report and review of the literature. *Pathol Int* 2004; **54**: 52-56 [PMID: 14674996 DOI: 10.1111/j.1440-1827.2004.01578.x]

9 **Zhang S**, Wang M, Xue YH, Chen YP. Cerebral metastasis from hepatoid adenocarcinoma of the stomach. *World J Gastroenterol* 2007; **13**: 5787-5793 [PMID: 17963312]

10 **Vardaman C**, Albores-Saavedra J. Clear cell carcinomas of the gallbladder and extrahepatic bile ducts. *Am J Surg Pathol* 1995; **19**: 91-99 [PMID: 7802141 DOI: 10.1097/00000478-199501000-00011]

11 **Nakashima H**, Nagafuchi K, Satoh H, Takeda K, Yamasaki T, Yonemasu H, Kishikawa H. Hepatoid adenocarcinoma of the gallbladder. *J Hepatobiliary Pancreat Surg* 2000; **7**: 226-230 [PMID: 10982619 DOI: 10.1007/s005340050181]

P- Reviewer Sheng Z **S- Editor** Huang XZ
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i22.3528

World J Gastroenterol 2013 June 14; 19(22): 3528-3530
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Reducing risk of transjugular intrahepatic portosystemic shunt using ultrasound guided single needle pass

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Received: February 26, 2013 Revised: April 11, 2013
Accepted: May 7, 2013
Published online: June 14, 2013

Abstract

Delayed liver laceration following transjugular intrahepatic portosystemic shunt (TIPS) is a serious and likely underdiagnosed complication. It is however an important complication following TIPS, which remains one of the most technically challenging interventional procedures performed. In addition to laceration, a number of complications regarding bleeding and perforation are well described following TIPS procedures. We feel the adoption of techniques such as ours and that of other authors described in the literature using an ultrasound-guided percutaneous transhepatic approach with a small caliber needle provides a safer and less traumatic procedure and should reduce complications of bleeding and almost completely eliminate the risk of liver laceration. Our procedure was successfully performed under conscious sedation rather than general anaesthesia further reducing the overall procedural risk to the patient.

Key words: Transjugular portal systemic shunt; Ultrasound guided; Haemorrhage; Complication; Reducing; Morbidity; Death; Liver; Laceration

Core tip: Transjugular intrahepatic portosystemic shunt (TIPS) for complications of portal hypertension is commonly formed by accessing a portal vein branch from a metal cannula wedged in a hepatic vein. A number of serious procedural complications including bleeding and perforation following TIPS have been described. We feel the adoption of techniques such as ours and that of other authors described in the literature using an ultrasound-guided percutaneous transhepatic approach with a small caliber needle provides a safer and less traumatic procedure and should reduce complications of bleeding and almost completely eliminate the risk of liver laceration.

Leong S, Kok HK, Govender P, Torreggiani W. Reducing risk of transjugular intrahepatic portosystemic shunt using ultrasound guided single needle pass. *World J Gastroenterol* 2013; 19(22): 3528-3530 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i22/3528.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i22.3528

TO THE EDITOR

We read with interest the excellent article by Liu *et al*[1] regarding the delayed liver laceration following transjugular intrahepatic portosystemic shunt (TIPS) for portal hypertension. This is a serious and likely underdiagnosed complication. It is however an important complication following TIPS, which remains one of the most technically challenging interventional procedures performed. In addition to laceration, a number of complications regarding bleeding and perforation are well described following TIPS procedures.

In their paper, Liu *et al*[1] correctly established a number of factors which contribute to bleeding and liver injury following TIPS: (1) liver cirrhosis with coagulopathy; (2) liver parenchyma and vascular injury during TIPS; and (3) early stent anticoagulation with low molecular weight heparin. While we fully agree with these postulations, we feel that another important factor is the

needle size used for percutaneous access and tract creation between the portal vein and the hepatic veins during TIPS. In our department, we have recently adopted a new technique utilising a 22-gauge microneedle and percutaneous liver puncture under direct ultrasound visualisation that dramatically decreases the risk of laceration.

This modification may help refine and improve the outcome of patients undergoing TIPS placement. The creation of the shunt between the portal and hepatic veins is traditionally performed under fluoroscopic guidance, and is considered the most difficult step in the establishment of a TIPS[2], and is usually successful only after several punctures[3]. This "blind" fluoroscopic procedure can be refined with fluoroscopic methods including wedged hepatic venography[4] and superior mesenteric artery arterial portography[5]. Ultrasound assisted TIPS which facilitates the creation of the portosystemic shunt have also been described including placement of metallic overlying skin markers[6], placement of metallic coils adjacent to or microwire placement within the portal vein[7]. These methods attempt to reduce the time spent achieving the portosystemic shunt and thus, the risk of bleeding complications.

More interestingly, the removal of the step requiring blind puncture of the portal vein has been described in the recent literature. Raza *et al*[8] described a single-pass technique to access the right portal and right hepatic veins under ultrasound guidance using an 18-gauge, 20-cm Chiba needle (Cook) with a technical success rate of 73% and no evidence of post-procedure puncture site haemorrhage. Liang *et al*[2] also achieved 100% technical success and no evidence of post-TIPS internal hemorrhage with a similar technique in patients with severely distorted liver parenchyma to obtain a left portal vein and inferior vena cava (IVC) shunt, using an 18-gauge, 20-cm needle. Whilst small in patient numbers, these studies highlight the feasibility of ultrasound guidance to reduce the number of punctures required to achieve a portosystemic shunt, which thus reduces the risk of bleeding.

To further reduce the risk of bleeding in these patients who are often coagulopathic, it is suggested to use a further modification of Raza *et al*[8] and Liang *et al*'s[2] technique using initial access with a 22-gauge, 20-cm Chiba needle (Cook) in an attempt to reduce the bleeding risk from the most challenging step of the TIPS procedure, especially in high risk patients. This method has also been described in a small series by Gazzera *et al*[9] in 8 patients with 100% technical success. This method requires pre-procedural drainage of ascites, if present, under ultrasound guidance with a 7-8 Fr pigtail catheter. Embolisation of the transhepatic needle tract can also be considered[8] especially in cases where ascites is present[2]. Where available, intravascular ultrasound may be useful to guide shunt creation especially in cases with portal vein thrombosis, distorted anatomy, Budd-Chiari syndrome or hepatic tumors[10].

We recently adopted a modified transhepatic single-needle pass for TIPS in our institution. It involved a 53-year-old male with cirrhosis undergoing TIPS for refractory ascites. His pre-procedure work up revealed a Child-Pugh B and Model for End-stage Liver Disease score of 14. Prior to the modified TIPS procedure, the ascites was drained under ultrasound guidance. Following this, both the neck and abdomen were prepared for a combined transjugular and transhepatic approach followed by administration of conscious sedation with intravenous midazolam and fentanyl. Using real time ultrasound guidance, the right portal vein (Figure 1) was punctured with a 22-gauge, 20-cm Chiba needle (Cook) close to the bifurcation of the main portal vein (MPV), with entry confirmed by aspiration of blood. Following this, the needle was advanced into the right hepatic vein under real-time ultrasound guidance, with confirmation of entry by blood aspiration. A 0.018-inch Nitinol guidewire was then advanced into the hepatic vein, IVC and right atrium. The tract was upsized with a 7-Fr co-axial introducer system. The introducer and stiffner was then removed allowing passage of a 0.035-inch Amplatz Ultrastiff guidewire (Cook) into the right atrium. The right internal jugular vein was the accessed under ultrasound guidance using a micropuncture set (Cook) comprising of a 21-gauge needle, 0.018-inch guidewire and 4-Fr co-axial catheter. This was then upsized to a 6-Fr long vascular sheath into the right atrium. At this point, a 25-mm diameter Amplatz Gooseneck Snare (ev3 Inc) was used to snare the transhepatic wire, achieving through and through access (Figure 2). The sheath was then advanced into the hepatic vein over the transhepatic-transjugular wire until resistance was met. A 6 mm × 20 mm and 8 mm × 20 mm angioplasty balloon catheter (Powerflex Pro, Cordis) was advanced over the wire and the hepatic and portal vein tract was dilated. The sheath was advanced into the dilated tract, and contrast was injected through the sheath to confirm intraportal position. A 0.035-inch, 260-cm long hydrophilic wire (Glidewire Advantage, Terumo, Japan) and 5-Fr Berenstein catheter was then introduced through the transjugular sheath and manipulated into the MPV and then into the superior mesenteric vein (Figure 3). The transhepatic–transjugular wire was removed at this stage, and the 6-Fr sheath was replaced with a 10-Fr sheath. The procedure was completed as a conventional TIPS with deployment of a 10 mm × 70 mm (Viatorr, WL Gore and Associates) TIPS endoprosthesis stent (Figure 4). The patient remained stable with an uncomplicated post-procedural course and was discharged after 3 d with a satisfactory baseline post-TIPS ultrasound.

We feel the adoption of techniques such as ours and that of other authors described in the literature using an ultrasound-guided percutaneous transhepatic approach with a small caliber needle provides a safer and less traumatic procedure and should reduce complications of bleeding and almost completely eliminate the risk of liver laceration. Finally, our procedure was successfully performed under conscious sedation rather than general anaesthesia further reducing the overall procedural risk to the patient.

In conclusion, the authors should be commended for publication of this important complication of liver laceration following TIPS, which remains one of the most technically challenging interventional procedures but we hope that adoption of a microneedle controlled approach will potentially eliminate such complications.

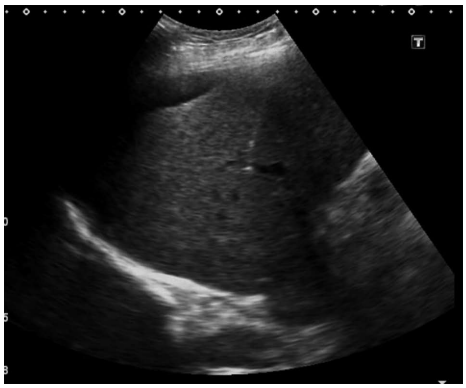

Figure 1 The right portal vein was punctured with a 22-gauge, 20-cm Chiba needle (Cook) close to the bifurcation of the main portal vein, with entry confirmed by aspiration of blood.

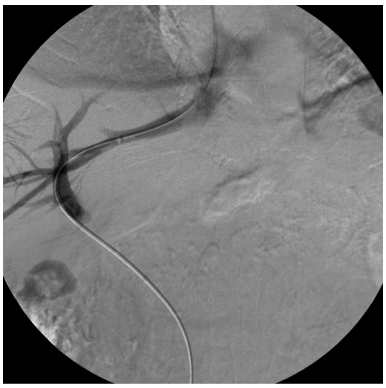

Figure 3 A 0.035-inch, 260-cm long hydrophilic guidewire (Glidewire, Boston Scientific) and 5 Fr Berenstein catheter was then introduced through the transjugular sheath and manipulated into the main portal vein and then into the superior mesenteric vein.

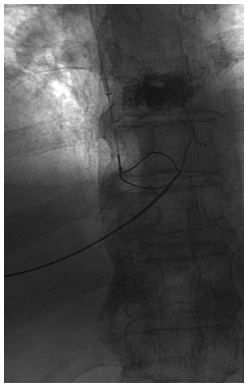

Figure 2 A 25-mm diameter Amplatz Gooseneck Snare (ev3 Inc) was used to snare the transhepatic wire, achieving through and through access.

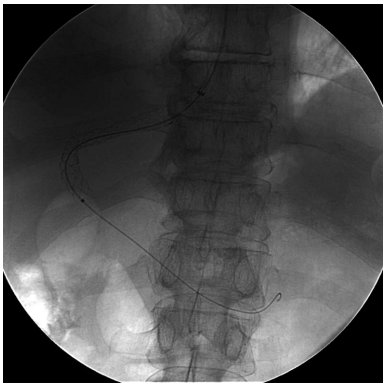

Figure 4 The procedure was completed as conventional transjugular intrahepatic portosystemic shunt with deployment of a 10 mm x 70 mm (Viatorr, WL Gore and Associates) transjugular intrahepatic portosystemic shunt endoprosthesis stent.

REFERENCES

1 **Liu K**, Fan XX, Wang XL, He CS, Wu XJ. Delayed liver laceration following transjugular intrahepatic portosystemic shunt for portal hypertension. *World J Gastroenterol* 2012; **18**: 7405-7408 [PMID: 23326153 DOI: 10.3748/wjg.v18.i48.7405]

2 **Liang HL**, Liu WC, Huang JS, Chen MC, Lai KH, Pan HB, Chen CK. TIPS in patients with cranial porta hepatis: ultrasound-guided transhepatic portohepatic-portocaval puncture in single needle pass. *AJR Am J Roentgenol* 2011; **196**: 914-918 [PMID: 21427345 DOI: 10.2214/AJR.10.4623]

3 **Zemel G**, Becker GJ, Bancroft JW, Benenati JF, Katzen BT. Technical advances in transjugular intrahepatic portosystemic shunts. *Radiographics* 1992; **12**: 615-622; discussion 623-624 [PMID: 1636029]

4 **Freedman AM**, Sanyal AJ, Tisnado J, Shiffman ML, Luketic VA, Fisher RA, Posner MP. Results with percutaneous transjugular intrahepatic portosystemic stent-shunts for control of variceal hemorrhage in patients awaiting liver transplantation. *Transplant Proc* 1993; **25**: 1087-1089 [PMID: 8442051]

5 **Rees CR**, Niblett RL, Lee SP, Diamond NG, Crippin JS. Use of carbon dioxide as a contrast medium for transjugular intrahepatic portosystemic shunt procedures. *J Vasc Interv Radiol* 1994; **5**: 383-386 [PMID: 8186613 DOI: 10.1016/S1051-0443(94)71508-6]

6 **Nöldge G**, Rössle M, Richter GM, Perarnau JM, Palmaz JC. [Modelling the transjugular intrahepatic portosystemic shunt using a metal prosthesis: requirements of the stent]. *Radiologe* 1991; **31**: 102-107 [PMID: 2041862]

7 **Roizental M**, Kane RA, Takahashi J, Kruskal J, Crenshaw WB, Perry L, Stokes K, Clouse ME. Portal vein: US-guided localization prior to transjugular intrahepatic portosystemic shunt placement. *Radiology* 1995; **196**: 868-870 [PMID: 7644659]

8 **Raza SA**, Walser E, Hernandez A, Chen K, Marroquin S. Transhepatic puncture of portal and hepatic veins for TIPS using a single-needle pass under sonographic guidance. *AJR Am J Roentgenol* 2006; **187**: W87-W91 [PMID: 16794144 DOI: 10.2214/AJR.05.1342]

9 **Gazzera C**, Fonio P, Gallesio C, Camerano F, Doriguzzi Breatta A, Righi D, Veltri A, Gandini G. Ultrasound-guided transhepatic puncture of the hepatic veins for TIPS placement. *Radiol Med* 2013; **118**: 379-385 [PMID: 22744357]

10 **Farsad K**, Fuss C, Kolbeck KJ, Barton RE, Lakin PC, Keller FS, Kaufman JA. Transjugular intrahepatic portosystemic shunt creation using intravascular ultrasound guidance. *J Vasc Interv Radiol* 2012; **23**: 1594-1602 [PMID: 23099001 DOI: 10.1016/j.jvir.2012.08.023]

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World J Gastroenterol 2013 June 14; 19(22): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

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Name of journal
World Journal of Gastroenterology

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

Launch date
October 1, 1995

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

Conference paper

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

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

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

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ISSN 1007-9327