ISSN 1007-9327
CN 14-1219/R

World Journal of Gastroenterology®

Indexed and Abstracted in:

Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, *Index Medicus*, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, *Nature Clinical Practice Gastroenterology and Hepatology*, CAB Abstracts and Global Health.
ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

Volume 14 Number 20
May 28, 2008
World J Gastroenterol
2008 May 28; 14(20): 3117-3280

Online Submissions
wjg.wjgnet.com
www.wjgnet.com
Printed on Acid-free Paper
世界胃腸病学杂志

A Weekly Journal of Gastroenterology and Hepatology

World Journal of Gastroenterology®

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2007-2009

Published by The WJG Press and Baishideng
Room 903, Ocean International Center, Building D
No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
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[1]Passed away on October 20, 2007
[2]Passed away on June 11, 2007

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Weekly Established in October 1995

National Journal Award 2005

Volume 14 Number 20
May 28, 2008

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World Journal of Gastroenterology
Volume 14 **Number** 20 **May** 28, 2008

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NAME OF JOURNAL
World Journal of Gastroenterology

RESPONSIBLE INSTITUTION
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PUBLISHING
The WJG Press and Beijing Baishideng BioMed Scientific Co., Ltd., Room 903, Ocean International Center, Building D, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
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OVERSEAS DISTRIBUTOR
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(Code No. 82-261)
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PUBLICATION DATE
May 28, 2008

EDITOR-IN-CHIEF
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SUBSCRIPTION
RMB 50 Yuan for each issue, RMB 2400 Yuan for one year

CSSN
ISSN 1007-9327
CN 14-1219/R

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doi: 10.3748/wjg.14.3117

World J Gastroenterol 2008 May 28; 14(20): 3117-3122
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Liver transplantation: Yesterday, today and tomorrow

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Received: October 15, 2007 Revised: March 15, 2008
Accepted: March 22, 2008
Published online: May 28, 2008

Abstract

With the advances in technical skills, management of postoperative complications and improvements in immunosuppressive drugs, liver transplantation is the standard treatment for many patients with chronic liver disease. Today, shortage of donor organs seems to be the major limiting factor for the application of liver transplantation. This review focuses on five issues that are challenging to clinical practice of liver transplantation and relevant to gastroenterologists. These include living donor liver transplantation, recurrent viral hepatitis, non-heart-beating donors, hepatocellular carcinoma, and ABO incompatible liver transplantation. Living donor and non-heart beating donor transplantations were initiated as a solution to increase the donor organ pool and it is expected that there will be an increase in the number of these donors. Recurrent hepatitis C and hepatocellular carcinoma following liver transplantation are among major problems and ongoing research in these diseases may lead to better outcomes in these recipients.

Key words: Liver transplantation; Hepatitis C virus; Hepatitis B virus; Hepatocellular carcinoma; ABO incompatibility; Living donor

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Abbasoglu O. Liver transplantation: Yesterday, today and tomorrow. *World J Gastroenterol* 2008; 14(20): 3117-3122 Available from: URL: http://www.wjgnet.com/1007-9327/14/3117.asp DOI: http://dx.doi.org/10.3748/wjg.14.3117

INTRODUCTION

Liver transplantation is one of the most important advances in medicine. First cadaveric liver transplantation was performed by Thomas Starzl in 1963 in Denver. After this failed trial, liver transplantation was successfully performed in humans in July 1967 again by Dr. Starzl. Although rejection was a major concern, many recipients from this early era have survived for more than 20 years using immunosuppression with azathioprine, prednisone, and antilymphocyte globulin (ALG)[1].

For clinical transplantation, the historical beginning was Medawar's recognition that rejection is an immune reaction[2] (Table 1). With the advances in immunosuppression, postoperative care and surgical technique, liver transplantation has become the golden standard in the treatment of many chronic liver diseases. Since then, the number of patients on the waiting list has increased and organ shortage appeared to be one of the major problems in clinical transplantation.

Raia of Brazil performed the first living donor liver transplantation (LDLT) in 1987 as a promising method to resolve the organ shortage, but the recipient died of a transfusion reaction despite a successful operation[3]. After this trial, LDLT has been performed by many other pioneer surgeons in other countries. In the last decade LDLT has become a widely accepted treatment modality. The most extensive experience in LDLT was initially gained in Asia. In countries such as Japan, where the availability of organs from deceased donor is limited, LDLT seems to be the only solution in the treatment of end stage liver diseases. According to the data of Japanese Liver Transplantation Society, the adult to adult LDLT is increasing per year. Despite this increase in adults, cases in children have reached a peak around 100 cases per year. The 1 and 5-year survival rates of all recipients were reported to be 81.8%, and 77%, respectively, while those of recipients of less than 18 years old was 85.6%, and 82.6% respectively. The prognosis of adult recipients was poor when compared to children[4]. It was suggested that the original disease recurrence such as hepatitis C and hepatocellular carcinoma (HCC) has influenced a significant decline in the survival of adult cases.

MAJOR ISSUES RELATED WITH LIVER TRANSPLANTATION

LDLT

The shortage of cadaveric liver organs has significantly

Table 1 History of liver transplantation[1,3]

Yr	Author	Application
1943	Gibson	Defined the immunologic nature of skin allograft rejection in humans
1955	Welch	First mention of liver transplantation in the literature in a dog study
1960	Medawar	Definition of acquired transplantation tolerance
1960	Starzl	Transplantation in dogs of multiple abdominal viscera
1963	Starzl	World′s first three attempts of liver transplantation in humans with maximum survival of 21 d
1968	Starzl	First long-term survival of four patients after liver transplantation
1978	Calne	Introduction of cyclosporine
1984	Bismuth	Reduced-sized adult liver transplanted into a small child
1987	Raia	First living donor liver transplantation
1988	Pichlmayr	Split one donor liver and two graft were used for two recipients
1988	Kalayoglu	Introduction of University of Wisconsin solution for preservation
1992	Starzl	Baboon to human xenotransplantation

inhibited further expansion of liver transplantation. Split liver transplantation has reduced waiting-list mortality in children, but not in adults. LDLT is currently the most effective alternative to overcome the organ shortage in adults. With the efforts of transplant surgeons in the establishment and popularization of LDLT, the number of LDLT has increased dramatically not only in Japan but also in Europe and the United States as well. Major advantages of LDLT include the good viability of the liver harvested from a healthy individual, the careful selection of the timing of the transplantation, and the potential good tissue matching. The reduced waiting period for a living donor organ may decrease the risks of decompensation or death before transplantation, thereby improving the overall chances for success. Disadvantages are the risk to healthy donors and also that, this modality has a potential psychological burden on the donor[5,6]. The surgical procedures for LDLT are technically more challenging and LDLT requires a full understanding of the hepatobiliary anatomy.

A wide range of complication rates are reported in the literature in donors after LDLT. Donor safety has a major importance in LDLT. Published reports on donor outcomes indicated a wide range of complication rates that varied between 9% and 67%[7,8]. In the Kyoto University experience 50 complications in 222 right lobe grafts have been encountered, including surgical complications is 18.5% and non-surgical complications is 3.2%[9]. On the other hand, the American Society of Transplant Surgeons reported a donor complication rate of only 10%. Thus it seems that donor morbidities have not been adequately reported and true extend of complications may be underestimated. A standardized system for reporting complications to a registry should be developed to allow meaningful data analysis. Donor mortality is also a major concern of LDLT. In the United States at least 3 deaths were confirmed. Another 3 deaths in Europe and 1 in Japan had been reported[10].

As living donation permits transplantation to take place independent of either waiting time or the severity of liver disease, the criteria required for LDLT may be modified when compared to deceased donor liver transplantation (DDLT). Estimation of liver volume needed in individual situations is an important factor in donor selection. Aged liver, steatotic liver, and special anatomic variants have the risk of a relatively poor graft quality. Recipient factors such as metabolic load, preoperative latent infections and other organ failures have negative impact on graft survival. The minimally required quantity of graft volume has not been fully clarified, which is one of the major issues of the adult to adult LDLT. The following two methods were developed to express the graft volume: First the ratio of graft volume (GV) in the standard liver volume (SLV) of the recipient, which is calculated by the recipient's height and body weight. Second, the ratio of graft weight in the recipient's weight (GRWR: graft to recipient weight ratio). The reported safe limit of small-for-size graft is from 30% to 40% in GV/SLV, while from 0.6 to 0.8 in GRWR[11-14].

Recipients with a small-for-size graft, suffer from graft dysfunction including hyperbilirubinemia, massive ascites, poor synthetic function that leads to serious conditions such as gastrointestinal bleeding and renal failure. When a graft size is conversely too large for a recipient such as a newborn infant, the graft necrosis occurs due to insufficient blood inflow into the graft.

As pointed out by Ghobrial and Bussuttil, future application of LDLT will be based on the accurate definition of risks imposed on donors compared with potential benefits realized by recipients[15]. As an example to this statement, the number of adult LDLT declined from approximately 400 in 2001 to 280 in 2002. Such a precipitous reduction may have occurred in response to the donor death in US in 2002 which raised increasing concerns for donor safety. While the number of LDLT in the US has declined, the number in Asia as a whole has continued to increase. LDLT accounted for less than 5% of liver transplants in the US but more than 95% of the transplants in Asia excluding mainland China. The overall number of LDLT procedures performed in Asian countries and areas with well-established programs (Japan, Korea, China Hong Kong and China Taiwan) has steadily increased over years[16].

In summary, the overall results with good patient and graft survival, together with acceptable donor morbidity and mortality has led to the acceptance of LDLT in the transplant community. To maintain this procedure as a treatment modality in the future, satisfactory risk-benefit analysis and long-term morbidities imposed on living donors should be further investigated.

Recurrent viral hepatitis

The most common single cause of late graft loss after liver transplantation is the recurrence of the disease for which the liver transplantation has been performed[17]. Until last decade, successful long-term outcome after liver transplantation in patients with chronic active hepatitis-B has been limited because of high rate of recurrent

of recurrent hepatitis. Long-term passive immunization with high-dose intravenous hepatitis B immunoglobulin (HBIG) led to a significant improvement in the prognosis of these patients. High-dose intravenous HBIG may prevent recurrent hepatitis B virus infection, but the cost has limited its widespread use in countries with endemic hepatitis B virus infection. Low-dose intramuscular HBIG plus nucleoside analogs such as lamivudine was shown to be equally effective and safe and in the long-term prophylaxis against recurrent hepatitis B at less than 10% the cost of the high-dose regimen[18,19]. Although lamivudine is effective in most of the patients, lamivudine resistance is becoming a major concern. With adefovir, a potent antiviral drug that became available in recent years, these patients with lamivudine-resistant tyrosine-methionine-aspartate-aspartate mutant can also be treated[20,21]. Currently, liver transplantation can be safely performed in chronic active hepatitis B patients with similar survival as for patients transplanted for other indications.

The recurrence of hepatitis C is also a great concern after transplantation. Although short-term graft and patient survival rates of chronic active hepatitis C patients are comparable to those observed in other patients undergoing liver transplantation, HCV recurrence is universal and is associated with poor graft and patient survival[22]. In contrast, survival after retransplantation for recurrent hepatitis C is poor and retransplantation for these patients is controversial[23,24]. In a previous study Abbasoglu *et al* showed that recurrent hepatitis was the most common cause of late graft loss in patients who had undergone liver transplantation for chronic active hepatitis C[17].

Treatment of recurrent hepatitis C, whether preemptive or not, is an important issue. Despite recent achievements in the treatment of hepatitis C infection with pegylated interferons and ribavirin, patients with recurrent hepatitis C after liver transplantation are difficult to treat. Virological response rates in prophylactic and therapeutic approaches of hepatitis C reinfection after liver transplantation are low compared to the pre-cirrhotic hepatitis C infection. Moreover, optimal treatment duration and dosage of recurrent infection with pegylated interferon in combination with ribavirin remains to be defined[25]. Despite side effects, long-term antiviral maintenance therapy might be an effective approach for preventing progression to severe allograft fibrosis and thereby improving long-term survival in liver transplant recipients with recurrent hepatitis C[26].

Two large studies have shown that the incidence and severity of hepatitis C recurrence do not differ between DDLT and LDLT recipients; however another study has found that the incidence of cholestatic hepatitis is significantly greater in LDLT recipients[24,27,28]. Several studies have identified a number of potential risk factors for recurrent hepatitis C infection including hepatitis C virus related factors (virus load, genotype) as well as coinfection with other viruses such as cytomegalovirus, hepatitis B virus and hepatitis D virus[29]. There are still no well-defined parameters that would predict which patients are at risk to develop recurrent hepatitis C and those who will not. Strategies including pre- and post-transplant antiviral therapy may further improve the results.

Non-heart-beating donors

The first liver transplantation from a non-heart beating donor (NHBD) was performed by Nakayama in Japan in 1964. NHBD livers are considered as a potential for expanding donor pool. The critical problem with NHBD livers is prolonged warm ischemia time. Despite calls for the use of hepatic grafts from NHBD, there are few studies examining long-term outcome. Although metabolism is slowed 1.5- to 2-fold for every 10℃ drop in temperature, considerable metabolic activity still occurs during cold preservation. In NHBD organs, the effects of cold ischemia are superimposed on the injury occurred during warm ischemia. The pattern of injury sustained during warm and cold ischemia is slightly different. Cold ischemia leads to initial injury to sinusoidal endothelial cells whereas warm ischemia mainly injures the hepatocytes[30]. It seems that the additional injury resulting from warm ischemia in NHBD donation requires alternative preservation strategies to minimize the ischemic injury. Donor warm ischemic time may predispose hepatic allografts to an increased incidence of ischemic type biliary strictures. Although graft and patient survival has been reported to be similar to that of heart beating donor transplants, caution is urged with the use of these organs[31].

Despite the increased risk of graft and patient survival, NHBD livers are being increasingly used with acceptable results. Abt *et al* analyzed data from the United Network for Organ Sharing database. In 144 NHBDs and 16 856 heart beating donors (NHBDs) the 1-year (70.2% *vs* 80.4%) and 3-year (63.3% *vs* 72.1%) graft survival were inferior in the NHBD group. The primary non-function risk after transplantation was also significantly higher (11.8 % *vs* 6.4%) in the NHBD group[32].

New strategies in organ preservation, normothermic recirculation, normothermic preservation, cytoprotection, and development of reliable markers to predict postoperative graft function may improve results in clinical transplantation with NHBD liver grafts. Based on the clinical studies and continued shortage of liver allografts, the use of NHBD organs are recommended, however, with several caveats. Careful donor (< 60 years of age) and recipient (stable, not intubated) selection, minimizing warm (< 30 min) and cold (< 8 h) ischemia, utilization of histology, and discarding organs with significant steatosis may provide acceptable results[32,33].

HCC

HCC is the most common primary liver cancer and most patients with HCC also suffer from coexisting cirrhosis. For the treatment of patients without cirrhosis, resection should be considered whenever possible. Hepatic reserve is the one of the major determinants of liver resection. When compared with resection, transplantation restores liver function and has the advantage of removing tissue with an oncogenic potential[34]. To obtain the optimal

benefit from the limited number of organs available, strict selection criteria has been developed to offer liver grafts to patients with the highest likelihood of survival after transplantation. In 1996 Mazzaferro *et al* showed that when strict criteria were applied, transplantation of patients with early HCC has resulted in excellent results with 4-year survival rate of 75%. This led to the development of Milan criteria from a retrospective analysis of 48 patients. This survival rate was achieved in patients with solitary tumor of less than 5 cm and those who have up to 3 tumor nodules each of which is smaller than 3 cm without vascular invasion or extra hepatic metastasis[35]. With the achievement of good results in HCC patients with more advanced tumors, the Milan criterion was expanded. Yao *et al* proposed UCSF criteria (solitary tumor smaller than 6.5 cm or 3 of fewer nodules with the largest lesion smaller than 4.5 cm or total tumor diameter less than 8.5 cm without vascular invasion[36]. In this study the expansion of Milan criteria did not impact on survival adversely. On the other hand, this approach reduced the availability of cadaveric grafts for patients with other liver diseases. The Barcelona Clinic Liver Cancer Group has proposed expanding the Milan criteria to single tumor of 7 cm or less, or 5 tumors of 3 cm or less, in patients who showed a partial response to any treatment lasting for more than 6 mo[37]. However organ shortage, higher drop-out rate, and less favorable results render these attempts to a controversial issue. With the expansion of listing criteria, liver transplantation could be performed in more advanced cancer patients but this lead in turn to poor survival rates. All patient selection criteria rely on radiological imaging to assess intrahepatic disease and exclude extra hepatic spread. It may be possible to improve patient selection by increasing the sensitivity of imaging studies and detection of micrometastasis[38].

About 50% of HCC patients who are initially candidates for liver transplantation will become ineligible, if the median waiting period exceeds 1 year[39,40]. As a result of tumor progression during the waiting period, LDLT gained popularity to transplant HCC patients in a better clinical condition without a long waiting time. Although controversial, it may be claimed that LDLT can be performed in patients with HCC that exceeds the Milan criteria as 3-year survival rate of greater than 50 has been showed in other studies[41]. In two studies it was shown that LDLT is superior to DDLT for patients with HCC meeting Milan criteria, when waiting times for organs from deceased donors exceed six months[42,43]. Despite the availability of LDLT tumor progression is still a major concern and strategies like chemoembolization and radiofrequency ablation to reduce tumor growth during waiting period have shown promising results[44]. Although many studies have shown that microvascular invasion and histological grade are significant risk factors for poor prognosis, these are difficult to know clearly before transplantation. Noninvasive markers to predict the prognosis of HCC may help better patient selection in the future[45].

ABO incompatible liver transplantation

Two antigen systems (ABO and HLA) play role in transplantation. In liver transplantation the ABO system is important while HLA system has a minor role. Crossing the ABO barrier in liver transplantation is usually not performed except for emergency conditions and results of ABO incompatible liver transplantation have been markedly inferior with an increased incidence of vascular and biliary complications and rejection, when compared to ABO compatible grafts. In children below the age of three years, ABO incompatible liver transplantations have been more successful[46]. In recent years, promising results with ABO incompatible liver transplantation using A_2 donors (subgroup of A which is less reactive and occur in approximately 20% of group A individuals) have been reported. In a Swedish study of 10 adult blood group O recipients who received A_2 cadaveric grafts, patient and graft survival was 10/10 and 8/10 respectively at 8.5 mo median follow up with tacrolimus based protocol and initial immunosuppression with antithymocyte globulin, interleukin-2-receptor antagonists or anti-CD20 antibody[47]. In 16 pediatric ABO-incompatible pediatric liver transplantation, Heffron *et al* reported one-year actuarial graft survival of 92% utilizing standard immunosuppression with selective post-operative plasmapheresis and without splenectomy[48]. Plasmapheresis may be useful by reducing the recipients' antibody titers before and after transplantation. ABO incompatible liver transplantation may be the only available option in LDLT, if the patients have no ABO identical or compatible donors. According to the Japanese Registry of LDLT Across ABO Blood Type Barrier, 97 ABO incompatible LDLTs were performed in Japan before 2005 and 5-year survival rate of the patients was 38% before 2001 and improved to 63% among patients who underwent transplantation after 2002[49].

Although recent studies support the concept of ABO-incompatible liver transplantation both in adults and children, further studies are needed to draw a conclusion. More well-designed, controlled clinical trials are necessary to establish optimal pretransplantation management protocols including immunosuppressive regimens in this group of patients.

CONCLUSION

Liver transplantation is the only definitive treatment modality of end stage liver diseases. Although LDLT has been widely performed with results similar to whole organ cadaveric transplantation, the benefits of the recipients *versus* the risks and long-term morbidities imposed on the donors require further studies. The overall reported donor mortality is 12 in about 6000 transplantations (0.2%)[50]. Recurrent viral hepatitis and HCC are among the major causes of late graft loss after liver transplantation. Current antiviral treatment for recurrent HCV offer limited chance of long-term success. To overcome organ shortage there is now a resurgence of interest in NHBD liver transplantation. Although ABO incompatible liver transplantation especially using A_2 donors is promising particularly in children, more studies are needed to draw a conclusion.

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S- Editor Li DL **L- Editor** Alpini GD **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3123

World J Gastroenterol 2008 May 28; 14(20): 3123-3128
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Strategy for treatment of nonerosive reflux disease in Asia

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Received: February 25, 2008 Revised: April 1, 2008
Accepted: April 8, 2008
Published online: May 28, 2008

Abstract

The paper is to review the clinical and pathophysiologic differences between of nonerosive reflux disease (NERD) and reflux esophagitis (RE), and to propose a treatment strategy for NERD, especially for patients in Asia. A Medline search was performed regarding the clinical and pathophysiologic differences between NERD and RE, and treatment of NERD and RE. The characteristics of NERD patients in Asia are as follows: (1) high proportion of female patients, (2) low frequency of hiatal hernia, (3) high frequency of *H pylori* infection, (4) severe glandular atrophy of the gastric mucosa, and (5) frequent resistance to proton pump inhibitor (PPI) therapy. In Asian NERD patients, exposure of the esophagus to acid is not increased, and esophageal motility is normal. These characteristics are similar to those of patients in Western countries. Our recommended first-choice treatment is administration of PPI in combination with a prokinetic agent. However, at present, because there is limited evidence regarding effective treatments for NERD, it is best to try several different treatment strategies to find the best treatment for each patient.

Key words: Nonerosive reflux disease; Asia; Treatment

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Hiyama T, Yoshihara M, Tanaka S, Haruma K, Chayama K. Strategy for treatment of nonerosive reflux disease in Asia. *World J Gastroenterol* 2008; 14(20): 3123-3128 Available from: URL: http://www.wjgnet.com/1007-9327/14/3123.asp DOI: http://dx.doi.org/10.3748/wjg.14.3123

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a condition that develops when reflux of stomach contents causes troublesome symptoms and/or complications[1]. GERD is more common in Western countries than in Asian countries, such as China, Korea, and Japan. Epidemiologic studies show a prevalence of GERD symptoms in Western countries ranging from 20% to 40%[2,3] and in Asian countries ranging from 5% to 17%[4]. The prevalence in Asian countries has increased gradually[4]. Esophagogastroduodenoscopy (EGD) is the gold standard for the diagnosis of erosive GERD [reflux esophagitis (RE)], and the Los Angeles (LA) classification of esophagitis is generally accepted as the best means for endoscopic assessment of GERD[5]. In Japan, the prevalence of RE (LA classification grades A, B, C, and D) is approximately 15%, and most of these cases are grade A or B[6]. The majority of GERD cases are cases of nonerosive reflux disease (NERD).

NERD was previously considered a mild/early type of RE that would progress to severe RE. However, it was reported that, regardless of therapy, only 2.7% of NERD patients develop RE after 3 years and only 3% of patients develop RE after 6 years[7]. A recent retrospective study of 2306 GERD patients found that these patients at least two separate upper endoscopic examinations during the 7-year (mean) follow-up period. Examinations revealed that 69% of the patients were unchanged, 21% were improved, and 11% became worse[8]. Another study[9] reported similar results. These studies suggest that NERD rarely progresses to RE over time. In addition, NERD is significantly more refractory to treatment than RE[3]. Therefore, it was recently suggested that the underlying mechanism of development of NERD is different from that of RE. Here we review the clinical and pathophysiologic differences between NERD and RE and propose a treatment strategy for NERD, especially for patients in Asia.

Table 1 Clinical characteristics of NERD and RE patients in Asia

	NERD	RE
Male/Female	0.59-1.65	1.18-7.13
Average age (yr)	45.2-57.5	49.0-59.7
Mean body mass index (kg/m^2)	22.1-23.1	21.7-24.2
Complication of hiatal hernia (%)	17.7-34.8	35.1-77.0
H pylori infection (%)	36.3-48.3	18.0-32.3
Glandular atrophy of the gastric mucosa (open-type) (%)	25.0-43.0	6.7-25.0
Efficacy of proton pump inhibitor (%)	29.5-64.0	55.4-90.3

METHODS

Studies on GERD were identified by computerized and manual searches of the available literature. The Medline search (1975-2007) was performed using the following medical subject headings: reflux disease and Asia. Papers published in English were considered.

CLINICAL AND ESOPHAGEAL MOTILITY CHARACTERISTICS OF NERD IN ASIA

Several researchers examined characteristics of NERD and RE patients in Asia[10-18]. The male/female ratios ranged from 0.59 to 1.65 in NERD patients. On the other hand, those of RE patients ranged from 1.18 to 7.13. A higher proportion of female patients was observed in NERD patients compared with RE patients. There were differences between NERD patients and RE patients in frequency of hiatal hernia, frequency of *H pylori* infection, grade of glandular atrophy of the gastric mucosa, and effect of proton pump inhibitor (PPI) therapy as well. Namely, compared with the RE patients, the characteristics of NERD patients in Asia are as follows: (1) higher proportion of female patients, (2) lower frequency of hiatal hernia, (3) higher frequency of *H pylori* infection, (4) severe glandular atrophy of the gastric mucosa, and (5) frequent resistance to PPI therapy (Table 1). In addition, Asian NERD patients are more frequently affected by functional dyspepsia, irritable bowel syndrome, and psychiatric diseases than RE patients[13]. These characteristics are similar to those of Western NERD patients. However, there are several other characteristics in Western NERD patients, such as younger age and less obese[3]. As the prevalence of *H pylori* infection in Asian populations has decreased to levels similar to those in Western populations, these additional characteristics may be observed in Asian patients in the near future.

With respect to esophageal motility, NERD patients have several characteristics that differ from those of RE patients. In NERD patients, the resting lower esophageal sphincter (LES) pressure is not decreased. In addition, exposure of the esophagus to acid is not increased, and esophageal motility is normal (Table 2)[19]. These characteristics are similar to those of patients in Western countries, although the grades of motility index abnormalities in Asian RE patients are lower than those in Western RE patients[20].

Table 2 Esophageal motility characteristics in NERD and RE patients in Asia

	NERD	RE
Resting LES pressure	Mildly increased	Moderately decreased
Reflux episodes/hour	Moderately increased	Moderately increased
Primary peristalsis	Normal	Moderately decreased
Secondary peristalsis	Mildly decreased	Moderately decreased
Acid clearance	Mildly delayed	Moderately delayed

LES: Lower esophageal sphincter.

It seems that there are differences in pathophysiology between Asian RE patients and Western RE patients, because the grades of motility index abnormalities are different between them. However, there seems no significant difference in pathophysiology between Asian NERD and Western NERD patients, because clinical and esophageal motility characteristics are considerably similar between them.

PATHOPHISIOLOGY OF NERD

The main pathophysiology of RE is excessive exposure of the esophagus to gastric acid. Approximately 90% of patients with RE can be cured with a PPI, which is the strongest type of gastric acid suppressor[3]. In contrast, only one-third of NERD patients can be cured with a PPI. Although the cause of NERD that is responsive to PPI may be excessive exposure of the esophagus to acid, PPI-resistant NERD may be associated with the factors described below.

Incomplete acid suppression

In some patients, even the highest approved dose of PPI cannot sufficiently suppress gastric acid secretion. In patients with insufficient gastric acid suppression, gastric juice may reflux, exposing the esophagus to acid. The time required for metabolism of PPI differs between patients possibly due to polymorphisms in the genes encoding metabolic enzymes, such as CYP2C19[21,22]. In patients with the rapid metabolic phenotype, administration of twice the approved dose of PPI and concomitant administration of PPI and H_2-receptor antagonist (H_2RA) may be more effective[23,24]. It has also been reported that administration of an aluminum- and magnesium-containing antacid may be effective for some NERD patients[25].

Esophageal hypersensitivity to acid

Some patients with severe RE do not have symptoms of acid regurgitation, even if severe esophageal acid exposure is confirmed[26]. However, many NERD patients have a normal level of esophageal acid exposure. Therefore, there appears to be significant esophageal hypersensitivity to acid exposure in PPI-resistant NERD patients, and symptoms may occur when gastric acid is refluxed[27]. Hyperosmotic foods, such as cake and chocolate, and alcoholic beverages may be the cause of this esophageal hypersensitivity[28]. Ingestion of such

foods and drinks may cause heartburn. It has been suggested that ingestion of hyperosmotic foods/drinks loosens the tight junctions between esophageal epithelial cells, and when gastric acid is refluxed, it easily intrudes between epithelial cells and stimulates the terminals of sensory nerves[26].

Esophageal hypersensitivity to esophageal wall distension

In NERD patients, heartburn symptoms are induced by distension of the esophageal wall by balloon dilatation or by pumping saline into the esophageal lumen[29]. These findings suggest the possibility that foods, air, and fluids that contain no acid may cause heartburn symptoms simply by distending of the esophageal wall.

Reflux of duodenal juice (bile and pancreatic juice)

PPI suppresses gastric acid excretion but has no effect on reflux itself. Therefore, in patients with duodenogastric reflux, duodenal juice (bile and pancreatic juice) may be refluxed into the esophagus. It is possible that the refluxed duodenal juice may affect the esophageal mucosa[30]. NERD patients frequently have functional dyspepsia[4], and significant duodenogastric reflux and delayed gastric emptying time in patients with functional dyspepsia have been reported[31,32]. These findings support the idea that reflux of duodenal juice into the esophagus causes NERD.

Esophageal motility abnormalities

It has been reported that NERD patients show normal resting LES pressure and primary contraction waves but significantly reduced frequency of secondary contraction waves[19,33]. This may be due to a reduced response to distension of the esophageal wall. Secondary contraction waves are stimulated by distension of the esophageal wall and act to discharge refluxed gastric acid and air into the stomach. Heartburn symptoms may be associated with reduced motility function in the esophageal wall.

Sustained esophageal contraction

Sustained contraction of the longitudinal muscles of the esophagus causes heartburn, and prolonged contraction may lead to chest pain. This phenomenon is called sustained esophageal contraction (SEC) and is identified by intraluminal ultrasonography[34,35]. SEC occurs just before the onset of heartburn symptoms. There are two types of SEC: SEC with or without subsequent acid reflux. Because patients with the latter type also have heartburn symptoms, the association of SEC with NERD is of great interest.

Psychological factors

NERD patients frequently have mental disorders[13]. Psychological factors are associated with response to treatment as well as symptoms[36]. A high level of anxiety is predictive for the nonresponse to acid suppression therapy.

Eosinophilic esophagitis

Eosinophilic esophagitits affects both children and adults and is characterized by symptoms of GERD and dense esophageal eosinophilia, both of which are unresponsive to PPI[37,38]. This disease is caused by food allergies or by aeroallergens. Effective treatment include systemic/topical corticosteroids, or specific food elimination. Esophageal stricture is a potential complication, and the natural history of the disease is still unknown. Eosinophilic esophagitis may be diagnosed as PPI-resistant NERD, but should be excluded from the diagnosis of NERD.

TREATMENT STRATEGY FOR NERD IN ASIA

At present, PPI-based step-down treatment is recommended for GERD patients[39,40]. In a meta-analysis, the relative risks of PPI and H_2RA treatment for NERD compared with placebo were 0.69 (95% confidence interval, 0.62-0.78) and 0.84 (0.74-0.95), respectively, indicating that PPI is a more effective treatment than H_2RA[41]. PPI treatment can eliminate NERD symptoms faster than H_2RA treatment. In addition, PPI treatment has been reported to be more cost-effective than other treatment[42].

Prokinetics such as mosapride, itopride, metclopramide, and domperidone are also effective for treatment of NERD[43-45]. Prokinetics are thought to work by reducing reflux of duodenal juice into the esophagus[31] and speeding absorption of PPI. In addition, mosapride improves esophageal motility, whereas metclopramide and domperidone do not have this ability[46]. Mosapride shortens bolus transit time in the esophagus, reduces the duration of the longest reflux episode and reflux fraction time, and enhances the contraction strength in the lower esophagus.

Reflux of stomach contents is related to transient LES relaxation (TLESR) in NERD patients[47]. Therefore, control of TLESR is another important point for NERD treatment. 5-HT_3, cholecystokinin (CCK)-A, and gamma-aminobutyric acid (GABA) receptors influence TLESR[48-50]. 5-HT_3 receptor antagonist, CCK-A receptor antagonist, and GABA receptor agonist reduce the frequency of TLESR. Mosapride is a selective 5-HT_4 receptor agonist, and the metabolite acts as a 5-HT_3 receptor antagonist[51,52]. Therefore, mosapride reduces the frequency of TLESR, leading to reduced gastric acid reflux in NERD patients.

Some NERD cases are refractory to PPI and/or prokinetics. In these patients, psychological factors may be associated with symptoms. In these patients, administration of an antidepressant and/or minor tranquilizer should be considered. However, evidence for the benefits of these agents in treatment of NERD is weak[53], and further studies are needed to clarify the effects of such medications on NERD.

For NERD patients with infrequent symptoms of heartburn, on-demand therapy with PPI (and/or prokinetics) is proposed as the best treatment option[54,55]. Additional studies of the effectiveness of this treatment regimen are needed.

Here we propose a new strategy for treatment of NERD in Asia based on the basic idea of step-down

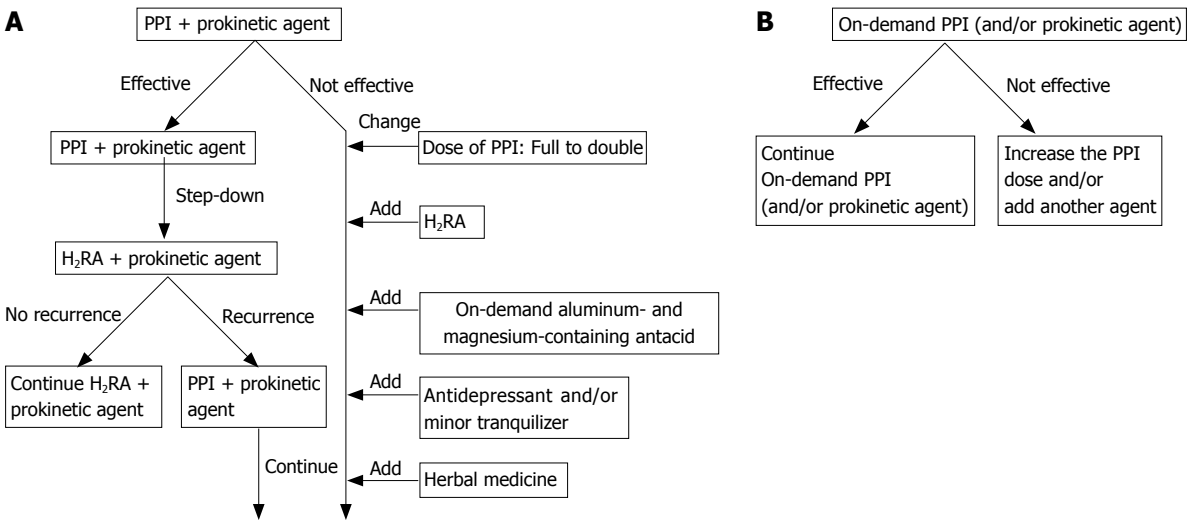

Figure 1 Proposed treatment strategy for NERD patients in Asia. **A**: Patients with moderate or severe symptoms; **B**: Patients with infrequent symptoms.

therapy (Figure 1). The recommended first-choice treatment is administration of PPI in combination with a prokinetic agent such as mosapride. PPI can cure only one-third of NERD patients, a prokinetic agent in conjunction with the PPI can increase the efficacy. NERD is frequently associated with functional dyspepsia that can be treated with prokinetic agents. In addition, because the quality of life of NERD patients is quite low, NERD patients need quicker and more effective treatment options[56]. If this treatment is not effective, twice the recommended dose of PPI or combined treatment with PPI and an H_2RA is recommended. PPI together with on-demand aluminum- and magnesium-containing antacid might be effective. If these treatments are not effective, administration of an antidepressant or minor tranquilizer should be considered. Herbal medicines such as rikkunshito may provide relief for some patients[57], and are often administered especially in Asian countries.

For patients with infrequent symptoms, on-demand treatment with PPI and/or a prokinetic agent is recommended. However, there is not sufficient evidence for a best treatment for NERD. Further studies are needed to clarify the efficacy of treatment. Large-scale, double-blind, randomized controlled trials of PPI *vs* PPI with a prokinetic agent are also needed to clarify the benefit of the prokinetic agent.

Further trials are needed to establish the strategy for treatment of NERD. At present, because there is limited evidence regarding effective treatments for the disease, it is best to try several different treatment strategies to find the best treatment for each patient.

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S- Editor Zhong XY **L- Editor** Ma JY **E- Editor** Lu W

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3129

World J Gastroenterol 2008 May 28; 14(20): 3129-3136
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Dr. Peter V Draganov, *Series Editor*

Intraductal biliary and pancreatic endoscopy: An expanding scope of possibility

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Received: February 21, 2008 Revised: March 23, 2008
Accepted: March 30, 2008
Published Online: May 28, 2008

Abstract

Intraductal endoscopy describes the use of an endoscope to directly visualize the biliary and pancreatic ducts. For many years, technological challenges have made performing these procedures difficult. The "mother-baby" system and other various miniscopes have been developed, but routine use has been hampered due to complex setup, scope fragility and the time consuming, technically demanding nature of the procedure. Recently, the SpyGlass peroral cholangiopancreatoscopy system has shown early success at providing diagnostic information and therapeutic options. The clinical utility of intraductal endoscopy is broad. It allows better differentiation between benign and malignant processes by allowing direct visualization and targeted sampling of tissue. Therapeutic interventions, such as electrohydraulic lithotripsy (EHL), laser lithotripsy, photodynamic therapy, and argon plasma coagulation (APC), may also be performed as part of intraductal endoscopy. Intraductal endoscopy significantly increases the diagnostic and therapeutic yield of standard endoscopic retrograde cholangiography (ERCP), and as technology progresses, it is likely that its utilization will only increase. In this review of intraductal endoscopy, we describe in detail the various endoscopic platforms and their diagnostic and clinical applications.

Key words: Intraductal endoscopy; Choledochoscopy; Cholangioscopy; Pancreatoscopy; Biliary endoscopy; Duodenoscope-assisted cholangiopancreatoscopy; Miniscope; Percutaneous choledochoscopy; Laparoscopic choledochoscopy; Lithotripsy

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Judah JR, Draganov PV. Intraductal biliary and pancreatic endoscopy: An expanding scope of possibility. *World J Gastroenterol* 2008; 14(20): 3129-3136 Available from: URL: http://www.wjgnet.com/1007-9327/14/3129.asp DOI: http://dx.doi.org/10.3748/wjg.14.3129

INTRODUCTION

Intraductal endoscopy describes the use of an endoscope to evaluate the biliary and pancreatic ducts. There are significant technological challenges encountered in creating a scope that allows direct visualization of these ducts. However, attempts have been made, and technology is developing that promises greater opportunity to provide improved diagnosis and therapy regarding lesions in the biliary and pancreatic ducts.

HISTORY AND TYPES OF SCOPES

Cholangioscopy was considered as early as the 1950's[1]. However, technology at that time caused severe limitations. In the 1960's, intraoperative cholangioscopy was first successfully utilized[2-4]. Peroral cholangioscopy (POCS) was initially described in the mid-1970's. One of the first reports demonstrated that a fiberscope of 8.8 mm diameter could be directly inserted through the mouth, into the biliary system after an endoscopic papillotomy, without the need of using a second scope as a guide[5]. This scope did provide a biopsy channel to obtain tissue samples. Other investigators also successfully demonstrated the use of POCS to directly visualize the biliary system during this time[6-10].

The idea of guiding a small caliber "baby" cholangioscope through the channel of a "mother" duodenoscope into the common bile duct (CBD) gained acceptance. This "mother-baby" system is also known as duodenoscope-assisted cholangiopancreatoscopy (DACP). However, use of the early cholangioscopes was difficult since

their optical fibers were prone to break easily from pressure applied with the elevator of the duodenoscope. Regardless, Urakami demonstrated successful access to the ductal system in 25 of 30 cases in 1980 by using this technique[11]. The University of Chicago published their experience with a conventional "mother-baby" system utilizing a set of Olympus scopes (TJF-M20 and CHF-B20) (Olympus Inc, Tokyo, Japan), where the "baby" scope had a diameter of 4.5 mm, two-way deflection and included an instrument channel[12]. This system was used in patients 18 times over a 3-year study period. Initially, they demonstrated a steep learning curve, when they intubated the papilla in only 2 of 5 cases. They subsequently found it was necessary to perform a papillotomy before the "baby" scope could be passed. After this adjustment, they were successful at intubating the papilla in 13 of 13 cases. While the 1.7 mm working channel on the cholangioscope did allow for diagnostic and therapeutic intervention, the system was found to be cumbersome to use. Average time of the procedure was around 2 h. Two endoscopists were required (i.e. one for each scope). The cholangioscopes continued to be fragile and prone to breaking. Further, these cholangioscopes only had two-way deflection at the tip as opposed to the typical four-way deflection offered by other endoscopes. These limitations led this group to conclude that while this "mother and baby" system certainly offered new endoscopic potential, it would best be utilized in only select patients at highly specialized tertiary referral centers. Another study at Case Western Reserve University further validated the use of this Olympus system by successfully visualizing the biliary tree in five patients[13]. The steerable properties of the cholangioscope combined with the presence of the accessory channel allowed it to have significant advantages over past attempts at POCS.

The search for a less cumbersome technique to directly visualize the biliary tree led to a small pilot study with an attempt to perform direct visualization of the biliary tree with an ultra-slim upper endoscope[14]. This technique used endoscopic retrograde cholangiography (ERCP) to place a super-stiff 0.035-inch diameter Jagwire (Boston Scientific Corp, Natick, Mass) in the CBD. Using the wire to maintain access, the duodenoscope was removed and an ultra-slim upper endoscope (GIF-XP 160, Olympus America Inc, Center Valley, PA) with an outer diameter of 5.9 mm was back loaded over the guidewire under fluoroscopic and endoscopic control into the duodenum and then across the ampulla of Vater into the CBD. Endoscopic sphincterotomy was required in order to permit passage of the endoscope into the CBD. This procedure was successful in providing direct cholangioscopy in 3 of 3 patients. Further studies will show whether this technique may have broader application. However, this technique can be performed by only one endoscopist, and the larger working channel (2.0 mm) of the endoscope allows for larger biopsies and the potential for more therapeutic applications.

Several miniscopes have been developed which allow the ability to examine the biliary and pancreatic ducts. The extreme small size of some of these scopes, ranging as small as 1 to 15 French in diameter, allowed for their delivery into even the smallest of ducts, and could allow access without the presence of papillotomy when the outer diameter of the scope is less than 2.5 mm[15-19]. While these very small scopes raise interesting possibilities, their use is limited by their fragility, lack of tip deflection and lack of an inner working channel. A fine-caliber flexible miniscope created by Soda[20], allowed access to the bile duct without necessitating sphincterotomy due to its external diameter of only 2.09 mm. However, unlike many other fine-caliber miniscopes, this scope did have a central working channel of 0.72 mm.

Slightly larger miniscopes with bi-directional angulation systems and instrument channels were developed by Olympus (CHF BP 30 with 3.4 mm diameter) and Pentax (FCP-9P with 3.1 mm diameter and FCP-8P with 2.8 mm diameter)[21]. Sander and Poesl developed a new miniscope (2.3 mm in diameter) for POCS (PolyDiagnost, Reichertshausen, Germany), with a less fragile, steerable tip, which had two different degrees of stiffness. This scope has a working channel measuring 1.2 mm (3.6 Fr), through which a probe for electrohydraulic lithotripsy (EHL) and a stone extraction basket can be passed[22]. These two authors demonstrated successful pancreatoscopies with their scope in 8 of 10 cases and successful choledochoscopies in 11 of 11 cases. The presence of the instrument channel in all three of these scopes allows for therapeutic applications. Also, common to these miniscopes is their ability to be introduced through a standard therapeutic duodenoscope, hence these scopes could become part of a DACP (DACP) system. However, none of these scopes had separate air/water channels, and it is frequently necessary to continuously irrigate the bile ducts due to stone debris or sludge obscuring the view. Thus, at times, nasobiliary drainage tubes have been inserted in the bile duct along with the cholangioscope in order to allow irrigation to be effectively performed during the cholangioscopy examination.

While some of the fine-caliber miniscopes have been used to perform pancreatoscopy, one group of Japanese researchers has focused on developing a miniscope specifically designed to perform pancreatic duct visualization. Kodama and others developed a prototype peroral electronic pancreatoscope (external diameter 2.1 mm) and found its images did provide fine detail of the pancreatic duct[23]. They utilized an ultraminiature charge-coupled device with sequential color wheel method to generate images. This initial prototype scope was limited by its lack of a working channel. The group continued their development and in 2004 published their experience with another peroral electronic pancreatoscope prototype with a 2.6 mm external diameter and an inner working channel of 0.5 mm[24]. This scope was successfully inserted into the pancreatic duct without sphincterotomy in 7 of 9 cases. A duodenoscope was required to insert the scope into the pancreatic duct, and two endoscopists were required to perform the case. However, images were obtained that

provided excellent visualization of the pancreatic duct and sampling of pancreatic fluid could be performed *via* the working channel.

Recently, the SpyGlass peroral cholangio-pancreatoscopy system (Boston Scientific Corp, Natick, Mass) has been introduced[25]. This system makes use of a reusable optical probe, a disposable access and delivery catheter (SpyScope), and disposable biopsy forceps. The outer diameter of the SpyScope is 10 French. This system offers several advantages over previous cholangioscopes. It allows for single-operator control of both the duodenoscope and the SpyScope because the SpyScope catheter is mounted on the duodenoscope by a silastic belt. The endoscopist can sequentially manipulate the controls of both the duodenoscope and the SpyScope with one hand; thus, the need for two endoscopists is eliminated. This system also uses 4-way tip deflection, which allows for improved access of tertiary ducts. Further, the irrigation channel (0.6 mm) is separate from the working channel (1.2 mm), which allows for sustained continuous irrigation regardless of whether the working channel is in use. These advances have allowed this system to be used clinically in a number of tertiary referral centers.

Clinical data regarding the SpyGlass system continues to be collected; however, an initial feasibility study is available[26]. In this study, 35 patients underwent cholangioscopy with the SpyGlass system. Procedural success defined as attaining the diagnostic or therapeutic goal of the procedure. Procedural success was documented in 91% (32 of 35 patients). Sphincterotomy was frequently required in patients, in that 8 of 10 patients with intact sphincters required sphincterotomy at the time of the SpyGlass procedure. SpyGlass directed biopsy yielded promising results in that 19 of 20 (95%) of optically guided biopsies yielded specimens with adequate tissue for histologic evaluation. EHL was successful in 5 of 5 (100%) of patients when performed *via* the SpyGlass working channel. Two patients (6%) experienced procedure-related complications, namely ascending cholangitis in one patient and cholangitis with intrahepatic abscess in the other patient. Both patients recovered without sequelae. While this initial data is promising, the prospective data currently being collected from clinical use of the SpyGlass system will provide a better analysis of its potential impact on cholangiopancreatoscopy.

DIAGNOSTIC APPLICATIONS

Intraductal endoscopy may be used for multiple diagnostic indications (Table 1). Direct visualization of the ducts may increase the ability to differentiate and diagnose lesions accurately in comparison with standard imaging and ERCP techniques. In 1999, Siddique reported an experience of 61 choledochoscopies performed *via* the transpapillary route for diagnostic purposes[27]. Importantly, this study showed that direct visualization provided additional unsuspected diagnostic information in 18 of the 61 (29.5%) patients, beyond that which had been achieved by previous workup. A Korean study reviewed cholangioscopic findings from 111 patients with benign or malignant bile duct tumors[28]. By evaluating mucosal changes, presence of neovascularization, and patterns of luminal narrowing, it was determined that bile duct tumors did indeed demonstrate unique optical characteristics, that could allow optical differentiation among adenocarcinoma, adenoma, hepatocellular carcinoma, mucin-hypersecreting cholangiocarcinoma, biliary cystadenocarcinoma, and squamous cell carcinoma. Thus, it was felt that cholangioscopy can provide additional information that would be useful in differentiating benign from malignant lesions and would help characterize the type of malignant lesion. Another Korean study of 63 patients[29] with indeterminate strictures reported that cholangioscopy could potentially improve the diagnosis of cholangiocarcinoma by allowing for the optical recognition of an irregularly dilated and tortuous vessel, the so-called "tumor vessel." They found that this "tumor vessel" was noted in 25 of 41 patients with malignancy (61%), while no patients with benign stricture had this characteristic appearance. The value of direct cholangioscopy could be seen best in this study by combining the optical observation of tumor vessel with percutaneous transhepatic cholangiography-guided biopsy resulting in a diagnosis of malignancy in 39 of 41 patients (96%). This is a significantly increased rate of preoperative diagnosis when compared with percutaneous transhepatic cholangiography-guided biopsy alone (80.4% sensitivity for diagnosis in this study). In 2005, data from 97 patients showed the additive value of combining direct POCS with standard ERCP[30]. The combination of POCS and ERCP improved the sensitivity of diagnosing malignant lesions from 58% to 93%. Additionally, POCS was especially useful in evaluating 21 filling defects of uncertain etiology which had been noted on ERCP cholangiogram. POCS was able to correctly diagnose all 8 malignant lesions and all 13 benign lesions (i.e. accuracy of diagnosis was 100%). In particular, 4 fixed and immobile bile duct stones had the appearance of

Table 1 Diagnostic uses of intraductal endoscopy

Optically guided biopsies of stricture
Indeterminate stricture
Dominant stricture in primary sclerosing cholangitis
Evaluate fixed filling defect noted on cholangiogram or other imaging
Differentiate benign *versus* malignant intraductal mass
Optical examination yields visual clues
Improved yield from tissue sampling under visual guidance
Precisely map intraductal tumor prior to resection
Collect significant fluid sample for cytology
Visually evaluate intraductal mucinous neoplasms
Visually evaluate choledochal cyst
Visually evaluate for post-liver transplant ductal ischemia
Visually evaluate for intraductal spread of ampullary adenoma
Evaluate with visual exam and tissue sampling for infection
Cytomegalovirus
Fungal infection

tumor on ERCP, but were diagnosed correctly as benign stones at a glance with POCS.

Biliary strictures, with the exception of those clearly following surgery or trauma, are frequently concerning for malignancy. Obtaining adequate tissue from these biliary strictures, which can provide definitive diagnosis, is often challenging. Traditionally, ERCP may be of assistance in characterizing the stricture by providing tissue sampling; however, the low yield rates of ERCP-based methods for securing the pathologic diagnosis of malignancy has been demonstrated in multiple studies. The diagnostic yield is variable in the range of 35% to 70%[31-43]. Percutaneous transhepatic cholangioscopy (PTCS) and POCS have both been used to obtain visually guided biopsies. However, a risk of percutaneous cholangioscopy is the potential for tumor seeding along the tract. In 1997, Sato published results obtained from 25 bile duct carcinomas showing carcinomas and invasive carcinomas were diagnosed histologically from biopsy specimens obtained with PTCS guidance in 96% and 91% of the cases, respectively[44]. However, the sensitivity of a single biopsy for diagnosis for invasive carcinoma was only 62%, which demonstrated the need for multiple biopsies in order to obtain a higher diagnostic yield. In 2003, Somogyi reported the feasibility of using POCS with visually-guided biopsy to successfully directly biopsy an intraductal papillary mucinous tumor within the common hepatic duct[45]. Cholangioscopy additionally allowed precise mapping of the tumor in preparation for surgical resection. A 2006 report further details the usefulness of cholangioscopy in patients with indeterminate pancreaticobiliary pathology by evaluating 62 patients[46]. If a lesion was initially observed with direct POCS, biopsies were obtained under direct visualization (cholangioscopy-directed) or through the duodenoscope (cholangioscopy-assisted). Overall in this study, sensitivity to detect malignancy by utilizing POCS was 89%, and specificity was 96%, which continues to mark a significant improvement over utilization of only ERCP techniques to obtain tissue. As mentioned previously, the SpyGlass system has also been used for optically guided biopsy[26]. The sensitivity and specificity for diagnosis utilizing SpyGlass-directed biopsy was 71% and 100%, respectively, in evaluation of 20 patients' intraductal lesions. Current multi-center trials will shed more light on the use of this new system.

Attempts have been made to utilize POCS in patients with primary sclerosing cholangitis (PSC). A study from the University of Colorado examined 41 PSC patients with POCS[47]. In order to evaluate dominant strictures, POCS-directed biopsies were obtained. In cases where the cholangioscopic biopsy forceps could not pass through the operating channel due to angulation, POCS-assisted biopsies were obtained. Impressively, tissue samples were adequate for histologic evaluation in 32 of 33 patients. The median follow-up period of 17 mo, has shown that this method of evaluation was able to successfully exclude cancer in 31 of 31 patients (100%) where biopsies were negative. The predominant difficulty in this study came due to limitations of technology with the cholangioscopes which were used (Olympus CHF BP30, Olympus CHF B160, Pentax FCP 9P), in that the stricture of interest could not be traversed in 14 cases. Another study detailing the use of POCS in PSC was published by a German group in 2006[48]. In this study, 53 PSC patients with dominant bile duct stenoses underwent transpapillary cholangioscopy and POCS-assisted tissue sampling in addition to ERCP. This study found that utilization of cholangioscopy was statistically significantly superior to ERCP for detecting malignancy in terms of its specificity (93% *vs* 51%) and accuracy (93% *vs* 55%). Thus, this group concluded that transpapillary cholangioscopy significantly increases the ability to distinguish between malignant and benign dominant bile duct stenoses in patients with PSC.

Direct pancreatoscopy can also play a diagnostic role in differentiating pancreatic duct lesions[49]. Pancreatoscopy can visualize chronic scarring and stenosis of the duct, pancreatic duct stones, and intraductal papillary-mucinous neoplasms (IPMN's) of the pancreas. In 1997, peroral pancreatoscopy was utilized to evaluate carcinoma *in situ* of the pancreas[50]. The carcinoma *in situ* in the main duct had the optical appearance of papillary mucosa, irregular mucosa, or nodular mucosa. Pancreatic juice collected during pancreatoscopy provided a better yield than traditional catheter collection, in that fluid collected during pancreatoscopy from all 11 patients with carcinoma *in situ* yielded positive cytology, while only 7 of 11 patients' cytology was positive when collected without direct pancreatoscopy. Thus, this study concluded that peroral pancreatoscopy and pancreatoscopic cytology are indeed useful for locating and diagnosing carcinoma *in situ* of the pancreas. In 1998, further evidence of the additive value of pancreatoscopy to supplement traditional diagnostic techniques was published[51]. In this report, pancreatoscopy was performed in 24 patients with intraductal mucinous neoplasms of the pancreas. Pancreatoscopy was able to detect 10 cases of intraductal mucinous neoplasms (IPMN's) that were not diagnosed with endoscopic ultrasound (EUS) or ERCP. Multiple other studies have evaluated the benefits of pancreatoscopy, especially in regard to evaluating intraductal mucinous neoplasms[52-58]. However, more recently, peroral pancreatoscopy has been combined with narrow-band imaging to emphasize certain image features often seen with IPMN's, such as mucosal structures and capillary vessels[59]. It is thought that the addition of narrow band imaging may aid in the diagnosis of the primary tumor and help in the determination of the extent of the tumor.

Other diagnostic uses of intraductal endoscopy include the evaluation of choledochal cysts[60-62]. Hemobilia of unknown etiology has been evaluated by cholangioscopy[63]. Infectious etiologies of bile duct pathology, such as cytomegalovirus (CMV) and fungal infections, have also been exposed by the use of direct cholangioscopy[27,64]. There also may be a role for evaluation of the biliary tree after liver transplant. A case report exists detailing the use of methylene blue-

Table 2 Current therapeutic applications of intraductal endoscopy

Stone extraction
Electrohydraulic lithotripsy (EHL)
Laser lithotripsy
Argon plasma coagulation (APC)
Photodynamic therapy
Nd-YAG laser ablation
Cystic duct stent placement

aided chromoendoscopy *via* POCS to optically diagnose extensive bile duct necroses and inflammation consistent with ischemic-type biliary lesions after transplant[65]. Other diagnostic uses of POCS will become evident as better technology allows for greater use of this modality.

THERAPEUTIC APPLICATIONS

Intraductal endoscopy is useful not only for diagnostic purposes, but it also has therapeutic applications (Table 2). Intraductal endoscopy has been frequently used to remove stones from within the ducts that cannot be removed by standard ERCP techniques in 5% to 10% of cases, due to size, location, or adherence to biliary epithelium[66]. EHL has been used in combination with POCS in multiple reports. EHL employs the use of a bipolar electrode in an aqueous medium. The probe is placed at the surface of the stone and directly observed using the cholangioscope. The probe emits spark discharges, which create a shock wave that fragments the stone[67]. Binmoeller reported, in 1993, that this technique was successful in removing stones where standard mechanical lithotripsy had failed in 64 of 65 patients[68]. Arya reported, in 2004, on experience with 94 patients who received POCS combined with EHL[69]. Of this group, 93 patients had failed previous standard stone extraction with ERCP. In this retrospective review, POCS combined with EHL was successful in performing stone fragmentation in 96% of cases, and stones were completely removed in 90% of cases. In both of these studies, there were no significant complications associated with the procedures. In elderly patients where biliary stone removal with traditional methods is unsuccessful, permanent biliary stenting has been attempted. However, Hui demonstrated in a prospective study of 36 high-risk patients with difficult CBD stones that POCS guided lithotripsy, when compared to stenting alone, allows for significantly less mortality and cholangitis[70]. Another study using EHL with POCS reported a 100% success rate for large bile duct stone removal after failure to remove the stone with a mechanical lithotriptor during ERCP[71]. In 2002, data from 36 patients who had strictly intrahepatic stones underwent POCS guided lithotripsy[72]. Indeed, this form of therapy was successful in these difficult cases to achieve complete stone removal in 64% of cases. Most recently, the SpyGlass-directed EHL system allowed for success in 5 of 5 patients, although after the initial procedure two patients did require repeat SpyGlass-directed EHL and one patient required repeat ERCP in order to achieve complete stone clearance[26].

Standard surgical management has been difficult for patients with gallstones which erode into the common hepatic duct and form a cholecystobiliary fistula (i.e. Mirizzi types 2-4). In 25 patients (23 patients with Mirizzi type 1 syndrome and two with Mirizzi type 2 syndrome), POCS combined with EHL allowed for successful treatment of the stone in all patients with type 2 Mirizzi syndrome, while it failed in both patients with type 1 Mirizzi syndrome[73]. Thus, it was felt that POCS guided therapy may offer a safe and effective alternative to surgery in patients with type 2 Mirizzi syndrome.

There are other therapeutic interventions which have been coupled with POCS. Multiple reports describe the use of cholangioscopy along with laser lithotripsy[12,74,75]. Laser lithotripsy may be used under fluoroscopic or direct cholangioscopy guidance. Current evidence indicates that POCS-guided laser lithotripsy is especially preferred in cases of intrahepatic stones or in patients with stones situated proximal to a bile duct stenosis[76]. Photodynamic therapy, under peroral cholangioscopic guidance, has also been utilized for patients with biliary tumors. In 1998, Ortner reported on the use of photodynamic therapy under cholangioscopic guidance to treat nonresectable Bismuth type Ⅲ and Ⅳ cholangiocarcinoma[77]. In this study, therapy was successful at restoring biliary drainage, improving mortality and enhancing quality of life. In 2003, Ortner reported results of a randomized trial of cholangioscopically guided photodynamic therapy with stenting *versus* stenting only for nonresectable cholangiocarcinoma[78]. The improvement of survival in the group receiving photodynamic therapy was so impressive that it was considered unethical to continue with randomization after the first 39 patients. Specifically, the photodynamic therapy group had median survival to 493 d, while the stenting only group had median survival to 98 d ($P < 0.0001$). Treatment with photodynamic therapy and stenting also led to improvement of cholestasis and quality of life compared with endoscopic stenting alone. Argon plasma coagulation (APC) has also been utilized under direct optical guidance to treat an intraductal papillary mucinous neoplasm involving the extrahepatic bile ducts[79]. However, in this case, after cholangioscopic evaluation, a thin gastroscope (Olympus GIF-H180, Olympus America Inc, Center Valley, PA) was introduced across the papilla into the bile duct, since the APC probe would not fit down the working channel of the cholangioscope. Other therapeutic applications reported in concert with cholangioscopy include Nd-YAG laser ablation of tumor stent ingrowth and biliary angiodysplastic lesions[27].

COMPLICATIONS AND SAFETY

There are no large trials specifically addressing the safety of intraductal endoscopy. Most information regarding safety and complications comes from individual case series, often with small numbers of patients enrolled. However, intraductal endoscopy is generally believed to

be a safe procedure with relatively few complications. Complications typically include minor bleeding at the time of sphincterotomy or lithotripsy[73]. There was one report of bile duct perforation following POCS guided EHL in 1993[68]. Obviously, the incidence of cholangitis is increased in patients with incomplete biliary drainage, from causes such as a biliary stricture or residual biliary stones; however, cholangitis has not been reported as a direct cause POCS[73]. Reports in the literature generally demonstrate a low threshold to give antibiotics in POCS guided procedures, but the use of antibiotics is based on the needs of an individual clinical situation. Pancreatitis has been reported in 2 of 52 (3.8%) of pancreatoscopy cases[49]. Complication rates will be better calculated as more intraductal endoscopic procedures are performed and further prospective data is collected.

COMPARATIVE PROCEDURES

There are two other significant methods which allow optical examination of the ductal systems and deserve brief mention due to their association with POCS. PTCS, also known as percutaneous choledochoscopy, and laparoscopic choledochoscopy have both been used extensively to for diagnostic and therapeutic purposes. While PTCS is more invasive than POCS, there are times when it allows excellent visualization, even in difficult anatomic situations where a POCS technique has failed[80]. Many of the same diagnostic and therapeutic techniques utilized with POCS are also used with PTCS, including targeted biopsy and management of stones with lithotripsy. One unique use of PTCS was documented, where a push-type sphincterotome was used *via* PTCS to create a papillary sphincterotomy and allow drainage of obstructing biliary stones in 3 patients who each had an endoscopically inaccessible papilla[81]. There are no reports of percutaneous pancreatoscopy. There have been no significant randomized studies directly comparing PTCS *versus* POCS. Generally, POCS is preferred as the initial therapy, due to its less invasive nature. However, if POCS is not available, or if POCS techniques fail, then PTCS may be used.

Laparoscopic choledochoscopy has been utilized to explore the CBD. Frequently, this technique has been utilized at the time of laparoscopic cholecystectomy, when intraoperative cholangiogram shows concern for retained CBD stones[82]. There are multiple surgical techniques which have been used to explore the CBD, but choledochoscopy *via* the cystic duct appears to be the safest and most effective approach, with success rates of 90%[83]. A benefit of this procedure is that the papilla may be left intact without sphincterotomy[84]. There is minimal experience with using laparoscopic techniques to perform pancreatoscopy; however, reports do exist[85].

CONCLUSION

Experience with intraductal endoscopy has shown its advantages over conventional ERCP in regards to the diagnosis and treatment of biliary and pancreatic disease. Direct optical examination may provide significant additional information about ductal lesions. Furthermore, the ability to guide instrumentation in the ducts under direct optical guidance provides significant advantages. As technology advances, the utilization of this endoscopic modality will only increase and new uses for this technology will likely develop.

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S- Editor Li DL **L- Editor** Rippe RA **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3137

World J Gastroenterol 2008 May 28; 14(20): 3137-3148
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Peter V Draganov, Dr, *Series Editor*

Pain management in chronic pancreatitis

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Received: October 28, 2007 Revised: January 30, 2008
Accepted: February 6, 2008
Published online: May 28, 2008

Abstract

Abdominal pain is a major clinical problem in patients with chronic pancreatitis. The cause of pain is usually multifactorial with a complex interplay of factors contributing to a varying degree to the pain in an individual patient and, therefore, a rigid standardized approach for pain control tends to lead to suboptimal results. Pain management usually proceeds in a stepwise approach beginning with general lifestyle recommendations. Low fat diet, alcohol and smoking cessation are encouraged. Analgesics alone are needed in almost all patients. Maneuvers aimed at suppression of pancreatic secretion are routinely tried. Patients with ongoing symptoms may be candidates for more invasive options such as endoscopic therapy, and resective or drainage surgery. The role of pain modifying agents (antidepressants, gabapentin, pregabalin), celiac plexus block, antioxidants, octreotide and total pancreatectomy with islet cell auto transplantation remains to be determined.

Key words: Chronic pancreatitis; Pain; Endoscopy; Endoscopic ultrasound; Pancreas

Peer reviewers: Tatjana Crnogorac-Jurcevic, MD, PhD, Cancer Research UK, Molecular Oncology Unit, Barts and The London School of Medicine and Dentistry, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom; Giuseppe Brisinda, MD, Department of Surgery, Catholic School of Medicine "Agostino Gemelli", Largo Agostino Gemelli, 8-00168 Rome, Italy

Gachago C, Draganov PV. Pain management in chronic pancreatitis. *World J Gastroenterol* 2008; 14(20): 3137-3148 Available from: URL: http://www.wjgnet.com/1007-9327/14/3137.asp DOI: http://dx.doi.org/10.3748/wjg.14.3137

INTRODUCTION

Chronic pancreatitis remains an enigma in the field of gastroenterology. Challenges can be encountered in defining the etiology and pathogenesis, in securing the diagnosis, and finally in providing adequate therapy. Chronic pancreatitis is a common problem, but the exact prevalence is unclear. Many patients suffering from chronic abdominal pain may indeed have unrecognized chronic pancreatitis. The prevalence in the developed world is reported from 0.4% to 5%[1].

In the western world alcohol abuse is the overwhelming etiologic factor. Of patients with chronic pancreatitis, 60% to 70% have 6 to 12 years history of heavy consumption of alcohol (150-175 g/d)[2]. Less common, but important etiological factors to consider, are ductal obstruction (from tumors and strictures), autoimmune, hypercalcemia, hyperlipidemia, toxins, and genetic. In a small number of cases, there is no identifiable causative factor and the pancreatitis is deemed idiopathic. It should not be surprising in view of this array of etiologic factors that there exist uncertainties in both diagnosis and ultimately treatment of chronic pancreatitis. Adding to the perplexity of this clinical situation are the multiple treatment options that can be provided by primary care physicians, gastroenterologists, interventional endoscopists, and surgeons. Despite the evolution of new medications and tools in the last two decades no clear consensus has emerged on the management of chronic pancreatitis. Most reports are either anecdotal or collected experiences of a single approach.

It is the purpose of this review to discuss the different modalities that are currently being used for the treatment of pain in chronic pancreatitis and to attempt to integrate them in a patient centered comprehensive approach.

PATHOPHYSIOLOGY OF PAIN IN CHRONIC PANCREATITIS

At least 85% of patients with chronic pancreatitis

develop pain at some point during the course of their disease. Painless chronic pancreatitis is rare, and more commonly late in the natural history of idiopathic chronic pancreatitis[3]. The frequency, severity and other characteristics of pain in chronic pancreatitis have a major impact on its management, the number of treatments, and the choice between medical and surgical interventions.

Several hypotheses exist as to the basis for pain in chronic pancreatitis; however, the exact mechanism is still not completely known. Possible mechanisms for pain include acute inflammation of the pancreas, increased pressure within the ductal system and parenchyma, neuritis, recurrent ischemia of the parenchyma; intra-pancreatic causes such as acute pseudocysts; and extra-pancreatic causes such as common bile duct or duodenal stenosis[4,5]. The relative contribution of each factor is unknown.

THERAPY OF PAIN IN CHRONIC PANCREATITIS

Medical therapy

Nonspecific supportive therapy: The first line in pain management is the use of medical therapy. The initial step of medical therapy usually is nonspecific supportive treatment. Supportive therapy is aimed at treating the concurrent symptoms and not the underlying factors in pain causation. Analgesic drugs are still the most commonly adopted method for pain relief. The obvious problem with this method of treatment is that patients often become dependant on heavy narcotic use. Most patients with chronic pancreatitis have their pain treated with analgesics on an episodic or continuing basis. Although the use of narcotics for the treatment of chronic pancreatitis is widespread, there are no controlled trials testing their efficacy as compared to the other modalities. Time intervals and doses of drug application must be adapted to the individual pain pattern. Although reluctance to use of narcotics is understandable, it should not be withheld if the treatment would otherwise not lead to adequate pain control[6].

There may be significant psychiatric, psychological, or psychosomatic contributions to the pain syndrome in these patients. Many physicians and surgeons use antidepressant medications as concomitant therapy, acknowledging the difficulty in assessing the psychological contributors to patients' pain syndrome. The benefits are anecdotal and variable in any individual experience and have never been rigorously assessed. It has been suggested that the natural path of chronic pancreatitis is toward progressive glandular insufficiency and calcification, and with the eventual 'burnout' would come spontaneous remission of pain[7]. There is a school of thought against conservative therapy. Pain is endured until burnout. This theory sheds light on the uncertainty regarding the duration of clinical pain, and if burnout is indeed a certainty and not solely a proposed hypothesis[8,9]. In conclusion, a strategy of waiting for spontaneous pain relief is not reliable and may be unreasonable advice for the patient with persistent or frequent severe pain.

Pancreatic enzymes: The presumed mechanism for pain relief after the administration of oral pancreatic enzymes is thought to involve the negative feedback inhibition to the pancreas. A cholecystokinin (CCK)-releasing peptide in the duodenum is normally denatured by pancreatic trypsin. In chronic pancreatitis, damage to acinar cells results in decreased secretion of pancreatic trypsin and consequently insufficient denaturing of the CCK-releasing peptide. This then leads to the potentiation and increased release of CCK, which causes pancreatic pain related to an increase in pancreatic enzyme output. When pancreatic enzymes are administered orally, there is more complete denaturing of the CCK-releasing peptide, thereby diminishing the release of CCK[10,11]. The results of studies examining the use of pancreatic enzymes that are administered orally to treat the pain of chronic pancreatitis have been variable, in part because of a high placebo response rate of over 35%, the potential for exogenously administered digestive enzymes to be inactivated by gastric acid and pancreatic proteases, and the lack of efficacy of enteric coated preparations[12-16].

In one of the earliest double-blind randomized trials of pancreatic enzymes, Isakson *et al* showed the pain relieving effect of oral enzyme preparations in a proportion of patients with chronic pancreatitis[16]. They took 19 patients with chronic pancreatitis, and treated them for 1 wk with a granulated pancreatic enzyme preparation (Pankreon®; five times daily 7.5 mL) or placebo and vice versa. Pain was evaluated using an analog scale and by questioning. A 30% pain reduction was seen after treatment with pancreatic extract compared to placebo. Fifteen of the nineteen patients had less pain during the week of treatment with pancreatic extracts. These results could not be confirmed by Halgreen, who conducted a 4-wk double-blind cross-over study with pancreatic enzymes (Pancrease®) in 20 chronic pancreatitis patients. There was no significant pain reduction[17]. In a placebo-controlled, double-blind, crossover study, pancrelipase (Viokase), in a dose of six tablets taken four times per day for one month, significantly reduced pain in 75% of patients with mild-to-moderate disease[15]. The best response was in young women with idiopathic chronic pancreatitis, whereas patients with advanced disease, including those with steatorrhea, had no response.

Of the 6 randomized trials published to date two studies using a non-enteric coated enzyme preparation reported benefit and four studies using an enteric-coated capsule showed no effect on pain in chronic pancreatitis. The conflicting study results led to investigators questioning the mechanism of negative feedback inhibition in the proximal small bowel[18]. As noted, the presumed mechanism for pain relief with administration of oral pancreatic enzymes is thought to involve feedback inhibition of the exocrine pancreas by the

degradation of CCK-releasing peptide in the duodenum. The administered enzymes would need to release activated serine proteases into the duodenum. This is much more likely with the non-enteric coated than the enteric-coated preparations, and hence the suspicion that the former are more effective. A meta-analysis of the six randomized, double-blind, placebo-controlled trials for the treatment of chronic pancreatitis with pancreatic enzymes showed no benefit in improving pain. The pooled estimate of the percentage of patients per study who preferred enzymes relative to placebo was 52% (95% confidence interval 45%-60%). This was not statistically different from 50%. Thus, this analysis demonstrates no significant benefit of pancreatic enzyme therapy to relieve chronic pancreatitis-associated pain[19]. It should be noted that this meta-analysis combines studies using enteric-coated and studies using non enteric-coated preparations. In that way, the potential benefit of non enteric-coated enzymes may have been negated by the lack of positive effect with non-enteric-coated preparation. The role of oral pancreatic enzymes in reducing pain in chronic pancreatitis, therefore, remains unclear. Additional studies are required to establish the effectiveness of this modality of treatment and to define whether certain subsets of pain: chronic *versus* intermittent pain; patients with or without exocrine insufficiency; alcoholic *versus* idiopathic pancreatitis; minimal *versus* extensive pancreatic duct changes; are more likely to benefit from enzyme therapy than others. Non-enteric coated enzymes are certainly safe and reasonable to try before considering more invasive or risky therapies.

Octreotide: Cholecystokinin-receptor antagonists or somatostatin analogues, such as octreotide, have been postulated to work on the negative feedback inhibition as well as hypertension of the pancreatic duct due to outflow obstruction. Inhibition of pancreatic secretion using somatostatin might, therefore, be effective in reducing pain in chronic pancreatitis. Octreotide is a synthetic somatostatin-analogue with an increased half-life, higher potency and the possibility of subcutaneous application. Experimental data suggest that octreotide increases the contractibility of the sphincter of Oddi, while somatostatin decreases it. This has, however, not consistently been demonstrated[20]. Normally, the release of cholecystokinin from specific intestinal cells is regulated by a cholecystokinin-releasing peptide in the proximal small intestine that is luminally active and trypsin-sensitive[13]. In chronic pancreatitis, exocrine insufficiency may lead to increased cholecystokinin-mediated stimulation of the pancreas. Theoretically, this process could be interrupted by the administration of cholecystokinin-receptor antagonists, or somatostatin. In a multicenter pilot study, octreotide, in a dose of 200 μg administered subcutaneously three times per day for 4 wk, reduced pain scores by 25% or more in 65% of patients with severe chronic pancreatitis[21]. On the other hand in a randomized, prospective, double-blind, placebo-controlled study conducted in Europe [100 mg subcutaneously (*sc*) every 8 h] administered to 10 patients for only 3 d was no more effective than placebo in relieving pain in chronic pancreatitis[22]. In a second study[23], octreotide (100 mg *sc* every 8 h for 3 wk) administered to six patients in a nonblinded fashion provided relief of pain in some but not all patients. In a third study[24], octreotide was administered to 84 patients for 4 wk in a randomized, prospective, double blind trial and showed a trend toward benefit at the highest dosage used (200 mg *sc* every 8 h). However, this effect did not reach statistical significance in this dose-ranging study. The longevity of the possible benefit was not established. Clearly further studies are needed before the use of octreotide can be widely adopted.

Antioxidant therapy: Bhardwaj *et al*[25] reported a decreased micronutrient intake (Vitamin E, riboflavin, choline, magnesium, copper, manganese and sulphur) in patients with chronic pancreatitis. This was due to diet modifications due to pain, as well as to a lower caloric intake. This points to the possibility that micronutrients deficiency may contribute to increased oxidative stress. In a comparison between patients with chronic pancreatitis and acute pancreatitis, the antioxidant profiles appeared to be different. Patients with chronic pancreatitis had significantly lower plasma concentrations of selenium, Vitamins A and E, beta-carotene, xanthine, beta-cryptoxanthine and lycopene in comparison with patients with recurrent acute pancreatitis[26]. Cullen *et al*[27] reported a decrease in antioxidant enzyme expression in pancreatic cells from normal pancreas to chronic pancreatitis to pancreatic cancer. Another observation concerning antioxidants is the altering of antioxidant status in chronic pancreatitis patients, which is worsened in patients with diabetes mellitus[28]. A 1-year clinical trial with 10 patients studied the effect of food supplementation using a complex containing l-methionine, beta-carotene, Vitamins C and E and organic selenium[29]. This resulted in a significant decrease in the intensity of pain as well as in days of hospital admission. Based on a placebo-controlled trial, followed by a retrospective cross-sectional study in 94 patients, some authors recommend antioxidant therapy consisting of supplements of methionine, Vitamin C and selenium[30].

Based on the observations that activation of oxygen free radicals can cause metabolic changes leading to pancreatic ischemia, antioxidant treatment with allopurinol seems a valid option. A trial with 13 patients with chronic pancreatitis investigated the effect of allopurinol on pain in a cross-over double-blind, randomized treatment trial[31]. Allopurinol, which is believed to reduce oxidative stress by inhibiting xanthine oxidase and thereby preventing the formation of oxygen derived free radicals, was given to 13 patients with pain occurring at least three times each week. Allopurinol was not effective in reducing pain or improving activities of daily living compared to placebo. In contrast, others showed that addition of allopurinol or dimethyl sulfoxide to intramuscular pethidine hydrochloride significantly

enhanced the efficacy of the analgesic regime[32]. This report suggests that removing oxygen free radicals in chronic pancreatitis may result in a beneficial therapeutic effect. The results of the most recent randomized trial presented only in abstract form showed that the combination of selenium, Vitamin C, β-carotene, Vitamin E, and methionine was significantly better in controlling pain compared to placebo[33]. In summary, there are conflicting data about the effectiveness of antioxidant therapy. A few trials show potential benefit, but further research is needed before it can become standard of therapy.

Endoscopic therapy

Endoscopists have shown that they can overcome pancreatic duct obstruction caused by ampullary stenosis, strictures, or stones. However, there have been no published validated guidelines for defining significant obstruction, and methodology for assessing patients before treatment and then judging the efficacy of that treatment. It should be noted though that the alternative to endoscopy, surgical sphincterotomy and sphincteroplasty, have already proven to be less efficient[34,35]. These interventions are hardly ever used now. This may also be due to the more acceptable rate of complication with endoscopic procedures, in conjunction with stent placement and stone extraction. For the present, the decision to perform endoscopy is based partially on subjective judgments that include assessment of the need for long-term narcotic therapy, marked diminution of the quality of life because of intractable pain, or major nutritional consequences of pain. When major pain episodes cannot be controlled by major, but acceptable maintenance analgesics, intervals of narcotics, or reasonable and brief periodic hospitalizations, a trial of interventional therapy can be justified. Among three recent studies involving stent therapy in 98 patients, at times associated with other interventional therapies such as lithotripsy and/or sphincterotomy[36-38], two studies[36,38] reported amelioration of pain and one did not[37].

The ideal treatment for patients with pancreatic-duct stones, dilated pancreatic ducts, and pain is not known. The stones can be easily removed coincidently with the performance of a surgical-drainage procedure, such as pancreaticojejunostomy. Alternatively, however, they can be fragmented by extracorporeal shock-wave lithotripsy (ESWL) and removed endoscopically after sphincterotomy of the pancreatic duct. Stones can be cleared by this approach in roughly 80 percent of patients, and approximately 50% of these have long-term relief of their symptoms[39,40]. Dumonceau *et al* conducted a randomized trial comparing pain relief after extracorporeal shock wave lithotripsy alone *versus* in combination with endoscopic drainage of the main pancreatic duct in patients with painful calcified chronic pancreatitis. Two years after trial intervention, 10 (38%) and 13 (45%) patients of the ESWL alone and ESWL combined with endoscopy group, respectively, had presented pain relapse. In both groups, a similar and significant decrease was seen after treatment in the number of pain episodes/year (mean decrease 3.7 episodes). There was no difference between the treatment groups and the treatment costs per patient were three times higher in the ESWL combined with endoscopy group compared with the ESWL alone group[41]. The claims for the efficacy of stone removal for pain relief should be considered in context with the observations that the presence or absence of stones does not necessarily correlate with the existence of pain. In the absence of randomized prospective trials comparing stone ablation either with placebo or with surgical decompression, it is difficult to assess the results of pancreatic stone removal.

An alternative involves the use of endoprostheses or stents placed in the pancreatic duct endoscopically. Reports indicate that 30%-76% of patients receiving such stents had symptomatic improvement over a period of 14 to 36 mo of observation[42-46]. Cremer *et al*[42], for example, noted initial improvement of symptoms in 94% of patients who were so treated for pancreatic-duct strictures and upstream ductal dilatation. In that group of patients, 53% remained free of symptoms over a mean follow-up period of 36 mo. Similarly, Grimm *et al*[43] showed that 57% of their patients were symptomatically improved by this treatment over a mean follow-up period of 19 mo. Although these results seem encouraging, a criticism is that most of the data reported to date were from relatively short term, nonrandomized studies. The issue is further complicated by the fact that pancreatic-duct stents may not be entirely harmless; for example, they may cause further pancreatic duct changes and potentiation of chronic pancreatitis[47-49]. Endoprosthesis occlusion and migration also seem to be relatively common.

Analyzing all the endoscopic modalities taken together it is usual to find a report of 80%-90% complete stone clearance and good immediate pain relief[47]. The long term results were not as favorable in the larger series. Delhaye *et al*[39] found that of 123 patients, only 60% experienced complete or partial pain relief during 14 mo follow-up. So far there are two randomized control trials comparing endoscopic therapy with surgery[50,51]. The study from Dite *et al* randomized 72 patients with large duct chronic pancreatitis to endoscopic therapy *versus* surgical lateral pancreaticojejunostomy. In addition, 68 patients were treated with endoscopy or surgery based on patient preference. The results between the randomized and nonrandomized study groups are similar. After 5 years of follow-up only 14% of the patients treated by endoscopy were pain free compare with 36% in the surgery group. The latest randomized controlled study comparing endoscopy with surgery (lateral pancreaticojejunostomy) enrolled 36 patients. The results are strikingly similar to the previous study. Pain was absent in 16% of patients treated with endoscopy and 40% in patients treated with surgery. Based on these trials it appears that surgery provides better pain relief compared to endoscopy, but even surgery fails to provide substantial pain relief in more than half of the patients[51].

Endoscopic treatment may have a place in the prevention of acute relapsing pancreatitis, more so than treatment of the pain of chronic pancreatitis. To avoid this potential problem, some have suggested that endoscopically placed pancreatic-duct stents should be used only for relatively short periods. This serves as a screening procedure, to identify those patients most likely to benefit from surgical drainage[42,45,52]. At present, endoscopically placed stents should be considered an unproved, but potentially useful approach to the treatment of chronic pancreatitis.

Kozarek and Traverso[53] have analyzed collected experiences and indicate that the likelihood of symptomatic improvement with combination endotherapy is reported to be 50%-85% at 15 to 25 mo. Successful pain relief has been correlated anecdotally with stone removal and subsequent decrease in diameter of the pancreatic duct. As a rule, the focus is on stones in the main duct and the morbidity of side-branch stones has not been defined. Better selection of patients for endotherapy may be helpful in order to maximize results. Due to its low degree of invasiveness, however, endotherapy can be offered as a first-line treatment, with surgery being performed in case of failure and/or recurrence.

Nerve blockade

Although this modality is thought to be medical management, it may be administered *via* endoscopic or interventional radiological means. Although widely used, there have been relatively few formally reported experiences with nerve blocks for long-term therapy of chronic pancreatitis. Leung *et al*[54] studied the use of celiac block in 23 patients with chronic pancreatitis. Twelve of the 23 had complete analgesia, whereas six had partial relief. There was no effect in five patients. The mean pain-free period in the chronic pancreatitis patients was only 2 mo, and the longest 4 mo. Benefit was least in patients with previous pancreatic surgery and repeat blocks were unhelpful.

Because of possible concerns about potential irreversible nerve injury, including very rare anecdotes of paraplegia from neurolytic agents, injection of steroids for the treatment of chronic pancreatitis has been recommended, instead of the use of alcohol injected into the celiac plexus (principally used in the treatment of cancer pain)[55,56]. In one study, steroid injection provided relief of pain (lasting two mo) in only 4 of 16 patients[57]. Eleven of the 12 patients who did not obtain relief were narcotic dependent, whereas none of the four who obtained relief were narcotic dependent. This finding emphasizes the complexity of treating pain in a population of patients with chemical dependencies and other abnormal psychological and psychosomatic behavior. In another report[58], which investigated the mode of delivering the nerve block, only 2 of 8 patients with a CT-guided celiac plexus block experienced relief of pain compared with 6 of 14 who were treated by endoscopic ultrasonography-guided celiac plexus block with 10 mL of bupivacaine. The benefit from endoscopic ultrasonography-guided celiac plexus block seemed to persist longer that CT-guided block. More importantly, paraplegia has not been described after endoscopic ultrasonography-guided celiac plexus block, probably because of the anterior transgastric approach taken during endoscopic ultrasonography-decreasing or even eliminating the risk of nerve or spinal cord injury. The same group of investigators more recently published their prospective experience with endoscopic ultrasonography-guided celiac plexus block with steroids in 90 patients with pain resulting from chronic pancreatitis[59]. A significant improvement in pain score occurred in 55% of the patients. The benefit persisted beyond 12 wk in 26% of patients and beyond 24 wk in only 10%. Younger patients (< 45 years) and patients with previous pancreatic surgery for chronic pancreatitis did not appear to benefit from the block.

The current evidence indicates that endoscopic ultrasonography-guided celiac plexus block is safe and well tolerated, with excellent temporary results in some patients. Unfortunately, reliable predictors of success are lacking. In the absence of long-term studies with follow-up in patients with chronic pancreatitis whose pain is chronic, the role of endoscopic ultrasonography-guided celiac plexus block should be limited to treating flares of chronic pain in patients with otherwise limited therapeutic options.

Surgical treatment

Duval pioneered efforts to treat the pain of chronic pancreatitis by surgical means in the 1950s with trans-duodenal sphincteroplasty and with caudal pancreato-jejunostomy (the Duval procedure). The results of this procedure were fraught with variable and usually poor results, perhaps only helping some of those patients with true recurrent acute pancreatitis[60]. A more extensive drainage procedure, lateral pancreatojejunostomy, described by Puestow and Gillesby[61] and subsequently modified by Partington and Rochelle[62], was applied to the subset of patients with dilated main pancreatic duct and became the first surgical treatment widely considered to be effective for pain in this disease. At that time, however, its application was hampered because there was no way to determine preoperatively if a patient with chronic pancreatitis had the dilated ducts required for this procedure because neither ERCP nor CT was available until the 1970s. Thus, at exploration an intraoperative pancreatogram was used to select who would be candidates for lateral pancreatojejunostomy. In those without dilated ducts, the remaining options were to perform a sphincteroplasty (which was largely abandoned because of its failure) or to do nothing further. In the 1960s, surgeons began performing pancreatic resections for chronic pancreatitis, initially distal pancreatectomies (with poor results) and later distal subtotal (95%) resections, which were relatively more effective for pain, but rendered most patients diabetic[63]. Proximal resections of the head of the pancreas (i.e. Whipple procedures) were not widely applied until the 1980s, when the associated operative morbidity and mortality fell substantially[64-66].

Patients whose pain persists in spite of aggressive noninvasive treatment should undergo endoscopic retrograde pancreatography to define the caliber and morphologic characteristics of their pancreatic ducts. Depending on the population being studied, up to half of these patients may have dilated ducts, frequently with areas of stricture-the "chain of lakes" or "string of pearls" appearance; the remainder have either ducts of normal caliber (2 to 4 mm in diameter) or small ducts that may lack side branches-the "tree in winter" appearance[67,68]. Ducts larger than 8 mm in diameter can be successfully decompressed by an internal surgical-drainage procedure, such as a longitudinal pancreaticojejunostomy (the modified Puestow procedure)[60,62], but smaller ducts are not amenable to internal surgical drainage or resection.

Like most surgical procedures currently in use, those for chronic pancreatitis gradually became part of the armamentarium without undergoing rigorous testing and were never compared against medical treatment or no treatment. The vast majority of patients are still operated on when they continue to have intractable pain despite medical treatment. There are very few controlled trials in the surgical literature on this disease. The two randomized controlled studies comparing surgery with endoscopic therapy are discussed in the endoscopic therapy section. Surgical options include decompression/drainage operations, pancreatic resections, and denervation procedures. As with endoscopic interventional therapy, objective transferable criteria for the need for surgical intervention have not been developed or agreed upon.

Decompression/drainage operations: At present, the ultimate role of these various invasive approaches to the treatment of patients with large-duct, symptomatic chronic pancreatitis has not been established. Given the information available at the present time, most physicians recommend longitudinal pancreaticojejunostomy for patients with pain and dilated ducts. This operation may also retard the progression of exocrine and endocrine insufficiency[69,70]. Surgical decompression of the obstructed main pancreatic duct was for a long time the gold standard[71]. Drainage procedures today are most commonly side to side pancreaticojejunostomy. This particular procedure preserves parenchymal function. Longitudinal pancreaticojejunostomy is also used based on the concept the ductal obstruction leads to distention and that this in turn gives rise to pain and should thus be favored if the duct is widened. Ebbehoj *et al*[72] were able to show a relationship between the degree of pain and intrapancreatic pressure. Pancreatic pressure was measured by a percutaneously placed needle preoperatively, postoperatively, and one year after pancreatic duct drainage. Patients whose pressure decreased after surgery and remained low were pain free, whereas those with recurrent pain had increased pressure.

Theoretically, any procedure that improves drainage, either by improving flow into the jejunum or stomach, might be expected to relieve pain. Pancreatic decompression results in immediate and lasting pain relief in a high proportion (80%-90%) of patients with non-alcoholic chronic pancreatitis[73]. These procedures have been less successful with alcoholic chronic pancreatitis with pain relief averaged at 60%[74]. Although early good results have also been reported after a lateral pancreaticojejunostomy in patients with alcoholic pancreatitis, when these patients are followed for 5 years only 38%-60% of them continue to be pain free[75]. These operations are predicated upon the presence of a widely dilated main pancreatic duct (generally taken as > 6 to 7 mm) and the presumption that the dilated ducts imply an abnormally high pressure in the duct system[75] and in the pancreatic parenchyma[72,76]. The operation most commonly performed is a variant of the Puestow procedure, which is actually the Partington-Rochelle modification (lateral pancreaticojejunostomy)[62].

Many of the studies of lateral pancreatico-duodenectomy find that short-term pain relief is achieved in about 80% of patients and that the operation can be performed with a very low morbidity and mortality (0%-5%). Although the short-term studies shine a positive light on the procedure, long-term follow-up studies show that pain not uncommonly recurs. As time goes by, pain recurs, perhaps related to progression of the pancreatic injury and fibrosis. Pain relief for greater than two years is achieved in only 60% of patients[77,78]. Strategies for salvage in patients with persistent or recurrent pain after drainage procedures include redoing or extending the pancreatojejunostomy and resection procedures[79]. Of patients undergoing pancreatic duct drainage procedures, 25%-66% require concomitant biliary or gastric drainage, because of functionally significant obstruction of the bile duct or duodenum[80,81]. Biliary or duodenal strictures have been reported to be more likely in patients with large-duct disease than in their counterparts without dilated ducts[81].

The only reported attempt made to compare pancreatic duct drainage with no intervention in the management of pain is that of Nealon and Thompson[70]. In a series of 143 patients with chronic pancreatitis, 85% of the 87 patients who were treated by pancreatic duct decompression achieved pain relief, whereas pain abated spontaneously in only 1.3% of the 56 nonoperative patients. The study was not randomized, however, the principal criterion to determine candidacy for the operation was the presence of a dilated pancreatic duct. Thus, what the study actually reports is the outcome of pancreatojejunostomy in patients with dilated ducts *versus* the natural history of patients with chronic pancreatitis and no duct dilation. The study also found that deterioration of pancreatic function was slower in their patients with dilated ducts than in those with small ducts. Although this effect was ascribed by the investigators to the protection or relief afforded by the surgical drainage procedure, the cause and effect relationship is uncertain because of the differences in the patient population.

The consensus, albeit based on evidence from collected experiences, states that pancreatic duct decompression *via* lateral pancreatojejunostomy (a Puestow-type operation) can be accomplished with low associated morbidity and mortality and that pain relief will be achieved in the

majority of patients. For most experienced pancreatic surgeons, it is the preferred surgical treatment option in patients whose main pancreatic duct measures 6 mm or more because of its simplicity, safety, and benefits, including the advantage that remaining pancreatic tissue and function are at least not compromised further by loss from resection.

Drainage of pancreatic pseudocysts provides another form of pancreatic decompression in conjunction and even in continuity with a lateral pancreatojejunostomy when the main duct is also dilated. Up to 39% of patients undergoing lateral pancreaticojejunostomy have evidence of pseudocysts disease at the time of surgery[82]. Pseudocysts are found in about 25% of patients with chronic pancreatitis and have a much lower rate of spontaneous resolution than those that are a consequence of an attack of acute pancreatitis[82-84]. They can be the source of pain indistinguishable from that of the underlying chronic pancreatitis. In one study, surgical drainage resulted in complete short-term pain relief in 96% of 55 patients, and 53% remained pain free after a median follow-up of 11 years[84]. Endoscopic drainage of pseudocysts into the stomach or duodenum may be an alternative, especially in patients who do not have associated duct dilation. Studies directly comparing surgical with endoscopic drainage of pseudocyst are lacking.

It should also be mentioned that there are numerous variations of the previously mentioned operations. Frey *et al*[85,86] combined a coring out of the pancreatic head with a lateral pancreaticojejunostomy. In his series, the pain relief after 5 years was complete or improved in 87% of cases. There is also one randomized series of patients comparing the Beger and Frey procedure[86-90], with no difference in decrease of pain, but less morbidity with the Frey procedure.

Resection procedures: The therapeutic principle of resection is based on the assumption that pain in chronic pancreatitis is predominantly caused by inflammation. This inflammation then becomes the nidus for qualitative and quantitative changes of nerve fibers. This is especially seen in the clinical scenario of normal sized ducts and masses of the head of the pancreas. Thirty percent of patients with chronic pancreatitis develop inflammatory enlargement of the pancreatic head with subsequent obstruction of the pancreatic duct, and sometimes also of the common bile duct and duodenum. In these cases a pancreaticoduodenectomy, "Whipple procedure", has been the procedure of choice for a long time, as it provides reasonably effective pain relief. These resections, however, have both immediate postoperative morbidity and long-term morbidity. Insulin dependent diabetes mellitus has an increase in the incidence from 20% preoperatively to 60% in the years that follow[81]. Also, postgastrectomy complications detract significantly from the overall quality of life. The long-term mortality rate and quality of life after this procedure in patients with chronic pancreatitis has not always been encouraging, and in some studies disappointing[71]. Distal pancreatectomy alone had poor results unless the disease is largely confined to the body and tail of the gland, e.g. with an occlusion of the mid-pancreatic duct or with a pseudocyst in the tail. By contrast, resection of the pancreatic head by either a conventional or pylorus-preserving pancreaticoduodenectomy will provide pain relief in up to 85% of patients, even if the disease extends into the distal pancreas. In order to deal with these undesirable consequences of the Whipple procedure, surgeons turned to the pylorus preserving pancreaticoduodenectomy (PPPD) and the "Beger procedure"[88-91]. Russel[92], in studying the results of preservation of the duodenum in total pancreatectomy compared with those of standard pancreaticoduodenectomy, found no difference in pain relief between the results of the two operations. He noted that 13 (14%) of the 32 still had severe pain after duodenum preserving total pancreatectomy, and that six required major analgesics. The purported benefits of better postoperative nutritional status and glucose control in the duodenum-preserving procedure were addressed in two randomized trials[93,94].

Frey and Amikura have recently reported a surgical modification that combines removing part of the anterior segment of the pancreatic head with longitudinal duct anastomosis to the jejunum[86]. A randomized trial[87] found little difference between the Frey procedure and the duodenum-preserving resection of the pancreatic head as described by Beger and Buchler[95].

Noteworthy in recent years has been the very low operative morbidity and mortality of pancreatic resection, which may be one reason for the larger numbers of patients with benign disease being referred for surgical treatment. In a recent series of 231 pancreatic resections, the most frequent indication being chronic pancreatitis, the operative mortality was 0.4%[66]. McLeod *et al*[96] studied the morbidity of the Whipple operation. Although the study focused on resections for neoplasms, the observations pertain as well to those for chronic pancreatitis and show satisfactory digestion, weight maintenance, and activity level in the great majority of patients. A study of quality of life after pancreatic resections found that diabetes and its complications had the greatest negative influence on everyday well-being[97].

Distal pancreatectomy[98] has a very limited role in management of pain, and only in patients with non-dilated pancreatic duct and pseudocysts involving the tail of the pancreas does this procedure seem to be associated with a good outcome[99]. Keith *et al*[100], analyzed the results of 80% distal pancreatectomy, pancreaticoduodenectomy and total pancreatectomy. After an average follow-up of 5 years, 9 years, and 6 years, respectively, he found that four of five patients after pancreaticoduodenectomy required narcotics. Thirteen of 32 patients had complete pain relief after 80% distal pancreatectomy. Finally total pancreatectomy is usually reserved as a last resort following a failed partial pancreatic resection.

Resection of pancreatic tissue results in the loss of

some exocrine and endocrine function and increases the possibility or hastens the onset of fat malabsorption and diabetes. Whereas only 20% of normal pancreatic tissue is required for clinically adequate function, the pancreas already damaged by chronic pancreatitis may have substantially reduced reserves even before resection. Because of the complete lack of insulin and glucagon after total pancreatectomy, very brittle diabetes may ensue and can be the source of considerable morbidity and even mortality. In an attempt to lessen these adversities, autotransplantation of either part of the organ[101] or of islet tissue[102] has been described. In the latter study, Farney *et al* obtained insulin independence in 20% of 24 patients at a mean follow-up of 5.5 years. A more extensive experience with islet cell autotransplantation was reported by the Minnesota group in 1995 comprising 48 patients[103]. Forty-seven of the 48 patients had small duct chronic pancreatitis. Only one postoperative death resulted, but 25% of patient's encountered complications. There were 8 deaths in the follow-up period, none apparently attributable to the operation. In follow-up, from 1 mo to 17 years, 39% of patients reported that pain was resolved, and 61% still had some degree of pain. Twenty of 39 evaluable patients (51%) had initial (less than 1 mo) insulin independence, but this dropped to 15 patients (38%) beyond 1 mo. A more recent European experience of 13 patients indicated sustained insulin independence in 5 of 9 surviving patients (4 late deaths) from 9 to 48 mo after surgery[104]. The latest studies suggest improvement in both the areas of brittle diabetes and in pain control. Rodriquez *et al*[105] recruited 22 patients who underwent pancreatectomy and autologous islet cell transplantation. All patients demonstrated C-peptide and insulin production indicating graft function. Forty-one percent were insulin dependent, and 27% required minimal amount of insulin or a sliding scale. Eighty-two percent no longer required analgesics postoperatively and 14% experienced a decrease in need for narcotics. Their success was attributed due to the provision of pancreatectomy and islet cell transplantation earlier in the course of the disease. Clayton *et al*[106] followed 40 patients who had pancreatectomy followed by islet cell transplantation. At 2 years post-transplant, 18 patients had a median HbA1c of 6.6% (5.2%-19.3%), fasting C-peptide of 0.66 ng/mL (0.26-2.65 ng/mL), and required a median of 12 (0-45) units of insulin per day. At 6 years, these figures were 8% (6.1%-11.1%), 1.68 ng/mL (0.9-2.78 ng/mL) and 43 U/d (6-86 U/d), respectively. The majority of patients (68%) no longer require opiate analgesia. Finally, Gruessner *et al*[107] performed 112 islet autotransplants at the time of total pancreatectomy. They found that islet autotransplants, at the time of total pancreatectomy in patients who had not had previous operations on the body and tail of the pancreas, were associated with > 70% of the recipients achieving complete insulin independence. In contrast, a previous distal pancreatectomy or a Puestow drainage procedure was associated with complete insulin independence in < 20%. Islet autotransplantation offers a valuable addition to surgical resection of the pancreas, as a treatment for chronic pancreatitis; and even in cases in which insulin independence is not achieved, the potential beneficial effects of C-peptide make the procedure worthwhile, particularly in early disease.

Many studies on pancreatic resection and even those on drainage procedures show that up to 15% of patients undergoing these surgical treatments for treatment of pain due to chronic pancreatitis will be found to have pancreatic cancer[7,79,99,108] and it has been shown that a chronic pancreatitis is in fact, a small, but real risk factor in the development of pancreatic cancer[109]. This is an important consideration to keep in mind during the diagnostic work-up and choice of operation. The morphology of the pancreas by CT imaging and by cholangiopancreatography may fail to discriminate between cancer and chronic pancreatitis. Cytological confirmation by fine-needle aspiration is helpful when positive, but the true diagnosis may become known only with resection (10% of cases). This consideration in some cases may determine the treatment strategy.

Surgical denervation: Most of the sensory nerves returning from the pancreas pass through the celiac ganglion and splanchnic nerves. It is hypothesized that interruption of these fibers may lessen pain. Mallet-Guy[110] reported an experience with 215 patients over 30 years whose principal treatment for pain was by sensory denervation. These patients first underwent abdominal exploration to document the absence of pancreatic ductal dilation or pseudocysts and to correct any associated biliary pathology; this was immediately followed by resection of the greater splanchnic nerve and celiac ganglion through a left translumbar approach. Although excellent long-term results are reported (90% of patients were pain-free, with 60% followed for more than five years), the heterogeneity of the patient population and the simultaneous use of biliary diversion procedures in many cases precludes meaningful conclusions. This treatment has not been widely accepted.

The celiac block can be done during laparotomy or percutaneously, usually from the back. The placement of the injection can be done simply by using anatomical landmarks or by checking the position with an imaging modality: fluoroscopy, scout X-ray films, ultrasonography, computed tomography, or at angiography. A nerve block with 25 mL of 50% alcohol on each side should be preceded by a positive diagnostic block with long acting local anesthesia, carried out at least 1 d earlier. The method aims at blockage of the splanchnic nerves before they reach the celiac plexus[111].

Stone and Chauvin reported on 15 patients with chronic pancreatitis who had previous unsuccessful operative procedures for pain[112]. Denervation was accomplished with a transthoracic left splanchnicectomy with concomitant vagotomy, and all 15 patients had immediate pain control. Five later suffered recurrent pain, but were successfully treated with a right splanchnicectomy. The long-term outcomes are not

known. The advent of thoracoscopic surgery has made this procedure more attractive, and a few small series have reported its feasibility and early results[113,114]. Maher *et al* recently reported on 15 patients with chronic pancreatitis, mostly idiopathic, with chronic pain measured by visual analogue pain scale[115]. Unilateral thoracoscopic splanchnic nerve resection in eight patients and bilateral in seven patients resulted in significant decreases in pain frequency and intensity, as well as in narcotic consumption. Overall, 80% of patients had good results or were improved, with a mean follow-up of 16 mo. A controlled trial comparing this procedure to other surgical options or to medical treatment is needed. Of note, pancreaticoduodenectomy and duodenum-preserving resection of the pancreatic head may well confer pain relief at least in part through denervation.

CONCLUSION

Pain is the most difficult to treat symptom of chronic pancreatitis. The current approach is largely based on data from studies of suboptimal quality and expert opinions. At present, a step wise strategy is recommended starting with life style modifications such as alcohol abstinence and low fat diet, then moving to high dose non-coated pancreatic enzymes and oral analgesic therapy. In patients with dilated main pancreatic duct unresponsive to medical therapy, endoscopy or decompressive surgery should be considered. Patients with debilitating pain, non-dilated pancreatic duct and inflammatory masses may be candidates for resective surgery. The role of pain modifying agents (antidepressants, gabapentin, pregabalin), celiac plexus block, antioxidants, octreotide and total pancreatectomy with islet cell auto transplantation remains to be determined.

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S- Editor Li DL **L- Editor** Rippe RA **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3149

World J Gastroenterol 2008 May 28; 14(20): 3149-3158
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Peter V Draganov, Dr, *Series Editor*

Pancreatic function testing: Here to stay for the 21st century

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Received: January 31, 2008 Revised: March 9, 2008
Accepted: March 16, 2008
Published online: May 28, 2008

Abstract

The diagnosis of Chronic Pancreatitis (CP) is based on the detection of abnormal structure or function of the diseased pancreas. The pancreatic function tests more accurately determine the presence of CP than tests of structure, especially for early stage disease. The function tests can be divided into two categories: non-invasive and invasive. The invasive "tube" tests can reliably detect mild, early CP, but are only available at a few referral centers and tend to be poorly tolerated by patients. The non-invasive tests are easy to obtain, but tend to perform poorly in patients with early, mild disease. Therefore, no one test is useful in all clinical situations, and a detailed understanding of the rational, pathophysiologic basis, strengths, and limitations of various tests is needed. This review highlights the role of various pancreatic function tests in the diagnosis of CP including fecal fat analysis, fecal elastase, fecal chymotrypsin, serum trypsin, the secretin stimulation test, the cholecystokinin (CCK) stimulation test, the combined secretin-CCK stimulation test, the intraductal and endoscopic secretin stimulation tests, and the functional magnetic resonance imaging of the pancreas after secretin stimulation.

Key words: Pancreatic function testing; Secretin stimulation test; CCK stimulation test; Fecal elastase; Endoscopic secretin stimulation test; Chronic pancreatitis

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Lieb II JG, Draganov PV. Pancreatic function testing: Here to stay for the 21st century. *World J Gastroenterol* 2008; 14(20): 3149-3158 Available from: URL: http://www.wjgnet.com/1007-9327/14/3149.asp DOI: http://dx.doi.org/10.3748/wjg.14.3149

INTRODUCTION

Gastroenterologists frequently encounter patients with Chronic Pancreatitis (CP), which is responsible for 86 000 annual admissions in the United States alone[1]. Even more frequently encountered is the patient with chronic abdominal pain and suspected CP based on equivocal imaging or laboratory findings. Although defined by irreversible histologic damage to the pancreas, histologic specimens are difficult and morbid to obtain. Therefore, in practice, the diagnosis of CP is based on the detection of abnormal structure or function (endocrine and exocrine) of the diseased pancreas. However, gross radiographic and endoscopic structural changes are insensitive and can be nonspecific - especially for early stage disease. Therefore, gastroenterologists are often forced to rely on tests of pancreatic function, the so-called, pancreatic function tests (PFT's), to diagnose CP. Arguably, these more accurately determine the presence of CP than tests of structure. Unfortunately, many of these PFT's themselves have significant drawbacks.

Several new PFT's have been introduced in the last 5-10 years, such as fecal elastase, Secretin-stimulated Magnetic Resonance Cholangio-pancreatography (S-MRCP), and endoscopic Pancreatic Function Testing (ePFT).

A few key points in using and interpreting PFT's are: first, they can be falsely positive for at least a few months after an attack of acute pancreatitis; second, negative PFT's do not exclude acute relapsing pancreatitis in patients who do not yet have structural or functional pancreas damage; third, although the best PFT's, especially the secretin-based stimulation tests, are more sensitive in the detection of CP than nonfunctional tests, rarely they still can miss early stage CP.

NORMAL PANCREATIC PHYSIOLOGY

In order to appreciate the utility of pancreatic function

testing, one has to understand the normal functioning of the pancreas. In the basal or fasting state, the pancreas excretes small amounts of protein - rich and mildly alkaline fluid. During a meal, gastric distension and acid production stimulate the duodenal S cells to release secretin into the blood, which signals the ductal cells of the small ducts of the pancreas to secrete a large volume of bicarbonate-rich, clear, watery fluid (so called *hydraulic secretion*). Similarly, the postprandial increase in amino acids and fatty acids in the duodenal fluid stimulates the I cells of the duodenum to secrete cholecystokinin (CCK, aka pancreozymin). CCK, in turn, signals the acinar cells of the pancreas to release enzyme-rich fluid into the pancreatic duct. This is so called *ecbolic secretion*[2]. For completeness, vago-vagal pathways also stimulate pancreatic secretion and modulate hormone release. These are primarily responsible for an increase in pancreatic secretion during the cephalic phase of digestion. The effects of these two hormonal systems (Secretin and CCK) are measurable and are abnormal in CP. For example, CCK levels are elevated in patients with early CP compared to controls, and these levels are often low in advanced disease[3]. In general terms, the chronically damaged pancreas produces decreased volume, bicarbonate, and enzymes in pancreatic juice in response to a stimulus than the normal pancreas. These decrements can be exploited during pancreatic function testing.

NATURAL HISTORY OF CP

Pancreatic function testing is clinically important for a number of reasons. First, CP is a heterogenous disease. Patients lie on a spectrum ranging from early, painful disease (so called minimal change, or small duct CP) with relatively preserved physiology to end stage disease with very little endocrine or exocrine function. Patients with early stage CP are very difficult to diagnose and distinguish from other causes of chronic abdominal pain. For example, conventional testing, such as pancreas-protocol computed tomography (CT) scans, Magnetic Resonance Imaging (MRI), and Magnetic Resonance Cholangio-Pancreatography (MRCP), generally detects patients with late stage CP, typically when 50% or more of the gland is fibrotic and has been essentially destroyed. Some experts suggest that the traditional pancreatic function tests may detect patients with as little as 30% damage to the pancreas[4].

Another reason that PFT's are useful is that clinical assessment of steatorrhea (exocrine dysfunction) is unreliable. Many patients can have steatorrhea with only a single formed bowel movement a day. Further complicating the prediction of steatorrhea is the often long course of acute pain relapses or early CP that occurs for many years before the development of steatorrhea. In natural history studies, the time to the development of steatorrhea is quite long, about 20 years. Part of this lag time is explained by the pancreas' extensive reserve of lipase secreting capacity. The pancreas has to lose ninety percent of its lipase production before steatorrhea is measurable by fecal fat testing. Yet, lipase depletion occurs earlier and is more profound than protease and amylase deficiency[5]. This fact can be exploited during pancreatic function testing. Part of lipase's vulnerability is its dependence on bicarbonate secretion by the pancreas to ensure a high duodenal pH - up to 7.5-9.0 for optimum activity. Endocrine dysfunction may occur at, or slightly after, the development of steatorrhea[6]. Certainly, the time course of exocrine and endocrine dysfunction varies depending on the etiology of the CP. As an extreme example, cystic fibrosis patients can present in infancy with failure to thrive due to exocrine failure[7].

PROBLEMS WITH STRUCTURAL (NONFUNCTIONAL) TESTS FOR CP

Besides the subtle progression of the natural history of CP as a reason for the utility of pancreatic function testing, many of the conventional tests in the detection of CP have a number of drawbacks.

CT

CT is fairly sensitive for the detection of advanced CP with calcification, atrophy, fat replacement, and ductal dilation. In some studies as high as 75%[8] to 80%[9]. However, others have found that when compared to better tests such as ERCP and Secretin-CCK function testing, CT is only 47% sensitive in the diagnosis of CP. The specificity of CT is considerably higher than the sensitivity, around 90%[10]. CT carries the additional benefit of evaluating the pancreas for other pathology (e.g. pancreatic cancer), and the whole abdomen for alternative explanation of the patient symptoms.

MRCP

MRCP is a fairly good test for the detection of advanced CP. However, even compared to the relatively insensitive endoscopic retrograde cholangiopancreatography (ERCP), MRCP is only about 75% sensitive for advanced disease and 25% for small duct CP[11]. Generally, MRCP detects many of the same changes that are seen on CT. An added benefit of MRCP is improved detection and characterization of biliary and pancreatic strictures compared to other noninvasive imaging tests. However, the visualization of the pancreatic duct (PD) can be difficult by MRCP, which depends on volume and flow in the pancreatic duct that is already quite low in CP. Non-occluding strictures can make visualization of the PD difficult. Generally, conventional MRCP, like CT, does not detect subtle side branch abnormalities of minimal change CP[12].

ERCP

This test involves cannulation of the pancreatic and biliary ducts. ERCP is generally considered the gold standard in the diagnosis of structural pancreatic duct diseases. In several studies, ERCP can even detect a very small number of patients with negative PFT's[13].

However, these changes can be seen in the normally aging pancreas, and, overall, the sensitivity of ERCP for small duct CP is significantly less than that of the best pancreatic function tests, even at the quarternary centers most proficient at ERCP[14,15]. Overall, ERCP has sensitivity of 66% for detecting minimal change CP and is 93% sensitive for late stage CP, compared to secretin stimulation testing[5]. In addition, ERCP is highly operator dependent. Furthermore, it is fairly invasive and carries a risk of up to 20% of acute post-ERCP pancreatitis which is greatest in the patients suspected of having minimal change CP (with non-dilated ducts). Recent preliminary data suggest that even a relatively mild episode of acute post ERCP pancreatitis may lead to CP when evaluated several years after the episode of post-ERCP pancreatitis[16].

Endoscopic ultrasound (EUS)

EUS is an excellent initial test of choice in the diagnosis of minimal change CP. It has relatively few risks, even if fine needle aspiration is used, and is as sensitive as MRCP in the detection of occult choledocholithiasis[17], and is superior to MRCP and transabdominal ultrasound in detecting cholecystolithiasis. However, it does have several drawbacks. It still requires sedation so a full day of work is missed - not only by the patient, but by a driver/chaperone - making it relatively expensive. EUS is highly operator dependent. In addition, even more than ERCP, EUS can be falsely positive due to the echotexture changes of the normal aging pancreas or in diabetics. Therefore, EUS is better at "ruling out" CP than it is at "ruling in" CP. Sensitivities and specificities of EUS vary from 90% and 85% *versus* histology for advanced disease[18] to 97% and 60%, respectively for EUS-FNA compared to ERCP[19], to 57% and 64%, respectively for plain EUS compared to secretin stimulation testing[20], to 83% and 80%, respectively in a mixed population of early and advanced disease compared to histology[21]. Much controversy surrounds the endosonographic definition of CP, with some groups still using 3 EUS criteria for CP, while most agreeing that 5 or more criteria must be present diffusely[22]. Unfortunately, to date no consensus exists on the exact EUS diagnostic criteria for CP.

PANCREATIC FUNCTION TESTS FOR CP

Noninvasive "tubeless" pancreatic function tests

In an effort to discover a sensitive and specific function test for CP that avoids risk and invasive procedures and that can be performed on outpatient basis, several tests have been developed, all of which suffer several severe shortcomings, but may be useful in diagnosing CP in a patient with a long alcohol history or with equivocal imaging findings. Generally, these tests only detect advanced CP with steatorrhea, but are fairly cheap and reliable.

Seventy-two hour fecal fat: The 72 h fecal fat collection was once a routine part of the workup for malabsorption, and it remains the gold standard for quantification of steatorrhea. However, it suffers from many drawbacks, including its nonspecificity for pancreatic disease. For example, bacterial overgrowth, short bowel syndrome, and small bowel mucosal disease (e.g. celiac disease and Crohn's disease) can present with steatorrhea. However, the diarrhea of CP tends to be less voluminous yet fattier than other diarrheal illnesses. In addition, the 72 h fecal fat is inaccurate when performed in the outpatient setting for several reasons. First, it is unrealistic to expect the patient to refrigerate 72 h worth of stool. Second, adherence to a standardized 100 g/24 h fat diet per day for a total of 6 d (the 3 d preceding the test and then the test itself) is difficult. Achieving at least 100 g/d, typical for a large fast food lunch of double cheeseburger and French fries with milkshake, is relatively easy (of course, false negatives can occur in the patient unable to consume that much fat due to pain, though, these are typically early CP patients, who do not yet have steatorrhea). However, quantification of daily fat intake with food diaries as an outpatient is unreliable, making calculation of the coefficient of fat absorption similarly unreliable. Third, for this test, the patient must be off of oral pancreatic enzymes supplements for about a week prior to collection. As a result, some patients have bloating, abdominal discomfort, and gas from malabsorption or are otherwise unwilling to stop the enzymes.

In our institution, for the above reasons, we reserve the 72 h stool collection for research purposes, during which time the patient is admitted to a metabolic ward with a dietician familiar with the protocol to monitor consumption and adjust later meals to account for what has not been consumed. A 72 h stool collection during a high fat diet showing more than 7 g/d fat in the stool is abnormal[23]. The levels of steatorrhea seen in CP tend to be much higher (often > 20 g/d). For practicality, most pancreatologists have abandoned this test. However, a modified 24 h protocol can be used for clinical purposes to monitor response to enzyme therapy in patients experiencing an unexplained increase in steatorrhea, especially in growing children with cystic fibrosis, despite alleged compliance with enzymes.

Spot fecal fat: Sudan staining of a random stool sample for fecal fat is relatively insensitive for fat malabsorption. Generally, it detects steatorrhea only at 25 g/d or more. As a stool collection, it suffers many of the drawbacks of the 72 h fecal fat, including patient embarrassment, need to stop pancreatic enzyme supplements, need to be on a high fat diet for several days before the collection, *etc.* Greater than 6 droplets of fat per high power field are indicative of steatorrhea. As in the case of the 72 h fecal fat analysis, fat substitutes in foods such as Olestra©/Olean© or drugs such as orlistat or ezetimibe can give false positive results.

Fecal chymotrypsin: In advanced CP, lower concentrations of pancreatic proteases reach the stool than in controls. Trypsin is the principal protease secreted by the

pancreas, however, it undergoes degradation in the distal small bowel so is not a good fecal marker for pancreas enzyme output[24]. On the other hand, several other proteases made by the pancreas, such as chymotrypsin are useful stool markers. As with all fecal protease assays, the fecal chymotrypsin should be thought of as a surrogate for the 72 h fecal fat rather than for the conventional, "tube," pancreatic function tests. Chymotrypsin evades degradation in stool by binding to insoluble debris in stool and is stable for several days at room temperature, enabling a sample to be shipped to a reference lab. A fecal chymotrypsin below 3 U/g of stool suggests advanced CP. This test is altered by exogenous pancreatic enzyme supplementation so is useful to monitor for compliance, but is not available in the United States[25]. The fecal chymotrypsin assay is of little clinical value to detect early stage CP, but it has a reasonable sensitivity for advanced disease of from 50% to 80%, increasing to 80%-90% in cystic fibrosis[26], with a specificity of 50%-100%[27-29]. As in all fecal protease assays, watery diarrhea, such as from short bowel syndrome, can give false positive results (low fecal chymotrypsin) by diluting the sample.

Fecal elastase (FE): Pancreatic elastase-1 is a pancreas-specific protease that is minimally degraded during intestinal transit. In fact, it is concentrated 6-fold in stool compared to duodenal juice[30,31]. The concentration of fecal elastase in stool measured by Enzyme Linked Immunosorbant Assay (ELISA) correlates well with duodenal amylase, lipase, and trypsin in both CP patients and controls[32]. Typically, a fecal elastase less than 100 mcg/g of stool indicates severe pancreatic insufficiency. A value between 100-200 mcg is indeterminant, but in the face of other evidence, is suggestive of CP. Values over 200 mcg are normal.

FE suffers from many of the same limitations of the fecal chymotrypsin assay, notably that it only detects patients with steatorrhea and severe CP that likely could have been detected by other means. In various studies, compared to conventional pancreatic function testing and ERCP, the sensitivity of FE varies from between 0%-65% for mild disease to 33%-100% for severe CP, with generally good specificity (from 29% to 95%)[33-37]. FE may be superior to fecal chymotrypsin. For example, in one small study the FE had a sensitivity of 64% for detecting CP compared to 25% for fecal chymotrypsin[38]. Also, like fecal chymotrypsin only a spot stool sample is required rather than a 24 h or 72 h collection. FE also does not cross react to exogenous porcine enzymes so patients can remain on therapy for the test. However, FE is more expensive than fecal chymotrypsin.

Serum trypsin: The serum trypsinogen (a.k.a. trypsin) assay is unique among pancreatic function tests in being a serum sample, making it convenient and relatively cheap. Low levels, less than 20 ng/mL, are specific for CP, but are sensitive only for advanced disease. Levels from 20-29 are indeterminant, but sometimes represent early CP[39]. Sensitivities for mild to severe CP patients combined range from 33%-65%, but specificity is quite high[40]. Sensitivity for exocrine dysfunction is quite high, at about 95%[39]. One added benefit is that trypsin levels over 150 ng/mL are indicative of pancreatic inflammation. For example, the trypsin can be positive for a relapse of CP even when amylase and lipase levels are normal. Conversely, it can help differentiate benign, chronically elevated amylase and lipase from pancreatic inflammation[41]. The test used in our institution is a Radio-Immune Assay (RIA), so it has the disadvantage of requiring several days to obtain a result. We typically obtain this test along with the fecal elastase and pancreatic protocol CT as an initial battery in all patients suspected of having CP referred to our clinic. However, like the fecal assays, it is basically a marker of advanced disease and steatorrhea.

Invasive, traditional, "tube" pancreatic function tests

Since first described in the 1930s and 1940s, several techniques have been developed to measure pancreatic function after physiologic or supraphysiologic stimuli[42,43]. The central theme of these tests is to collect and quantitate the quality of pancreatic secretions to determine pancreatic secretory capacity.

Secretin stimulation test (SST): In a technique more widely publicized by Dreiling[44], a double lumen, 26 Fr, oro-duodenal tube with both gastric and duodenal ports is introduced fluoroscopically, stiffened with a guidewire, with only topical anesthesia (benzocaine spray and viscous lidocaine) applied to the posterior pharynx. The weighted tip should be advanced close to the ligament of Treitz and the tapered radiopaque portion of the tube should be positioned at the pylorus. Placement can be hampered by multiple factors including patient discomfort, nausea, gastroparesis, and pyloric stenosis.

We place both the gastric and duodenal ports to low constant suction by an electric flywheel pump whose gauge measures 2-5 inches Hg (51-127 mmHg). However, the suction produced by these pumps may be lower than the gauge suggests: our lab has found that standard wall units are too strong and inconsistent and may result in adherence of the tube to the duodenal wall with clogging of the ports. Constant vigilance is required to prevent clogging of ports which decreases yield of duodenal fluid. During experiments with Polyethylene Glycol (PEG) labeled with carbon 14 (^{14}C), 85% or more of duodenal fluid can be collected with this double lumen "Dreiling" tube with relatively little reflux of duodenal contents into the stomach[45,46]. We then measure basal duodenal and basal gastric pH and volume over 15 min. Next, we give a bolus of intravenous (IV) secretin, because bolus administration has been shown to be equivalent[47] or superior[48] to continuous infusion. The typical dose of porcine secretin is a 1 U/kg IV bolus. This is a supraphysiologic dose, but is usually well tolerated other than some flushing. However, the cost of secretin is fairly high. One study showed that an even higher dose of secretin (4-5 U/kg) might be more sensitive[49]. We now use synthetic human secretin at

dose 0.2 mg/kg which has been shown to be equivalent to porcine secretin[50]. We then measure three parameters of the duodenal fluid collected over one hour in four 15 min aliquots: volume, pH, and bicarbonate concentration in mEq/L measured by back titration with hydrochloric acid. Others have found that automated analyzers are almost as good as the standard labor intense back titration[51]. The gastric pH and volume at the end of the study are also recorded. The highest concentration of bicarbonate obtained among the four 15 min aliquots is the peak bicarbonate concentration. For completeness, a microscopic exam is performed on the duodenal aspirate for Giardia, Gram stain, and Crystals. Then, the bicarbonate output (the product of bicarbonate concentration and volume) for that hour long post-stimulation period is calculated. The tube is then removed and the patient can resume normal activities and can drive home. Standardized ranges are 80-130 mEq/L for the peak bicarbonate, 1.5-5.7 mL/kg for the volume/kg of patient weight, and 10.1 to 37.0 mEq/h for the bicarbonate output. If the peak bicarbonate is less than 80 mEq/L, the patient is very likely to have CP. If the volume is low and proper position of the collecting tube is reconfirmed, we typically state that the patient should be evaluated further for a pancreatic duct obstruction.

The SST is arguably the most sensitive test for CP. Classically, bicarbonate is thought to be produced by small pancreatic ducts[2]. Consequently, one might anticipate that the SST would be the most sensitive test to diagnose small duct, minimal change CP. This hypothesis was upheld in several studies. The SST, when compared with histology, is 75% sensitive in detecting early stage CP, and up to 97% for late stage disease with a specificity of 90%[52,53]. Compared to SST, ERCP has about a 66% sensitivity for early disease, though it comes close to SST for late stage disease[4,54].

In addition, several histologic studies suggest bicarbonate production may be the best way to diagnose early CP. A study in dogs indicates that the maximal bicarbonate output is closely related to functional pancreatic mass[55]. In addition, an early study by Dr. Dreiling found an excellent correlation between findings on histology and findings of the SST. The SST picked up 20/24 patients (83%) who had CP by pathology whereas ERCP was only 17/24 (71%) sensitive. All underwent SST first, followed by ERCP, and 24 went on to exploratory laparotomy[54].

However, the SST does have some shortcomings, notably difficulty with tube placement and that false positives can be seen for several months after an attack of acute pancreatitis. This is the reason we delay EUS, S-MRCP, fecal elastase testing, fecal fat testing, and SST for several months after an attack of acute pancreatitis.

CCK stimulation testing: In use almost as long as the SST, the classical CCK stimulation test is a useful test, developed and used primarily at the Mayo Clinic in Rochester, Minnesota. Because this test measures ecbolic (enzyme) output, it is, in theory, a measure of the processes that lead to steatorrhea, and could be less sensitive than SST. However, it is still one of the most sensitive tests for the presence of CP. One study of normal controls in Japan found no differences between the SST and the CCK stimulation test[56]. The CCK stimulation test has a number of drawbacks including the need for placement of two specialized 2-lumen tubes with simultaneous gastric and duodenal aspiration and duodenal perfusion of a solution containing mannitol and PEG. CCK is also administered under constant infusion at 40 ng/kg per hour, but it can be given as a bolus[57,58]. Caerulein, which is found on the skin of tree frogs and can be produced synthetically, can substitute for CCK. Caerulein is, in fact, many times more potent a secretogogue than CCK[59]. Bombesin can also substitute for CCK[60].

In the classical CCK stimulation test, as in most tube tests, the basal 20 min aspiration of duodenal and gastric contents is discarded. The gastric and duodenal ports are continually withdrawn under low intermittent suctioning and duodenal fluid is collected over 80 min into four 20 min aliquots. Also during the first 20-40 min, the contraction of the gallbladder by CCK (and resultant flow of bile into the duodenum) affects the measurement of pancreatic output. In addition, as CCK can delay gastric emptying[61], and is thought to cross the blood brain barrier and mediate central pain mechanisms[62], symptoms of nausea and vomiting are common during infusion and more common than symptoms from secretin infusion[63]. The classic CCK stimulation test also requires measurement, and constant intestinal perfusion, of a nonabsorbable marker, and recovery rates vary significantly[64]. If the illustration in the *Gastroenterology* article which first described it is still in use today, it has fewer aspiration ports in the duodenum than the conventional Dreiling tube, and uses pressure suctioning of 40 mmHg[65] which, as mentioned above, may be somewhat different than the suction used at University of Florida with the conventional Dreiling tube.

A modified version of this test using a conventional Dreiling tube, placed under light sedation, and measuring only lipase by a hospital based lab assay was found to be very sensitive in patients with both early (Cambridge 2) and late stage CP by ERCP (Cambridge 3 and 4)[66]. However, no one has compared this test directly to the SST. In addition, as we shall discuss later in the section on endoscopic secretin stimulation testing, use of sedation may affect recovery of secretions and cost.

Combined secretin-CCK (secretin-pancreozymin) stimulation testing: This test is used mostly in Europe and Japan and allows measurement of both bicarbonate and enzyme production by the pancreas. In theory, the simultaneous administration of Secretin with CCK has the potential to dilute the measurement of enzyme activity by watery, bicarbonate solution. However, CCK can also be given before[67] or after[57] Secretin. It also shares one of the drawbacks of the CCK stimulation test: increased bile secretion into the duodenum.

In one study of the Secretin-CCK test, the peak bicarbonate - rather than CCK-related parameters - was correlated nearly linearly to the severity of histologic changes in CP. Also in this study, the second and third best measures of histologic damage were the amylase activity and the total volume, respectively. In that study, the secretin-CCK test was 67% sensitive for various stages of CP, which is somewhat less than other studies of the SST. However, this study used stringent requirements for the diagnosis of CP. All 3 parameters (peak bicarbonate, volume of duodenal secretions, and amylase output) had to be decreased in order to qualify as CP. Applying our cutoffs for peak bicarbonate, only, to this data would give greater sensitivity with only some loss of specificity[4,68].

Another study found that the trypsin activity in pancreatic fluid was not as sensitive a measure of CP as the peak bicarbonate during Secretin-CCK testing[69]. A recent, and probably the largest, study of Secretin-CCK stimulation testing supported this finding, mostly in cystic fibrosis patients. In this study, 336 CCK-Secretin tests were reviewed. Using enzyme (trypsin) activity alone (cutoff < 50 U/kg per hour) would have had 25% false positives if enzyme recovery were not corrected for losses (if a marker had not been used); i.e. 25% of patients with good enzyme activity would have been falsely classified as pancreas-insufficient[70].

A third study of 19 alcoholic CP patients and 6 patients with idiopathic CP who underwent CCK-secretin testing and went on to surgery for refractory symptoms, 18/18 of whom had an abnormal ERCP, found that the peak bicarbonate concentration and output were the best measures of small duct dilation seen on histology. In addition, peak bicarbonate output was the best measure of acinar atrophy with a Spearman correlation coefficient of -0.71 (P between 0.001 and 0.01) and the chymotrypsin output was also significantly correlated (Spearman, -0.57), but with a higher P of between 0.01 and 0.02. Peak volume also correlated fairly well with acinar atrophy (Spearman -0.44, P between 0.02 and 0.05), but peak bicarbonate concentration was weaker (Spearman -0.17, $P > 0.05$). In summary, this study found that the hydraulic parameters (volume, peak bicarbonate concentration, peak bicarbonate output) were overall better predictors of abnormal histology than the ecbolic parameters (chymotrypsin)[2].

These studies indicate that the CCK portion of the Secretin-CCK stimulation test adds little information in the diagnosis of CP that the secretin stimulation portion alone (or perhaps the classic SST) already provides. However, the Secretin-CCK test is certainly a more sensitive measure of pancreatic enzyme production than the bentiromide test, which tests primarily protease production by the pancreas[71].

Perfusion testing: Researchers in the Gastroenterology Division at the University of Florida in Gainesville over the last 25 years have developed and implemented a method of measuring endogenous and exogenous pancreatic enzyme activity in the duodenum of patients with CP analogous to the Mayo clinic CCK methodology. This "perfusion test" enables quantification of delivery of exogenous pancreatic enzymes to the duodenum. Some notable differences between this perfusion test and the CCK stimulation test include use of a standardized meal rather than CCK to stimulate the pancreas, use of a modified Dreiling tube attached to a 7 Fr Dobhoff tube, placed without sedation under fluoroscopy, and the perfusion of radiolabeled Carbon-14 Polyethylene Glycol (PEG) to enhance assessments of recovery. This perfusion test measures endogenous enzyme production in the fasted and fed states with a standardized Ensure® meal. Volume of both gastric and duodenal collections, pH, and enzyme activity are recorded over a 3 h period. The test is then repeated immediately after intake of an exogenous pancreatic enzyme[72]. The inconvenience and time required for this test render it useful only for the research setting.

Intraductal SST: In the intraductal secretin stimulation test, typically the main pancreatic duct is cannulated using ERCP techniques and then pancreatic fluid is collected, after the administration of secretin alone, or secretin followed by CCK. The patient is sedated without anticholinergic medications such as diphenhydramine (Benadryl®) or opiates, usually with benzodiazepines. Typically, the pancreatic fluid collected in this manner has a higher bicarbonate concentration than in the classical SST, around 130 mEq/L for controls, and less then 105 mEq/L for CP patients, owing to lack of contamination by bile and duodenal content. Some of the disadvantages of this test include the complication rate of ERCP, the need for sedation, and the relatively short time periods of collection (usually 15 min, as limited by sedation and fluoroscopy room time). An advantage of the intraductal test is that pure pancreatic juice is collected without contamination with bile or duodeno-gastric contents and that it can be used in patients with Billroth I and Ⅱ gastric resections.

One group showed that the intraductal test could not reliably differentiate between 19 CP patients, 14 "early CP" patients, and 14 controls[73], despite a long intraductal collection period of 60 min. The investigators used extra CCK with secretin after the initial secretin boluses in 15 patients. They used only 70 U maximum of secretin and did not adjust for weight of the patients. In addition, their aspiration catheter was prefilled with a dye to assist in identifying the start of the collection, which may have been problematic due to mixing. Also their "early CP" patients had only acute relapsing pancreatitis with no evidence of chronicity by imaging or conventional pancreatic function testing.

For the analyses, it appears they combined the patients with "early CP" and those with CP. They found that this combined group of CP patients produced significantly less volume of pancreatic secretions than controls after stimulation with 1 CU of secretin and 70 CU of secretin but not after 4 CU of secretin. CP patients also had significantly less bicarbonate concentrations only after 4 CU of secretin compared to

controls. Bicarbonate output was decreased significantly at all time points for CP patients compared to controls. Interestingly, at only the first minute time point, in patients with CP, the protein content of fluid was higher but not significantly so, than controls, perhaps due to concretions of inspissated enzymes in this group from PD stasis. However, after 70 CU of secretin, the protein output of CP patients was significantly less than controls.

A second, larger study of 12 patients with CP and 33 controls (22 normals and 11 with other nonpancreatic GI disorders), which used only a 20 min collection time, found that the sensitivity of the intraductal test peak bicarbonate compared to SST was 100% with a specificity of only 66%. Volume had an 88% sensitivity and a 91% specificity for CP by SST[74].

The most recent study of the intraductal secretin stimulation test was less favorable. In this comparison of the intraductal secretin stimulation test and SST, in which 19 patients served as their own control, the sensitivity of the intraductal secretin stimulation test compared to the conventional SST was only 80%, with a very poor specificity of 20%. Against pancreatogram, the intraductal test was 100% sensitive but only 55% specific[75]. This group used three 5-min collections (as is customary for most intraductal secretin stimulation tests) and the first was discarded. Based on these results, we do not recommend the use of the intraductal secretin stimulation test for routine diagnostic or research purposes.

Endoscopic secretin stimulation testing (eSST): An alternative to traditional pancreatic function testing is to sedate the patient, and collect duodenal juice under endoscopic guidance from a polyethylene tube passed through the biopsy channel of a standard upper endoscope after stimulation with secretin[51,76] or the combination of secretin-CCK[77]. This offers the advantage of patient comfort and sedation. The eSST has been extensively studied by a group of investigators from the Cleveland Clinic. The overall impression is that the eSST has the potential to yield results similar to the conventional SST. This comes to no surprise since the two tests are very similar with the main differences being the use of sedation in the eSST and the use of the endoscope to collect duodenal secretions rather than a Dreiling tube for the conventional SST. However, it should be noted that the eSST and the SST have only been directly compared in one small cross over study of healthy controls only, without any CP patients, in which the SST group also received sedation, which we do not do and could have confounded the results in favor of the eSST[78].

Unfortunately, the eSST has several disadvantages. Although the eSST is technically easy to perform, it is impractical, and to date it has not gained acceptance. The main problem appears to be that occupying an endoscopy room and keeping the patient sedated for more than one hour are cost-prohibitive. Although the Cleveland Clinic group has shown that a 45 min endoscopic collection is reasonable with good sensitivity with some loss of specificity[79], we have shown that a full 60 min is necessary for full sensitivity and specificity of the classical secretin stimulation test[80]. Furthermore, patients and their escorts will also have to miss a whole day of work. In addition, medications used for sedation may have effects on pancreatic secretions[81]. Opiates may constrict the sphincter of Oddi, and propofol contains 5% triglyceride which may have effects on pancreatic secretion. Although one small study of normal subjects did not find an effect of light sedation on secretion during endoscopic secretin stimulation testing, it used fairly low doses - 2.5 mg of midazolam and 62.5 mg of meperidine[82]. However, we have found that greater amounts of sedatives are required in most patients with chronic abdominal pain who are referred for evaluation of possible CP.

"Enhanced imaging" pancreatic function tests (S-MRCP): Because of some of the shortcomings of conventional MRCP in the diagnosis of pancreatic disorders, some have investigated the use of MRI with secretin stimulation to increase the flow and volume in the pancreatic duct. The filling of the duodenum can be semi-quantitated to assess for CP. One possible problem with this technique is that it measures volume of pancreatic flow rather than bicarbonate concentration. In theory, obstructive lesions, or sphincter of Oddi spasm could give positive results in the absence of true CP[83]. In addition, MR images are acquired over at most 30 min, which is often an insufficient length of time during secretin stimulation and which may lead to reduced sensitivity.

One German study of 18 CP patients, defined by ERCP, many of whom had previously undergone pancreatic duct stenting and removal, and 5 diseased controls exemplified some of these issues with S-MRCP. This study, even on these patients with obviously advanced CP, showed a 69% sensitivity of S-MRCP with 1 CU/kg of secretin and 90% specificity as compared to relatively insensitive pancreatic function tests, such as the fecal elastase and ^{13}C Mixed Chain Triglyceride Breath Test (MCT-BT)[83].

Another method that S-MRCP uses to assess for CP is parenchymal enhancement during gadolinium infusion (also used during conventional MRI, but which is not used during conventional MRCP). To assess for parenchymal enhancement, T1-weighted sequences with fat suppression are crucial. Also important is the pattern of gadolinium enhancement of the parenchyma: CP patients show decreased enhancement in the arterial phase and increased enhancement in the early venous phase, which are thought to be due to decreased pancreatic blood flow. On T2 imaging, enhancement is seen in CP patients compared to controls, indicating fatty or fibrous replacement of the parenchyma. After 0.5 IU/kg of secretin, the reduced T2 signal changes showed a good correlation with the Lundh test, a pancreatic function test using meal based stimulation. This study also showed a good correlation between

duodenal diameter after S-MRCP and the Lundh test. Patients with severe CP had an average increase in duodenal diameter of 1.7 mm. In mild CP the increase was 4.7 mm, and in controls, 14 mm. However, in this study, the patient population was not well defined. They did have a cohort with mild pancreatitis but again we do not know the criteria used to establish this[84].

Another group, this time from Japan, has distinguished S-MRCP, which they reserve to look for duct changes, from "Secretin-Stimulated, Diffusion Weighted MRI" which focuses on secretin-induced changes within the parenchyma of the gland. This new type of MRI calculates the Apparent Diffusion Coefficient (ADC) which measures diffusion of water molecules in the microcirculation. They claim that this type of MRI is even more sensitive than S-MRCP and that it evaluates local and regional pancreatic exocrine function. They also measured changes in alcoholic patients, known not to have structural pancreatic disease by conventional CT. Notably these patients did not undergo pancreatic function testing or ERCP[11].

CONCLUSION

Most pancreatic function tests have high sensitivity and specificity to accurately diagnose patients with advanced CP. The noninvasive tests tend to perform poorly in patients with early, mild disease. Some specialized invasive "tube" tests can reliably detect mild, early CP but are only available at a few quarternary referral centers. S-MRCP and Diffusion Weighted, Secretin Stimulated MR are promising technologies but, for the near future, are not likely to provide the same discriminating power as the best "tube" tests. The quest for a simple, noninvasive, cheap, and accurate pancreatic function test continues.

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S- Editor Li DL **L- Editor** Rippe RA **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3159

World J Gastroenterol 2008 May 28; 14(20): 3159-3164
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Analysis of surgical and perioperative complications in seventy-five right hepatectomies for living donor liver transplantation

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Received: February 15, 2008 Revised: April 17, 2008
Accepted: April 24, 2008
Published online: May 28, 2008

Abstract

AIM: To present an analysis of the surgical and perioperative complications in a series of seventy-five right hepatectomies for living-donation (RHLD) performed in our center.

METHODS: From January 2002 to September 2007, we performed 75 RHLD, defined as removal of a portion of the liver corresponding to Couinaud segments 5-8, in order to obtain a graft for adult to adult living-related liver transplantation (ALRLT). Surgical complications were stratified according to the most recent version of the Clavien classification of postoperative surgical complications. The perioperative period was defined as within 90 d of surgery.

RESULTS: No living donor mortality was present in this series, no donor operation was aborted and no donors received any blood transfusion. Twenty-three (30.6%) living donors presented one or more episodes of complication in the perioperative period. Seven patients (9.33%) out of 75 developed biliary complications, which were the most common complications in our series.

CONCLUSION: The need to define, categorize and record complications when healthy individuals, such as living donors, undergo a major surgical procedure, such as a right hepatectomy, reflects the need for prompt and detailed reports of complications arising in this particular category of patient. Perioperative complications and post resection liver regeneration are not influenced by anatomic variations or patient demographic.

Key words: Right hepatectomy; Surgery; Living-related liver transplantation; Surgical complications

Peer reviewers: Paulo Ney Aguiar Martins, MD, PhD, Surgery Department, Transplantation Division, Harvard Medical School, Massachusetts General Hospital, Boston 02129, United States; Dr. Adam G Testro, Department of Gastroenterology and Liver Transplantation, Austin Health Institution, Heidelberg 3032, Australia

Gruttadauria S, Marsh JW, Vizzini GB, di Francesco F, Luca A, Volpes R, Marcos A, Gridelli B. Analysis of surgical and perioperative complications in seventy-five right hepatectomies for living donor liver transplantation. *World J Gastroenterol* 2008; 14(20): 3159-3164 Available from: URL: http://www.wjgnet.com/1007-9327/14/3159.asp DOI: http://dx.doi.org/10.3748/wjg.14.3159

INTRODUCTION

Lortat-Jacob reported the first anatomic right hepatectomy in 1952[1]. Since then, and particularly in the past two decades, hepatic surgery has achieved important technical breakthroughs, such as intermittent portal triad clamping, total vascular exclusion, preoperative portal vein embolization with two-stage hepatectomy, and sophisticated methods of parenchymal transection.

An increased interest in the outcomes of right hepatectomy for adult to adult living-related liver transplantation (ALRLT) has likely contributed to these breakthroughs[2].

Although surgical techniques of excellence and major improvements in perioperative management are now a

reality in referral centers for liver surgery, there are still several issues that make this major surgical procedure extremely worrisome when performed in healthy individuals, such as living donors.

In particular, there is still no definite consensus regarding the amount of liver that can be safely resected[3], a crucial point for the recipient and perhaps more important for the healthy donor.

Recent studies have emphasized that in living-related liver transplantation, results and survival appear to correlate with stratification in the volume of the liver allograft transplanted, expressed either as a graft-to-body weight ratio or as a percentage of the standard liver volume of the recipient[4].

Clearly, living-related liver transplantation (LRLT) represents the natural evolution of other surgical procedures, namely reduced-size liver transplantation and split-liver transplantation[5], and is based on the segmental anatomy of the liver and on its peculiar capacity to regenerate.

This procedure represents a major challenge for the centers involved, though it has been widely reported that it is a valuable option for decreasing mortality rates and drop out from waiting lists[6,7].

However, potential risk for the donor makes this procedure unique, and when complications in the healthy donor arise, the implications for the medical community are potentially devastating[8]. A recent systematic review[9] that focused on adult donor outcomes concluded that there are small but real risks when using the right lobe for living donors, though it also claimed that nearly all donors returned to normal function within 6 mo. Moreover, due to the short history of ALRLT, the long-term risks for the living donor are still largely unknown.

Numerous single-institution series have reported their complications for liver living-related donors[10-15] and a recent large study from the U.S. reported an analysis of administrative data on a group of 433 right- and left-lobe living donors identified as those at risk for perioperative complications[16].

The ethical debate over the potential risk to the donor[17] renders this field of surgery controversial and, as a result, we believe, worthy of reports on all single center experiences.

The aim of this study is, in fact, to present an analysis of the surgical and perioperative complications in a series of seventy-five consecutive right hepatectomies for living related liver transplantation (RHLD) performed in our center.

MATERIALS AND METHODS

From January 2002 to September 2007, we performed 75 RHLD, defined as removal of a portion of liver corresponding to Couinaud segments 5-8, in order to obtain a graft for ALRLT. Two left-hepatectomies, corresponding to Couinaud segments 2-4, were performed for the same purpose during the initial phase of our experience, but are not reported in this study. The number of cases per year has been progressively increasing, with a peak reached in 2006, when 24 RHLD were performed. The trend has continued through this year (2007), with 17 RHLD performed so far. ALRLT represented the 20% of our total liver transplant activity over the last 2 years.

Donor selection and characteristics

All living donors went through a complete evaluation process, managed by a multidisciplinary team consisting of clinical psychologists, hepatologists, anesthesiologists, transplant surgeons, referring physicians and family doctors. The evaluation process was completed in 3 d, with blood work, ultrasound and consults on the first day; Volumetric Angio computed tomograghy (CT) Scan and Cholangio nuclear magnetic resonance imaging (MRI) on the second day; and liver biopsy on the third day.

Initially, the work-up included endoscopic retrograde cholangiography; this has since been replaced by Cholangio MRI.

Beginning in 2002, a total of 254 potential living donors were evaluated; 165 (65%) were excluded, and of those accepted for living donation, 12 (5%) are still undergoing work-up. At first we were more restrictive; as a result, all patients with aberrant vascular or biliary anatomy, or steatosis greater than 10%, were rejected. Then 20% macrovesicular steatosis was categorized as the upper limit.

Of the 75 living donors accepted, the ages ranged between 18 and 54, and all were biologically or emotionally related to the recipients. There were 46 ABO identical couples and 29 compatible couples (Table 1). These demographic data are quite similar to those reported online by the European Liver Transplant Registry concerning the activity of 135 institutions in 35 European countries in the period 1991-2005.

The CT-scan-calculated graft to recipient body-weight ratio was always above 0.8%, and all anatomic anomalies of the vascular and biliary system were detected by preoperative imaging (Table 1).

Two anti-hepatitis B core positive donors were immediately accepted[6] in accordance with the far-eastern experience, and were transplanted in two recipients with end stage liver disease secondary to hepatitis C virus, treated after transplant with lamivudine 100 mg/d.

Seven other donors were initially excluded because of their elevated body weight, which was a body mass index (BMI) of > 30. After nutritional assessment (nutritional and dietary past history, and life-style evaluation) the dietician arranged a personal diet, moderately hypocaloric (carbohydrates 55%-57%, proteins 17%-19%, and lipids 24%-27%) and encouraged the donor to perform physical activity. Acceptable monthly weight loss was considered approximately 2-4 kg, with a final BMI of < 30 kg/m^2. After 3 mo of a low calorie diet all seven living donors had a protocol liver biopsy that showed hepatic steatosis of < 20%, and were therefore considered eligible for donation.

Surgical complications were stratified according to the Clavien classification of postoperative surgical complications[18] (Table 2).

The perioperative period was defined as within 90 d

Table 1 Demographic, anatomic and surgical characteristics of 75 RHLD (mean ± SD)

Characteristics	*n*	Percent (%)
Age		32.27 ± 9.29
Range		[18;54]
Classes		
0-20	6	8
21-40	53	70.67
41-60	16	21.33
Sex		
Male	35	46.67
Female	40	53.33
Height (cm)		169.05 ± 8.86
Weight (kg)		68.19 ± 11.79
Donor relationship		
Biologically related	65	86.67
Sibling	10	13.33
Child	51	68.00
Parent	4	5.33
Not biologically related	10	13.33
Spouse	5	6.67
Other nonbiological	5	6.67
Donors		
ICU length of stay (d)		
Average		1.66
Range		[1;5]
Total length of stay (d)		
Average		8.26
Range		[6;14]
Length of donor surgery (h)		7.90 ± 1.75
Graft weight (g)		784.57 ± 158.15
GRBWR		1.43 ± 0.44
Bile ducts		
Double bile ducts	50	67
Single bile duct	25	33
Hepatic veins		
1	58	77
2	17	23
Hepatic arteries		
1	73	97
2	2	3
Portal veins		
1	65	87
2	10	13

GRBWR: Graft to recipient body weight ratio; ICU: Intensive care unit.

of surgery. Detailed descriptions of this surgical technique have been previously reported elsewhere[6,19].

Postoperative management and follow-up

After surgery, all donors were extubated before leaving the theater, and transferred to the intensive care unit (ICU) for at least 24-h monitoring. Deep venous thrombosis prophylaxis was based on early administration of low molecular heparin, started as soon as the prothrombin activity reached 50%, together with compression devices and early mobilization. Liver function tests were checked daily for at least 7 d, and then weekly for the first 2 mo. The subcutaneous administration of low molecular heparin was discontinued 14 d after surgery.

In order to guarantee optimal analgesia and early mobilization, all but two donors underwent epidural catheter placement immediately before surgery. Catheter removal was performed after 72 h, and after having normalized the coagulation parameters. Antimicrobial prophylaxis changed over time: the first 13 donors received piperacillin tazobactam for the first 72 h, after which prophylaxis consisted of ceftriaxone.

A CT scan of the abdomen was performed 2 mo after surgery, with volumetric analysis of the liver.

Three months after surgery all the donors were seen at the outpatient clinic for check up.

Statistical analysis

Data are expressed in mean ± SD for continuous variables and as percentage for categorical variables. Data were compared with chi-square test or Fisher's exact test 2 tailed for categorical variables and Student's *t*-test for continuous variables; $P < 0.05$ were considered significant.

All statistical analyses were performed using SPSS (SPSS Inc., Chicago, Ill, United States).

RESULTS

None of patients manifested any complications from pre-operative liver biopsy.

No living donor mortality was present in this series. No donor operation was aborted and no donors received any blood transfusion.

After the first 9 cases, we started to reinfuse the blood aspirated during surgery with the Cell Saver System (median: 250 mL; min: 0; max 1680).

Length of surgery, length of stay in the ICU, and total hospitalization are reported in Table 1, while all complications, codified according to the Clavien system, and their management, are reported in Table 2.

Twenty-three (30.6%) living donors presented one or more episodes of complication in the perioperative period. All these complications were resolved within the perioperative period.

Two donors (I.D. 1 and 12) had a small re-laparotomy because the intra-abdominal drain could not be removed.

One donor (I.D. 6) experienced a transient partial portal vein thrombosis, asymptomatically detected by ultrasound and completely resolved with low molecular heparin.

Two donors (I.D. 17 and 18) developed complications graded Ⅳ by the Clavien system. They were both admitted to the ICU: in one case for monitoring of an acute pancreatitis following an endoscopic retrograde cholangiopancreatography (ERCP) performed because of a biliary leak, and in the second case for monitoring of a pulmonary embolism with no cardiac derangements.

Five patients (I.D. 13, 14, 17, 20, 21) presented complications that required multiple treatments: i.e. percutaneous drainage and stent placement.

Two patients (I.D. 19 and 21) presented two discrete, unrelated complications each: pleural effusion plus intra abdominal collection in one case and pleural effusion plus biliary leak in the other case.

Seven patients (9.33%) (I.D. 5, 9, 13, 14, 17, 20, 21) out of 75 developed biliary complications, which were the most common complications in our series. However, all of them were successfully treated by interventional procedures with removal of all stents or catheters within 6 mo

Table 2 Classification of surgical complications in RHLD

Patients ID	Complications/Treatment	Classification of surgical complications Clavien annals of surgery 2004	Frequency (%) of complication for every classification grade
1	JP retained in the abdomen/Relaparotomy	Grade Ⅲb	13.04
2	Edema, ascites/None	Grade Ⅰ	21.74
3	Prolonged hyperbilirubinemia/None	Grade Ⅰ	
4	Fluid collection/Percutaneous drainage	Grade Ⅲa	17.39
5	Biliary leak /ERCP with stent placement	Grade Ⅲb, d	21.74
6	Transient portal vein thrombosis/Enoxaparin	Grade Ⅱ	13.04
7	Bilateral massive pleural effusion/Percutaneous drainage	Grade Ⅲa	
8	Colitis by CD/Metronidazole	Grade Ⅱ	
9	Biliary leak/ERCP (stent placement)	Grade Ⅲb, d	
10	Mild pleural effusion/None	Grade Ⅰ	
11	Intrabdominal fluid collection/Percutaneous drainage	Grade Ⅲa	
12	JP retained in the abdomen/Relaparotomy	Grade Ⅲb	
13	Intrabdominal fluid collection; biliary leak/Percutaneous drainage; ERCP (sphincterotomy)	Grade Ⅲb	
14	Intrabdominal fluid collection; biliary leak/Percutaneous drainage; ERCP (sphincterotomy and stent placement)	Grade Ⅲb, d	
15	Intrabdominal fluid collection/Percutaneous drainage	Grade Ⅲa	
16	Prolonged hyperbilirubinemia/None	Grade Ⅰ	
17	Intraabdominal fluid collection; Biliary leak/Percutaneous drainage, ERCP (sphincterotomy, stent placement X 3, acute pancreatitis, PTBD placement)	Grade Ⅲb, d/ Grade Ⅳ	4.35
18	Pulmonary embolism and iliac vein thrombosis/Anticoagulation	Grade Ⅳ	4.35
19	Moderate pleural effusion; intraabdominal fluid collection/Percutaneous drainage; percutaneous drainage	Grade Ⅲa- Grade Ⅲa, d	4.35
20	Biliary leak/ERCP (Sphincterotomy, endoscopic stent placement failure); PTBD	Grade Ⅲb, d	
21	Intraabdominal fluid collection; biliary leak, moderate pleural effusion/Percutaneous drainage; ERCP (sphincterotomy, endoscopic stent placement); percutaneous drainage	Grade Ⅲb, d	
22	Prolonged hyperbilirubinemia/None	Grade Ⅰ	
23	Fever/Antibiotic treatment	Grade Ⅱ	

Table 3 CT scan calculated donors mean liver volume (mean ± SD)

CT scan calculated		
Total liver volume	Right lobe volume	Remnant liver volume
1538.94 ± 277	954.67 ± 219.6	584.28 ± 121.67
	CT scan calculated right lobe volume 2 mo after surgery into the recipient	CT scan calculated remnant liver volume 2 mo after surgery
	1511.60 ± 257.88	1065.08 ± 195.24
	98% regeneration	69% regeneration

from surgery.

CT-scan-calculated donor mean total liver volume, mean right lobe volume, mean remnant liver volume, plus mean liver volume 2 mo after surgery in the donor and in the recipients, are reported in Table 3.

Mean value of donor liver volume was restored to 98% of the preoperative mean volume within 2 mo of surgery in the recipient and to 69% of the preoperative mean volume in the donor.

There was an 18% difference (P = 0.0001) between CT-scan-calculated right lobe donor mean volume (954.67) and right lobe weight mean value (784.56) on the back table.

There were no differences in distribution of anatomical variations in the groups of complicated and uncomplicated RHLD (Table 4). In addition, there were no differences between the complicated and uncomplicated RHLD regarding the baseline and post regeneration mean value of calculated liver volumes (Table 5).

Table 4 Distribution of anatomic variations in the complicated and uncomplicated groups of RHLD, *n* (%)

Anatomic variations	23 complicated RHLD	54 RHLD without complications	*P* value
Double bile ducts	15 (65.21)	36 (66.67)	
Single bile duct	8 (34.78)	18 (33.33)	0.78
Hepatic veins			
Single	21 (91.30)	39 (72.22)	
Double	2 (8.69)	15 (27.78)	0.09
Hepatic arteries			
Single	22 (95.65)	53 (98.15)	
Double	1 (4.34)	2 (1.85)	0.82
Portal veins			
Single	20 (86.95)	46 (85.19)	
Double	3 (13.04)	8 (14.81)	0.54

All patients returned to their own activity after this perioperative period.

DISCUSSION

Donor safety has to be the first priority during the entire process of living-related transplantation, from the first day of evaluation through the entire follow-up period.

Therefore, an accurate and comprehensive step-by-step work-up protocol for donor evaluation has been designed in our center in order to ensure donor safety and, additionally, to confirm that the donor is capable of providing a suitable graft for the recipient.

In our experience, use of routine liver biopsy, though not generally accepted in all centers, allowed the exclu-

Table 5 CT scan calculated donors mean liver volume in the complicated and uncomplicated groups of RHLD (mean ± SD)

	23 complicated RHLD	54 RHLD without complications	*P* value
CT scan calculated total liver volume	1517.7 ± 292.4	1547.20 ± 292.43	0.68
CT scan calculated right lobe volume	957.67 ± 226.37	953.50 ± 219.16	0.94
CT scan calculated remnant liver volume	560.05 ± 89.19	593.70 ± 131.69	0.28
CT scan calculated remnant liver volume 2 mo after surgery	1078.6 ± 201.65	1059.57 ± 194.42	0.72

sion of potential donors who otherwise would have been considered fit to donate based on other tests[20].

On the other hand, the biopsy allowed us to enroll donors who were anti-hepatitis B core positive.

Moreover, the routine use of liver biopsy as a screening tool in the living donor work-up allowed us to explore more safely the very common problem of steatosis.

The usefulness of steatotic livers depends on the percentage of fat, as livers with moderate to severe steatosis decrease graft and patient survival (with an additional unpredictable risk for liver donor regeneration). A BMI of > 30 may reliably predict a higher degree of steatosis in most donors. In order to enlarge the pool of living donor livers, but also to improve post-transplant outcomes, we made an attempt to lower the percentage of steatosis, rather than to turn down such overweight donors, by applying a short-term treatment of diet and exercise in all living-donor candidates with hepatic steatosis. After RHLD, no such donors experienced life-threatening complications or died. No long-term clinical impairment of treated donors has been observed and, after donation, all of them have returned to previous activity.

A strategy of careful evaluation of the living donor performed by an interdisciplinary team cannot be overemphasized.

A wide range of living donor complication rates are reported in the literature, with an estimated risk of mortality and morbidity after RHLD of 0.4% and 35%, respectively.

Overall, the complication rates range from 0% to 67%, with an overall crude complication rate of 31%[21]. The literature has reported 11 deaths, and 2 liver transplants in donors who have undergone RHLD. Additionally, one donor is in a persistent vegetative state after donation[22].

Organ shortage is a dramatic problem which can be limited by the rational use of ALRLT. So, based on our previous experience with liver resection[2] and use of partial livers from deceased donors[23], we began the living-related liver transplant program. Moreover, our partnership with one of the most active living-related liver transplant programs in the world[24] has allowed us to gain experience rapidly in this controversial field of surgery.

In our series, 30.6% of living donors developed a complication in the perioperative period, this not different from data recently reported in the literature[24]. In this group, RHLD with complications, there was no major incidence of anatomic variants, or difference in terms of liver regeneration after surgery, when compared with patients who did not develop any complications.

Additionally, our data regarding CT-scan-calculated liver volume confirmed that volumetric imaging may overestimate the actual liver volume[24].

Biliary complications (9.33%) were the most common complications after RHLD in our series, though no patients had to undergo repeated laparotomy for this reason. In two cases, after the failure of the endoscopic treatment, we were able to resolve the biliary leak due to a combined "rendezvous" procedure between endoscopist and interventional radiologist, who were able to pass an internal external transhepatic biliary drainage.

None of the 75 live donors in this series, regardless of their post-operative course, manifested any regrets about live donation.

In conclusion, this study reports the largest Italian experience with RHLD, focused on perioperative complications and on donor safety, which must be the first priority in right-lobe living-related donation.

Strict donor selection, detailed informed consent validated by the Italian law, together with a growing volume of cases performed every year, have allowed us to safely perform right hepatectomies for living donation in our center.

The need to define, categorize and record complications when healthy individuals, such as living donors, undergo a major surgical procedure like a right hepatectomy, reflects the need for prompt and detailed reports of complications arising in this particular category of patient.

Perioperative complications and post resection liver regeneration are not influenced by anatomic variations or patient demographic.

ACKNOWLEDGMENTS

The authors would like to thank Antonella Vassallo, Domenico Biondo and Warren Blumberg for their help in editing this paper.

COMMENTS

Background

Adult to Adult living related liver donors play an essential role in filling the gap of transplants needed due to a heavy shortage of cadaveric donations. Considering that living related donors are healthy individuals at baseline, it is imperative to ensure good outcomes and return to quality of life.

Research frontiers

The study improved measures to assure safety in the healthy donor, improved overall diagnostic capability by non-invasive tools in the donor work-up, and provided possibility of expanding Milan Criteria for recipient of living-related liver transplantation (LRLT). It indicated improvements in prevention of biliary complications and small-for-size syndrome.

Innovations and breakthroughs

Authors designed an accurate, comprehensive step-by-step work-up protocol for donor evaluation to ensure donor safety and to confirm that the donor is capable of providing a suitable graft. Their research has proven the necessity of evaluat-

ing the overall health of both the donor and recipient at many different levels from biopsy to body mass index. It indicated liver biopsy in the exclusion of potential donors otherwise considered fit to donate. These biopsies assess the quality of the donation to ensure the likelihood of success of the transplant and the health of both the donor and the recipient. These biopsies also confirm the true donor status of Hepatitis B, thereby allowing us to enroll donors who had false positive serum tests. They also test body mass index in order to prescribe a diet and exercise program to heavier donors to allow their inclusion. Their experience shows that heavier donors, when subjected to an exercise and diet program, all return to previous activity. In fact, no life threatening complications, long term impairments, or deaths have occurred in these donors. It indicated improvements in prevention of biliary complications and small-for-size syndrome.

Applications

It would be applied in improving in prevention of biliary complications and measures to assure safety in the healthy donor.

Peer review

This is an important issue that needs reporting. The authors performed a single institution series-report study of 75 patients stratifying them into two groups, complicated (23) and uncomplicated resections (54) to try to identify factors that might have influenced outcome. For this purposed, they analyzed anatomical variations and the liver remnant volume. With high wait list mortality and rather static donor levels, ALRLT is an option that needs serious consideration and historically the high rate of donor complications has held units back from moving forward with this procedure.

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S- Editor Li DL **L- Editor** Li M **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3165

World J Gastroenterol 2008 May 28; 14(20): 3165-3173
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Treatment and survival in a population-based sample of patients diagnosed with gastroesophageal adenocarcinoma

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Supported by The Health Research Board of Ireland, N01-PC-35133, N01-PC-35135, N01-PC-35136, N01-PC-35137, N01-PC-35138, N01-PC-35139, N01-PC-35141, N01-PC-35142, N01-PC-35143, N01-PC-35145, N02-PC-15105, N02-PC-15106 and N02-PC-15107
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Received: September 27, 2007 Revised: April 12, 2008
Accepted: April 19, 2008
Published online: May 28, 2008

Abstract

AIM: To examine the extent of use of specific therapies in clinical practice, and their relationship to therapies validated in clinical trials.

METHODS: The US National Cancer Institutes' Patterns of Care study was used to examine therapies and survival of patients diagnosed in 2001 with histologically-confirmed gastroesophageal adenocarcinoma (n = 1356). The study re-abstracted data and verified therapy with treating physicians for a population-based stratified random sample.

RESULTS: Approximately 62% of patients had stomach adenocarcinoma (SAC), while 22% had gastric-cardia adenocarcinoma (GCA), and 16% lower esophageal adenocarcinoma (EAC). Stage Ⅳ/unstaged esophageal cancer patients were most likely and stage Ⅰ-Ⅲ stomach cancer patients least likely to receive chemotherapy as all or part of their therapy; gastric-cardia patients received chemotherapy at a rate between these two. In multivariable analysis by anatomic site, patients 70 years and older were significantly less likely than younger patients to receive chemotherapy alone or chemoradiation for all three anatomic sites. Among esophageal and stomach cancer patients, receipt of chemotherapy was associated with lower mortality; but no association was found among gastric-cardia patients.

CONCLUSION: This study highlights the relatively low use of clinical trials-validated anti-cancer therapies in community practice. Use of chemotherapy-based treatment was associated with lower mortality, dependent on anatomic site. Findings suggest that physicians treat lower esophageal and SAC as two distinct entities, while gastric-cardia patients receive a mix of the treatment strategies employed for the two other sites.

Key words: Adenocarcinoma; Esophageal adenocarcinoma; Gastroesophageal; Gastric adenocarcinoma; Survival; Chemotherapy; Radiotherapy

Peer reviewers: Emad M El-Omar, Professor, Department of Medicine & Therapeutics, Aberdeen AB25 2ZD, United Kingdom; Dr. Bernardino Rampone, Department of General Surgery and Surgical Oncology, University of Siena, viale Bracci, Siena 53100, Italy; Dino Vaira, Professor, Department of Internal Medicine and Gastroent, University of Bologna, S.Orsola-Malpighi Hospital-Nuove Patologie, Pad. 5-via Massarenti 9, Bologna 40138, Italy; Shotaro Nakamura, MD, Department of Medicine and Clinical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

Cronin-Fenton DP, Mooney MM, Clegg LX, Harlan LC. Treatment and survival in a population-based sample of patients diagnosed with gastroesophageal adenocarcinoma. *World J Gastroenterol* 2008; 14(20): 3165-3173 Available from: URL: http://www.wjgnet.com/1007-9327/14/3165.asp DOI: http://dx.doi.org/10.3748/wjg.14.3165

INTRODUCTION

The incidence and mortality of esophageal and gastric-cardia adenocarcinoma (GCA) has increased dramatically since the 1970s in western countries, while that of stomach cancer has decreased[1-3]. Gastroesophageal

adenocarcinomas have a poor prognosis[4-8]. However, numerous randomized clinical trials (RCTs) have evaluated, and continue to evaluate, the survival benefit of various treatment regimens.

Surgery remains standard care for early stage esophageal cancer. The MAGIC trial, a large phase Ⅲ European RCTs found that patients with resectable lower esophageal or gastric adenocarcinomas treated with peri-operative chemotherapy had better progression-free and overall survival rates compared to surgery only[9]. This benefit was supported by the Fédérale Nationale des Centres de Lutte Contre Le Cancer (FNLCC) for patients with gastroesophageal adenocarcinoma who received pre-operative chemotherapy compared to surgery alone[10]. However, RCTs evaluating pre-operative chemoradiation compared to surgery alone have had conflicting results; some indicate better survival for esophageal cancer patients[11,12]. For locally advanced esophageal cancer, a phase Ⅲ RCT, RTOG 85-01, demonstrated improved survival in patients who received chemoradiotherapy compared to radiation alone[13], although most of these patients had squamous esophageal cancer. Another small RCT in patients with locally advanced esophageal cancer found chemoradiation superior to radiation alone[14]. These trials support the use of definitive chemoradiotherapy for locally advanced disease and its potential use for some patients with resectable disease. Current National Comprehensive Cancer Network (NCCN) guidelines for patients who are medically unfit for surgery or have unresectable disease recommend radiation and concurrent chemotherapy as treatment or best supportive care if patients cannot tolerate chemotherapy[15].

Surgery is also the standard of care for early stage gastric cancer. A US Intergroup phase Ⅲ trial, INT-0116, demonstrated post-surgical chemoradiation improved overall and disease-free survival in patients with stomach adenocarcinoma (SAC) and GCA[16]. The MAGIC and FNLCC trials also included patients with SAC and GCA. RCTs have also evaluated various chemotherapy treatments for patients with advanced or metastatic stomach and gastric-cardia cancer and have demonstrated improved survival for particular regimens[17,18]. The current NCCN guidelines for patients with metastatic stomach cancer recommend chemotherapy as treatment or best supportive care for those unable to tolerate chemotherapy[19].

Few studies have examined community-based patterns of care for these cancers. A study on esophageal adenocarcinoma (EAC) and squamous cell carcinoma patients diagnosed between 1996 and 1999 found that chemoradiation was most frequently given although patients with chemoradiation followed by surgery had better survival compared to definitive chemoradiation[20]. Research suggests, however, that community-based use of treatment and the observed survival of patients in the community can vary depending on clinical and non-clinical factors[21-26].

We present a population-based study, analyzing the receipt of various treatment strategies among a stratified random sample of patients with gastroesophageal adenocarcinoma. This study aims to determine whether treatment strategies used in routine community practice are based on anatomic location or cancer origin and to examine community-based use of specific chemotherapy regimens, especially those evaluated in RCTs. Finally, we assess factors that influence treatment receipt and patient survival.

MATERIALS AND METHODS

We included individuals aged ⩾ 20, newly diagnosed during 2001 with histologically-confirmed lower esophageal (EAC), GCA and SAC. Patients were ineligible if diagnosis was by death certificate only, autopsy, if they had a previous cancer diagnosis, other than non-melanoma skin, or were simultaneously diagnosed with another cancer. Patients were sampled from the National Cancer Institute's Surveillance, Epidemiology and End-Results Program (SEER) including Atlanta, Detroit, Seattle, New Mexico, Iowa, Louisiana, New Jersey, Connecticut, Utah, and California (Los Angeles County, San Francisco/Oakland, San Jose/Monterey, and greater California). Individuals were stratified by registry and race/ethnicity, and randomly sampled within strata. Non-Hispanic blacks, Hispanics, Asians/Pacific Islanders and Native Americans were over-sampled to obtain more stable estimates.

Data from medical records were re-abstracted to verify patient demographics, tumor characteristics, and treatment. Abstractors from each registry were centrally trained to ensure consistency of abstracting and coding. Because therapy is frequently provided in an outpatient setting, each patient's physician was contacted to verify treatment received, and provide names of any other physician who may have administered therapy. That physician was then contacted. All co-morbid conditions recorded at the hospitalization for most definitive treatment were abstracted. These were coded centrally by a single Registered Health Information Technologist and analyzed using the Charlson score[27]; an index of nineteen conditions, weighted according to the adjusted risk of one-year mortality.

We included 1411 cases. Patients were grouped by anatomic sites based on ICD-O2 codes; EAC (ICD-O: C15.2, C15.5, n = 165), GCA (the portion of the stomach surrounding the gastroesophageal junction) (C16.0, n = 246), and SAC (C16.1-C16.9, n = 1000) and stage, Ⅰ-Ⅲ and Ⅳ/unstaged. Because of small numbers (18) of Ⅳb EAC, these were grouped with Ⅳ/unstaged.

Treatments were defined as the receipt of surgery, radiotherapy, chemotherapy, in any combination. Non-adenocarcinoma cases were excluded from the therapy analyses (n = 55); 1356 adenocarcinoma patients were included in the treatment analyses.

Data analyses were performed using Stata 8.0 and SUDAAN (Research Triangle Institute, Research Triangle Park, NC). Analyses were weighted to reflect the SEER population from which the sample was drawn. Multivariable analyses were conducted using logistic

and multinomial logistic regression. Cancer survival was analyzed using Cox regression models with a maximum two-year follow-up (through December 2003). All *P*-values were two-sided.

RESULTS

Approximately 62% of patients had SAC, 22% GCA, and 16% lower EAC (Table 1). Median age was highest (76 years) for stage Ⅰ-Ⅲ SAC patients and lowest (67 years) for stage Ⅳ/unstaged GCA (data not shown).

Lower EAC

Over 12% of stage Ⅰ-Ⅲ patients with EAC received surgery alone (Table 2). About 27% of patients with stage Ⅰ-Ⅲ EAC received tri-modality therapy (surgery, radiation and chemotherapy), while 36.5% of these patients received chemotherapy plus radiation therapy with no surgery. One-quarter of stage Ⅰ-Ⅲ EAC patients received no chemotherapy. The most frequently administered agent was 5-FU, frequently with cisplatin. Few patients with late/unstaged EAC received surgery, in any combination. Chemoradiotherapy, however, was given to nearly 47% of these patients. In multinomial logistic regression, age ⩾ 70 was associated a 70%-80% decreased use of chemotherapy and chemoradiation in patients with EAC (Table 3).

Non-Hispanic blacks and Asian/Pacific Islanders with EAC had significantly higher hazards of cancer deaths than non-Hispanic white patients (Table 4). Patients age < 70 with a Charlson score of ⩾ 1 had a significantly increased risk as did those with late/unstaged disease. EAC patients who received chemotherapy had better survival, although not statistically significant. In a separate model, EAC patients who received chemoradiation had decreased hazards (HR = 0.69, 95% CI = 0.43-1.06 model not presented). The prognostic factors in the Cox proportional hazards model containing chemoradiation were otherwise the same as those significantly associated with death in the model which adjusted for chemotherapy.

GCA

Patients with GCA received therapies at a rate between that of EAC and SAC patients. Surgery alone was provided to 34% of stage Ⅰ-Ⅲ GCA patients (Table 2). One-quarter of stage Ⅰ-Ⅲ GCA patients received trimodal therapy. Nearly twice as many GCA patients as EAC patients but less GCA than SAC patients received no chemotherapy. Fewer patients received chemoradiotherapy compared to EAC patients. 5-FU was the most frequently used chemotherapeutic agent. Nearly twice as many late/unstaged GCA patients as EAC received no therapy. In multinomial logistic regression, age ⩾ 70 was associated with a 70%-80% decrease in chemotherapy alone or chemoradiotherapy (Table 3). Women and patients with a Charlson Score of ⩾ 1 were significantly less likely to receive chemotherapy, but not chemoradiation. In the Cox proportional hazards models patients, with late/unstaged disease or poorly/undifferentiated tumors had an increased risk of cancer deaths while married individuals had a decreased risk (Table 4).

SAC

Of the three anatomic sites, patients with SAC were most likely to receive surgery alone (Table 2). Nearly 50% of stage Ⅰ-Ⅲ SAC patients received surgery alone. Less than 20% of stage Ⅰ-Ⅲ SAC patients received trimodal therapy. Fewer SAC patients than EAC or GCA patients received chemotherapy. As with the other two anatomic sites, 5-FU was most frequently administered. Of the three anatomic sites, late/unstaged SAC patients received no definitive cancer treatment most often. In multinomial regression, age ⩾ 70 was associated with 80% less chemotherapy alone or chemoradiation (Table 3). Late/unstaged disease was associated with decreased use of chemoradiation but a substantial increased use of chemotherapy alone. Proportional hazards models for cancer deaths showed that in non-surgical patients, late/unstaged disease or a poor/undifferentiated tumor was associated with increased risk of cancer death (Table 4). However, patients receiving chemotherapy had a significantly decreased risk. Among surgical patients, a Charlson Score of ⩾ 1, regardless of age and having late/unstaged disease was associated with increased hazards. Lower risks were seen among Asian/Pacific Islanders, and a non-significant decreased risk among patients who received chemotherapy (Table 4). Patients who received chemoradiation had a statistically significant decreased risk both with (HR = 0.56, 95% CI = 0.35-0.89) and without surgery (HR = 0.62, 95% CI = 0.43-0.92) (model not presented) but all other prognostic factors had similar associations with hazard ratios as the Cox models which adjusted for chemotherapy.

DISCUSSION

RCTs have demonstrated that certain treatment strategies and regimens improve survival for patients with esophageal and gastric cardia adenocarcinoma. Variation in gastroesophageal cancer survival, however, has sometimes been attributed to case mix[28]. We therefore selected adenocarcinoma cases only and categorized patients by anatomic site to assess rates of treatment and survival among a population-based sample of patients treated in the community. We found significant differences in treatment and survival by anatomic site, stage, age, and race/ethnicity. This study highlights the considerably varied approach that community physicians take to treat adenocarcinomas at each anatomic site.

Lower EAC

While there is no consensus definition of the optimal therapy for patients with resectable EAC, clinical trials have indicated survival improvements when surgery is supplemented with additional therapies. Of the three cancer sites investigated in the current study, stage Ⅰ-Ⅲ EAC patients had the lowest rates of surgery alone

Table 1 Percentage distribution (weighted for the sampling fraction) of clinical and non-clinical characteristics for gastroesophageal cancer patients diagnosed in 2001 NCI: Patterns of care study (n = 1411) (Wt%)

	Lower esophagus		Gastric cardia		Stomach	
	I-III (n = 86)	IV-V (n = 79)	I-III (n = 119)	IV-V (n = 127)	I-III (n = 491)	IV-V (n = 509)
Age						
< 70	57.5	46.4	57.2	54.0	36.7	42.4
⩾ 70	42.5	53.6	42.8	46.0	63.3	57.6
Marital status						
Other	39.9	43.6	37.9	41.0	46.4	48.9
Married	60.1	56.4	62.1	59.0	53.6	51.1
Race						
NH White	93.9	87.8	78.0	75.1	51.0	47.9
NH Black	1.0	3.3	4.3	5.8	13.4	14.2
Hispanic	4.1	7.9	11.6	12.0	15.8	20.5
A/PI	1.0	1.0	6.2	6.8	19.1	17.0
NA/AI	0.0	0.0	0.0	0.4	0.6	0.3
Charlson score						
Zero	72.3	81.0	77.7	86.5	79.5	78.9
1+	27.7	19.0	22.3	13.5	20.5	21.1
Vital status Dec 2003						
Deceased	69.6	88.8	70.0	88.3	53.9	89.9
Histology						
Adeno, NOS	92.8	81.0	79.5	70.4	50.4	50.7
A. intestinal	0.0	3.7	9.1	4.1	14.6	7.2
A. diffuse	0.0	0.0	1.8	4.4	4.0	3.0
Signet	6.0	12.0	8.0	18.9	20.6	28.9
Mucinous	1.2	2.4	0.9	1.0	5.5	5.5
Papillary	0.0	0.9	0.7	0.0	0.9	1.5
Tubular	0.0	0.0	0.0	1.0	2.2	0.6
Linus plastica	0.0	0.0	0.0	0.2	1.8	2.6
Linitis plastica/Signet ring						
Linitis plastica	3.9	0.0	0.0	4.1	5.3	7.2
Signet	13.9	13.1	15.2	22.4	24.4	33.6
No mention	81.6	86.9	78.3	71.3	69.5	58.5
Unknown	0.6	0.0	6.5	2.2	0.8	0.7
Intestinal metaplasia in resected tumor						
None	31.3	28.5	36.7	17.9	25.4	24.3
Metaplasia	7.9	3.0	18.4	5.9	31.2	9.6
No mention	36.6	29.9	30.6	37.5	30.1	28.0
Unknown	24.2	38.6	14.3	38.8	13.3	38.1
Grade						
Well differentiated	4.1	12.0	2.9	4.9	6.9	2.3
Moderate	18.9	39.6	31.3	33.4	28.1	19.4
Poor/Undif	57.1	28.5	59.0	51.3	56.5	60.7
Unknown	19.8	20.0	6.8	10.4	8.4	17.7
Barrett's esophagus						
No	21.3	27.7	39.1	19.9	43.4	28.4
Yes	33.2	9.3	11.0	3.8	0.0	0.6
Other	3.9	1.1	1.3	0.0	0.0	0.0
No mention	16.8	21.8	27.8	35.2	43.7	31.5
Unknown	24.8	40.1	20.7	41.1	12.9	39.5
History of Barrett's						
No history	19.3	22.6	27.5	25.6	29.4	29.4
History	22.7	14.0	6.0	8.7	1.7	0.5
No mention	54.5	60.0	59.5	61.8	67.2	67.9
Unknown	3.4	3.4	7.0	3.9	1.8	2.1
H pylori						
Negative	22.6	39.2	32.3	29.6	37.6	33.1
Positive	2.1	13.5	10.0	15.9	18.6	15.9
No mention	73.3	41.9	51.1	52.6	41.4	49.0
Pernicious anemia						
No history	25.9	33.1	26.9	31.5	31.1	28.7
Pernicious Anemia	0.6	0.0	8.2	0.4	6.1	3.9
No mention	71.5	62.0	60.4	65.4	59.6	64.9
Unknown	2.0	4.9	4.5	2.7	3.2	2.6
History of ulcers						
No history	23.5	32.6	28.2	29.5	19.6	23.1
Peptic ulcers, NOS	7.9	11.6	5.8	4.7	12	10.5
Duod/pyloric ulcer	2.1	1.7	0.4	1.3	1.1	2.9
Gastric ulcer	4.3	0	8.6	9.6	22.7	13.3
Other	6.5	7.1	0.4	1.4	0.9	0.8
No mention	55.1	44.7	50.1	51	40.6	46.9
Unknown	0.6	2.4	6.6	2.4	3.1	2.5

American Indians/Native American are included in Table 1 for completeness of reporting. Histology groupings were created according to the following: Adenocarcinoma-NOS = 8140, 8210, 8255, 8261 (n = 842), Adeno-Intestinal = 8144 (n = 122), Adeno-Diffuse = 8145 (n = 45), Signet Cell = 8490 (n = 295), Mucinous/mucin-producing = 8480 + 8481 (n = 48), Papillary/Serous = 8260 + 8460 + 8461 (n = 12), Tubular = 8211 (n = 17), Linitis plastica = 8142 (n = 23).

Table 2 Percentage distribution (weighted for the sampling fraction) of treatment characteristics and survival for gastroesophageal adenocarcinoma patients diagnosed in 2001 NCI: Patterns of care study (n = 1356)[1] (Wt%)

	Lower esophagus		Gastric cardia		Stomach	
	I-III	IV-V	I-III	IV-V	I-III	IV-V
Therapy received						
Surgery only	12.3	1.3	34	2.2	49.6	15.7
Radiation only	8.1	3.5	4.7	7.2	2	3.3
Chemotherapy only	0.5	24	1.2	21.9	3.6	22.1
Surgery and radiation	0.8	0	2.6	0.9	3.9	0.4
Surgery and chemo	2.5	1.3	8.6	4.8	3.2	5.6
Surgery, rad, chemo	27	4.6	25	11.2	19.2	6.1
Chemo and radiation	36.5	46.8	9.8	12.4	1.5	3.4
None	12.4	18.7	14.1	39.4	17.1	43.4
Chemotherapy						
No chemo	25.1	21.5	48.7	40.8	64.9	54.5
Single agent	5.4	25.5	11.4	9.9	10.3	8.4
Multi-agent	61.1	50.9	31.4	39.6	17.1	29
Refused	5.9	1.5	4.3	7.2	4	4.3
Rec, unknown if given	1.9	0	1	2.1	1.8	2.3
Unknown	0.6	0.6	3.2	0.4	2	1.5
Chemotherapy agent						
5-FU	58.6	37.9	35.7	34.7	23.2	26.4
Doxorubicin	0.5	0	0.3	1	0.5	3.4
Capecitabine	1.2	0	0.7	7.4	0.6	4
Cisplatin	38.7	37.2	22	17.2	2.8	10.8
Etoposide	1	0	0.7	10.7	1.4	5.8
Irinotecan	0.7	12.9	0.5	5.9	1.6	5.6
Leucovorin	4.1	0.9	6.1	21.8	12.1	15.1
Mitomycin-C	13.7	4.3	0.5	0.6	0.2	1.8
Oxaliplatin	No one					
Epirubicin	0	0	0	1	0	1.2
Paclitaxel	11.8	20.8	5.9	7.3	2.2	4.6
Docetaxel	0	1.6	0.7	2.1	0.6	1.4
Chemotherapy plus surgery (with or without radiation)						
No	70.5	94.2	68.3	84	77.7	87.8
Pre-op	23.7	4.9	16.8	6.4	0.3	0.5
Post-op	5.8	0.9	9	8.5	20.3	11.2
Unknown	0	0	5.9	1.1	1.7	0
Surgery plus chemotherapy and radiation						
No	73	95.4	75	88.5	80.9	93.9
Pre-op	21.9	4.6	13.4	4.3	0.2	0.5
Post-op	5.1	0	8.6	6.9	17.4	5.6
Unknown	0	0	3	0	1.5	0
Median survival time (mo)						
Non-surgical pts	13	8	8	6	5	4
Surgical patients	22	13	19	13	26	6

[1]13 American Indians/Native Americans excluded.

Table 3 Therapy among patients with gastroesophageal adenocarcinoma by anatomic site, 2001: Multinomial logistic regression for the receipt of chemoradiation (Chemo + RT) and chemotherapy alone

Site Characteristic	Lower esophagus					Gastric-cardia					Stomach				
	Chemo + RT		Chemo			Chemo + RT		Chemo			Chemo + RT		Chemo		
	OR	95% CI	OR	95% CI	*P*	OR	95% CI	OR	95% CI	*P*	OR	95% CI	OR	95% CI	*P*
Age					0.05					< 0.001					< 0.001
< 70	1		1			1		1			1		1		
⩾ 70	0.3	0.1-0.96	0.2	0.03-0.9		0.2	0.1-0.4	0.3	0.1-0.7		0.2	0.1-0.4	0.2	0.1-0.3	
Race/ethnicity					0.33					0.64					0.24
Non-hispanic white	1		1			1		1			1		1		
Non-hispanic black	1.1	0.1-10.2	0.9	0.1-14.5		0.5	0.1-1.7	1	0.2-6.7		1.3	0.6-2.5	0.6	0.3-1.0	
Hispanic	0.1	0.01-0.9	0.3	0.02-3.6		0.4	0.1-1.6	1.6	0.3-7.4		1.2	0.6-2.5	0.6	0.3-1.4	
Asian/Pacific Islander						0.5	0.1-1.9	1.4	0.4-4.9		1.7	0.9-3.4	0.8	0.4-1.4	
Gender					0.32					0.01					0.64
Male	1		1			1		1			1		1		
Female	1.2	0.5-3.3	0.3	0.04-2.1		1.2	0.4-3.3	0.3	0.1-0.8		1.3	0.8-2.1	1.1	0.7-1.8	
Marital status					0.28					0.86					0.74
Not married	1		1			1		1			1		1		
Married	2.1	0.7-6.5	1	0.3-3.9		1	0.4-2.6	1.4	0.4-4.6		1.2	0.7-2.0	1.2	0.7-1.9	
Stage					0.12					0.01					< 0.001
Ⅰ-Ⅲ	1		1			1		1			1		1		
Ⅳ & unknown	1	0.4-2.7	3.6	0.9-13.6		0.7	0.3-1.7	3.8	1.3-11.4		0.6	0.4-0.9	5.2	3.0-8.8	
Differentiation grade					0.57					0.36					0.38
Well/Moderately differentiated	1		1			1					1		1		
Poorly/Undifferentiated	1.2	0.4-3.6	0.9	0.2-3.7		0.8	0.3-1.9	1	0.3-3.1		1.3	0.7-2.2	1.3	0.7-2.5	
Unknown	0.6	0.2-1.9	0.2	0.03-1.4		1.5	0.3-7.3	0.2	0.03-1.8		0.7	0.3-2.0	1.6	0.8-3.5	
Charlson score					0.25					0.02					0.35
0	1		1			1		1			1		1		
1+	2	0.6-6.7	0.6	0.1-3.1		0.4	0.1-1.3	0.1	0.02-0.6		0.7	0.4-1.2	0.9	0.5-1.7	
H pylori					0.02					0.004					0.65
No	1		1			1		1			1		1		
Yes	0.1	0.02-0.8	0.4	0.03-5.6		1	0.2-4.2	0.4	0.1-2.1		1.2	0.6-2.5	1.3	0.6-2.8	
Unknown	0.1	0.03-0.4	0.2	0.03-0.8		0.2	0.1-0.7	0.8	0.3-2.3		1	0.6-1.7	1.5	0.9-2.5	

Model also adjusted for registry.

as their primary treatment, but highest rates of pre-operative chemotherapy and chemoradiation as well as definitive chemoradiation. This may reflect the significant morbidity associated with esophageal surgery[29-32]. However, toxicities associated with pre-operative chemotherapy or chemoradiation can preclude a patient from further treatment[33].

RCTs and a meta-analysis have suggested a survival benefit associated with pre-operative and adjuvant chemoradiation compared to surgery alone[11,33-37]. The US-Intergroup trial, CALGB-9781, closed early due to poor accrual, but an intent-to-treat analysis on the 56 enrolled patients, demonstrated better median survival in favor of trimodal therapy[12]. In our study, over a quarter of stage Ⅰ-Ⅲ EAC patients received trimodality therapy. The MAGIC and FNLCC phase Ⅲ trials support the use of perioperative or preoperative chemotherapy; however, we found that few (2.5%) stage Ⅰ-Ⅲ EAC patients received surgery and chemotherapy as primary treatment. Chemoradiation was the treatment strategy received by the largest percentage of patients with stage Ⅰ-Ⅲ EAC (36.5%) and late/unstaged EAC patients (47%).

GCA

Optimal therapy for GCA is not clear. Most RCTs have included patients with this cancer in trials conducted for either or both of the other two anatomic sites[18,38]. Reflective of this, we found that GCA patients seemed to receive treatment at a rate that fell midway between the other two anatomic sites. In the current population-based study, stage Ⅰ-Ⅲ GCA patients were most frequently treated with surgery alone (34%) or trimodal therapy (22%). For late/unstaged disease less than 25% received chemotherapy alone and a significant percentage received no therapy (39%).

SAC

In contrast to EAC and GCA patients, SAC patients received surgery alone most frequently (50% of stage Ⅰ-Ⅲ and 16% of stage Ⅳ/unstaged disease) and radiotherapy and chemotherapy less frequently than the other two anatomic sites. Although the MAGIC and FNLCC trials demonstrated a survival advantage for patients with gastroesophageal adenocarcinoma[9], this was not evident in the current population-based study, where less than 20% of stage Ⅰ-Ⅲ SAC patients received chemoradiation with surgery.

With respect to advanced disease, several RCTs for SAC have demonstrated survival benefits for chemotherapy compared to best supportive care for stage Ⅳ (late-stage) disease[17]. However, we found that only 22% of patients with late/unstaged SAC received chemotherapy alone, with an additional 15% receiving chemotherapy with surgery, with surgery and radiation, or as chemoradiation. Furthermore, for late/

Table 4 Cox proportional hazards model for cancer death among lower esophageal and GCA patients overall (Model 1) and among SAC patients who did or did not receive surgery (Model 2)

	Model 1						Model 2					
	Lower esophagus (n = 164) With & Without surgery			Gastric-cardia (n = 241) With & Without surgery			Stomach					
							No surgery (n = 461)			With surgery (n = 490)		
Characteristic	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age & co-morbidity			0.02			0.44			0.97			0.01
< 70, Charlson score = 0	1			1			1			1		
< 70, Charlson score = 1	2.7	1.4-5.2		1	0.4-2.1		0.9	0.6-1.4		2	1.1-3.7	
70+, Charlson score = 0	1.5	0.9-2.6		1.4	0.9-2.3		0.9	0.6-1.4		1.5	0.9-2.5	
70+, Charlson score = 1	1.5	0.7-3.3		1.1	0.5-2.4		1	0.6-1.6		2.4	1.4-4.0	
Race			< 0.001			0.08			0.39			0.003
Non-Hispanic White	1			1			1			1		
Non-Hispanic Black	3.4	1.8-6.7		0.8	0.5-1.2		1.1	0.8-1.5		1.1	0.6-1.7	
Hispanic	1	0.5-2.0		1.3	0.8-2.2		0.8	0.5-1.2		1.3	0.8-2.1	
Asian/Pacific Islander	6.4	2.9-14.4		0.4	0.2-1.0		0.9	0.6-1.2		0.5	0.3-0.9	
Gender			0.78			0.87			0.84			0.18
Male	1			1			1			1		
Female	0.9	0.6-1.5		1	0.6-1.4		1	0.8-1.4		0.8	0.5-1.1	
Marital Status			0.43			0.01			0.39			0.33
Not married	1			1			1			1		
Married	0.8	0.5-1.3		0.6	0.4-0.9		1.2	0.8-1.6		1.2	0.8-1.9	
Stage			< 0.001			< 0.001			0.004			< 0.001
Stage Ⅰ-Ⅲ	1			1			1			1		
Stage Ⅳ & unknown	2.5	1.5-4.2		2.6	1.6-4.0		1.7	1.2-2.3		5	3.4-7.4	
Differentiation grade			0.11			0.049			0.01			0.14
Well/Moderately	1			1			1			1		
Poorly/Undifferentiated	1.8	1.0-3.3		1.7	1.1-2.6		1.8	1.2-2.6		1.4	0.9-2.2	
Unknown	1.7	0.9-3.2		1.3	0.6-2.8		1.5	1.0-2.3		0.7	0.3-1.7	
Chemotherapy			0.09			0.68			< 0.001			0.12
No	1			1			1			1		
Yes	0.6	0.4-1.1		1.1	0.7-1.8		0.6	0.4-0.8		0.7	0.5-1.1	

unstaged disease, SAC patients received no therapy most frequently (43%).

Overall, our results do suggest that RCT-validated therapies have been incorporated into community practice, albeit at low levels. However, a significant percentage of patients, especially those with stage Ⅳ/unstaged disease, across all anatomic sites received no cancer-directed therapy. Our findings also highlight that the sequence and combination of chemotherapy, radiotherapy and surgery in the adjuvant setting was distinct for each anatomic site. For example, of stage Ⅰ-Ⅲ EAC patients who received surgery plus chemotherapy and radiation, 81% received this therapy pre-operatively [most frequently with 5-fluorouracil (5-FU) and cisplatin], while of stage Ⅰ-Ⅲ SAC patients who received surgery plus chemotherapy and radiation, 91% received this therapy post-operatively (most frequently with 5-FU and leucovorin). These sequences of therapy as well as the chemotherapeutic agents selected were also consistent with RCTs conducted in these disease sites[11].

Chemotherapeutic agents

Overall, the most frequently administered chemotherapeutic agents in our study were 5-FU, cisplatin, and leucovorin. Newer agents (paclitaxel, irinotecan) have been investigated in phase Ⅱ trials[39,40] for use in EAC patients. We found that these drugs were used in community practice (Table 2). Use of these compounds was much lower among patients with SAC and GCA cancers. No patients received oxaliplatin, possibly because these cases were diagnosed in 2001 and findings advocating oxaliplatin for esophageal cancer were only presented in 2006[38]. Specific chemotherapeutic agents used alone or in combination with surgery and radiation are listed in Table 5. Whether patients received chemotherapy alone, chemoradiation, or trimodal therapy, the majority of patients received 5-FU in combination with another chemotherapeutic agent.

Age disparities

Less frequent treatment of elderly patients has been widely reported[21-25,41]. Sabel *et al*[31] reported that 50% of patients age < 70 and 32% of those age ⩾ 70 were suitable for surgery at diagnosis. Similar to this, we found that in stage Ⅰ-Ⅲ EAC, 30% of patients aged ⩾ 70 compared to 51% of those age < 70 underwent cancer-directed surgery and 56% of gastric-cardia patients aged ⩾ 70 compared to 80% of those age < 70. The age-related treatment decline is likely attributable to a number of factors: (1) Potentially higher morbidity among elderly patients; (2) Compromised treatment options due to delayed presentation by elderly patients; (3) Increased anesthesiological risk[31,42]; and (4) A higher prevalence of co-morbidities.

Median age at diagnosis for stomach cancer is approximately 70 years[43], an age when patients have a reasonable life-expectancy[43]. Selected medically-fit elderly patients do as well as younger patients after surgical or adjuvant therapy[44,45]. Our models indicate

Table 5 Percentage distribution (weighted for the sampling fraction) of chemotherapy agents by selected therapeutic combinations gastroesophageal adenocarcinoma patients diagnosed in 2001; NCI: Patterns of care study (n = 1356)[1] (Wt%)

	Lower esophagus		Gastric-cardia		Stomach	
	I-III	IV-V	I-III	IV-V	I-III	IV-V
Chemotherapy only						
Etoposide + Doxorubicin + Cisplatin	1 patient	0	0	0	0	0.6
5-FU only	0	3.2	0	3.3	15.9	5.1
Mitomycin only	0	16.7	0	0	0	0
Paclitaxel only	0	2.1	19.2	3.3	0	0
Capcitabine only	0	0	0	0	0	4.4
Gemcitabine only	0	0	0	3.1	0	1.2
5-FU + 1 agent	0	18	56	16.6	25.5	18.8
5-FU + 2 agents	0	4	24.8	40	26.6	23.3
5-FU + 3 agents	0	0	0	7.9	0	15.6
5-FU + 4 agents	0	0	0	2.6	0	2.2
Irinotecan + Paclitaxel	0	20.6	0	1.2	0	0.6
Irinotecan + Cisplatin	0	15.4	0	1.7	0	8.4
Irinotecan + Cisplatin + Paclitaxel	0	0	0	0	20.4	0
Other	0	20	0	20	11.7	19.7
Radiation and chemotherapy						
5-FU only	2.1	9.2	34.8	29.2	5.6	20.6
Cisplatin only	0	17.1	0	3.3	0	0
5-FU + Leucovorin	0	1.2	0	0	0	14.3
5-FU + Cisplatin	50.6	28	61.5	32.6	7.9	0
5-FU + Mitomycin	25.1	0.7	0	0	0	0
5-FU + Irinotecan	0	0	0	0	30.1	0
Paclitaxel + Carboplatin	0	13.6	0	4.7	0	0
Chemo, NOS	4.6	8.9	0	7.5	20	13.1
Other	17.6	21.3	3.7	22.7	36.4	52
Surgery, radiation & chemotherapy						
5-FU only	5	0	9.4	0	32.4	26.5
5-FU + Leucovorin	13.3	0	17.6	31.7	46.5	40.9
5-FU + Cisplatin	41.1	15.6	27.7	7.3	2.3	0
5-FU + Paclitaxel + Carboplatin	15.3	44.6	1.6	0	0.7	0
Other agents/Combos	25.3	39.8	43.7	61	18.1	32.6

[1]13 American Indians/ Native Americans excluded; NOS: Not otherwise specified.

that in EAC or GCA patients, being age < 70 with a Charlson score of 1+ was significantly and inversely associated with surgery (data not shown). Older patients (⩾ 70) were also less likely to have surgery even with a Charlson score of 0. In our multinomial models, being age ⩾ 70 was associated with a decreased use of chemotherapy or chemoradiation even after adjusting for co-morbid conditions. Although we saw this disparity in the use of chemotherapy or chemoradiation by age group, there was no evidence of treatment-related differences by racial/ethnic groups. This suggests that in a community-based setting, age, in addition to co-morbidity, influences whether or not a patient receives surgery, chemotherapy, and chemoradiation.

Survival

Survival from gastric adenocarcinoma is extremely poor[46]. Theuer *et al*[46] reported that patients aged ⩾ 70 had higher risk of death even after adjusting for clinical, non-clinical and treatment-related factors. In contrast, we observed that in GCA and non-surgical SAC patients, age and co-morbidity were not significant predictors of survival, perhaps due to the poor prognosis for these patients. We noted higher mortality in non-Hispanic blacks and Asian/Pacific Islanders with EAC. Such racial disparities have been reported in other cancers[47,48], however the underlying cause for this poorer survival is not clear.

A US-based survey of 59 radiotherapy facilities indicated improved survival associated with pre-operative chemoradiation for patients with esophageal cancer[20]. In the current study, chemotherapy and chemoradiation were associated with decreased mortality for SAC and EAC patients, although not GCA patients. This suggests that the receipt of chemotherapy and/or radiotherapy may improve outcome in these poor prognosis cancers. This analysis was not a randomized study of therapy and although we adjusted for Charlson comorbidity score and additional potential confounders, patients who had better baseline health and who were selected for chemotherapy, may have been more likely to respond to such treatment or may have had better survival regardless of the use of chemotherapy.

In conclusion, RCTs have demonstrated that specific treatment strategies prolong survival in certain patient groups. We note that the use of these therapies was very low in US community-based practice despite their demonstrated survival benefits. Our study shows lower mortality among patients with EAC and SAC who received chemotherapy and significant disparities in terms of age in treatment receipt. Our findings highlight the distinctly individualized approach taken by community physicians in treating adenocarcinoma at these three anatomic sites. Community physicians appear to differentiate gastroesophageal adenocarcinoma as two distinct entities (i.e. EAC and SAC) and use different treatment strategies and chemotherapeutic agents for each, while patients with GCA are treated with a mixture of those employed for the other two anatomic sites. Improvements in community-based treatment of gastroesophageal adenocarcinoma will require better differentiation of treatments for the different anatomic sites and more extensive incorporation of those treatments proven effective in clinical trials. Future RCTs should be designed and appropriately powered to account for differences related to the anatomic site or origin of the tumor as well as the underlying tumor biology.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contributions of the participating SEER registries and staffs. Without their dedicated efforts this research could not be conducted.

COMMENTS

Background

Randomized clinical trials (RCTs) have demonstrated that specific treatment strategies prolong survival in certain patient groups with gastric, gastro-esophageal and esophageal adenocarcinomas. However, the extent of use of these treatments in routine clinical practice is not clear. Research suggests that community-based use of treatment and the observed survival of patients in the

community can vary depending on clinical and non-clinical factors.

Research frontiers

To determine whether treatment strategies used in routine community practice are based on anatomic location or cancer origin. To examine community-based use of specific chemotherapy regimens especially those evaluated in RCTs. To assess factors that influence treatment receipt and patient survival.

Innovations and breakthroughs

We document relatively low community-based use of treatments tested in RCTs in patients with gastroesophageal adenocarcinoma. The use of these therapies was very low despite their demonstrated survival benefits. Our study shows lower mortality among patients with esophageal adenocarcinoma (EAC) and stomach adenocarcinoma (SAC) who received chemotherapy and significant disparities in terms of age in treatment receipt. Community physicians appear to take an individualized approach in treating adenocarcinoma at these three anatomic sites; differentiating between gastric and EAC and using different treatment strategies and chemotherapeutic agents for each, while patients with gastric cardia adenocarcinoma are treated with a mixture of those employed for the other two anatomic sites.

Applications

Improvements in community-based treatment of gastroesophageal adenocarcinoma will require better differentiation of treatments for the different anatomic sites and more extensive incorporation of those treatments proven effective in clinical trials. Future RCTs should be designed and appropriately powered to account for differences related to the anatomic site or origin of the tumor as well as the underlying tumor biology.

Peer review

This is a retrospective study of a large number of patients with gastroesophageal adenocarcinoma focusing on treatment modalities and survival. This is an excellent and relevant study, which was well conducted and presented.

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S- Editor Li DL **L- Editor** Alpini GD **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3174

World J Gastroenterol 2008 May 28; 14(20): 3174-3178
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

In vitro activity of moxifloxacin and piperacillin/sulbactam against pathogens of acute cholangitis

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Received: January 10, 2008 Revised: March 31, 2008
Accepted: April 7, 2008
Published online: May 28, 2008

Abstract

AIM: To analyze the *in vitro* activity of moxifloxacin and piperacillin/sulbactam against pathogens isolated from patients with acute cholangitis.

METHODS: In this prospective study a total of 65 patients with acute cholangitis due to biliary stone obstruction (n = 7), benign biliary stricture (n = 16), and malignant biliary stricture (n = 42) were investigated with regard to spectrum of bacterial infection and antibiotic resistance. Pathogens were isolated from bile cultures in all study patients. In 22 febrile patients, blood cultures were also obtained. *In vitro* activity of moxifloxacin and piperacillin/sulbactam was determined by agar diffusion.

RESULTS: Thirty-one out of 65 patients had positive bile and/or blood cultures. In 31 patients, 63 isolates with 17 different species were identified. The predominant strains were *Enterococcus species* (26/63), *E.coli* (13/63) and *Klebsiella species* (8/63). A comparable *in vitro* activity of moxifloxacin and piperacillin/sulbactam was observed for *E.coli* and *Klebsiella species*. In contrast, *Enterococcus species* had higher resistances towards moxifloxacin. Overall bacteria showed antibiotic resistances *in vitro* of 34.9% for piperacillin/sulbactam and 36.5% for moxifloxacin.

CONCLUSION: *Enterococcus species*, *E.coli* and *Klebsiella species* were the most common bacteria isolated from bile and/or blood from patients with acute cholangitis. Overall, a mixed infection with several species was observed, and bacteria showed a comparable *in vitro* activity for piperacillin/sulbactam and moxifloxacin.

Key words: Cholangitis; Acute cholangitis; Endoscopy; Antibiotics; Moxifloxacin; Piperacillin; Sulbactam; Biliary stricture; Resistance; Bacterial pathogens

Peer reviewers: Dr. Pietro Invernizzi, Department of Medicine, Surgery and Dentistry, University of Milan, Milan 20142, Italy; Sharon DeMorrow, Assistant Professor, Research and Education/Medicine, Scott and White Hospital, Temple 76504, United States

Weber A, Huber W, Kamereck K, Winkle P, Voland P, Weidenbach H, Schmid RM, Prinz C. *In vitro* activity of moxifloxacin and piperacillin/sulbactam against pathogens of acute cholangitis. *World J Gastroenterol* 2008; 14(20): 3174-3178 Available from: URL: http://www.wjgnet.com/1007-9327/14/3174.asp DOI: http://dx.doi.org/10.3748/wjg.14.3174

INTRODUCTION

Acute cholangitis, first described by Charcot in 1877 is a frequent and potentially serious complication in patients with bile duct obstruction. Ductal obstruction leads to a raised intrabiliary pressure with cholangiovenous reflux and bacteremia, which may progress to septicaemia[1]. Ductal stones, benign or malignant biliary strictures are reasons for the obstruction. Biliary decompression by endoscopic or percutaneous transhepatic procedures and selection of appropriate antibiotics are crucial in the therapy of these patients[2-5]. The efficacy of antibiotics in the treatment of biliary infections depends on the microbiological activity against the most common pathogens and the excretion of the antibacterial agents in the obstructed biliary tract. In case of complete obstruction of the common bile duct, no significant biliary excretion of the antibiotics occurs, so that biliary

bactericidal concentrations cannot be achieved[6,7]. However, recently a sufficient biliary concentration of the fluoroquinolone moxifloxacin in patients with obstructive cholangitis was reported[8]. Because bacteremia may progress to septicaemia, a high level of serum concentrations of the antibiotic agents is also important for the treatment of biliary tract infections. Although acute cholangitis is a common clinical problem associated with a high level of morbidity and mortality, there is no standardized approach for therapy of this disease. The selection of antibacterial agents is based on the severity of the disease, the expected biliary pathogens or the activity of antibacterial agents against the isolated bacteria from blood or bile cultures. Broad spectrum antibiotics, active against gram negative and gram positive organisms, are the preferred treatment[2,9-11]. Therefore, in case of severe cholangitis, the mostly preferred drug is piperacillin, a broad spectrum penicillin. In a prospective randomised trial including patients with acute cholangitis, equal clinical efficacy was observed with piperacillin alone compared to ampicillin plus tobramycin[12]. The combination of piperacillin with the β-lactamase inhibitor sulbactam might be an alternative procedure when the resistance pattern shows a relatively high incidence of ureidopenicillin-resistant *E.coli* or *Klebsiella species*[13]. Because of increasing resistance and allergic reactions against penicillin, other antibacterial agents for the treatment of acute cholangitis become necessary. Moxifloxacin is characterized by an enhanced activity against gram positive, gram negative and in anaerobic organisms and by a sufficient concentration in the obstructive bile duct. Therefore it may be an alternative antibacterial treatment in patients with acute cholangitis. To address this question, we performed a prospective trial to analyze the *in vitro* activity of moxifloxacin and piperacillin/sulbactam against pathogens isolated from patients with acute cholangitis.

MATERIALS AND METHODS

Study population

The study included 65 consecutive patients suffering from acute cholangitis who were treated between February 2004 and November 2005 in the Department of Gastroenterology at the Technical University of Munich. All of the following criteria had to be fulfilled: (1) clinical diagnosis of acute cholangitis, (2) elevated cholestasis parameter (bilirubin > 3 mg/dL), (3) elevated infection parameters (leucocytes > 12 G/L, c-reactive protein > 3 mg/dL) or fever (> 38.5℃), and (4) age 18-90 years. Exclusion criteria were as follows: (1) primary sclerosing cholangitis, (2) liver cirrhosis, (3) liver transplantation, (4) acquired immunodeficiency syndrome (AIDS), (5) primary immunodeficiency syndrome, (6) therapy with glucocorticoids and other immunosuppressant drugs, (7) leucopenia (leucocytes < 1 G/L), and (8) infection focus other than acute cholangitis.

Isolation of bacteria

From all patients included in this study, bile samples for culture were taken. Bile was obtained by endoscopic retrograde cholangiography (ERC) or by percutaneous transhepatic biliary drainage (PTBD). ERC and biliary drainage were performed with a standard videoduodenoscope OlympusTFJ 160-R. Endoscopic sphincterotomy (EST) was conducted using an Olympus papillotome introduced over a Terumo guide wire. At ERC, intraductal bile was collected before contrast agent injection by passing a sterile standard ERC catheter into the obstructed bile duct and aspirating bile into a sterile 10 mL syringe. In case of PTBD, 2-4 mL bile was collected into a sterile 10 mL syringe after penetration of the bile duct with the puncture needle. Thereafter, a percutaneous transhepatic biliary catheter was inserted by the Seldinger technique. Because of the percutaneous placement of this catheter, bile could be obtained all the time in case of fever, chills and increasing infection parameters (leucocytes, c-reactive protein). In 22 febrile patients (temperature > 38.5℃), blood cultures were also obtained. Typically, 10 mL of blood was obtained and transferred into aerobic and anaerobic culture broth (BacTec system, Becton Dickinson, Heidelberg, Germany).

Microbiological investigation

In case of positive blood- and/or bile cultures, the *in vitro* activity of moxifloxacin and piperacillin/sulbactam was performed by agar diffusion assay test.

The bile/specimen sampled was examined for aerobic and anaerobic bacteria. In each case, 50-100 μL bile/specimen were both transferred into liquid nutrient media (glucose broth, thioglycollate broth) and spread on solid culture media (Columbia sheep blood agar, chocolate agar, McConkey agar, Schädler anaerobic agar, Schädler KV anaerobic agar, and Sabouroud agar). Subsequently, the culture media were incubated at 37℃. The aerobic cultures were incubated for 48 h, with the first readout taken after 24 h. The anaerobic cultures were monitored for the first time after 48 h and processed further as required. To identify bacteria in the blood, one aerobic and one anaerobic blood culture bottle (BacTec system, Becton Dickinson, Heidelberg, Germany) were each inoculated with 10 mL of venous blood. The blood cultures were incubated at 37℃ for 5 d. For control purposes and to exclude failure of automatic detection of the BacTec system each flask was subcultivated under aerobic (chocolate agar in 10% CO_2) and anaerobic conditions (Schädler anaerobic agar) at the end of the incubation period. Cultivable germs were identified using the ATB, API or VITEK system (BioMérieux, Nürtingen, Germany). In order to identify antimicrobial inhibitors approximately 10 μL of fluid specimen were placed in the depression of an agar plate containing a suspension of spore forming bacteria. With an antibiotic being present and taking effect in the specimen a clear inhibition zone was to be seen around the point of application. Colony forming units were

Table 1 Patient characteristics, physical and laboratory parameters on admission

		Standard values	Scale unit
Number of patients	65	-	-
Mean age	68 ± 12.3	-	-
Gender			
Male	32	-	-
Female	33	-	-
Bilirubin	7.9 ± 7.4	< 1.2	mg/dL
Alkaline phosphatase	675 ± 510	40-120	U/L
γ-Glutamyltransferase	697 ± 682	< 66	U/L
Aspartate aminotransferase	193 ± 300	10-50	U/L
Alanine aminotransferase	136 ± 147	10-50	U/L
Leucocytes	16.9 ± 10.7	4-9	G/L
C-reactive protein	17.3 ± 9.5	< 0.5	mg/dL

not determined in this study. Antibiotic susceptibility testing was performed using both the disk diffusion test or the MIC test using the VITEK system (BioMérieux, Nürtingen, Germany) or the Etest system (AB Biodisk, Solna, Sweden) according to the recommendations of the CLSI (Clinical Laboratory Standards Institute; formerly NCCLS/National Committee for Clinical Laboratory Standards).

RESULTS

During the study period from February 2004 to November 2005, a total of 65 consecutive patients with acute cholangitis were included in the current clinical trial. The patients had the following characteristics: mean age 68 ± 12.3 years, 32 male and 33 female, bilirubin 7.9 ± 7.4 mg/dL, alkaline phosphatase 675 ± 510 U/L, γ-glutamyltransferase 697 ± 682 U/L, aspartate aminotransferase 193 ± 300 U/L, alanine aminotransferase 136 ± 147 U/L, leucocytes 16.9 ± 10.7 G/L, c-reactive protein 17.3 ± 9.5 mg/dL (Table 1).

Obstruction of the bile duct was caused by gallstones in 7/65 (10.8%) patients, benign strictures in 16/65 (24.6%) patients and malignant strictures of the biliary tract in 42/65 (64.6%) patients.

Thirty-one out of 65 patients had positive bile- and/or blood cultures. Sixty-three bacterial isolates and 17 different bacterial species were identified from 31 patients. The predominant isolated bacteria were *Enterococcus species* (26/63), *E.coli* (13/63), and *Klebsiella species* (8/63). Thereby, three quarter (74.6%) of the isolated bacteria were obtained from these predominant species, while the remaining quarter (25.4%) consisted of 7 different types. Within the group infected with *Enterococcus species*, *Enterococcus faecium* and *Enterococcus faecalis* were most frequent with 8 and 7 isolates, respectively. Bacteriobilia was documented in 22/65 patients and was polymicrobial in 17 patients (77.3%). Positive blood culture were obtained in 13/65 patients and was polymicrobial in only 1 patient (7.7%).

The resistance pattern of the isolated pathogens was investigated by an *in vitro* activity assay. Table 2 gives an overview of all bacterial pathogens and their resistance patterns regarding moxifloxacin and piperacillin/sulbactam. In summary, 34.9% (22/63) of all isolated pathogens were resistant, 6.4% (4/63) were intermediately resistant, and 58.7% (37/63) were susceptible to piperacillin/sulbactam. In comparison to these results 36.5 % (23/63) of all isolated pathogens were resistant, 9.5% (6/63) intermediate resistance, and 54% (34/63) susceptible to moxifloxacin (Figure 1).

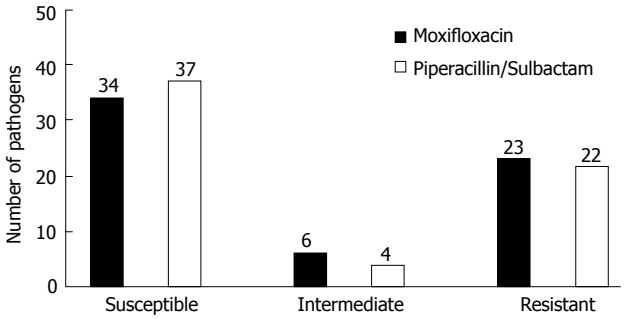

Figure 1 Comparison of *in vitro* activity of moxifloxacin and piperacillin/sulbactam in all isolated bacterial pathogens.

DISCUSSION

Acute cholangitis is an infection of the obstructed biliary tract with a wide spectrum of pathogens. Common microbial populations associated with cholangitis include gram-negative bacteria like *E.coli* and *Klebsiella species*. Gram-positive organisms, mainly *Enterococcus species* and anaerobes, are also found[14-21]. While previous works found *E.coli* infection in 20.9% and *Enterococcus species* in 20.9%[17], our current results reveal that the most common isolates are *Enterococcus species* [41.3% (26/63)], *E.coli* [20.6% (13/63)] and *Klebsiella species* [12.7% (8/63)]. In addition to this a lot of other bacterial pathogens were isolated by blood and/or bile cultures (Table 2). Thus, the shift towards the higher rate of *Enterococcus species* and the high prevalence of *Klebsiella* infections might be related to the use of wide-spectrum antibiotics used in the past years.

Establishment of biliary drainage is the mainstay of therapy for patients with acute cholangitis. Endoscopic sphincterotomy with subsequent biliary drainage is the therapy of choice, but in case of therapy failure percutaneous transhepatic bile drainage is an alternative method for biliary drainage[22-24]. Nevertheless, once endoscopic and/or percutaneous transhepatic procedures have been performed, the spectrum of bacterial infection might change, and increased frequency of mixed infections has been reported[17]. Our current data are in line with this observation and reveal polymicrobial infections of the biliary tract in 17 out of 22 patients.

Overall, our results indicate that bacterial pathogens could only be isolated in 48% of the patients. Antibiotic treatment has to start early during the infectious process. In clinical practice, it is not possible to isolate bacterial pathogens in all patients and the time to receive the resistance pattern creates a delay of several days. Therefore, knowledge of bacterial spectrum and resistance pattern of antimicrobial agents are essential for the treatment of patients suffering from acute cholangitis.

Table 2 Resistance pattern for moxifloxacin and piperacillin/sulbactam in all pathogens

	Moxifloxacin			Piperacillin/Sulbactam		
Pathogens	**Susceptible**	**Intermediate**	**Resistant**	**Susceptible**	**Intermediate**	**Resistant**
Enterococcus species	9	4	13	16	1	9
Enterococcus NS	2	4	2	7		1
Enterococcus faecium	1		7	2	1	5
Enterococcus faecalis	4		3	5		2
Enterococcus casseliflavus	2			2		
Enterococcus gallinarum			1			1
Escherichia coli	8	1	4	11		2
Klebsiella species	5	1	2	4	1	3
Klebsiella pneumoniae	3	1	2	2	1	3
Klebsiella oxytoca	2			2		
Enterobacter species	5			3		2
Enterobacter cloacae	3			3		
Enterobacter NS	2					2
Pseudomonas aeruginosa	2		1		1	2
Aeromonas species	1		1			2
Aeromonas hydrophila/caviae	1					1
Aeromonas NS			1			1
Citrobacter freundii	2			1	1	
Coagulase neg. Staphylococcus			2			2
Gram negative rod NS	1			1		
Streptococcus anginosus	1			1		

NS: Not specified.

Finally, it has to be mentioned that in patients with an obstructed biliary tract, the biliary excretion of several antibiotic agents is limited[6,25]. Recently, it was reported that moxifloxacin, a fluoroquinolone, can reach clinically significant concentrations in obstructed biliary tract[8]. Therefore it may be a superior treatment in patients with acute cholangitis that suffer from biliary obstruction. Until now, no data about antimicrobial activity of moxifloxacin against pathogens of acute cholangitis exists. Therefore, we isolated pathogens from patients with acute cholangitis and analyzed the *in vitro* activity of moxifloxacin and piperacillin/sulbactam. Our data show a comparable *in vitro* activity of moxifloxacin and piperacillin/sulbactam in patients with acute cholangitis. Kiesslich *et al*[26] reported a resistance rate of 71.8% (28/39) for piperacillin and 76.7% (33/43) for ampicillin (both without β-lactamase inhibitors) in bacteria isolated from obstructed biliary tract during endoscopic retrograde cholangiography. In this study, the resistance rate for other fluoroquinolones ciprofloxacin and levofloxacin was 19.0% (8/42) and 2.2% (1/45), respectively. In agreement with these results, 96% (122/127) sensitivity to ciprofloxacin and 29% (37/127) sensitivity to ampicillin was reported in other studies[27].

The *in vivo* benefit of fluoroquinolones in patients with biliary tract infections was investigated in several clinical trials. Karachlios *et al*[28] performed a prospective, randomized trial with ofloxacin in one, and ceftriaxone in the other group. The clinical cure or improvement of clinical symptoms was the same in both groups. In another prospective randomized trial, an adequate clinical benefit was shown for ciprofloxacin mono therapy in comparison to a triple therapy with ceftazidime, ampicillin and metronidazole[29]. Also levofloxacin, a newer enantiomer of ofloxacin showed an adequate clinical effect when compared to ceftriaxone[30]. In this prospective randomized trial, patients of both study groups received metronidazole additionally.

Although, moxifloxacin and piperacillin/sulbactam appears to have a comparable *in vitro* activity against pathogens of acute cholangitis, moxifloxacin may have a clinical benefit due to its extensive biliary excretion in obstructed biliary tract. Randomized clinical trials should be performed to evaluate clinical outcome of moxifloxacin in patients with acute cholangitis.

COMMENTS

Background

Cholangitis is a frequent and potentially serious complication in patients with bile duct obstruction. Biliary decompression by endoscopic intervention and selection of appropriate antibiotics are crucial for therapy of these patients. The use of broad-spectrum penicillin is generally accepted. Because of increasing resistance and allergic reactions against penicillin, other antibacterial agents for the treatment of acute cholangitis are essential moxifloxacin is characterized by an enhanced activity against gram-positive and -negative anaerobic organisms as well by a sufficient concentration in the obstructive bile duct. Therefore it may be an alternative antibacterial treatment for acute cholangitis.

Research frontiers

To our knowledge, no study exists investigating the *in vitro* activity of moxifloxacin against pathogens isolated from patients with acute cholangitis. The current study was designed to analyze the *in vitro* activity of moxifloxacin and piperacillin/sulbactam against pathogens of acute cholangitis.

Innovations and breakthroughs

The predominant pathogens isolated from patients with acute cholangitis were *Enterococcus species*, *E.coli* and *Klebsiella species*. A comparable *in vitro* activity of moxifloxacin and piperacillin/sulbactam was observed for *E.coli* and *Klebsiella species*. In contrast, *Enterococcus species* had higher resistances towards moxifloxacin. Overall bacteria showed antibiotic resistances of 34.9% for piperacillin/sulbactam and 36.5% for moxifloxacin.

Applications

These data suggest that moxifloxacin can be used as an alternative antibiotic therapy in patients with cholangitis that show allergic reactions to piperacillin/sulbactam. Additionally, due to the extensive excretion of moxifloxacin in the obstructed biliary tract it may have a clinical advantage compared to the

standard therapy. Randomized controlled trials should be performed to evaluate the clinical outcome of moxifloxacin in patients with acute cholangitis.

Terminology

Acute cholangitis with the triad of jaundice, fever and abdominal pain: was first described by Charcot in 1877. It is a frequent and potentially serious complication in patients with bile duct obstruction due to ductal stones, benign and malignant bile duct strictures. Bile duct obstruction leads to a raised intrabiliary pressure with cholangiovenous reflux and bacteraemia, which may induce sepsis.

Peer review

This manuscript evaluates the relative resistance of bacterial cultures isolated from patients suffering acute cholangitis to piperacillin/sulbactam (the current antibiotic therapy) versus moxifloxacin. It is well designed, performed and written. It is of clinical relevance.

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S- Editor Li JN **L- Editor** Alpini GD **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3179

World J Gastroenterol 2008 May 28; 14(20): 3179-3182
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Are acute exacerbations of chronic inflammatory appendicitis triggered by coprostasis and/or coproliths?

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Received: January 18, 2008 Revised: April 22, 2008
Accepted: April 29, 2008
Published online: May 28, 2008

Key words: Chronic inflammatory appendicitis; Coprostasis; Coproliths; Recurrent appendicitis

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Sgourakis G, Sotiropoulos GC, Molmenti EP, Eibl C, Bonticous S, Moege J, Berchtold C. Are acute exacerbations of chronic inflammatory appendicitis triggered by coprostasis and/or coproliths? *World J Gastroenterol* 2008; 14(20): 3179-3182 Available from: URL: http://www.wjgnet.com/1007-9327/14/3179.asp DOI: http://dx.doi.org/10.3748/wjg.14.3179

Abstract

AIM: To examine the role of coprostasis and coproliths in recurrent appendicitis.
METHODS: We evaluated four hundred and twenty seven consecutive pathology reports of all appendectomy specimens from January 2003 to December 2004. Findings were categorised as showing acute appendicitis, acute recurrent appendicitis, subacute recurrent appendicitis, chronic appendicitis, or appendices without inflammation. All patients had presented with acute right lower quadrant pain. In 94 instances, there was a history of recurrent similar episodes in the past.
RESULTS: Of the 427 histology reports, 294 were interpreted as showing acute appendicitis, 56 acute recurrent appendicitis, 34 subacute recurrent appendicitis, 28 chronic appendicitis, and 15 non-inflamed appendices. Coprostasis was observed in 58 patients (13.58%) and the presence of coprolith in 6 (1.4%). Coprostasis, and age, were among the predictors in the final model.
CONCLUSION: Coprostasis but not coproliths seems to be a contributing factor to acute exacerbations of chronic inflammatory appendicitis.

INTRODUCTION

Despite the disrepute associated with the term "recurrent appendicitis," there is evidence to suggest that such appendectomies are associated with improvement of the symptoms that lead to admission of the patients[1-4]. The pathophysiology of recurrent inflammation of the appendix is uncertain. Acute appendicitis is thought to be associated with obstruction of the appendiceal lumen, leading to bacterial overgrowth, inflammation, ischaemia, and ultimately perforation. Some authors have thus hypothesized that recurrent lower quadrant pain can be due to either incomplete obstruction of the lumen of the appendix, or a disproportionate production of mucus[5,6]. Recurrent symptom alleviation after appendicectomy in a proportion of our patients compelled us to examine the potential role of coprostasis and coproliths in recurrent appendicitis.

MATERIALS AND METHODS

Patients

We evaluated prospectively all consecutive pathology reports for appendicectomy specimens at the Department

of General Surgery, Marien Hospital Bottrop, Germany from January 2003 to December 2004. Specimens were categorized as acute appendicitis, acute recurrent appendicitis, subacute recurrent appendicitis, chronic appendicitis, and appendices showing no signs of inflammation. The presence of a coprolith (thickened "stone-like" faeces) and coprostasis (appendiceal lumen filled with faeces, completely impacted and not just the presence of a little stool) was noted. Clinical details were supplemented by review of selected case notes.

Specimens were doubly evaluated and classified into five categories by two pathologists separately and the presence of coprostasis and coprolith was also recorded. In those cases with discrepancy in the diagnosis or in the presence of a coprolith or coprostasis specimens (n = 4) were reviewed by an experienced independent pathologist.

Patients were divided in 5 groups: Group A for acute appendicitis, group B for subacute recurrent appendicitis, group C for acute recurrent appendicitis, and group D for chronic inflammation of the appendix. Histology reports with normal non-inflamed appendices were classified as group E. Definitions of the pathologic entities were as follows:

Acute appendicitis (group A): At early stages the serosa is intensely erythematous due to congestion of the subserosal blood vessels. In advanced stages few intact crypts exist lined with intact mucosal epithelium, lamina propria is hypercellular due to neutrophil infiltration, hemorrhage and ulcers are found at the surface caused by the sloughing off of the inflammatory necrotic tissue.

Subacute recurrent appendicitis (group B): This entity is characterized by lympho-follicular hyperplasia, discrete granulocytes, mucocutaneous infiltration and hyperaemic serosa.

Acute recurrent appendicitis (group C): In different sections of the appendix, there can be recognized relative diffuse, inflammatory mucocutaneous and appendiceal wall infiltrations. Primarily neutrophils exist that spread out also within the subserosal tissue. Erosive lesions are additionally observed.

Chronic inflammatory (group D): This is characterized by the presence of unequivocal mural granulation tissue, with or without frank fibrosis, partial or total obliteration of the lumen by fibrous tissue and hyperplasia or atrophy of the lymphoid tissue.

Statistical analysis

Statistical methods included nonparametric Yates correction chi-square, Fisher's exact test (two tails) for categorical variables, and Mann-Whitney U test for quantitative variables. The Random Forest test (data mining procedure) was used to disclose the variables for use in regression analysis. The General Discriminant analysis model was used to evaluate the discriminating effect of coprostasis and coproliths on the defined groups. A Receiver Operating Characteristic (ROC) curve was used to define the ideal cut-off separator for continuous predictor variables. A significance level of 0.05 was assigned. Statistica release 7 (Statsoft) was used for statistical analysis.

Table 1 Incidence of coprostasis and coproliths among groups (%)

Pathology classification	Coprostasis (n = 58)	Coprolith (n = 6)
Acute	33/294 (11.22)	3/294 (1.02)
Subacute recurrent	12/34 (35.20)	1/34 (2.94)
Acute recurrent	9/56 (16.07)	1/56 (1.78)
Chronic	3/28 (10.71)	0/28 (0.00)
No inflammation	1/15 (06.66)	1/15 (6.66)

RESULTS

There were 427 appendectomy pathology reports, corresponding to 265 females and 162 males. Mean patient age was 24.40 ± 17.16 years (range, 4-89 years). All patients were referred for acute right lower quadrant pain. In 94 cases, there was a reported history of recurrent similar episodes in the past. Among these 94 patients 56 had acute recurrent, 34 subacute recurrent and 4 chronic appendicitis.

Of the 427 histology reports, 294 were diagnostic of acute appendicitis, 56 of acute recurrent appendicitis, 34 of subacute recurrent appendicitis, 28 of chronic appendicitis, and 15 of non-inflamed appendices.

Coprostasis was observed in 58 patients (13.58%) and the presence of a coprolith was noted in 6 (1.4%) cases. The incidence and the respective percentages of coprostasis and coprolith among separate histology groups are shown in Table 1.

Associated findings were noted in 6 patients: sigmoid cancer in one, corpus luteum cyst in one, and Meckel's diverticulum in four. Yersinia infection was observed in 1 patient of group B and in one patient of group C. Parasitic infections were diagnosed in 3 patients of group A. Among the 15 cases of group E (non-inflamed appendices), there were four diverticular ruptures, two Meckel's diverticulitis, one carcinoid tumor, one mesenteric arterial embolism, three adnexitis, two tubo-ovarian abscesses, one endometriosis, and one bilateral ovarian biopsy negative for malignancy.

Prominent pathologic findings were encountered more frequently among group A patients. Appendiceal lumen dilatation greater than 10 mm was noticed in 12 patients of group A, one of group B, and 2 of group C. Forty five appendices of group A were gangrenous, and 38 were perforated. This contrasts with the appendices of patients within groups B and C, which were neither gangrenous nor perforated. The incidence of peri-appendicitis was higher in group A (205/294, 69.38%) than in group B (3/34, 8.82%) and group C (28/56, 50%; Table 2). Appendiceal plastrons were documented in 10 patients of group A and one of group D. There were none among patients of groups B and C.

Table 2 Pathological findings

	Acute (A: n = 294)	Sub-acute recurrent (B: n = 34)	Acute recurrent (C: n = 56)	Chronic (D: n = 28)	P
Gangrenous	45	0	0	0	A *vs* B, P = 0.0101, A *vs* C, P = 0.0007
Perforated	38	0	0	0	A *vs* B, P = 0.0199, A *vs* C, P = 0.0019
Peri-appendicitis	205	3	28	3	A *vs* B, P = 0.0001, A *vs* C, P = 0.0498
Abscess	14	0	0		
Other					
Oxyuriasis	3	0	0	0	
Yersinia	0	1	1	0	

Table 3 Forward stepwise regression analysis model, only age and coprostasis were among predictors in the final model

	Steps	Degrees of freedom	F to remove	P to remove	F to enter	P to enter	Effect status
Age	Step number 1	3			4.472079	0.005081	Entered
Gender		3			0.880568	0.453150	Out
Coprostasis		3			3.277140	0.023264	Out
Coprolith		3			0.681613	0.564865	Out
Other pathology		3			1.437535	0.234958	Out
Age	Step number 2	3	447207	0.005081			In
Gender		3			0.914989	0.435791	Out
Coprostasis		3			3.039213	0.031532	Entered
Coprolith		3			1.140316	0.335493	Out
Other pathology		3			1.256381	0.292311	Out
Age	Step number 3	3	421918	0.007020			In
Coprostasis		3	303921	0.031532			In
Gender		3			0.729353	0.536341	Out
Coprolith		3			1.119171	0.343986	Out
Other pathology		3			1.059992	0.368677	Out

The presence of coproliths did not discriminate among groups. Summary of stepwise regression; variable appendicitis forward stepwise P to enter, < 0.05; P to remove, > 0.05.

In order to find the potential role of coprostasis and coprolith as predictors of the various appendicitis classes as described in methods section, we applied Random Forest classification test (this Data Mining technique - Random Forest algorithm developed by Breiman - can be used for classification problems in order to predict a categorical dependent variable). Importance (from high to low) was attributed to Age = 1, Gender = 0.32, Coprostasis = 0.25, Oxyuriasis and Yersinia cases = 0.12 and Appendicolith = 0.05.

Taking into account the potential predictors suggested from the Random Forest test we proceeded for further analysis. Coprostasis, age, gender and oxyuriasis and Yersinia cases were prognostic factors among the four groups (excluded was the "No inflam-mation" group of patients) by univariate analysis. The presence of a coprolith did not achieve statistical significance. Coprostasis (P = 0.0032), age (P = 0.0077), and oxyuriasis and Yersinia cases (P = 0.0354), but not the presence of coprolith, were also found to be predictive variables by forward stepwise regression analysis. The level of significance for "coprostasis" in each group is reported in Table 3. The null hypothesis was rejected in groups D (P = 0.0351) and E (P = 0.0496), but substantiated in groups A (P = 0.6885), B (P = 0.0796) and C (P = 0.1311), implying coprostasis as an etiologic factor in acute, subacute recurrent and acute recurrent appendicitis.

A further Discriminant forward stepwise analysis was employed in order to find the predictive model only for subacute recurrent and acute recurrent cases (Table 3). Only age and coprostasis were among predictors in the final model.

A Receiver Operating Characteristic (ROC) curve was used to select the optimum decision threshold for patient age. Patients less or equal to 40 years had a higher prevalence of subacute (29/5, P = 0.0012) and acute recurrent (45/11, P = 0.0003) appendicitis. Subacute and acute recurrent appendicitis was also found to be more prevalent in females (27/7, P = 0.0083; 39/17, P = 0.0241).

DISCUSSION

The perception that acute appendicitis might subside spontaneously and re-emerge with bouts of right lower quadrant pain (so-called recurrent appendicitis) has met debate and disbelief. Nonetheless, 10% of patients presenting with acute appendicitis report previous similar physical findings that settled without surgery[7,8]. Subsequent appendectomy is remedial[3].

It has been assumed that the likely pathophysiologic

mechanism of recurrent appendicitis is either incomplete obstruction of the lumen of the appendix or disproportionate mucus production. Except for two cases of yersiniosis associated with coprostasis, we did not observe any disorder involving the gastrointestinal system (such as inflammatory bowel disease, sarcoidosis, tuberculosis, polyarteritis nodosa, endometriosis, parasitosis, changes in neuroendocrine cells) that could account for the chronic or recurrent inflammation of the appendix.

Our study specifically addressed the presence of coprostasis, as opposed to previous series in which only coproliths were considered as causative of either acute or recurrent disease. A radiographically visualized coprolith was considered by many authors as a specific and unquestionable indicator of appendicitis due to obstruction[9]. A growing body of evidence however, suggests that luminal obstruction is not an indispensable factor in the development of appendicitis. Arnbjorusson and Bengmark[10] measured intraluminal pressures in acute appendicitis, and concluded that obstruction was the result rather that the cause of the inflammatory process. The incidence of coproliths in our series was 1.21%. The frequency of coprolith in acute appendicitis according to others ranges from 0.02%[11,12] up to 65%[13]. Our present study also rejects the role of coproliths as causative factors in recurrent appendicitis and imposes the use of high fiber diet after an atypical episode.

The reasons we followed this methodology to trace the relationship of coprostasis to the recurrent appendicitis were: (1) The distinction between subacute and acute recurrent appendicitis helps to better delineate the role of coprostasis as a causative factor since these are two sequential phases of the same entity from its initiation to the well established clinical presentation. (2) The use of data mining procedure disclosed the variables for use in regression analysis. The Random Forest test consists of a collection (ensemble) of simple tree predictors, each capable of producing a response when presented with a set of predictor values. For classification problems, this response takes the form of a class membership, which associates (classifies) a set of independent (predictor) values with one of the categories present in the dependent variable. (3) We had to establish first the predictors of all the patients' cohort and subsequently to insert them in analysis for the recurrent appendicitis (subacute or acute) group of patients only in order to avoid a type Ⅱ error.

According to previous reports, the cut-off age after which the incidence of acute appendicitis declines is about thirty years. Based on our observations, the cut-off age in recurrent appendicitis is around forty.

In summary, it seems from our data that coprostasis rather than coproliths could be a contributing factor to acute exacerbations of chronic inflammatory appendicitis, and that its clinical and histologic findings are milder than those of acute appendicitis.

COMMENTS

Background

According to our experience, pain associated with chronic or recurrent appendicitis decreases after elective appendicectomy in selected patients. There is little information about the causal factor. Some authors have hypothesized that recurrent lower quadrant pain can be due to either incomplete obstruction of the lumen of the appendix or to a disproportionate production of mucus.

Research frontiers

We have recently hypothesized that coprostasis and or coproliths may be the contributing factor to acute exacerbations of chronic inflammatory appendicitis.

Innovations and breakthroughs

The present data provide circumstantial evidence that adds to the standing of 'chronic or recurrent appendicitis' as a separate clinical entity. Its clinical and histologic findings are milder than those of acute appendicitis and the cut-off age in recurrent appendicitis is around forty. Coprostasis and not coproliths are the contributing factor to acute exacerbations of chronic inflammatory appendicitis.

Applications

High fiber diet after an atypical recurrent episode is of potential clinical relevance.

Peer review

This study raises an interesting hypothesis about the role of coprostasis in acute exacerbations of "chronic inflammatory appendicitis" by examining the histologic findings of various manifestations of this entity. It also clarifies the definition and the clinical relevance of this disreputed term.

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S- Editor Zhong XY **L- Editor** Negro F **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3183

World J Gastroenterol 2008 May 28; 14(20): 3183-3187
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Retrospective analysis of old-age colitis in the Dutch inflammatory bowel disease population

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Received: December 25, 2007 Revised: March 16, 2008
Accepted: March 23, 2008
Published online: May 28, 2008

Abstract

AIM: To describe the characteristics of Dutch patients with chronic inflammatory bowel disease (IBD) first diagnosed above 60 years of age-a disease also known as old-age colitis (OAC) and to highlight a condition that has a similar appearance to IBD, namely segmental colitis associated with diverticular disease (SCAD).

METHODS: A retrospective longitudinal survey of patient demographic and clinical characteristics, disease characteristics, diagnostic methods, management and course of disease was performed. The median follow-up period was 10 years.

RESULTS: Of a total of 1100 IBD patients attending the Department of Gastroenterology, 59 (5%) [median age 82 years (range 64-101); 25 male (42%)] were identified. These patients were diagnosed with ulcerative colitis (n = 37, 61%), Crohn's disease (n = 14, 24%), and indeterminate colitis (n = 8, 15%). Remission was induced in 40 (68%) patients within a median interval of 6 mo (range 1-21) and immunosuppressive therapy was well tolerated. Histological evaluation based on many biopsy samples and the course of the disease led to other diagnosis, namely SCAD instead of IBD in five (8%) patients.

CONCLUSION: OAC is not an infrequent problem for the gastroenterologist, and should be considered in the evaluation of older patients with clinical features suggestive of IBD. Extra awareness and extensive biopsy sampling are required in order to avoid an erroneous diagnosis purely based on histological mimicry of changes seen in SCAD, when diagnosing IBD in the presence of diverticulosis coli.

Key words: Inflammatory bowel disease; Old-age colitis; Segmental colitis

Peer reviewer: Yoshihide Fujiyama, Professor, Internal Medicine, Division of Gastroenterol, Shiga University of Medical Science, Tsukinowa, Seta, Otsu 520-2192, Japan

Hadithi M, Cazemier M, Meijer GA, Bloemena E, Felt-Bersma RJ, Mulder CJ, Meuwissen SGM, Peña AS, van Bodegraven AA. Retrospective analysis of old-age colitis in the Dutch inflammatory bowel disease population. *World J Gastroenterol* 2008; 14(20): 3183-3187 Available from: URL: http://www.wjgnet.com/1007-9327/14/3183.asp DOI: http://dx.doi.org/10.3748/wjg.14.3183

INTRODUCTION

Inflammatory bowel disease (IBD), a lifelong uncontrolled inflammation of the intestinal mucosa, is broadly subdivided into ulcerative colitis, Crohn's disease, and in 10%-15% of patients, indeterminate or unclassified colitis, when a definitive diagnosis of ulcerative colitis or Crohn's disease cannot be made at colonoscopy, colon biopsy or colectomy[1,2].

The pathogenesis of IBD remains obscure. While it is clear that there are genetic, environmental, and immunological factors involved in the pathogenesis of IBD, the exact contribution of each and the sequence of events that culminates in clinically apparent IBD remains the subject of intense investigation.

Although IBD may occur at any age, the peak age of onset is 15-30 years old and approximately 10% of cases occur in individuals < 18 years old[3]. Old-age colitis (OAC) refers to patients older than 60 years, who are affected by a broad group of colonic diseases, such as infection, carcinoma, drug-induced disease, vasculitis, microscopic colitis, ischemic colitis, and IBD. Diagnosis of IBD in older patients may be difficult because it can easily be confused

with other forms of colitis commonly occuring at this age.

Earlier reports have indicated that both ulcerative colitis and Crohn's disease have a bimodal age distribution, with a second, smaller peak incidence occurring in individuals aged 50-70 years[4-7]. Two recent studies have shown that 21%-23% of ulcerative colitis occurs after the age of 50 years and 5% after 70 years[8,9]. This age group comprises around 12% (range 8%-20%) and 16% (range 7%-26%) of all newly diagnosed patients with ulcerative colitis and Crohn's disease, respectively[10].

An additional group of disorders called segmental colitis associated with diverticular disease (SCAD) has been found to masquerade as IBD on both a clinical and histological basis[11], since colonic diverticula, most often involving the sigmoid colon, commonly affect middle-aged and elderly individuals[12,13].

In this retrospective cohort, we attempted to differentiate the broad nomer OAC and to describe the characteristics of Dutch IBD patients older than 60 years. In addition, we wanted to highlight one of the conditions that is similar to IBD, namely SCAD, since an overlap of IBD and diverticular disease has long been recognized and is not infrequent in clinical practice[14].

MATERIALS AND METHODS

An IBD database review of more than 1100 patients, covering the years 1990 to the current time, provided 64 cases with OAC. The diagnosis of IBD was determined according to conventional endoscopic, radiological and histological criteria[15-17]. Medical records of each patient in this study were reviewed for the following information: sex, age, diagnosis, duration of disease, presenting symptoms, medications (including non-steroid anti-inflammatory drugs), anatomic location of disease, coexistence of diverticulosis, extraintestinal manifestations, laboratory results, radiological results, histopathological examinations, previous medical and surgical treatment strategies, remission rate and development of refractory disease, postoperative morbidity and mortality, overall outcome, and development of malignancy. Dedicated gastroenterological pathologists revised histological specimens from all subjects. Extraintestinal manifestations included erythema nodosum, pyoderma gangrenosum, peripheral arthritis, sacroiliitis/spondylitis, and episcleritis or uveitis. Refractory disease was defined as patients who were not adequately controlled with conventional therapy or immunosuppressive agents, or who required surgical intervention[18]. The diagnosis of colonic diverticular disease was established by colonoscopy, barium enema, or both. SCAD was considered when colitis was restricted to a diverticular segment of the left colon (excluding the rectum); the rectum and proximal colon were endoscopically and histologically normal; and when there was no recurrence of segmental colitis following surgical resection of the affected segment[11].

Statistical analysis

Descriptive analysis was performed and continuous data were expressed as the median (range) and categorical data as numbers (percentage). Differences in erythrocyte sedimentation rate, serum albumin and hemoglobin were compared by using one way analysis (ANOVA). $P \leqslant 0.05$ was considered statistically significant. Sensitivity and predictive values of radiographic examinations were calculated by using 2 × 2 tables when the diagnosis was based on endoscopic and histological results. Statistical analysis was performed using the Statistical Software Package version 11.0 (SPSS Inc., Chicago, IL, USA).

Table 1 General characteristic of patients with OAC

Characteristic	Total (n = 59)
Age, median (range, yr)	82 (64-101)
Men, n (%)	25 (42)
Body mass index, median (range)	21 (17-29)
Patient subsets, n (%)	
Ulcerative colitis	37 (63)
Crohn's disease	14 (24)
Indeterminate colitis	8 (13)
Presenting symptoms, n (%)	
Rectal bleeding	35 (65)
Diarrhea	27 (50)
Abdominal pain	23 (42)
Weight loss	18 (33)
Constipation	7 (13)
Fever	5 (9)
Extra-intestinal symptoms, n (%)	9 (17)
NSAIDs, n (%)	13 (22)
Diverticulosis coli, n (%)	36 (61)

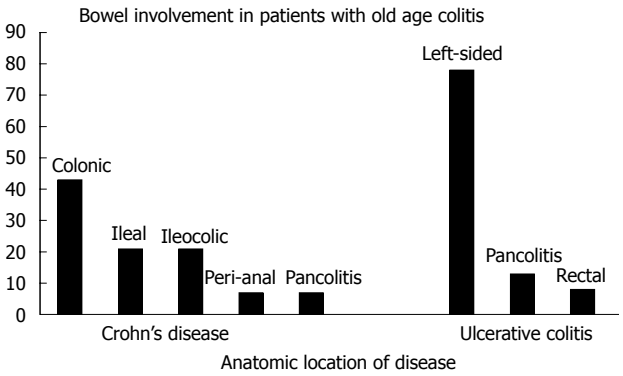

Figure 1 Bowel segment involvement in OAC.

RESULTS

Patients and disease characteristics

A total of 64 patients with OAC were identified. Five patients were excluded because the required histopathological studies were not available, and the remaining 59 patients were included in the analysis. Table 1 summarizes the general and disease-related characteristics and Figure 1 illustrates the bowel involvement in patients with Crohn's disease and ulcerative colitis. Colonic involvement was the rule in all eight patients with indeterminate colitis. Seven patients, including one with indeterminate colitis presented with pancolitis, and one patient had fistulous Crohn's disease. Extra-intestinal

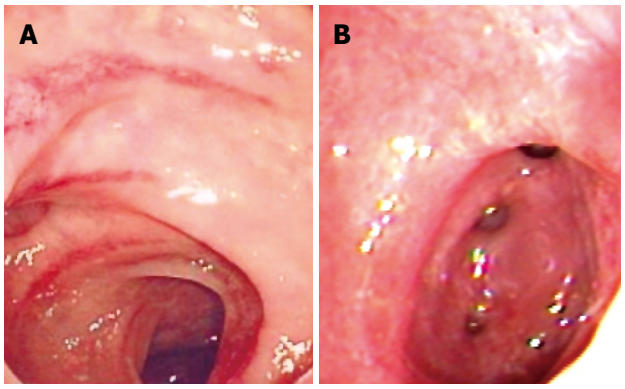

Figure 2 Endoscopic images showing signs of mucosal inflammation of sigmoid segment affected by diverticulosis coli, the inflammatory signs are stressed along the crests of the colonic folds in (**A**), and diffusely spread in (**B**).

manifestations included peripheral arthritis (n = 5), uveitis (n = 2) axial spondylitis (n = 1), and erythema nodosum (n = 1).

High erythrocyte sedimentation rate was found in 41 (69%) patients at presentation, exceeding in frequency decreased serum albumin or anemia (30% and 29%, respectively, $P < 0.001$). A total of 51 radiographic examinations were performed including barium enema (n = 36), small bowel follow-through (n = 8), and abdominal computerized tomography (n = 7), and depicted features suggestive of IBD in 17 (29%) patients (sensitivity 0.36 and PPV 0.79). An average of five (range 3-17) biopsies were obtained from separate segments of the colon and usually from affected and normally appearing mucosa on each endoscopical examination. Histology of surgically removed bowel segments was further evaluated in eight patients.

Infiltration of inflammatory cells was confined to the mucosa in the majority of patients [35 (95%) patients with ulcerative colitis, 10 (71%) with Crohn's disease, and seven (87%) with indeterminate colitis], and extended from the mucosa with reactive involvement of muscularis propria in the remaining patients. The inflammatory infiltrate was transmural in one patient with Crohn's disease. Crypt abscesses were identified in 26 (70%) patients with ulcerative colitis, four (29%) with Crohn's disease, and four (50%) with indeterminate colitis. Granulomas on the other hand, were recognized in three (8%) patients with ulcerative colitis, five (36%) with Crohn's disease, and four (50%) with indeterminate colitis.

Management and course of the disease

Mesalazine preparations, corticosteroids and azathioprine were administered to either induce or maintain remission in a total of 43 (73%), 23 (40%) and seven (12%) patients, respectively. No marked side effects were reported during a median follow-up period of 10 years (range 1-14). Remission was induced in 27 (73%), seven (50%) and six (75%) patients with ulcerative colitis, Crohn's disease and indeterminate colitis, respectively, within a median interval of 6 mo (range 1-21).

Eighteen (30%) patients were considered to have refractory disease that necessitated surgical intervention, such as sigmoid resection, partial colectomy, pancolectomy, or ileal or ileocecal resection. However, postoperative recurrence was documented in two patients with Crohn's disease and postoperative morbidity and mortality was 66% and 6%, respectively. Two patients died due to terminal cholangiocarcinoma, one patient postoperatively (*Klebsiella pneumonia* sepsis), and three patients due to causes unrelated to IBD, with an overall mortality of 10%.

SCAD

Diverticulosis coli was present in 61% of patients with OAC. Five (8%) patients showed features that were suggestive of a diagnosis of SCAD. All five had endoscopic as well as histopathological features of colitis that affected the sigmoid colon, with sparing of the rectum and proximal colon. Endoscopic examinations showed either a circumferencial localization of erythema, granularity and friability, and sparing of the ostia, as shown in Figure 2A, or diffuse periosteal distribution of erythema, as shown in Figure 2B. Two patients underwent surgery and remained in remission without maintenance treatment. The other three patients were treated initially with a course of mesalazine preparations and were further maintained in remission by increasing daily fluid intake and using fiber-rich laxative preparations. These patients were retrospectively considered to have SCAD and not IBD as initially diagnosed.

DISCUSSION

This retrospective cohort study showed that 5% of IBD patients who attended our referral center were aged > 60 years old, and could be categorized as having OAC. On the other hand, 8% of patients with OAC have retrospectively non-IBD colitis that the so-called OAC implicates a broader diagnosis than IBD.

With respect to the predominance of ulcerative colitis, anatomic location in Crohn's disease, presenting symptoms, management, and postoperative morbidity and mortality, our findings were in agreement with earlier observations[10,19-21]. Unlike our findings, higher incidence rates of IBD in older patients and higher frequency rates of isolated proctitis within the ulcerative colitis subgroup have been reported[22-24]. The difference in these rates is very likely related to the retrospective nature of the studies. Noticeably, two patients developed cholangiocarcinoma and none developed colorectal carcinoma during the follow-up period. The available data disallowed further disclosure of underlying sclerosing cholangitis. The use of immunosuppressive agents in this older population appears to warrant broadened application, even if there is little objective data on which to base this practice[25]. The use of infliximab as an anti-inflammatory treatment in patients with IBD has been reported to be safe, including in those aged > 60 years old[26]. However, there appears to be a significant risk of deleterious and fatal adverse events when infliximab is used in older patients[27]. More

safety data about the use of biological agents in older populations are needed, especially when more new agents with proven efficacy are evolving.

Some attribute one-third of the small incidence peak of IBD in this age group to ischemia[28]. However, the chronic course of the disease and the emergence of refractory colitis made the diagnosis of ischemic colitis unlikely in our study, along with the histological findings that were also not supportive of a diagnosis of ischemic colitis.

A recognized pitfall in clinical practice appeared in this series; misdiagnosing SCAD as IBD in 8% of patients[29-33]. SCAD has long been recognized as an example of the overlap of IBD and diverticular disease[14,16,29,30]. The pathogenesis of this apparently distinct form of colitis is unclear[14].

Factors such as age and the high predilection of Crohn's disease for distal localization in older patients contribute to the simultaneous occurrence of both disorders in this population[34]. Differentiating between IBD and SCAD imposes a challenging task to the clinician as well as the pathologist. Clinical evaluation, laboratory tests, radiological results and endoscopic examinations (especially in diffuse type), in addition to histological studies, may be misleading. Sometimes even intestinal resection cannot provide the clinician with a definitive diagnosis. Luminal mucosal inflammation, although unusual, may occur in diverticular disease due to redundancy and mucosal prolapse[35]. When the luminal inflammation appears in what is called crescentic fold disease, a diagnosis of SCAD seems more probable[36]. A diagnosis of SCAD becomes more difficult when the inflammation affects a colon segment diffusely. The histology of SCAD may closely mimic ulcerative colitis and the hallmarks of Crohn's disease[16,37]. To complicate the issue, many cases of SCAD seem to respond post aut propter favorably to treatment with mesalazine preparations similar to that given for IBD[30]. That is why a definitive diagnosis may remain obscure for a long time, and only the course of the disease may bring to light the underlying nature of the disorder. Newly emerging instruments in the diagnostic panel such as serological markers[38-40], advanced radiological examinations such as contrast-enhanced magnetic resonance imaging[41], wireless capsule video endoscopy[42], and double-balloon small enteroscopy[43] may facilitate an early and correct definite diagnosis. These diagnostic modalities appear to be valuable for patients who have indeterminate colitis or who are failing medical therapy. The multiple harvest of biopsy specimens at each endoscopic session seems to be helpful in differentiating colitis in segmental fashion, especially SCAD that can be cured by limited resection of affected segments, although this conclusion is based on limited data from this retrospective study.

In summary, OAC is not an infrequent problem for the gastroenterologist and should be considered in the evaluation of older patients with clinical features suggestive of IBD. This entity is broader than IBD alone and therefore more challenging. Extra awareness is required in order to avoid an erroneous diagnosis purely based on histological mimicry of changes seen in SCAD when diagnosing IBD in the presence of diverticulosis coli, and taking multiple biopsies from each part of the colon is recommended.

COMMENTS

Background

Inflammatory bowel disease (IBD) is a lifelong uncontrolled inflammation of the intestinal mucosa that mainly affects the young age group but also older individuals.

Research frontiers

Diagnosis of IBD in older patients may be difficult because it can easily be confused with other forms of colitis commonly occuring in this age group, such as segmental colitis associated with diverticular disease (SCAD).

Applications

Old-age colitis (OAC) is not an infrequent problem for the gastroenterologist and should be considered in the evaluation of older patients with clinical features suggestive of IBD. Extra awareness is required in order to avoid an erroneous diagnosis.

Terminology

OAC refers to patients older than 60 years affected by a broad group of colonic diseases, such as infection, carcinoma, drug-induced disease, vasculitis, microscopic colitis, ischemic colitis, and IBD. SCAD has been found to masquerade as IBD on both a clinical and histological basis.

Peer review

The authors described the characteristics of Dutch patients with chronic IBD with a first diagnosis above 60 years of age, also known as OAC, and highlighted one of the conditions that has the appearance of IBD, namely SCAD. This is an interesting study. The authors concluded that OAC is not an infrequent problem for the gastroenterologist and should be considered in the evaluation of older patients with clinical features suggestive of IBD.

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S- Editor Li DL **L- Editor** Kerr C **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3188

World J Gastroenterol 2008 May 28; 14(20): 3188-3194
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Effect of probiotic *Lactobacillus rhamnosus* GG intervention on global serum lipidomic profiles in healthy adults

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Received: February 2, 2008 Revised: April 15, 2008
Accepted: April 22, 2008
Published online: May 28, 2008

Abstract

AIM: To investigate the effect of three weeks' intervention with a probiotic *Lactobacillus rhamnosus* GG (LGG) bacteria on global serum lipidomic profiles and evaluate whether the changes in inflammatory variables (CRP, TNF-α and IL-6) are reflected in the global lipidomic profiles of healthy adults.

METHODS: We performed UPLC/MS-based global lipidomic platform analysis of serum samples (n = 26) in a substudy of a randomised, double-blind, placebo-controlled 3-wk clinical intervention trial investigating the immunomodulatory effects of probiotics in healthy adults.

RESULTS: A total of 407 lipids were identified, corresponding to 13 different lipid classes. Serum samples showed decreases in the levels of lysophosphatidylcholines (LysoGPCho), sphingomyelins (SM) and several glycerophosphatidylcholines (GPCho), while triacylglycerols (TAG) were mainly increased in the probiotic LGG group during the intervention. Among the inflammatory variables, IL-6 was moderately associated by changes in global lipidomic profiles, with the top-ranked lipid associated with IL-6 being the proinflammatory LysoGPCho (20:4). There was a weak association between the lipidomic profiles and the two other inflammatory markers, TNF-α and CRP.

CONCLUSION: This was the first study to investigate the effects of probiotic intervention on global lipidomic profiles in humans. There are indications that probiotic LGG intervention may lead to changes in serum global lipid profiles, as reflected in decreased GPCho, LysoGPCho and SM as well as mainly increased TAG.

Key words: Probiotic; *Lactobacillus rhamnosus* GG; Lipidomic; Inflammatory mediators; Healthy adults

Peer reviewers: Laura E Matarese, MS, RD, LDN, FADA, CNSD, Thomas E. Starzl Transplantation Institute, UPMC Montefiore, 7 South, 3459 Fifth Avenue, Pittsburgh, PA 15213, United States; Dr. Raymund Rabe Razonable, Division of Infectious Diseases, Mayo Clinic Institution, 200 First Street SW, Rochester 55905, United States; Dr. Francesco Costa, Dipartimento di Medicina Interna, U.O. di Gastroenterologia, Università di Pisa, Via Roma, Pisa 67-56122, Italy

Kekkonen RA, Sysi-Aho M, Seppänen-Laakso T, Julkunen I, Vapaatalo H, Orešič M, Korpela R. Effect of probiotic *Lactobacillus rhamnosus* GG intervention on global serum lipidomics profiles in healthy adults. *World J Gastroenterol* 2008; 14(20): 3188-3194 Available from: URL: http://www.wjgnet.com/1007-9327/14/3188.asp DOI: http://dx.doi.org/10.3748/wjg.14.3188

INTRODUCTION

The new global metabolic profiling techniques or 'metabolomics', have made it possible to measure large numbers of different metabolites, and are currently being applied to increase our understanding of the health and disease continuum[1]. High-dimensional lipid analysis technologies (lipidomics) provide an opportunity to measure lipids on a broad scale[2]. The majority of lipid pathways involved in lipid metabolism are known, but new lipid metabolites are being discovered all the time. It is not fully known how different pathways affect individual

metabolic health and how changes in the regulation of these pathways can influence major metabolic and inflammatory diseases like diabetes, cardiovascular and inflammatory diseases and obesity[2]. The new analytical capacity of lipidomics as a branch of metabolomics can increase our understanding of lipid biology, improve the characterisation of global lipid profiles and result in the identification of previously unknown changes in lipid metabolism[3].

One study has evaluated the transgenomic metabolic effects of two probiotic lactobacilli in mice[4], but as far as we know, the effects of probiotics on global lipidomic profiles in humans have not been characterised before. Previously, probiotics have been shown to possess immunomodulatory effects in *in vitro* assays, animal models and clinical trials[5,6], and their effects have been studied mainly in specific conditions such as allergy[7,8] and inflammatory diseases[9].

In the present study, we characterised the effect of the probiotic *Lactobacillus rhamnosus* GG (LGG) on global serum lipidomic profiles and investigated whether the changes in inflammatory variables (CRP, TNF-α and IL-6) are reflected in the lipidomics profiles of healthy adults.

MATERIALS AND METHODS

Subjects

The subjects were healthy adults (n = 26, 14 females, 12 males) with a mean age of 42 years (range 23-55) and a mean BMI of 24 kg/m^2 (range 20-30). The subjects were recruited to the study by an advertisement in the Helsinki area. The inclusion criteria were being healthy (no chronic illnesses), taking regular exercise (at least three times per week), and not participating in any other clinical trial. The exclusion criteria were milk allergy (due to the nature of the study products), use of antibiotics during the 2 mo before the study, acute gastrointestinal disorders during the 2 mo before the study, gastrointestinal diseases and related medication, pregnancy, and lactation. Before entering the study, the subjects gave their written informed consent. The study protocol was approved by the Ethics Committee of the Hospital District of Helsinki and Uusimaa.

Intervention

The present study was a substudy of a randomised, double-blind and placebo-controlled parallel group intervention study investigating the immunomodulatory effects of probiotic bacteria with four treatment groups; placebo, LGG, *Bifidobacterium animalis* ssp. *lactis* Bb12 and *Propionibacterium freudenreichii* ssp. *shermanii* JS[10]. Only the placebo (n = 15) and LGG (n = 11) groups were included in the present pilot study since the LGG exhibited the best anti-inflammatory potential in the original study. Prior to the intervention period, there was a 3-wk run-in period during which no probiotic-containing products were allowed. For 3 wk thereafter, the subjects consumed either a 250 mL milk-based fruit drink containing LGG bacteria (ATCC 53103, 6.2 × 10^7 cfu/mL) or a similar placebo drink without probiotic bacteria daily. A list of probiotic-containing products was given to the subjects, and they were asked not to consume any other probiotic-containing products at any point during the study. Otherwise they were allowed to eat freely. Venous blood samples from the antecubital vein were taken at baseline and after the 3-wk intervention. The blood samples were stored at -70℃ for global lipidomic analyses.

Inflammatory variables and serum lipids

Serum levels of C-reactive protein (CRP) were measured by a high-sensitivity particle-enhanced immunoturbidimetric CRP assay using a Tina-quant CRP (latex) high-sensitivity reagent and a Roche Hitachi 912 analyser (Roche Diagnostics GmbH, Mannheim, Germany) with a detection limit of 0.04 mg/L. All samples were over the detection limit. Cytokine levels (TNF-α, IL-6) in serum were determined using Quantikine HS, Human TNF-α/TNFSF1A (Catalog Number HSTA00D) and IL-6 (HS600B) immunoassays purchased from R&D Systems (Minneapolis MN, USA) according to the manufacturer's instructions. The detection limit was 0.5 pg/mL for TNF-α and 0.16 pg/mL for IL-6. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride concentrations were measured with their respective enzymatic kits from Roche Diagnostics using an autoanalyser (Roche/Hitachi 912 Automatic Analyzer). Low-density lipoprotein (LDL) cholesterol concentrations were calculated using Friedewald's equation[11].

Sample preparation for global lipidomic analysis

An aliquot (10 μL) of an internal standard mixture containing 11 lipid classes and 0.05 mol/L sodium chloride (10 μL) was added to the serum samples (10 μL). The lipids were extracted with chloroform/methanol (2:1, 100 μL). A standard mixture containing 3 labelled standard lipids was added (10 μL) to the extracts. The sample order for LC/MS analysis was determined by randomization.

Global lipidomics analysis by UPLC/MS

Lipid extracts were analysed on a Waters Q-Tof Premier mass spectrometer combined with an Acquity Ultra Performance LC™ (UPLC). The column, which was kept at 50℃, was an Acquity UPLCTM BEH C18 10 mm × 50 mm with 1.7 μm particles. The binary solvent system included (A) water (1% 1 mol/L NH_4Ac, 0.1% HCOOH) and (B) LC/MS grade (Rathburn) acetonitrile/isopropanol (5:2, 1% 1 mol/L NH_4Ac, 0.1% HCOOH). The gradient started from 65% A/35% B, reached 100% B in 6 min and remained there for the next 7 min. The total run time including a 5 min re-equilibration step was 18 min. The flow rate was 0.200 mL/min and the injected amount 0.75 μL. The temperature of the sample organiser was set at 10℃.

The lipid profiling was carried out on Waters Q-Tof Premier mass spectrometer using ESI+ mode. The data were collected at mass range of m/z 300-1200 with a scan duration of 0.2 s. The source temperature was

set at 120℃, and nitrogen was used as desolvation gas (800 L/h) at 250℃. The voltages of the sampling cone and capillary were 39 V and 3.2 kV, respectively. Reserpine (50 μg/L) was used as the lock spray reference compound (5 μL/min; 10 s scan frequency).

Data were processed using MZmine software, version 0.60[12]. Lipids were identified using an internal spectral library or by tandem mass spectrometry using UPLC/MS/MS as described previously[13]. The normalisation of lipidomic data was performed as follows: All monoacyl lipids except cholesterol esters, such as monoacylglycerols and monoacylglycerol-PL, were normalised with GPCho (17:0/0:0); diacyl lipids except ethanolamine PL were normalised with GPCho (17:0/17:0); ceramides with Cer (d18:1/17:0); the diacyl ethanolamine phospholipids with GPEtn (17:0/17:0); and the TG and cholesterol esters with TG (17:0/17:0/17:0). Other (unidentified) molecular species were calibrated with GPCho (17:0/0:0) for a retention time of < 300 s, GPCho (17:0/17:0) for between 300 s and 410 s, and TG (17:0/17:0/17:0) for higher retention times. Only the identified lipid molecular species were included in further data analyses.

Lipid nomenclature

Lipids from the global lipidomic analysis were named according to Lipid Maps (http://www.lipidmaps.org). For example, lysophosphatidylcholine (LysoGPCho) with 16:0 fatty acid chain was named monoacyl-glycerophosphocholine GPCho (16:0/0:0). In cases where the fatty acid composition could not be determined, the total number of carbons and double bonds was marked. For example, a phosphatidylcholine species PCho (16:0/20:4) is represented as GPCho (36:4). However, GPCho (36:4) could also represent other molecular species, for example, GPCho (20:4/16:0) or GPCho (18:2/18:2).

Statistical analysis

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS/DA) were utilised as modelling methods for clustering and discrimination[14]. PLS/DA is a pattern recognition technique that correlates variation in the dataset with class membership. The resulting projection model gives latent variables (LVs) that focus on maximum separation ("discrimination"). The random subsets cross-validation method[15] and Q2 scores were used to develop the models. The VIP (variable importance in the projection) values[16] were calculated to identify the most important molecular species for the clustering of specific groups. PLS/DA and PCA analyses were performed using Matlab, version 7.2 (Mathworks, Natick, MA, USA) and PLS Toolbox, version 4.0, of the Matlab package (Eigenvector Research, Wenatchee, WA, USA. All other analyses were performed using R statistical language (http://www.r-project.org/).

Comparisons between the levels of selected molecular species were performed using the paired Wilcoxon test. For the PLS/DA analyses as well as paired univariate analyses, the data were first log-transformed for each lipid so that $X = \log (z_2/z_1)$, where z_2 was the lipid concentration at 21 d and z_1 at baseline. With such transformation, the distribution of data was closer to normal and the within-person changes could better be analysed. Chance detection plotting was used to account for multiple hypothesis testing in univariate comparisons. The chance detection plot described how many lipids show more significant differences at random than those actually observed.

In order to assess whether any of the inflammatory variables were explained by global lipidomic profile data, we regressed global lipidomic profile data on selected inflammatory variables using an elastic net method[17]. The method selects an optimal subset of lipids, based on predictive performance of the regression model using extensive bootstrap-based cross-validation. The model is selected by minimum cross-validation-error criterion, which balances the bias against the variance of the estimates. For these analyses, the data were first log-transformed for each lipid/clinical variable so that $X = \log (z_2/z_1)$.

RESULTS

Serum lipids

The mean (SD) baseline value (mmol/L) for total cholesterol was 5.1 (1.1), for LDL cholesterol 3.1 (1.0), for HDL cholesterol 1.5 (0.4) and for triglycerides 1.0 (0.4) in the placebo group and, in the LGG group, 5.4 (1.2), 3.3 (1.0), 1.5 (0.4) and 1.4 (1.1), respectively. The mean (SD) change (mmol/L) during the 3-wk intervention in total cholesterol was 0.2 (0.5), in LDL cholesterol 0.1 (0.5), in HDL cholesterol 0.1 (0.2) and in triglycerides 0.0 (0.5) in the placebo group and 0.0 (0.4), 0.1 (0.3), 0.0 (0.2) and 0.0 (0.6) in the LGG group, respectively. There were no significant differences in serum lipids during the intervention.

Global lipidomic analysis

The global lipidomic analysis led to 407 identified lipid species, corresponding to 13 different lipid classes. PCA analysis revealed that no major outliers exist in the data, thus confirming that any changes detected in further analyses would not be due to specific outliers. PLS/DA analysis revealed that the LGG and the placebo groups differed at baseline, and therefore only the within-person changes were utilised in the later statistical analyses. PLS/DA analysis on log-transformed data indicated that the global lipidomic profiles of the groups were separable (Figure 1A). In the LGG group, significant changes ($P < 0.05$) in lipids were observed during the intervention using paired Wilcoxon test, although when accounting for multiple hypothesis testing by using the chance detection plot, no lipid changes were found to be significant within the 95% confidence interval. However, the VIP analysis revealed some common trends in the lipidomic profile data. Decreased LysoGPCho and sphingomyelins (SM), mainly decreased glycerophosphatidylcholines (GPCho) and mainly increased triacylglycerols (TAG) were among the most important

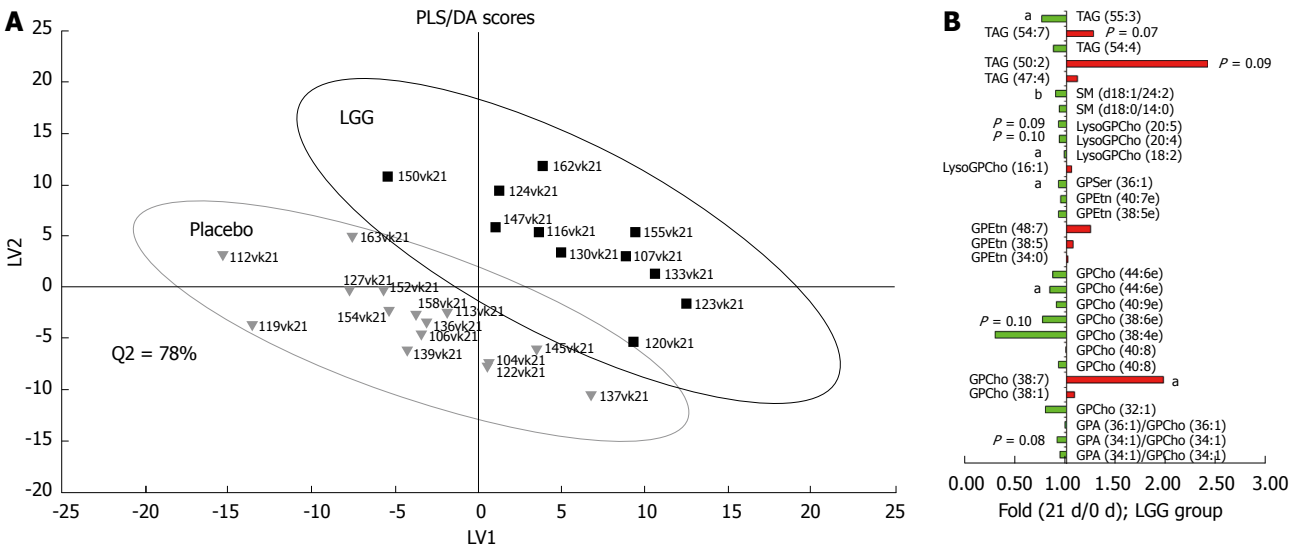


Figure 1 **A**: Partial least squares discriminant analysis (PLS/DA) of global serum lipidomic data during the probiotic intervention in healthy adults. The labels in the picture indicate subject ID numbers; **B**: Fold changes for the top 30 ranking lipids contributing to the PLS/DA model based on VIP analysis (variable important in the projection) ($^aP < 0.05$, $^bP < 0.001$).

variables contributing to the separation between the LGG and the placebo groups (Figure 1B).

Associations between global lipidomics profiles and inflammatory variables

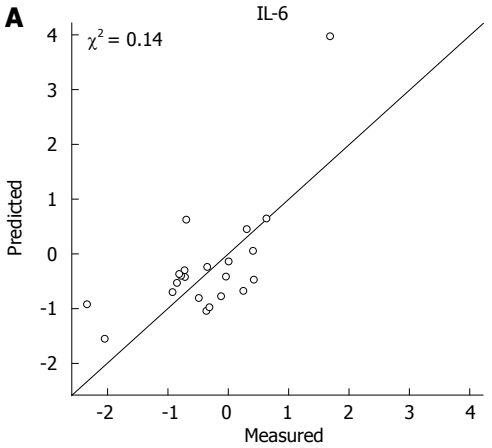
In order to investigate whether the changes in inflammatory variables during the 3-wk intervention were reflected in global lipidomic profiles, we regressed the lipidomic profile data on measured serum TNF-α, IL-6, and CRP values (Figure 2). The results revealed that a reasonably good model based on global lipidomic profiles was found for the proinflammatory cytokine IL-6, while the regression model was poor for CRP and TNF-α. The top-ranking lipid associated with the changes in IL-6 was the proinflammatory LysoGPCho (20:4) (Figure 2).

DISCUSSION

This study is the first to apply lipidomic techniques to analyse the global lipidomic profiles of healthy adults after a probiotic intervention. The lipidomic platform has already been applied in multiple studies investigating the pathophysiology of different diseases[18-21]. In the present study, we characterised the effect of probiotic LGG on global serum lipidomic profiles and investigated whether the changes in inflammatory variables (CRP, TNF-α and IL-6) were reflected in global lipidomic profiles in healthy adults. We observed that the probiotic LGG intervention may lead to changes in global lipidomic profiles.

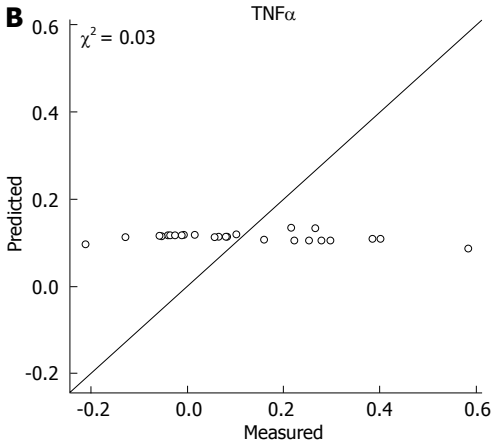
We found a trend towards decreased LysoGPCho after the probiotic LGG intervention. LysoGPCho, derived from phosphatidylcholines, are mediators that affect numerous functions in many types of cells, from proliferation and survival to migration and secretion. They are also involved in oxidative metabolism, angiogenesis, and carcinogenesis[22]. LysoGPCho is a major atherogenic lipid of oxidised LDL[23], and it has been associated with vascular inflammation, endothelial dysfunction and coronary atherosclerosis[24]. LysoGPCho induces an increase in several inflammatory cytokines (IL-1β, IL-6, TNF-α) in human peripheral mononuclear cells (PBMCs)[25]. Therefore, the reduction of LysoGPCho in the present study could be related to our, and previous, results showing a decreased production of TNF-α in PBMCs in healthy adults[10,26]. Interestingly, a high LysoGPCho level has been connected also to inflammatory bowel disease (IBD)[27,28], impaired mucosal barrier function and increased gut permeability[29-32]. LGG has not been effective in treating Crohn's disease[33,34], but it has been shown to maintain remission in patients with ulcerative colitis[35]. In addition, LGG normalises gut permeability[36,37] and enhances mucosal integrity and epithelial cell survival[38,39]. Taken together, the decrease in LysoGPCho after LGG intervention observed in the present study may be one of the metabolic events behind the beneficial clinical effects of LGG seen in ulcerative colitis and in normalised gut permeability.

In the present study, we also observed a decrease in SM after the LGG intervention. SM is a major membrane sphingolipid and the precursor of important signalling molecules like ceramide and sphingosine[40]. Recent studies reveal that metabolites of SM are critically important for the initiation and maintenance of diverse aspects of immune cell activation and also function as regulators of inflammatory responses[41-43]. High concentrations of sphingolipids and lipids of the SM/ceramide pathway have been connected to inflammatory processes in the development of atherosclerosis[44] and IBD[45,46]. The harmful effects of these lipids may be partly mediated via the production of reactive oxygen species in cells[42,47]. As in the case of LysoGPCho, the generation of ceramide by sphingomyelinases from SM and epithelial oxidative stress might contribute to



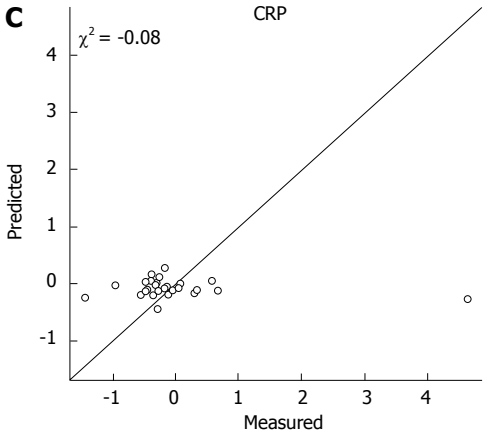
Top-ranking lipids

Lipid	*P*-value	Fold
LysoGPChO (20:4)	0.13	0.86
GPCho (34:5)	0.96	1.03
GPCho (50:7)	0.51	1.02
GPEtn (34:2e)	0.24	1.16
GPCho (36:1)	0.72	1.00
SM (d18:1/20:4)	0.38	0.88
TAG (51:2)	0.76	1.02
GPCho (38:7)	0.01	1.93
GPCho (38:4e)	0.12	0.21
GPSer (36:1)	0.03	0.91



Top-ranking lipids

Lipid	*P*-value	Fold
SM (d18:1/24:1)	0.41	0.89
GPCho (40:3)	0.92	1.23
GPCho (40:4)	0.44	1.14



Top-ranking lipids

Lipid	*P*-value	Fold
GPCho (36:1)	0.72	1.00
GPEtn (338:5)	0.18	0.72
GPEtn (38:0)	0.54	1.07

Figure 2 Cross-validated regression model prediction for IL-6 (**A**), TNF-α (**B**) and CRP (**C**) based on the global lipidomic profile data with the top-ranking lipids explaining the changes in inflammatory variables during the probiotic intervention.

the disturbed barrier function seen in diseases such as IBD[45]. Therefore, the decrease in SM seen after LGG intervention in the present study may also contribute to the beneficial effects on gut barrier function seen in the previous intervention studies with LGG[36-39].

Although we observed some common trends in the global lipidomic profiles after LGG intervention, one should notice that, when accounting for multiple hypothesis testing using the chance detection plot, no lipid changes were found to be statistically significant. This suggests that the study was either underpowered for investigations of global lipidomic profile changes in the described setting, or the observed baseline differences in the global lipidomic profiles dominated over responses to the intervention, masking potential effects of the LGG intervention. One thus cannot exclude the possibility that some of the significant changes were detected by chance. Furthermore, this pilot study was conducted with healthy individuals alone, whereas the effect of LGG intervention on global lipidomic profiles should also be investigated in subjects suffering from inflammatory conditions or disturbed gut barrier function before further conclusions can be drawn.

In conclusion, there are indications that probiotic LGG intervention may lead to changes in global lipidomic profiles reflected in decreased LysoGPCho and SM, mainly decreased GPCho and mainly elevated TAG. These changes may contribute, for example, to the

metabolic events behind the beneficial effects of LGG on gut barrier function seen in previous studies. IL-6 was moderately associated with the changes in lipidomic profiles. Lipidomics may provide powerful tools for identifying new biomarkers behind the clinical effects of probiotic intervention trials and for establishing relationships between molecular profiles and other known data from the same individual.

ACKNOWLEDGMENTS

The authors wish to thank Netta Lummela, for her expertise during the study visits, and all volunteers.

COMMENTS

Background

The new global metabolic profiling technique 'metabolomics' has made it possible to measure a large number of metabolites, and is currently being applied to increase the understanding of the health and disease continuum. The new analytical capacity of lipidomics as a branch of metabolomics can increase the understanding of lipid biology, improve the characterisation of global lipid profiles and result in the identification of previously unknown changes in lipid metabolism. Probiotics have been mostly studied in the prevention and treatment of different gastrointestinal diseases and allergy, but the mode of action of probiotics is poorly understood.

Innovations and breakthroughs

This study is the first to apply lipidomic techniques to analyse the global lipidomic profiles of healthy adults after a probiotic intervention. Lipidomic analysis showed that there were decreases in the levels of lysophosphatidylcholines (LysoGPCho), sphingomyelins (SM) and several glycerophosphatidylcholines (GPCho), and increases in triacylglycerols (TAG) in the probiotic LGG group. These changes may contribute, for example, to the metabolic events behind the beneficial effects of LGG on gut barrier function seen in previous studies.

Applications

Metabolomics and lipidomics may help to understand the action mechanisms of different agents, such as probiotics.

Terminology

Lipidomics is a branch of metabolomics which enables identification of lipids in a large scale. Probiotic bacteria are defined as living microorganisms that have beneficial effects on human health.

Peer review

This was an interesting paper and overall it was well written and well-presented. However, this is a small study. One good point is the randomized cohort. The study population was healthy individuals and the results may not be applicable to a population with lipid-related disease states. There were multiple comparisons and one thus cannot exclude the possibility that some of the significant changes were detected by chance.

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S- Editor Zhong XY **L- Editor** Lalor PF **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3195

World J Gastroenterol 2008 May 28; 14(20): 3195-3200
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Factors that influence outcome in non-invasive and invasive treatment in polycystic liver disease patients

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Supported by The scholarship provided by the "Secretaría de Relaciones Exteriores (SRE)" of the Government of Mexico
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Received: November 26, 2007 Revised: March 7, 2008
Accepted: March 14, 2008
Published online: May 28, 2008

Abstract

AIM: To evaluate the factors that influence outcome of both non-invasive and invasive treatment of polycystic liver disease.

METHODS: Analysis of clinical files of patients with complete follow-up from July 1986 to June 2006.

RESULTS: Forty-one patients (male, 7; female, 34), 47.8 ± 11.9 years age, and 5.7 ± 6.7 years follow-up, were studied. Alkaline phosphatase (AP) elevation (15% of patients) was associated with the requirement of invasive treatment (IT, P = 0.005). IT rate was higher in symptomatic than non-symptomatic patients (65.4% *vs* 14.3%, P = 0.002), and in women taking hormonal replacement therapy (HRT) (P = 0.001). Cysts complications (CC) were more frequent (22%) in the symptomatic patients group (P = 0.023). Patients with body mass index (BMI) > 25 (59%) had a trend to complications after IT (P = 0.075). Abdominal pain was the most common symptom (56%) and indication for IT (78%). Nineteen patients (46%) required a first IT: 12 open fenestration (OF), 4 laparoscopic fenestration (LF) and 3 fenestration with hepatic resection (FHR). Three required a second IT, and one required a third procedure. Complications due to first IT were found in 32% (OF 16.7%, LF 25%, FHR 66.7%), and in the second IT in 66.7% (OF 100%). Follow-up mortality rate was 0.

CONCLUSION: Presence of symptoms, elevated AP, and CC are associated with IT requirement. HRT is associated with presence of symptoms and IT requirement. Patients with BMI > 25 have a trend be susceptible to IT complications. The proportions of complications are higher in FHR and second IT groups. RS is more frequent after OF.

Key words: Hepatic cysts; Open fenestration; Laparoscopic fenestration; Hepatic resection; Recurrence of symptoms; Hormonal replacement therapy

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Barahona-Garrido J, Camacho-Escobedo J, Cerda-Contreras E, Hernández-Calleros J, Yamamoto-Furusho JK, Torre A, Uribe M. Factors that influence outcome in non-invasive and invasive treatment in polycystic liver disease patients. *World J Gastroenterol* 2008; 14(20): 3195-3200 Available from: URL: http://www.wjgnet.com/1007-9327/14/3195.asp DOI: http://dx.doi.org/10.3748/wjg.14.3195

INTRODUCTION

Polycystic liver disease (PLD) is an autosomic dominant disease related to chromosome 19 alterations in patients with hepatic involvement alone and in chromosomes 4 and 16 in those with renal cysts[1]. At autopsy, the prevalence appears to be 0.13%-0.6%, and the association to renal cysts about 30%[2-4]. PLD is generally asymptomatic and incidentally diagnosed. Abdominal pain, distension, early satiety, nausea and vomiting are common and hepatic function is rarely affected. Ultrasound (US) and computed tomography (CT) are common diagnostic methods. PLD is considered when more than 5 cysts are observed in the liver that typically appear anechoic, round and smooth-walled with distal echo enhancement in the US[5] (Figure 1A); and in CT with homogeneous fluid density and without wall or content enhancement after contrast administration[6]

(Figure 1B). Invasive treatment (IT) such as cyst aspiration with sclerotherapy, open fenestration (OF), laparoscopic fenestration (LF), fenestration plus hepatic resection (FHR), and hepatic transplantation in selected cases are preferred[1,4,7-10]. Symptoms are controlled with surgical liver volume reduction[11]. In a Mexican population, surgery for PLD has shown to modify quality of life[12]. We report a descriptive analysis of 41 patients with clinical and imaging diagnosis of PLD that have a complete follow-up during July 1986 to June 2006, making special emphasis on factors that influence outcome of both non-invasive and invasive treatment.

MATERIALS AND METHODS

Materials

This is a descriptive study of all patients diagnosed with PLD diagnosis from July 1986 to June 2006 at the National Institute of Health Sciences and Nutrition "Salvador Zubirán". Forty-nine clinical records with PLD diagnosis were reviewed; however, 8 patients were excluded from the analysis because the follow-up was not completed.

Methods

Variables as gender, age at diagnosis, time of diagnosis delay, BMI, symptoms, diagnosis method, cyst diameter, cyst complications (CC), liver function tests (LFT: bilirubin, transaminases, AP, lactic dehydrogenase, gamma glutamyl transpeptidase, albumin, prothrombin time, glucose and complete blood count at diagnosis, 1 and 6 months, and at 1, 5, 10, 15 and 20 years), comorbidity, extrahepatic cysts, hormonal replacement therapy (HRT) intake, IT requirement, IT complications, recurrence of symptoms (RS), and follow up procedures and outcome were analyzed.

Statistical analysis

Statistical data are expressed as mean ± SD. Numerical variables were analyzed by *t*-test and the categorical with χ^2 test or Fisher's exact test. A P value $\leqslant 0.05$ was accepted as being statistically significant. The SPSS 13.0 software (SPSS Inc., Chicago, Illinois, 2004) statistical program was used for the analysis.

RESULTS

Demographical data

A total of 49 patients with PLD were evaluated in our institution. Eight patients lost follow-up and were not included for the analysis. Forty-one (male 7, female 34) complete patients files were included (Figure 2). The mean age at diagnosis was 47.8 ± 11.9 years (range 27-82) and the mean follow-up time was 5.7 years. Eighteen (44%) patients had familiar history of PLD. Demographical data is shown in Table 1.

Hormonal replacement therapy is associated with symptoms occurrence

We found that 23.8% of postmenopausal women were taking HRT. In those patients the prevalence of symptoms at diagnosis was significantly higher than those without HRT (100% *vs* 43.8%, $P = 0.039$; OR = 2.286; 95% CI, 1.311-3.984). The requirement of IT also was higher in the HRT group than in non-HRT (80% *vs* 6.3%, $P = 0.001$; OR = 20; 95% CI, 35-115). The size of the major cyst was not associated with HRT ($P > 0.05$). The oral contraceptive intake was not associated with occurrence of symptoms ($P > 0.05$).

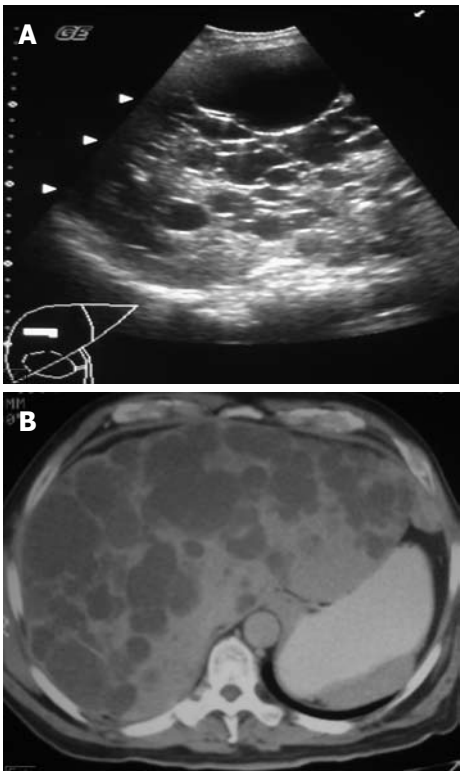

Figure 1 US (**A**) and CT (**B**) images showing numerous hepatic cysts in a 47-year-old asymptomatic male patient.

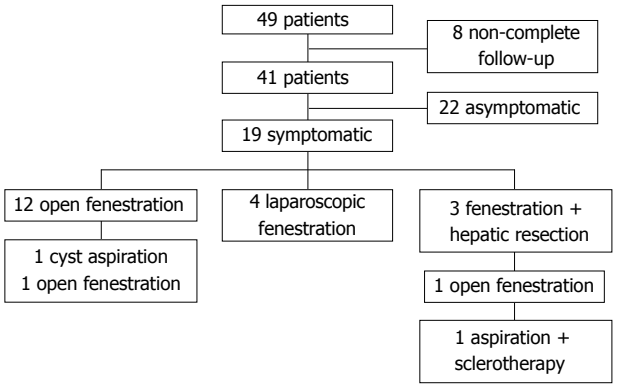

Figure 2 Clinical course and interventions of 49 PLD patients (July 1986-June 2006).

Symptoms, diagnosis, and PLD complications

Based on the initial symptoms the mean time of diagnosis delay was 2.8 (range 0-9) years. We found that 65.4% of the symptomatic *versus* 14.3% of non-symptomatic patients at diagnosis required IT ($P = 0.002$, OR = 11.333, 95% CI, 2.068-62.105). The symptoms described were abdominal pain 56%, early satiety 42%, increase of the abdominal perimeter 34%, and nausea 12%. Two patients

Table 1 Demographical data, extrahepatic cysts and comorbidity

N (Male/Female)	41 (7/34)
Age at diagnosis (range, yr)	47.8 ± 11.9 (27-82)
Mean diagnosis retard time (range, yr)	2.8 (0-9)
Mean follow up time (yr)	5.7
Familiar history of PLD	44%
Body mass index at diagnosis	
⩽ 25	41%
> 25-⩽ 30	44%
> 30	15%
Largest cyst diameter (range, cm)	8.2 ± 4.9 (2-25)
Extrahepatic cysts	
Renal cysts	68%
Pancreatic cysts	15%
Spleen cysts	8%
Ovary cysts	2%
Comorbidity	
Arterial hypertension	46%
Chronic renal insufficiency	24%
Dyslipidemia	15%
Hypothyroidism	7%
Type 2 diabetes	5%
Gastroesophageal reflux	5%

(4.9%) had portal hypertension manifested by esophageal varices and splenomegaly, and none of them was found with ascites or encephalopathy.

During the follow up time, symptomatic patients at diagnosis developed more CC than non-symptomatic (29.6% *vs* 0, $P = 0.023$). At this time, nine (22%) patients had CC: cyst infection 5 (55.6%), cyst bleeding 3 (33.3%), and cholangitis 1 (11.1%), at a mean time of 4 months (range 0-9, Table 2). In this group of patients, four (44.4%) had a complication at the time of diagnosis (cyst infection 2, cyst bleeding 2).

Renal cysts were present in 68% of PLD patients (Table 1). Liver CC were seen in 25% of patients with renal cysts, and in 7.7% of patients without them ($P > 0.05$).

At diagnosis, overweight (BMI > 25 but ⩽ 30), was found in 44%; and obesity (BMI > 30), in 15%. Patients with BMI > 25 had a trend to develop surgical complications ($P = 0.075$) such as abdominal pain, bleeding or infection (Table 1), and no significant association was found with the presence of symptoms, largest cyst diameter, CC, or IT requirement ($P > 0.05$).

Diagnosis was achieved by US in 78% and by CT in 22%. The largest cyst mean diameter was 8.2 ± 4.9 cm (range 2-25) and no association to symptoms or requirement of IT ($P > 0.05$) were found. The largest cyst mean diameter was 12.3 ± 8.8 cm in postmenopausal women taking HRT *vs* 8.3 ± 3.9 cm in those without HRT ($P > 0.05$).

Elevated alkaline phosphatase at diagnosis is associated with IT requirement

No significant alterations in bilirubin, transaminases, lactic dehydrogenase, gamma glutamyl transpeptidase, albumin, prothrombin time, glucose, and in complete blood count, were found at diagnosis or during follow up.

Table 2 Outcome of PLD patients

Symptomatic patients at diagnosis	27 (66%)
Abdominal pain	15
Early satiety	12
Increase of abdominal perimeter	9
Nausea	3
Complications during follow up	9 (22%)
Cyst infection	5
Cyst hemorrhage	3
Cholangitis	1
Invasive treatment patients	19 (46%)
Open fenestration	12
Laparoscopic fenestration	4
Fenestration + hepatic resection	3
Overall symptoms recurrence	4 (17%)
Open fenestration	3
Laparoscopic fenestration	0
Fenestration + hepatic resection	1
Surgery complications	6 (32%)
Bleeding	3
Infection	2
Pain	1

Because we considered IT requirement as an end point in PLD patients, we studied its association with elevated levels of LFT at diagnosis, finding that AP was elevated (⩾ 132 IU/mL) in 15.5% of patients. During follow-up, 100% of patients in the elevated AP group required IT *versus* 35.5% in the normal AP group ($P = 0.005$, OR = 2.818, 95% CI, 1.753-4.530).

Invasive treatment and complications

Abdominal pain was the most common indication for surgery (78%). Other indications were satiety (10%), cyst hemorrhage (5%), and cyst infection (5%).

Nineteen patients (46%) required IT (OF 12, LF 4, FHR 3) to control symptoms (Figure 2), at a mean time of 19 months (range 0-85) after PLD diagnosis. Mean age for the first IT was 47 ± 10 years.

Because of RS, 3 patients required a second IT at a mean time of 19 months (range 8-24): 2 (16.7%) in the OF group (cyst aspiration 1, OF 1) and 1 (33.3%) in the FHR group (OF 1). The last patient required a third IT (aspiration + sclerotherapy) 6 months later. None of the patients in the LF group showed RS.

Complications due to the first IT were found in 6 (32%) patients. Three (16.7%) patients had complications in the OF group (bleeding 3), 1 (25%) in the LF group (severe abdominal pain 1), and 2 (66.7%) in the FHR group (bleeding 1, infection 1; Table 2). No significant difference in complications between OF and LF was found.

Two (66.7%) patients after a second IT (OF 2) developed important complications (hemorrhage 1, pleural effusion 1), and the one who required a third IT had severe abdominal pain. Follow up mortality rate was 0.

DISCUSSION

This is one of the largest series published to date and includes a large follow-up time in both symptomatic and non-symptomatic patients with a detailed description

and associations to anthropometric and biochemical data, HRT intake, CC, IT requirement, IT complications, and outcome.

As in other case series[1,9,10], female gender predominated (83%), suggesting a possible role of estrogens in the development of liver cysts.

Data shows selective increase in liver cyst and parenchymal volume in female patients receiving postmenopausal estrogen therapy[13], but it is not clear if it correlates with symptoms and IT requirement. The association found between HRT, the presence of symptoms and IT requirement supports that HRT has an important role in the development of symptoms and so in the requirement of IT in PLD patients. Interestingly HRT was not associated to the size of the major cyst, suggesting that symptoms are not due to the size of cysts but maybe to the number of cysts or the liver volume occupied by them. As in other liver diseases, PLD may contraindicate HRT. Further and prospective studies are recommended to confirm such associations.

As others centers that inform that the diagnosis is more common during the fourth and the fifth decade of life[9,14], in Mexican patients seems to be equal.

No other series report the time evolution of PLD patients. We found a diagnosis delay time near to 3 years. This data may indicate that symptoms at the beginning of the disease are absent or mild and appear or increase as time advances, so patients search for medical attention.

Symptoms predicted IT requirement and were associated to a higher incidence of CC (bleeding, infection, *etc*). Most of the series report that the majority of patients are asymptomatic at diagnosis[1], but we found a high prevalence of symptoms (66%) in Mexican patients. We found a higher prevalence of abdominal pain (56%) than reported in other studies (36.5%)[9].

Hepatic failure in PLD patients is rare and few cases have been reported[15,16]. We found 2 (4.9%) cases of portal hypertension and none with ascites or encephalopathy. The cause of portal hypertension in these patients is not clear, but might be due to the mass effect that comprises vessels of the portal circulation. A reported rate of 2.5% for portal hypertension has been described[9]. Despite hepatomegaly, portal hypertension and its complications (ascites, variceal bleeding, *etc*) remain quite rare[1], interestingly; we report a higher rate of portal hypertension in our patients, maybe due to a longer follow-up time.

In our knowledge this is one of the largest follow-up in a PLD case series (5.7 years). Bistritz *et al*[9] reported that in 40 patients with a follow-up time of 4.69 years, 22.5% had cyst bleeding, 12.5% cyst rupture, 12.5% cyst infection, and 2.5% developed portal hypertension. During follow-up symptomatic patients of our study developed similar incidence of CC. We found a significant association between prevalence of symptoms and the development of CC during follow-up ($P = 0.023$). It suggests that symptomatic patients have increased risk factors that predispose CC. The presence of renal cysts did not significantly increase the incidence of liver CC ($P > 0.05$).

The association between anthropometric data and symptoms in PLD has not been studied. We found a trend to surgical complications in patients with BMI > 25 ($P = 0.075$), and no significant association with symptoms, largest cyst diameter, CC, or IT requirement. The BMI is not a reliable data because it is influenced by the large amount of hepatic weight in PLD patients, so other anthropometric measurement must be achieved in future studies, especially to quantify fat tissue. It is known that fat tissue is a hormonal-active tissue, though may influence the clinical presentation and outcome of PLD patients, as happens with HRT.

By far, US is reported as the most used method for diagnosis[1]. Our finding supports that US is a good and reliable method to achieve PLD diagnosis and brings important data such as number of cysts, cyst diameter and cyst complications. It is unclear if the cyst diameter is associated to symptoms or IT requirement. We found that despite a large cyst diameter, symptoms or requirement of IT are not related to it. It suggests that what determines the occurrence of symptoms or indicates surgical therapy is not the cyst diameter, but the number of cysts or the liver volume occupied by them. Further studies are necessary to determine the clinical value of these measures, including measure of liver volume.

The LFT in our patients usually were normal and were not associated to symptoms or outcome. We did not find significant alterations at diagnosis or during follow up. In his review, Arnold[1] described that LFT are often normal, but in symptomatic patients, AP levels may be elevated in 30%-47%, GGT in 60%-70%, aspartate aminotransferase in up to 27%, and bilirubin in 17%. We studied those variables, and only the elevation of AP (⩾ 132 mg/dL) at diagnosis was significantly associated with requirement of IT. This association suggests that elevated AP may be an important serological marker of disease activity and could be used to indicate IT to control symptoms.

Symptoms and complications are reported indications for IT. According to Chen[4] and Que *et al*[17] these indications include abdominal distention, abdominal pain, early satiety, fatigue, supine dyspnea, infected cysts, dialysis hypotension, bile duct obstruction, severe ascites and uterine prolapse. We found similar indications in our patients, being pain the most frequent.

RS is frequent with the majority of IT modalities. The reported rate of RS for cyst aspiration is up to 100% and probably does not provide definitive therapy[18]. The RS for OF is less common. One of the largest series is the one reported by Koperna *et al*[19] who described a RS rate of 21%, but also rates between 11%-33% had been reported[20-23]. For LF, a recent case series of 6 PLD patients reported by Garcea *et al*[24] showed 16% of RS, but also has been reported in up to 4.5%-71%[22,25]. In our patients, the RS after a first IT was low (16.7% for OF, 0% for LF, and 33.3% for FHR). The RS rate for OF and for FHR was as expected, interestingly the non-RS after LF is much lower than reported[23,24,26]. We think those findings may be due to patient selection criteria.

For OF the reported morbidity rate is 0%-56%[21,27],

for LF 0%-54%[21,28], and for FHR varies from 20% to 100%[29,30]. The higher rate of complications in the LF group compared to the OF group may be due to procedure selection criteria. The rate of complications after FHR was high but as expected. Randomized studies are necessary to know the real rate of complications and RS with each procedure, but as the prevalence of the disease is low, studies are difficult to perform.

In summary, the presence of symptoms at diagnosis, and CC during follow-up time is associated with IT requirement. The HRT is associated to the presence of symptoms and IT requirement. The prevalence of symptoms in PLD patients is high and abdominal pain is the most common. Patients with BMI > 25 have a trend to suffer from complications after IT. Cyst diameter is not associated with the presence of symptoms or need for IT. The AP elevation was associated with IT requirement, suggesting that AP may be a marker of disease severity. In near half of the patients, a first IT is performed and complications are frequent, especially in the FHR group, but the proportion of complications due to the second IT is higher. The RS is more frequent after OF, but this fact may be due to patient selection bias.

COMMENTS

Background

Polycystic liver disease (PLD) is generally asymptomatic and incidentally diagnosed. For symptomatic patients invasive treatment (IT) such as cyst aspiration with sclerotherapy, fenestration with or without hepatic resection, and hepatic transplant are options of treatment. There are no known associations that could help clinicians to determine the outcome of invasive or non-invasive treatment.

Research frontiers

Larger and prospective studies are required in order to find other variables that may affect outcome. It is important to evaluate the physiological basis of the impact of hormonal replacement therapy (HRT) on the outcome of PLD patients.

Innovations and breakthroughs

Knowledge of factors associated with IT requirement, complications, and recurrence of symptoms.

Applications

Helpful to determine outcome in both invasive and non-invasive treatment of PLD patients.

Peer review

This is a retrospective study investigating the association for invasive or noninvasive treatment of polycystic liver disease and biochemical abnormalities. It is a well-written and well-designed paper.

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S- Editor Zhong XY **L- Editor** Kremer M **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3201

World J Gastroenterol 2008 May 28; 14(20): 3201-3206
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Hepatitis B virus prevalence and transmission risk factors in inflammatory bowel disease patients at Clementino Fraga Filho university hospital

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Received: January 28, 2008 Revised: April 12, 2008
Accepted: April 19, 2008
Published online: May 28, 2008

Abstract

AIM: To evaluate the prevalence of hepatitis B virus (HBV) infection in inflammatory bowel disease (IBD) patients that followed up in our hospital and try to identify the possible risk factors involved in this infection transmission.
METHODS: This was a cross-sectional study for which 176 patients were selected according to their arrival for the medical interview. All these patients had already IBD diagnosis. The patient was interviewed and a questionnaire was filled out.
RESULTS: In the group of 176 patients whom we examined, we found that 17% (30) were anti-HBc positive. Out of 30 patients with positive anti-HBc, 2.3% (4) had positive HBsAg and negative HBV-DNA. In an attempt to identify the possible HBV infection transmission risk factors in IBD patients, it was observed that 117 patients had been submitted to some kind of surgical procedure, but only 24 patients had positive anti-HBc (P = 0.085). It was also observed that surgery to treat IBD complications was not a risk factor for HBV infection transmission, since we did not get a statically significant P value. However, IBD patients that have been submitted to surgery to treat IBD complications received more blood transfusions then patients submitted to other surgical interventions (P = 0.015).
CONCLUSION: There was a high incidence of positive anti-HBc (17%) and positive HBsAg (2.3%) in IBD patient when compared with the overall population (7.9%).

Key words: Inflammatory bowel disease; Hepatitis B virus; Prevalence; Risk factors

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Tolentino YFM, Fogaça HS, Zaltman C, Ximenes LLL, Coelho HSM. Hepatitis B virus prevalence and transmission risk factors in inflammatory bowel disease patients at Clementino Fraga Filho university hospital. *World J Gastroenterol* 2008; 14(20): 3201-3206 Available from: URL: http://www.wjgnet.com/1007-9327/14/3201.asp DOI: http://dx.doi.org/10.3748/wjg.14.3201

INTRODUCTION

The hepatitis B virus (HBV) infection is a worldwide public health problem. There are two billion people infected by HBV, and among these more than 350 million have chronic infection. Patients with chronic infection have a high death risk for hepatic cirrhosis or liver cancer. These two diseases are responsible for about 1 million people dying every year, in spite of the infection incidence falling recently[1-4].

In developed countries the sexual route is responsible for 30% of infections and is the main route of HBV transmission[3-5].

Health professionals, such as surgeons, pathologists, dialysis and chemotherapy technicians, have a high risk of acquiring HBV infections through small skin lesions or through accident with instruments that cut or perforate[6].

Patients with inflammatory bowel disease (IBD)

have high risk of infection by hepatitis viruses B or C[7] because during the course of their disease, they need blood transfusions, and sometimes surgical and endoscopic procedures for diagnosis and treatment[8-10]. Biancone *et al* observed that in Crohn's disease (CD), 2/3 of the patients will need an intestinal resection and almost 50% will need multiple surgeries[11]. It is important to confirm this data to alert health professionals about prevention and early diagnosis of HBV infection, because the steroids and immunosuppressant drugs used in IBD treatment worsen the HBV liver disease. Few studies exist to verify if these drugs influence HBV infection in IBD patients[12-15].

The Clementino Fraga Filho University Hospital is a reference center for IBD diagnosis and treatment. As it is not known exactly what the HBV infection rate in this group of patients in this institution, we decided to do this study.

The first aim of this study was to evaluate the prevalence of HBV infection in IBD patients that followed up in the hospital. The second aim is to evaluate the possible risk factors involved in HBV infection transmission in this patients group.

MATERIALS AND METHODS

This study was carried out between May 2002 and November 2004, for which 176 patients were recruited. All these patients had clinical, laboratory, radiological, endoscopic and histopathological IBD diagnosis. Included were patients of both sexes, at least 18 years old, for whom medical records were kept by the hospital and who live in Rio de Janeiro State. Patients with infectious, ischemic, actinic, and uncertain colitis were excluded.

The patients were selected, weekly, according to their order of arrival for the medical interview in the hospital IBD ambulatory. After, if the patient allowed us to include him/her in the study, he/she signed an informed consent term. Next, the patient was interviewed and during this interview, a questionnaire was filled out to obtain identification data such as age, sex and IBD type.

In order to identify possible risk factors for HBV infection transmission in this population, the patients were questioned about blood transfusion histories, surgical and endoscopic interventions, dialysis[16], use of endovenous illicit drugs[17], acupuncture treatment, the presence of tattoos[7] or "piercings" and if they engaged in promiscuous sex (defined as more than 3 sexual partners in a year or sexual intercourse with prostitutes)[18].

After the interview, 25 mL of blood were obtained from the patient and the material was submitted to the following analyses: qualitative test for total core antibodies; anti-HBc (Kit Diasorin S.p.A.-Italy); qualitative test for HBV antigen; HBsAg (Kit ELISA-Diasorin S.p.A.-Italy) and qualitative PCR-DNA for HBV (which can detect up to 10 particles/serum milliliter), this last analysis being only for patients with positive anti-HBc, patients with positive HBsAg, and for 14 (8%) patients with negative anti-HBc and HBsAg chosen at random.

Table 1 HBV infection transmission risk factors

Risk factors (n = 176)	n (%)
Blood transfusion	47 (26.7)
Surgery	117 (66.5)
Dialysis	0
Endovenous drug use	8 (4.5)
Tatoo	4 (2.3)
Acupuntura	7 (4.0)
"Piercings"	1 (0.6)
SPL	3 (1.7)
Digestive endoscopes	175 (99.4)

Table 2 Anti-HBc and HBsAg result distribution according to IBD type (n = 176)

	Anti-HBc	HBsAg
Positive	17 UC (56.7%) 13 CD (43.3%)	4 (2.3%)
Negative	146 (83%)	172 (97.7%)

Statistical analysis was processed by the SAS® software system. Differences were considered significant for an alpha risk of 5%.

Our objective was to verify if there is a significant association between a positive anti-HBc result and any of the risk factors analyzed. For this purpose the following methods were applied: for proportions comparison (qualitative variables) the chi-square test was used (χ^2) or the exact Fisher test. For numeric variables comparison (quantitative) between two groups, the t-test was used for independent samples or the Mann-Whitney test, when the variable did not present normal distribution due to great dispersion or for the ordinal nature of the data.

RESULTS

In our sample there were 68 (38.6%) men and 108 (61.4%) women. There were 102 (58.0%) CD patients and 74 (42.0%) UC patients.

There were surgical procedure histories in 117 patients (66.5%). Blood transfusion was reported by 47 patients (26.7%). Eight patients (4.5%) confirmed the use of endovenous illicit drugs. None of the patient had undergone dialysis treatment and only 3 patients affirmed having a promiscuous sexual life (Table 1).

Forty-nine patients were without treatment; 7 patients used immunosuppressant drugs; 74 used steroid drugs; while 46 patients used both.

Table 2 shows that among the 176 patients, 30 patients (17%) had positive anti-HBc: 17 (56.7%) with UC and 13 (43.3%) with CD.

Among the 30 patients with positive anti-HBc, 4 had positive HBsAg.

The 30 patients with positive anti-HBc and the 14 patients with negative anti-HBc randomly selected were submitted to the PCR HBV-DNA qualitative test. All of these patients had negative PCR HBV-DNA results. The four patients with positive HBsAg were also

Table 3 Risk factors according to anti-HBc result-frequency and percentile

Risk factors		Anti-HBc Positive *n*	Positive %	Negative *n*	Negative %	*P*
Sex	Men	12	40.0	56	38.0	0.86
	Women	18	60.0	90	61.6	
Digestive endoscopy	Yes	16	53.3	91	62.3	0.35
	No	14	46.7	55	37.7	
Retosigmoidoscopy	Yes	13	43.3	50	34.0	0.34
	No	17	56.7	96	65.8	
Blood transfusion	Yes	10	33.3	37	25.3	0.36
	No	20	66.7	109	74.7	
Surgery	Yes	24	80.0	93	63.7	0.085
	No	6	20.0	53	36.3	
Surgery to treat IBD complications	Yes	8	33.3	47	50.5	0.13
	No	16	66.7	46	49.5	

Table 4 Non-numeric variables risk factors for HBV transmission

Variable	Anti-HBc	*n*	Mean	SE	Minimum	Maximum	*P*
Age (yr)	Positive	30	47.7	11.9	26	78	0.001
	Negative	146	39.0	13.9	18	84	
Diagnose time (mo)	Positive	30	114.1	109.3	8	372	0.37
	Negative	146	89.9	90.4	1	600	
Colonoscopy number	Positive	30	2.3	1.9	0	10	0.52
	Negative	146	2.1	1.6	1	8	

submitted to qualitative PCR HBV-DNA tests and they also had negative results. Among these patients, those with positive anti-HBc and HBsAg tests are considered inactive HBV bearers.

Table 3 supplies the frequency (*n*) and the risk factor percentile (%) according to anti-HBC results and the corresponding *P* value. The statistical analysis was accomplished by the χ^2 test or by the exact Fisher test.

It was observed that sex, digestive endoscopy, retosigmoidoscopy, and blood transfusions were not considered probable risk factors in HBV infection transmission.

When we calculated surgery only, we observed that 24 patients (80%) with positive anti-HBc were submitted to some type of surgical intervention while the other 20% (6 patients) with positive anti-HBc did not undergo any surgical intervention ($P = 0.085$). We also tried to stratify patients submitted to surgery into two groups: those who underwent surgery to treat IBD complications and those who underwent other surgeries. The *P* value was not significant ($P = 0.13$). Dialysis, endovenous illicit drug use, tattoos, acupuncture, piercing and a sexual promiscuous life style, were not analyzed due to low frequency of observed cases.

Table 4 shows that patients with positive anti-HBc have an average age significantly older ($P = 0.001$) than patients with negative anti-HBc. A significant difference was not observed for disease diagnosis time ($P = 0.37$) neither for number of colonoscopies ($P = 0.52$) among both positive and negative anti-HBc groups.

One hundred and seventeen patients were submitted to some form of surgery. We analyzed the relationship between blood transfusion and surgery carried out to treat IBD complications, and it was observed that patients submitted to surgeries to treat IBD complications needed more transfusions ($P = 0.015$) than patients submitted to other types of surgery, as illustrated in Figure 1.

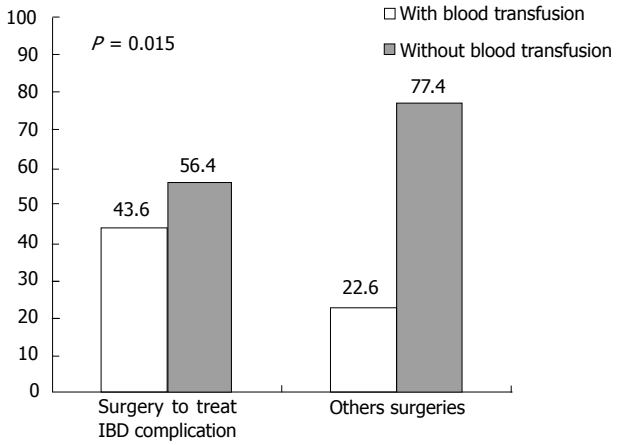

Figure 1 Blood transfusion need according to surgery complexity.

DISCUSSION

In this study we observed that positive anti-HBc prevalence was 17% (30 patients) in a sample of 176 patients. This data shows that positive anti-HBc prevalence in IBD patient groups is larger when compared with the overall Brazil population (7.9%) and with the Rio de Janeiro state population (2.5%) figures[18].

In the literature, we found only one case-control study that evaluated the HBV prevalence in IBD patients. In that study, the anti-HBc prevalence was larger in CD (10.9%) and ulcerative colitis patients (11.5%) when compared with control group individuals (5.1%)[11]. Our study had very similar results for CD and ulcerative colitis for positive anti-HBc prevalence. These results are probably because IBD patients are frequently exposed to surgical interventions and/or endoscopies as well as necessary blood transfusion that can be a means of transmitting HBV[19,20].

Among 30 patients with positive anti-HBc result, 2.3% (4) had positive HBsAg with negative HBV-PCR DNA, patients that are considered HBV inactive bearers. This prevalence is considered high when we compare it with a Brazil Health Ministry study in 2006 in the central west, Northeast and Brasília regions that shows an HBsAg prevalence of 0.5%.

In addition to identifying HBV prevalence, we also tried to identify the possible risk factors for HBV transmission that could increase HBV infection prevalence among IBD patients. Considering 5% to be a significant threshold, we found that such factors as: (1) Sexual activity; (2) Digestive endoscopy; (3) Retosigmoidoscopy and (4) Blood transfusion were ruled out as possible risk factors for HBV infection of IBD patients.

Despite the fact that our sample contained 108 female patients corresponding to 61.4% of the total

sample, when we compared the positive anti-HBc percentile in women and in men, we did not obtain *P* value with statistical significance ($P = 0.86$), even when we separated the male and female group according to IBD type, both groups being very similar in this respect. Biancone had a different result in his study. He demonstrated that female status was an important factor to be considered in HBV infection in CD patients[11].

Studies in the last 5 years have verified the possibility of HCV and HBV transmission mainly through endoscopic procedures during therapeutic interventions. Studies demonstrated the presence of HBV-DNA in endoscopic channels that were not submitted to appropriate disinfection processes[20]. In our sample procedures, such as digestive endoscopy and retosigmoidoscopy, we did not discover any evidence of HBV transmission risk factors (digestive endoscopy $P = 0.35$ and retosigmoidoscopy $P = 0.34$).

Considering blood transfusion is an important viral hepatitis transmission route[7,21], it has already been demonstrated by Long *et al* in 2000 and Biancone *et al* in 2001 that blood transfusion was an important risk factor in HCV transmission among IBD patients[11,22]. However, we were not able to demonstrate that blood transfusion was a risk factor for HBV infection transmission in our group because in our sample only 10 out of 47 patients with positive anti-HBc received blood transfusions while the other 20 with positive anti-HBc did not have blood transfusion histories ($P = 0.36$).

When we analyzed surgery as a possible risk factor, despite of the fact of not having a *P* value smaller than 0.05, we observed that 80% (24) of patients with positive anti-HBc had been submitted to some surgical procedure while the other 20% (6) did not undergo any surgical procedures ($P = 0.085$).

Biancone *et al* showed that surgery, and mainly surgical procedures to treat IBD complications, were an important risk factor in HCV transmission among IBD patients[11]. We can try to explain the Biancone *et al* discoveries if we take into consideration that gastrointestinal surgeries to treat IBD complications are high complexity operations[10] and probably need blood transfusions during surgical procedure, which could cause a bias in the statistical analysis because the surgery itself was not the cause of transmission but the transfusion. The possible risk factor for HCV infection transmission in these cases was blood transfusion that patients received during these procedures. In our study we separated patients according to surgery type: group 1-patients submitted to surgery to treat IBD complications, and group 2-patients submitted to other surgical interventions; we did not find a significant *P* value ($P = 0.13$). However, as can be seen in Figure 1, our hypothesis that patients submitted to surgeries to treat IBD complications received more blood transfusions than patients submitted to other surgical interventions was confirmed ($P = 0.015$).

When we compare our results with Spijkerman *et al*'s study, our data is divergent because according to that study high complexity surgeries (i.e. surgeries with more than one hour of duration, surgeries with a larger incidence of postoperative complications and those with a higher risk of complication requiring further surgery or more blood transfusions) are associated with a higher risk of HBV infection transmission[19]. However, in this study it was demonstrated that the HBV infection was transmitted through an HBV infected surgeon during surgery.

For the other qualitative variables: dialyses, endovenous illicit drug use, tattoos, acupuncture, "piercings" and sexually promiscuous lifestyle, the associations were not analyzed because we had low frequencies of observed cases.

In quantitative-variable analysis (age, disease diagnosis time and number of colonoscopies), the *P* value results have statistical significance ($P = 0.001$). The average age of patients with positive anti-HBc was higher (47.7 years) than patients with negative anti-HBc (39.0 years). In the literature, the positive anti-HBc prevalence was associated with ages older than 50 years in CD and in UC[11]. These data were found, we believe, because older patients probably have a longer disease duration time and therefore have had more time to develop complications requiring surgical and endoscopic interventions. However, we were not able to prove the veracity of these assumptions.

Biancone *et al* have shown ($P = 0.37$) that disease duration time (number of months since IBD diagnosis) is associated with incidence of positive anti-HBc in UC patients[1].

Steroids, immunosuppressant drugs and the anti-TNF antibodies (anti-necrosis tumor antibodies-Infliximab®) in IBD patients[23,24], as some studies have demonstrated, can influence the course of hepatic disease when used in HBV infected patients, mainly patients with positive HBsAg and anti-HBc and negative HBV-DNA (called inactive bearers)[12-14]. It is also important to note that in patients with positive anti-HBc and negative HBsAg, the HBV can replicate because the virus stays inside the hepatocytes although there is an apparent serologic cure[23]. These studies show that immunological suppression caused by these drugs could cause viral replication and spread infection inside hepatocytes. When these drugs were suspended and the immunological reaction was restored, the infected hepatocytes were be destroyed quickly and there was an increase in the transaminases levels ("flare") and an accentuated viremia reduction[15,25]. Two cases of fulminant hepatitis were identified after use of Infliximab® in rheumatoid arthritis patients infected by HBV[26] and one case of hepatic insufficiency and death in a CD patient treated with Infliximab®[27,28]. The reactivation of HBV can happen also to inactive bearers submitted to transplants or in cancer patients who are submitted to chemotherapy. Such patients need higher immunosuppressant drug doses than do IBD patients[12].

Patients with positive HBsAg and anti-HBc and negative HBV-PCR DNA have increased risk of reactivating their HBV infections. Therefore, the use of lamivudine is recommended before immunological suppression therapies[29]. Lau and collaborators demon-

strated that patients with lymphoma infected by HBV who were submitted to chemotherapy did not have HBV infection reactivated when they used lamivudina one week before chemotherapy was begun[30].

In conclusion, our study demonstrated that there were high incidences of positive anti-HBc (17%) and positive HBsAg (2.3%) in IBD patients in Clementino Fraga Filho University Hospital when compared with the overall population (7.9%).

These data show that it is important to have an early diagnosis of HBV infection in diagnosed IBD patients before any IBD treatment is initiated using steroids, immunosuppressant drugs, or anti-TNF antibodies, as that IBD treatment may worsen quiescent HBV hepatic disease. We also recommend HBV vaccination in this group of patients.

COMMENTS

Background

Hepatitis B virus (HBV) infection is considered a worldwide public health problem. Inflammatory bowel disease (IBD) patients have a high risk of acquiring HBV infection because they sometimes need blood transfusions, invasive surgical and endoscopic procedures. The objective of this study is to verify the seroprevalence of HBV infection and to identify the infection transmission risk factors in IBD patients at Clementino Fraga Filho University Hospital.

Innovations and breakthroughs

The statistical analysis cannot identify one possible risk factor for HBV transmission but the study found among the IBD patients 4 persons with positive HBsAg who were called inactive bearers. Studies show that immunological suppression caused by steroids, immunosupressants drugs and the anti-TNF antibodies (anti necrosis antibodies-Infliximab) in IBD patients can influence the course of hepatic disease once used in HBsAg positive patients. Theses drugs would take a viral replication and infection spread inside hepatocytes. It has already been related to 1 case of hepatic insufficiency and death in a Crohn' s disease (CD) patient and 1 case of fulminant hepatitis in rheumatoid arthritis patient, both with positive HBsAg and treated with these drugs. In patients with positive HBsAg lamivudine use would be recommended before immunological suppression.

Applications

After this study, we recommend HBV vaccination for IBD patients that have never been infected by HBV and also recommend lamivudine for patients with positive anti-HBc and needs to use steroids and immunomodulators.

Peer review

This article did not identify one risk factor for HBV infection transmission in IBD patients but it shows us that these patients have high risk of acquiring this infection because they need invasive procedures. IBD patients that have been infected already must receive lamivudine before immunological suppression. It is very interesting.

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S- Editor Li DL **L- Editor** Li M **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3207

World J Gastroenterol 2008 May 28; 14(20): 3207-3211
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Contrast-enhanced intraoperative ultrasonography equipped with late Kupffer-phase image obtained by sonazoid in patients with colorectal liver metastases

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Received: March 8, 2008 Revised: April 16, 2008
Accepted: April 23, 2008
Published online: May 28, 2008

Key words: Contrast-enhanced intraoperative ultrasonography; Late Kupffer-phase image; Sonazoid; Colorectal cancer liver metastases; Occult hepatic lesions

Peer reviewer: Kenji Miki, MD, Department of Surgery, Showa General Hospital, 2-450, Tenjin-cho, Kodaira, Tokyo 187-8510, Japan

Nakano H, Ishida Y, Hatakeyama T, Sakuraba K, Hayashi M, Sakurai O, Hataya K. Contrast-enhanced intraoperative ultrasonography equipped with late Kupffer-phase image obtained by sonazoid in patients with colorectal liver metastases. *World J Gastroenterol* 2008; 14(20): 3207-3211 Available from: URL: http://www.wjgnet.com/1007-9327/14/3207.asp DOI: http://dx.doi.org/10.3748/wjg.14.3207

Abstract

AIM: To find occult metastases during hepatectomy in patients with colorectal cancer liver metastases (CRCLM), contrast-enhanced intraoperative ultrasonography (CE-IOUS) was performed using a new microbubble agent, sonazoid, which provides a parenchyma-specific contrast image based on its accumulation in the Kupffer cells.

METHODS: Eight patients with CRCLM underwent CE-IOUS using sonazoid before hepatectomy. The liver was investigated during a late Kupffer-phase imaging, which is a valuable characteristic of sonazoid.

RESULTS: CE-IOUS using sonazoid provided the early vascular- and sinusoidal-phase images for 10 min followed by the late Kupffer-phase image up to 30 min after the injection of sonazoid. IOUS did not provide new findings of metastatic lesion in the 8 patients. However, during the late Kupffer-phase image of sonazoid, a metastatic lesion was newly found in two of the 8 patients. These newly detected lesions were removed by an additional hepatectomy and histopathologically diagnosed as a metastasis.

CONCLUSION: CE-IOUS using sonazoid can allow surgeons to investigate the whole liver with enough time and to find new metastases intraoperatively.

INTRODUCTION

Hepatic resection is the only treatment offering a chance of long-term survival to patients with colorectal cancer liver metastases (CRCLM)[1-4]. However, a total of 75% of patients with CRCLM who undergo liver resection will develop recurrence and the main site of recurrence is the liver[5]. In addition, 65% to 85% of all recurrences appear within the first 2 years[5]. Therefore, occult liver metastases may present at the time of hepatectomy and can be undetected preoperatively by computed tomography (CT), magnetic resonance image (MRI), or positron emission tomography (PET)[6].

Intraoperative ultrasound (IOUS) is now considered as a standard method to determine the resection margin or to find preoperatively undetected tumors[7,8]. Recently some authors reported that a contrast-enhanced IOUS (CE-IOUS) was more sensitive than conventional IOUS to identify new lesions and subsequently to influence surgical management[9,10].

Sonazoid (perfluorobutane, GE Helthcare, Oslo, Norway) is a new microbubble agent[11] that provides a parenchyma-specific contrast image based on its accumulation in the Kupffer cells in the liver[12-14]. Sonazoid was

recently approved for clinical use in Japan, and it presents with a late Kupffer-phase image with a long duration following a vascular- and a sinusoidal-phase images[14]. SonoVue (Bracco spA, Milan, Italy) has been already used as a microbubble agent in CE-IOUS[9,10], but it does not have the Kupffer-phase image[13]. The present brief clinical report shows our experience of CE-IOUS using sonazoid in patients with CRCLM.

MATERIALS AND METHODS

Examination of IOUS and CE-IOUS was performed using an Aplio-XV (Toshiba, Tokyo, Japan) and a micro-convex probe (PVT-375BT, 3.5 MHz, Toshiba). CE-IOUS was performed under a pulse inversion harmonic (PIH) imaging capability (Toshiba). A bolus intravenous injection of sonazoid [0.015 mL/kg body weight (0.12 μL microbubble/kg body weight as perflubutane microbubble)] was performed *via* the peripheral venous line followed by 10 mL of normal saline flush. Immediately after the administration of sonazoid, the portal veins, hepatic veins, and the normal liver parenchyma were uniformly enhanced. Hepatic metastases were identified as a dark contrast free filling defect during an early vascular phase image lasting 3 min after the injection of sonazoid. Approximately 10 min after the injection, the liver was scanned again to observe a late Kupffer-phase image. The hepatic metastases were identified as filling defects clearer than those observed at the vascular phase (Figure 1). The late Kupffer-phase image lasted at least for 30 min.

Eight patients with CRCLM underwent CE-IOUS in addition to IOUS. The number and size of metastases identified on preoperative CT, MR, and percutaneous contrast-enhanced ultrasonography (CE-US) were compared with those detected by IOUS and CE-IOUS.

RESULTS

CE-IOUS using sonazoid provided the early vascular- and sinusoidal-phase images for 10 min followed by the late Kupffer-phase image up to 30 min after the injection of sonazoid. Figure 1 shows IOUS and CE-IOUS images of a metastasis at the Segment 8. The lesion was detected as an unclear slightly hypoechoic mass by IOUS (Figure 1A), but the lesion was shown as a clear hypoechoic mass during the late Kupffer-phase (Figure 2B).

Between December 2007 and February 2008, eight patients underwent CE-IOUS. Preoperatively detected sites of liver metastases by CT, MRI, and CE-US were listed, and some differences among CT, MRI and CE-US existed as shown in Table 1. Preoperative CT seemed superior to MRI (patient No. 1 and 4). In addition, preoperative CE-US did not seem useful for finding metastases at the Segment 7 (patient No. 1, 2, and 5). Mainly based on the preoperative findings of CT, surgical methods were planned preoperatively in the eight patients (Table 2). IOUS did not provide new findings of metastatic lesion in the eight patients. Indeed, IOUS could not show some metastatic lesions detected by CT or MRI (Table 1, patient No. 1 and 2). However, CE-IOUS confirmed all hepatic lesions detected by CT or MRI. In addition, metastatic lesions were newly found by CE-IOUS in two of the eight patients. These newly detected lesions were removed by an additional hepatectomy and histopathologically diagnosed as a metastasis.

In the patient No. 1 (Table 1), a small hypoechoic lesion with 6 mm in diameter at the Segment 4 was newly detected by the CE-IOUS at the late Kupffer-phase view (Figure 2A) although IOUS did not show this lesion. This small lesion was resected and histopathologically confirmed as a metastatic nodule (Figure 2C).

In the patient No. 2 who preoperatively presented with liver metastases at the Segment 3 (Table 1), another lesion at the Segment 7 was pointed out as a metastasis with an ill-defined mass by preoperative CT and MRI (Figure 3A and B). Preoperative percutaneous CE-US using sonazoid could not show the lesion at the Segment 7 because of the attenuation of echogenecity. During the surgery, IOUS did not show the metastasis at the segment 7, but CE-IOUS showed a well-demarcated mass at the Segment 7 (Figure 3C). This lesion was resected by a partial hepatectomy and histopathologically confirmed as a metastasis. In addition, CE-IOUS detected a new small lesion at the Segment 6 which was not pointed out by CT or MRI preoperatively (Figure 4). This lesion at the Segment 6 was also removed and histopathologically confirmed as an occult metastasis.

DISCUSSION

The importance of CE-IOUS in patients with CRCLM has been shown by two recent studies[9,10]. Indeed, Torzilli *et al* reported that new metastatic lesions, which were not detected by preoperative examinations and IOUS, were detected in 5 out of 24 patients (21%) using CE-IOUS[9]. They also reported that the modification rate of hepatectomy by CE-IOUS alone was 21% in the patients with CRCLM. Leen *et al* showed that additional new hepatic metastases were detected in 11 out of 57 patients (19%) and the planned surgical methods were converted in these patients[10]. In the present study, an occult metastatic lesion was newly detected in two of the eight patients using CE-IOUS and removed by an additional hepatectomy. These metastatic lesions were not detected by preoperative CT, MRI, preoperative percutaneous CE-US, or IOUS.

Sonazoid is a novel microbubble-based ultrasound contrast agent, and is classified as a second-generation agent in which the perfluorocarbon gas has enough intravascular stability *in vivo*[15,16]. Watanabe *et al* showed that microbubbles of sonazoid were taken up by Kupffer cells immediately after intravenous injection and existed as microbubbles for 30 min within Kupffer cells, and that the hepatic parenchyma-specific contrast by sonazoid was due to the distribution of the microbubbles in Kupffer cells[14]. Therefore, sonazoid has a unique "late Kupffer-phase image" in addition to "early-vascular phase image" and "sinusoidal-phase image". This late Kupffer-phase image can provide high echogenic contrast

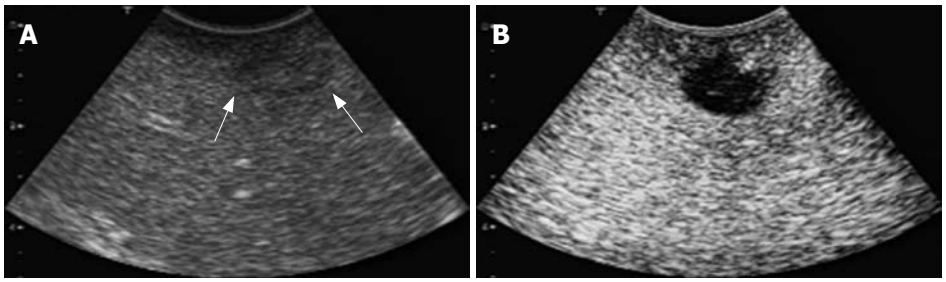

Figure 1 IOUS and CE-IOUS views of a metastasis at the Segment 8. **A**: The metastatic lesion was unclearly detected as a slightly hypoechoic mass; **B**: CE-IOUS view of the same lesion. The metastatic lesion was shown as a distinct hypoechoic mass at the late Kupffer-phase.

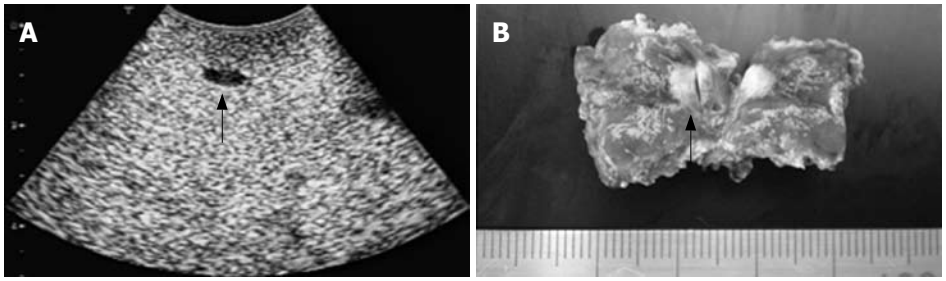

Figure 2 An occult metastasis. **A**: An occult metastasis at the segment 4 only detected by CE-IOUS. A clear hypoechoic mass (approximately 6 mm in diameter; black arrow) was newly detected at the delayed Kupffer phase. This metastatic lesion could not be found by CT, MRI, and IOUS. **B**: Macroscopic view of this metastasis (arrow).

Table 1 Preoperatively diagnosed sites of liver metastases by CT, MRI and CE-US, intraoperatively found lesions by IOUS and CE-IOUS, and intraoperatively newly found metastases by CE-IOUS

Patient	Preoperatively diagnosed metastases			Intraoperatively found lesions		
No.	By CT	By MRI	By CE-US	By IOUS	By CE-IOUS	By CE-IOUS
1	S5, S5-6	S5, S5-6, S7	S5, S5-6	S5, S5-6	S5, S5-6, S7	S4
2	S3, S7	S3, S7	S3	S3	S3, S7	S6
3	S6, S6	S6, S6	S6, S6	S6, S6	S6, S6	(-)
4	S6-7, S1	S6-7	S6-7, S1	S6-7, S1	S6-7, S1	(-)
5	S7	S7	(-)	S7	S7	(-)
6	S7	S7	S7	S7	S7	(-)
7	S7-6, S3	S7-6, S3	S7-6, S3	S7-6, S3	S7-6, S3	(-)
8	S3, S4, S8	S3, S4, S8	S3, S4, S8	S3, S4, S8	S3, S4, S8	(-)

Table 2 Preoperatively planned surgical methods mainly performed surgical methods, and methods of additional surgery according to the findings of CE-IOUS

Patient No.	Preoperatively planned surgery	Mainly performed operative procedures	Methods of additional surgery based the findings of CE-IOUS
1	Enucleations at S5, S5-S6, and S7	Bisegmentectomy of S5 and S6 Enucleation at S7	Enucleation at S4
2	Left lateral sectionectomy, partial resection of S7	Left lateral sectionectomy, partial resection of S7	Enucleation at S6
3	S6 segmentectomy	S6 segmentectomy	(-)
4	Right posterior sectionectomy, S1 partial resection	Right posterior sectionectomy, S1 partial resection	(-)
5	S7 partial resection	S7 partial resection	(-)
6	S7 segmentectomy	S7 segmentectomy	(-)
7	Posterior sectionectomy, left lateral sectionectomy	Posterior sectionectomy, left lateral sectionectomy	(-)
8	Enucleations at S3, S4, and S8	Enucleations at S3, S4, and S8	(-)

enhancement in the liver parenchyma[14]. On the other hand, other microbubble contrast agents such as Imavist and SonoVue provide parenchyma-specific contrast by transient mechanical slowdown of microbubbles within the sinusoid, and these two contrast agents are hardly phagocytosed by the Kupffer cells[10,13,17]. Therefore, Imavist and SonoVue cannot provide the late Kupffer-phase image, and the parenchyma-specific contrast image of these two microbubble agents can be seen during only 3 min to 5 min after the injection. Therefore, as previously described, SonoVue needs repeated injections during CE-IOUS to perform a whole liver examination[9,10]. Therefore, sonazoid seems a superior microbubble contrast agent for CE-IOUS in patients with CRCLM since a whole precise liver investigation by CE-IOUS, in which determination of surgical margin and examination of occult metastases should be investigated. Therefore, CE-IOUS needs more than 5 min, and the present brief

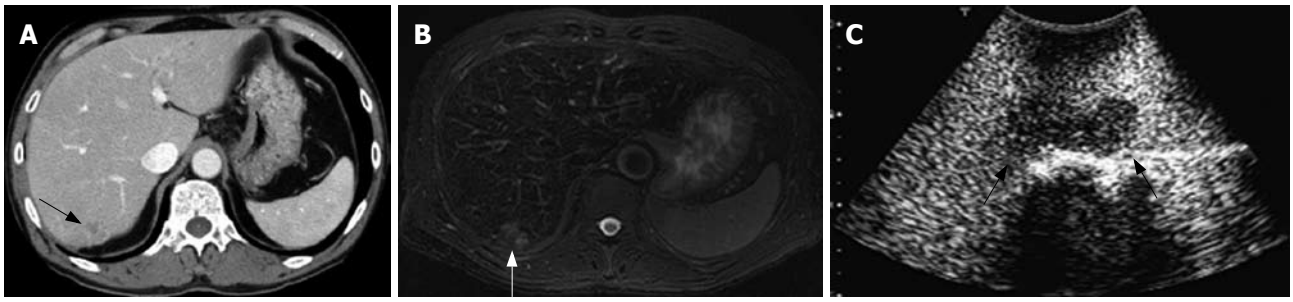

Figure 3 Preoperative CT and SPIO-MRI, and CE-IOUS. **A**: An enhanced-CT view and an ill-defined low density mass was detected at the segment 7 (arrow); **B**: A SPIO MRI view and an ill-defined high intensity mass was detected at the segment 8 (arrow); **C**: CE-IOUS view at the delayed Kupffer phase and a well-demarcated hypoechoic mass was detected by CE-IOUS.

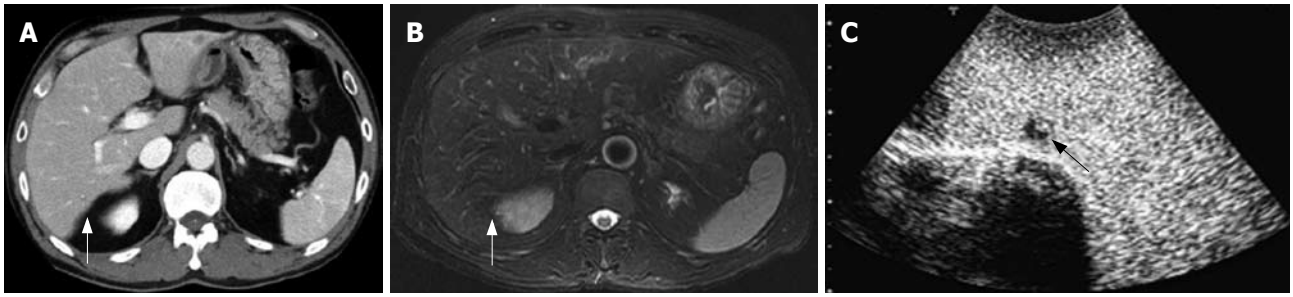

Figure 4 Preoperative CT and SPIO-MRI, and CE-IOUS. **A**: An enhanced-CT could not detect any lesion at the Segment 6 (arrow); **B**: A SPIO MRI view could not detect any lesion at the Segment 6 (arrow); **C**: CE-IOUS view at the delayed Kupffer phase and a small hypoechoic mass partially containing a isoechoic lesion was detected by CE-IOUS.

clinical experience of CE-IOUS confirmed the usefulness of sonazoid during surgery in patients with CRCLM. In addition, the duration of the approximately 30 min of the late-Kupffer phase image using sonazoid seems useful to perform preoperative percutaneous CE-US compared to SonoVue because the limiting time of SonoVue image (5 min) does not seem convenient to perform preoperative CE-US. Indeed, a routine preoperative CE-US in our institution, in which only the late Kupffer-phase image is performed, can be performed between 10 min and 30 min after the injection of sonazoid. However, based on our experience, small metastases at the Segment 7 seem hardly visualized by percutaneous CE-US using sonazoid because of the attenuation of echogenecity as shown in the present case No. 2.

Sonazoid has been reported as a safe medicine. Indeed, the incidence of adverse effects of sonazoid was shown in 25 out 397 patients (6.3%) in a clinical phase Ⅱ study performed in Japan. The main side effects were headache (1.0%) and diarrhea (1.0%), but no anaphylactic shock due to sonazoid was reported unlike with contrast-enhanced CT. The image mechanism of CE-IOUS using sonazoid seems similar to superpara-magnetic iron oxide-enhanced magnetic resonance image (SPIO-MRI) because both images are based on the phagocytosis by Kupffer cells. However, sonazoid is much less expensive compared to SPIO-MRI. Regardless of sensitivity rate of sonazoid for detecting small metastases compared to SPIO-MRI, CE-IOUS is useful to perform intraoperative liver biopsy of newly detected lesions and to determine an additional hepatectomy.

In conclusion, CE-IOUS using sonazoid can allow surgeons to investigate the whole liver with enough time (at least 30 min of the late Kupffer-phase image) and to find new metastases intraoperatively.

COMMENTS

Background

Contrast-enhanced intraoperative ultrasonography (CE-IOUS) seems more sensitive than conventional IOUS to identify new occult lesions during hepatectomy in patients with colorectal cancer liver metastases (CRCLM). Sonazoid (perfluorobutane, GE Helthcare, Oslo, Norway) is a new microbubble agent that provides late Kupffer-phase image, which cannot be obtained by conventional contrast mediums.

Research frontiers

No study has investigated the intraoperative efficacy of the late Kupffer-phase image of sonazoid in patients with CRCLM.

Innovations and breakthroughs

CE-IOUS using sonazoid enabled whole liver investigation at least for 30 min of the late Kupffer-phase image. Occult metastases, which had not been detected preoperatively, were newly found in some patients and removed by an additional hepatectomy.

Applications

CE-IOUS using sonazoid can reduce intrahepatic recurrence after hepatectomy in patients with CRCLM.

Peer review

This article presented the clinical significance of CE-IOUS using sonazoid during hepatectomy for colorectal cancer liver metastases. CE-IOUS for detection of liver metastases requires stable image for enough time to perform repeated whole liver scans. Sonazoid seems to be suitable for this purpose. This article is worthy for publication in *WJG* with minor revision.

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S- Editor Zhong XY **L- Editor** Negro F **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3212

World J Gastroenterol 2008 May 28; 14(20): 3212-3217
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Incidence of reflux esophagitis and *Helicobacter pylori* infection in diabetic patients

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Received: January 9, 2008 Revised: March 30, 2008
Accepted: April 6, 2008
Published online: May 28, 2008

Abstract

AIM: To investigate the incidence of reflux esophagitis (RE) and *H pylori* infection in the diabetic patient.
METHODS: The incidence of RE and *H pylori* infection were investigated in 85 patients with diabetes mellitus and the results were compared with controls.
RESULTS: The incidence of RE in diabetic patients was 17.6%. Although this tended to be higher in diabetic patients, there were no statistically significant differences between diabetic patients and controls. The incidence of *H pylori* infection in diabetic patients was 53.7% but no statistically significant difference was seen between diabetic patients and controls in the incidence of *H pylori* infection.
CONCLUSION: No significant differences could be seen between diabetic patients and controls in the incidence of RE and *H pylori* infection.

Key words: Diabetes mellitus; Reflux esophagitis; *Helicobacter pylori*

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Ariizumi K, Koike T, Ohara S, Inomata Y, Abe Y, Iijima K, Imatani A, Oka Y, Shimosegawa T. Incidence of reflux esophagitis and *Helicobacter pylori* infection in diabetic patients. *World J Gastroenterol* 2008; 14(20): 3212-3217 Available from: URL: http://www.wjgnet.com/1007-9327/14/3212.asp DOI: http://dx.doi.org/10.3748/wjg.14.3212

INTRODUCTION

At present, the frequency of lifestyle-related illnesses such as diabetes mellitus and obesity is increasing due to the westernization of the Japanese diet. Diabetic patients are now estimated at 7 400 000 in Japan[1] and diabetes is showing a world-wide tendency to increase[2].

In gastroesophageal reflux diseases (GERD), frequent gastroesophageal acid reflux causes such symptoms as heartburn, water brash, chest pain, and esophageal discomfort, lowering the quality of life of patients. Also, GERD damages the esophageal mucosa through erosion and the development of ulcers, mainly in the lower esophagus, leading to reflux esophagitis (RE). The incidence of RE has been on the rise in recent years, and today, it is one of the most common chronic diseases for adults in Europe and the United States[3]. While the incidence of RE in Japan is considered low as compared with Europe and the United States, the incidence of RE in Japan has increased due to the westernization of the Japanese diet, the rapidly growing elderly population, and lower *H pylori* infection rates[4].

Some investigators have reported that the incidence of RE is high in diabetic patients[5,6], although few reports have examined the incidence of RE in diabetic patient in Japan. There are some reports that hiatal hernia, age, *H pylori* infection and body mass index (BMI) are considered to affect the outbreak of RE[7,8]. Recent studies have reported that *H pylori* infection was less prevalent in patients with RE than those without RE, and was considered to suppress the onset of RE by inducing gastric mucosal atrophy and lowering gastric acid secretion[9,10].

Some investigators have reported that the incidence of *H pylori* infection in diabetic patients is higher than controls[11-14], though other investigators have reported

no such significant differences between these groups[15,16].

The objective of the present study is to examine the incidence of RE and *H pylori* infection in diabetic patients.

Patient demographics, incidence of GERD, incidence of columnar lined esophagus (CLE), serum gastrin concentration, and pepsinogen (PG) Ⅰ/Ⅱ (an index of gastric mucosal atrophy based on serologic finding) were also evaluated.

MATERIALS AND METHODS

Study design

A total of 85 consecutive patients with diabetes mellitus who visited the Department of Diabetes and Metabolic Diseases at Tohoku University Hospital from December 2002 to September 2003 were included in the present study. Patients who had other severe complications, had taken PPI or H2 receptor antagonists within four wk, or had undergone esophagogastrectomy were excluded from the present study. Nine hundred and forty four patients who had undergone endoscopy at the same period and another 67 age and sex-matched non-diabetic subjects without upper GI tract disorders were also included. Informed consent was obtained from each patient. The study protocol was approved by the ethical committee of the Tohoku University Graduate School of Medicine.

The subjects were divided into two groups, a well glycemic controlled group and a poorly controlled group. Patient demographics, incidence of RE, incidence of GERD, incidence of CLE, incidence of *H pylori* infection, severity grade of RE, serum gastrin concentration and PG Ⅰ/Ⅱ were investigated between the good glycemic controlled and poorly controlled group. The incidence of RE, GERD, CLE and the severity grade of RE were compared with 944 patients who had undergone endoscopy at the same study period as controls.

The incidence of RE and GERD were assessed due to HbA1c, disease duration, diabetic complications, and BMI. *H pylori* infection status was investigated between DM patients and 67 age and sex-matched non-diabetic subjects without upper GI tract disorders.

Patient demographics

The following factors were investigated: gender, age, height, body weight, BMI, type of DM [insulin dependent DM (IDDM) or non insulin dependent DM (NIDDM)], duration of DM, presence of hiatal hernia, with insulin therapy, with calcium antagonists, with complications (retinopathy, nephropathy and neuropathy). All patients were examined by an ophthalmologist for retinopathy. Nephropathy was diagnosed if albuminuria was > 0.3 g/L or there was evidence of chronic renal failure. Neuropathy was diagnosed if the patients had sensory abnormalities, vibration hyposensitivity, orthostatic hypotension, or impotence.

Good and poorly glycemic controlled groups: The good glycemic controlled group had hemoglobin A1c (HbA1c) 6.4% or less, and the poorly controlled group had an HbA1c value of 6.5% or more.

Assessment of RE: Subjects were diagnosed as having RE of grade A to D by the Los Angeles Classification[17].

Diagnosis of hiatal hernia: In the present study, hiatal hernia was defined as a hernia in which the gastric mucosa could be seen by endoscopy circumferentially from the esophageal hiatus.

Diagnosis of CLE: CLE was defined as the replacement of the normal squamous lining of the lower esophagus by columnar epithelium.

Assessment of *H pylori* infection: In the present study, patients were diagnosed as having *H pylori* infection if they tested positive to at least one of the following tests: biopsy of the mucosa of the gastric body and gastric antrum along the greater curvature during endoscopy, rapid urease test, and serum *H pylori* antibody test. Patients were diagnosed as being free of *H pylori* infection if they tested negative to all tests.

PG Ⅰ/Ⅱ and gastrin level: PG Ⅰ/Ⅱ[18] and gastrin level[19] were measured to assess gastric mucosal atrophy. Each blood sample was centrifuged, and the sera were stored frozen at -20℃ until testing.

Diagnosis of GERD, incidence of reflux symptoms: GERD was diagnosed with a self-administered questionnaire (QUEST). When the sum of the scores was 4 or more, the patient was considered as having GERD[20,21]. All patients were interviewed by investigators regarding their symptoms related to RE such as heartburn, burning in the upper abdomen, gastro-esophageal regurgitation, fullness, abdominal distension, anorexia, nausea, abdominal pain, and difficulty in swallowing food.

Statistical analysis

Of the various patient background factors, gender, type of DM, presence of hiatal hernia, with insulin therapy, with calcium antagonists, with diabetic complications, incidence of RE, reflux symptoms, GERD, CLE and *H pylori* infection status were compared between diabetic patients and controls by a chi-square test. Age, height, body weight, BMI, duration of DM, sum of the QUEST score, PG Ⅰ/Ⅱ and gastrin level were expressed as mean ± SD, and a one-way ANOVA test was used to compare these parameters between diabetic patients and controls. The Mann-Whitney's *U* test was used to compare RE severity. The significance level was set at < 5%.

RESULTS

Of the 85 diabetic patients, there were 79 NIDDM and 6IDDM patients, 45 (52.9%) men and 40 (47.1%)

Table 1 Patient characteristics

	Well controlled group (%)	Poorly controlled group (%)	*P*
Gender (Male/Female)	(20/16)	(25/24)	0.84
Type of DM (IDDM/NIDDM)	(1/35)	(5/43)	0.36
Age (yr)	65.9 ± 10.3	60.0 ± 11.9	0.02
Height (cm)	160.3 ± 8.7	156.9 ± 17.6	0.29
Body weight (kg)	61.4 ± 16.5	61.1 ± 17.9	0.93
BMI	23.5 ± 5.6	23.3 ± 3.1	0.77
Duration of DM (yr)	12.9 ± 10.5	16.7 ± 10.3	0.10
Hiatal hernia	33.3 (12/36)	22.4 (11/49)	0.38
Insulin therapy	33.3 (12/36)	61.2 (30/49)	0.02
Calcium antagonist	13.9 (5/36)	24.5 (12/49)	0.35
Retinopathy	16.7 (6/36)	32.7 (16/49)	0.16
Nephropathy	2.3 (1/36)	8.2 (4/49)	0.56
Neuropathy	13.9 (5/36)	20.4 (10/49)	0.45

Table 2 Comparison between diabetic patients and controls

	Diabetic patients (%)	Controls (%)	*P*
RE	17.6 (15/85)	10.3 (97/944)	0.056
Los angeles classification			
A	80.0 (12/15)	44.3 (43/97)	0.01
B	20.0 (3/15)	48.5 (47/97)	
C	0	6.2 (6/97)	
D	0	1.0 (1/97)	
Incidence of CLE	32.9 (28/85)	37.7 (356/944)	0.45

CLE: Columnar lined esophagus.

women. The mean age ± SD was 62.5 ± 11.5 years (range 28-85 years, median 64 years). The mean durations of DM was 15.1 ± 10.5 years (range 0-44 years, median 14 years).

Thirty-six patients comprised the well glycemic controlled group and 49 patients comprised the poorly controlled group. In the poorly controlled group, age and the number of patients with insulin therapy were higher than in the well controlled group. Among each group, there were no significant differences in gender, type of DM (IDDM or NIDDM), height, body weight, BMI, duration of DM, presence of hiatal hernia, with calcium antagonists, or diabetic complications (Table 1).

In the diabetic patients, the incidence of RE was 17.6% (15/85), and in the 944 controls who had undergone endoscopy at the same period in our division, the incidence of RE was 10.3% (97/944). Though the incidence of RE tended to be higher in diabetic patients, the difference was not significant between diabetic patients and controls (P = 0.056). Under the Los Angeles classification, all diabetic patients were grade A or B, and the severity grade of RE was statistically lower in diabetic patients than controls. The incidence of GERD and reflux symptoms in the diabetic patients were 32.9% (28/85) and 36.6% (31/85), respectively. The incidence of CLE was 32.9% (28/85), and the length all were less than 3 cm long. In the 944 controls, the incidence of CLE was 37.7% (356/944) (more than 3 cm: 11 patients, less than 3 cm: 345 patients) (Table 2).

Table 3 Comparison between good and poorly glycemic controlled groups

	Well controlled group (%)	Poorly controlled group (%)	*P*
RE	19.4 (7/36)	16.3 (8/49)	0.93
Incidence of GERD	25.0 (9/36)	38.8 (19/49)	0.27
Symptoms	30.1 (11/36)	41.3 (20/49)	0.46
QUEST score	2.06 ± 3.16	3.37 ± 4.22	0.12
Los angeles classification			
A	85.7 (6/7)	75.0 (6/8)	0.62
B	14.3 (1/7)	25.0 (2/8)	
C	0	0	
D	0	0	
Incidence of CLE	27.8 (10/36)	36.7 (18/49)	0.52
PG Ⅰ / Ⅱ	4.07 ± 2.61	4.94 ± 2.31	0.19
Gastrin	166.0 ± 126.9	137.6 ± 122.8	0.40

CLE: Columnar lined esophagus.

Comparison between well and poorly glycemic controlled groups

The incidences of RE in the well glycemic controlled group and poorly controlled group were 17.6% (15/85) and 10.3% (97/944), respectively. The incidences of RE in patients with HbA1c under 5.7, 5.8 to 6.4, 6.5 to 7.9, and higher than 8.0 were 27.3% (3/11), 16% (4/25), 14.6% (6/41), and 25% (2/8), respectively. The incidences of GERD for the same patient groups were 36.4% (4/11), 20% (5/25), 34.1% (14/41), and 62.5% (5/8), respectively. The incidence of RE and GERD did not show any particular tendency.

Among the well and the poorly glycemic controlled groups, there were no significant differences in the frequency of RE, GERD, reflux symptoms, CLE, sum of the QUEST score, severity grade of RE, PG Ⅰ / Ⅱ or gastrin level (Table 3).

Comparison between groups receiving and not receiving calcium antagonists

The incidences of RE in patients with and without calcium antagonists were 23.5% (4/17) and 16.2% (11/68), respectively. The incidences of GERD for the same groups were 29.4% (5/17) and 33.8% (23/68), respectively. There were no significant differences between patients receiving and not receiving calcium antagonists. The incidences of RE in the well and poorly glycemic controlled groups were 16.7% (6/36) and 18.3% (9/49), respectively. The incidences of GERD were 25.0% (9/36) and 38.8% (19/49), respectively. There were no significant differences between the well and the poorly glycemic controlled groups.

The incidence of RE and GERD in patients according to disease duration

The incidences of RE in patients with disease durations of less than 6 years, 6 to 11 years, 11 to 16 years, and more than 16 years were 11.8% (2/17), 10.5% (2/19), 21.4% (3/14) and 22.9% (8/35), respectively. The incidences of GERD for the same durations were 23.5% (4/17) 42.1% (8/19), 42.9% (6/14) and 28.6% (10/35),

respectively. The incidence of RE tended to rise with increased duration of the disease. The incidence of GERD did not show any particular tendency.

The incidence of RE and GERD with and without complications

Of the diabetic patients, 22 had retinopathy, 5 had nephropathy and 15 had neuropathy. The incidences of RE in DM patients with and without complications were 20% (6/30) and 16.5% (9/55), respectively. The incidences of GERD were 33.3% (10/30), and 32.7% (18/55), respectively. There were no significant differences between patients with and without diabetic complications.

The incidence of RE and GERD in non-obese and obese patients

The incidences of RE in non-obese (BMI less than 25) and obese patients (BMI higher than 25) were 17.2% (10/58) and 18.5% (5/27), respectively. The incidences of GERD were 29.3% (17/58) and 40.7% (11/27), respectively. There were no significant differences between non-obese and obese patients. There were also no BMI-related differences between patients with and without RE (23.3 ± 2.8 *vs* 23.4 ± 4.5) and between those with GERD and without GERD (23.8 ± 6.4 *vs* 23.2 ± 2.7).

H pylori infection

Of the 85 diabetic patients, measurement of their *H pylori* infectious status could be performed in 67 patients, of whom 53.7% (36/67) had *H pylori* infection. Of the age and sex-matched controls, 68.7% (46/67) had *H pylori* infection, with no significant differences seen in *H pylori* infection status between diabetic patients and controls ($P = 0.11$).

The incidences of RE in *H pylori* (+) and *H pylori* (-) patients were 19.4% (7/36) and 9.7% (3/31), respectively. The incidences of GERD in *H pylori* (+) and *H pylori* (-) patients were 27.8% (10/36) and 35.5% (11/31), respectively. No significant differences in the incidence of RE and GERD could be demonstrated between *H pylori* (+) and *H pylori* (-) patients.

DISCUSSION

The incidence of RE has been on the rise in recent years, and today, it is one of the most common chronic diseases for adults in Europe and the United States[22].

Today, the incidence of RE is reported to be 10%-20% in Europe and the United States[23-25], and 14%-16% in Japan[26-28]. While the incidence of RE in Japan is considered low as compared to Europe and the United States, the incidence of RE in Japan has increased due to the westernization of the Japanese diet, the rapidly growing elderly population, and lower *H pylori* infection rates[26-28].

Parkman[5] has reported that the incidence of RE in patients was 20% (4/20). Antwi[6] reported an incidence of 40.7% (22/54), though in these reports, glycemic control in many of the patients was poor and many had neuropathy. In the present study, the incidence of RE in diabetic patients was 17.6% (15/85), and the incidence of RE in the 944 controls was 10.3% (97/944). The incidence of RE tended to be higher in diabetic patients, there were no significant differences between diabetic patients and controls.

In Japan, with respect to the severity of RE, most patients are reported to have mild esophagitis (Grade A or B under the Los Angeles classification[26,27]. In the present study, all patients had mild esophagitis.

In Japan, Nishida has previously reported that the incidence of GERD diagnosed with the QUEST questionnaire in diabetic patients was 25.3%, and significantly higher than that of controls[29]. In this previous study, they used the QUEST questionnaire to investigate GERD, though they did not perform gastrointestinal endoscopy to investigate RE. In the present study, we used the QUEST questionnaire and additionally performed gastrointestinal endoscopy to investigate GERD and RE. To the best of our knowledge, the present study is the first study to investigate both GERD and RE at the same time for diabetic patients in Japan. In the present study, the prevalence of GERD was 32.9%.

HbA1c is an index of DM control over some months beforehand. Complications of DM such as retinopathy, nephropathy and neuropathy occur as a result of poor control of diabetes for several years. So it is conceivable that DM-related complications are more appropriate than HbA1c as an index of the diabetic control. In the present study, there were no significant differences between patients with and without complications in the incidence of RE and GERD.

Some patients with DM, particularly those with NIDDM, are obese, which increases the intra-abdominal pressure, and may worsen RE[30]. In the present study, there were no significant differences between obese patients and non-obese patients in the incidence of RE or GERD. There were also no BMI-related differences between patients with and without RE and those with and without GERD. In the present study, obesity was not a risk factor for RE or GERD in the diabetic patient.

DM induces complications such as retinopathy, nephropathy and neuropathy. Diabetic neuropathy occurs in all sensory, motor and autonomic nerves. Some investigators have reported that in diabetic patients, RE can be induced in the presence of lowered LES pressure, abnormal esophageal motility, increased transient lower esophageal sphincter relaxation (TLESR), lowered acid clearance of the esophagus and prolonged gastric emptying time due to the dysfunction of autonomic nerves or the vagal nerve[31-35]. Prolonged gastric emptying time sometimes induces an unexpected hyper or hypoglycemic status, especially in the patients using insulin or oral hypoglycemic agents. Disorder of the sensory nerves raises the perception threshold level, and some patients cannot feel reflux symptoms, possibly leading to stricture of the esophagus. In some patients, RE can be discovered for the first time during routine endoscopy. In the present study, the incidence of RE tended to be higher in diabetic patients, although the differences between diabetic patients and controls were not significant.

It would be important to test for the presence of RE in diabetic patients during the daily examination.

The incidence of *H pylori* infection in diabetic patients has attracted some controversy. Some investigators have reported that the incidence of *H pylori* infection in diabetic patients is higher than controls[9-13]. Some investigators have reported that in DM patients, due to impaired immune function and impaired gastrointestinal motility, they were prone to *H pylori* infection[36-38]. On the other hand, some previous studies show a lower incidence of *H pylori* in diabetic patients than controls[39], whereas in other studies, there was no difference in the prevalence of *H pylori* infection between diabetic patients and controls[11,14]. In some studies, *H pylori* infection was assessed by only one or two methods taken from among a biopsy of the mucosa, the rapid urease test, and the presence of serum *H pylori* antibodies. In the present study, *H pylori* infection was investigated by all three methods, so the incidence of *H pylori* infection in DM patients the present study is considered to be accurate, being recorded as 53.7%, but with no significant differences between diabetic patients and controls.

In conclusion, our results indicated that there were no differences in the incidences of either RE or *H pylori* infection between diabetic patients and controls.

ACKNOWLEDGMENTS

We are grateful to Akira Tamura, Yasushi Ishigaki, Kanji Hirai, Yoshimitsu Ishihara, Kazuma Takahashi, Yoshinori Hinokio and Susumu Suzuki in the division of Diabetes and Metabolic Diseases for their assistance.

COMMENTS

Background

Some investigators had reported that the incidence of reflux esophagitis (RE) and *H pylori* infection is high in diabetic patients. Only a few reports, however, have examined the incidence of RE and *H pylori* infection in diabetic patients in Japan. The present study was designed to investigate the incidence of RE and *H pylori* infection in the diabetic patient.

Research frontiers

We investigated the incidence of gastroesophageal reflux diseases (GERD), RE and *H pylori* infection in the diabetic patient. The present study is the first study to investigate both GERD and RE at the same time for diabetic patients in Japan.

Innovations and breakthroughs

We used the questionnaire (QUEST) questionnaire to investigate GERD and performed gastrointestinal endoscopy to investigate RE at the same time for diabetic patients.

Applications

In the present study, the incidence of RE tended to be higher in diabetic patients. It would be important to test for the presence of RE in diabetic patients during the daily examination.

Peer review

This manuscript indicated that there were no differences in the incidences of either RE or *H pylori* infection between diabetic patients and controls. The study was well performed and the conclusion was clinically useful.

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S- Editor Yang RH **L- Editor** Negro F **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3218

World J Gastroenterol 2008 May 28; 14(20): 3218-3223
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Reactive oxygen species and chemokines: Are they elevated in the esophageal mucosa of children with gastroesophageal reflux disease?

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Supported by A Grant Obtained from Marmara University School of Medicine, SAG-TUS-300505-0214
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Received: December 15, 2007 Revised: March 20, 2008
Accepted: March 27, 2008
Published online: May 28, 2008

Abstract

AIM: To determine the role of inflammatory cytokines and reactive oxygen species (ROS) in childhood reflux esophagitis.
METHODS: A total of 59 subjects who had complaints suggesting GERD underwent esophagogastroduodenoscopy. Endoscopic and histopathologic diagnosis of reflux esophagitis was established by Savary-Miller and Vandenplas grading systems, respectively. Esophageal biopsy specimens were taken from the esophagus 20% proximal above the esophagogastric junction for conventional histopathological examination and the measurements of ROS and cytokine levels. ROS were measured by chemiluminescence, whereas IL-8 and MCP-1 levels were determined with quantitative immunometric ELISA on esophageal tissue. Esophageal tissue ROS, IL-8 and MCP-1 levels were compared among groups with and without endoscopic/histopathologic esophagitis.
RESULTS: Of 59 patients 28 (47.5%) had normal esophagus whereas 31 (52.5%) had endoscopic esophagitis. In histopathological evaluation, almost 73% of the cases had mild and 6.8% had moderate degree of esophagitis. When ROS and chemokine levels were compared among groups with and without endoscopic esophagitis, statistical difference could not be found between patients with and without esophagitis. Although the levels of ROS, IL-8 and MCP-1 were found to be higher in the group with histopathological reflux esophagitis, this difference was not statistically significant.
CONCLUSION: These results suggest that the grade of esophagitis is usually mild or moderate during childhood and factors apart from ROS, IL-8 and MCP-1 may be involved in the pathogenesis of reflux esophagitis in children.

Key words: Gastroesophageal reflux disease; Reflux esophagitis; Reactive oxygen species; Interleukine-8; Monocyte chemoattractant protein-1

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Tutar E, Ertem D, Unluguzel G, Tanrikulu S, Haklar G, Celikel C, Ademoglu E, Pehlivanoglu E. Reactive oxygen species and chemokines: Are they elevated in the esophageal mucosa of children with gastroesophageal reflux disease? *World J Gastroenterol* 2008; 14(20): 3218-3223 Available from: URL: http://www.wjgnet.com/1007-9327/14/3218.asp DOI: http://dx.doi.org/10.3748/wjg.14.3218

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of

the most frequently encountered gastrointestinal pathologies in children with a prevalence of 8% during infancy. When the presence of heartburn as a presenting symptom was considered, the prevalence rises up to 40% among adults[1-4]. Early diagnosis and treatment is of importance as GERD results in serious problems that have negative influences on the quality of life. Since there is a wide range of symptoms regarding GERD and the results of GER questionnaires (GERQ) are controversial together with the incoherencies among other investigative methods; diagnosis of the disease is difficult[5].

Oxidative stress has been associated with several disease states like atherosclerosis, pulmonary fibrosis, cancer, neurodegenerative diseases and aging[6-7]. Furthermore, reactive oxygen species (ROS) are reported to play a role in the pathogenesis of several gastrointestinal diseases such as inflammatory bowel disease and peptic ulcer[8-11]. In studies carried out on animal models of esophagitis as well as those on human esophageal tissue, ROS that are generated in the process of reflux esophagitis were found to be responsible for the esophageal tissue damage, and this finding was further supported by the studies showing that tissue damage could be prevented with the use of antioxidants[12-19].

Several chemokines have been shown to increase significantly in inflammatory disease states like pulmonary diseases, viral meningitis, asthma, rhinitis, a topical dermatitis, ulcerative colitis and Crohn's disease[20-24]. While IL-8 has chemotactic activity for neutrophils, monocyte chemoattractant protein-1 (MCP-1) is effective in the activation of monocytes, macrophages as well as lymphocytes[25]. In studies conducted in adults, chemokine levels were found to be significantly high in the esophageal tissues of the patients with esophagitis as compared to controls; furthermore a significant correlation was reported between the severity of the reflux esophagitis and chemokines levels[26-30]. However, there is only limited data on the levels of ROS and tissue cytokines in the pathogenesis of reflux esophagitis in children. In a study including 10 children with reflux symptoms, IL-6 levels of the esophageal tissue was found to be higher than that of the normal resulting in a claim by the researchers that cytokines could be important in the pathogenesis of the reflux disease[31].

The aim of this study is to investigate the roles of chemokines and ROS in reflux esophagitis in children by measuring the levels of ROS, IL-8 and MCP-1 in the esophageal tissues of children having endoscopic and histopathological esophagitis.

MATERIALS AND METHODS

Patient selection, endoscopy and histopathology

Consecutive children who underwent upper gastro-intestinal endoscopy between September 2005 and January 2006 were prospectively considered for the study. Patients who had complaints related to GER such as vomiting, epigastric pain, regurgitation, retrosternal pain, dysphagia and persistent wheezing were included in this study. Patients with a history of non-steroidal anti-inflammatory drug, proton pump inhibitor, H2 receptor antagonist or antibiotics use or those having severe chronic co-morbidities like diabetes mellitus, renal diseases or neurological diseases were excluded from the study. The study was approved by the local ethics committee of Marmara University School of Medicine, and informed consent forms were obtained from first degree relatives of all the patients.

Same endoscopy team performed the upper gastrointestinal system endoscopy in all patients with a pediatric fiberoptic gastroscope having an inner diameter of 2.8 mm (Fujinon EG 200 HR, Japan). Following an appropriate fasting time based on age of the patients, sedation was established by iv administration of meperidine (2-4 mg/kg) and midazolam (0.2-0.4 mg/kg) and endoscopy was performed. The degree of endoscopic esophagitis was evaluated according to Savary-Miller classification[32]. Patients with and without endoscopic esophagitis formed the first group (Group Ⅰ). During endoscopy, 4 esophageal biopsy samples were obtained from 20% proximal part of esophagogastric junction for conventional histopathological examination and the measurements of ROS and cytokine levels. At the end of the study, histopathological examination of the biopsy samples was carried out at the same time by the same pathologist who was blind for the clinical and laboratory findings of the patient. The diagnosis of histopathological esophagitis was based on the classification by Vandenplas[33] and the cases with or without histopathological esophagitis formed the second group (Group Ⅱ). For simplifying histopathological evaluation; Stage 1a, 1b and 1c were grouped as mild, Stage 2 and 3 as moderate and finally Stage 4 and 5 as severe esophagitis.

The third tissue sample was rinsed with 0.9% physiological saline and placed in eppendorf tubes for measurement of ROS levels in fresh tissues in the biochemistry laboratories. Other tissue samples were stored at -80℃ for the measurement of tissue IL-8 and MCP-1 at the end of the study.

For conventional histopathological examination, 3 samples from the antrum, 2 from the corpus and 2 from the duodenum were obtained from all patients, and these were placed in 10% formaldehyde and sent to pathology for examination. The presence of *H pylori* was confirmed with a positive rapid urease test and histopathological identification of *H pylori*. Gastric biopsy samples were evaluated with a modified Sydney scoring system that allowed for identifying the presence of gastritis, its severity and the density of *H pylori*.

Biochemical measurements

For the measurement of free oxygen radicals on esophageal tissue, chemiluminescence method was employed. The measurements were carried out with Mini Lumat LB 9506 Luminometer (EG&G, Berthold, Germany) at room temperature on fresh tissue samples. In the method employed, lucigenin corresponds to superoxide radical, whereas luminol identifies the total

value for hydroxyl radical (OH^-), hydroperoxyl radical (HO2') and peroxyl (RO2') radical. The tissues were first placed into 3 mL of PBS solution, on top of the tissues luminol or lucigenin (Sigma Chemicals, USA) were added at a concentration of 4 mmol/L as enhancers and measurements were obtained.

IL-8 and MCP-1 levels were measured by the quantitative immunometric sandwich enzyme linked-immunosorbent assay (ELISA) method. For IL-8 (Diaclone Research, France) and MCP-1 (Biosource, California, USA) measurements, the tissue samples were placed into eppendorf tubes and kept at -80℃. Cytokine measurements were performed on the same day with IL-8 and MCP-1 from the same samples. The tissues obtained were homogenized with phosphate buffered physiological saline to prepare 10% homogenates. After the homogenates were centrifuged at 1000 × *g* for 10 min, the supernatant obtained was used for measuring IL-8 and MCP-1 levels. As IL-8 and MCP-1 levels were to be calculated based on total amount of tissue protein, protein measurements were also performed on biopsy samples. The measurement of protein in esophageal homogenates was performed according to the "Bicinchoninic acid" method. For the procedure; bicinchoninic acid solution (Sigma B 9643, Sigma Chemicals) and 4% $CuSO_4$, protein coloring reagent (0.2 mL 4% $CuSO_4$ on 10 mL bicinchoninic acid solution) were used. Ten μL of homogenate was added onto 200 μL of protein coloring reagent. After mixing, the tube was kept at 37℃ for 30 min. It was brought back to room temperature and the absorbance of the coloured complex was measured at 562 nm. The values were expressed as pg/mg tissue protein. IL-8 and MCP-1 measurements were carried out with EL × 800 BIO-TEK Instruments, Inc./USA brand ELISA device in line with the instructions provided in the commercial kits.

Statistical analysis

In the statistical evaluation of the findings obtained from the study SPSS (Statistical Package for Social Sciences) for Windows 10.0 program was used. The Kolmogorov Smirnov test was used to compare the distributions of luminol, lucigenin, MCP-1 and IL-8 to parameters with normal distribution. As IL-8 and MCP-1 parameters had limit values, logarithmic conversion was used and the values were identified accordingly. In the comparisons of two groups with normal distributions, Student-*t* test was used. In the evaluation of the parameters that do not have a normal distribution, the Kruskal Wallis test was used. Chi-square test, McNemar test, Kappa analysis and diagnostic screening tests (sensitivity, specificity) were used to compare qualitative data. *P* level of < 0.05 was evaluated as being statistically significant.

RESULTS

A total of 152 children underwent endoscopy during the study period and 59 out of 152 subjects who had complaints suggesting GERD included in the study. ROS levels were measured in 54 and cytokine levels in 55 out of 59 patients. Mean age of the patients in the study was 8.9 ± 4.4 years (age range 1.5-17 years). Of 59 patients 28 (47.5%) had normal esophagus whereas 31 (52.5%) had endoscopic esophagitis according to Savary Miller classification. In histopathological evaluation, 80% of the cases had mild or moderate degree of esophagitis. Of 31 patients having endoscopic esophagitis, 29 (93.5%) also had histopathological esophagitis whereas of 28 patients not having endoscopic esophagitis 18 (64.3%) had histopathological esophagitis. The sensitivity of endoscopic esophagitis for prediction of histopathological esophagitis was significantly high (93.6%, *P* = 0.0083). However, endoscopy had a low specificity in the diagnosis of histopathological esophagitis in children. Kappa correlation rate between the 2 methods was 30.1%: PPV, 61.7% and NPV, 83.3%.

When esophageal tissue ROS and chemokine levels were compared among groups with and without endoscopic esophagitis, statistical difference could neither be found between the stages of endoscopic esophagitis nor between the patients with and without esophagitis (Table 1).

When a comparison was made in terms of luminol and lucigenin levels of cases with and without histopathological esophagitis, there was a difference of statistical significance between the two groups (*P* = 0.049 and *P* = 0.044, respectively). While luminol levels did not differ among normal and patients with mild esophagitis, luminol levels of patients with moderate esophagitis were significantly higher than the normal patients (Table 2). Since there was not a statistically significant difference in luminol levels between children with mild and moderate esophagitis, two groups were combined, however, groups with and without histopathological esophagitis did not differ for either luminol or lucigenin measurements (*P* > 0.05).

Patients with histopathological esophagitis and normal were compared for MCP-1 and IL-8 levels. The highest cytokine levels were identified in patients with moderate esophagitis while lowest levels were found in normal children. However, this difference did not reach a statistical evaluations (*P* > 0.05, Table 3).

In order to investigate the effect of presence of *H pylori* gastritis, the patients having esophagitis were classified and patients with and without *H pylori* gastritis were compared. Patients with *H pylori* gastritis and those not having gastritis did not show any statistically significant difference between their levels of ROS and chemokines.

DISCUSSION

Gastroesophageal reflux when untreated in children may be related to some serious complications such as esophagitis, failure to thrive, esophageal strictures, Barrett esophagus and adenocarcinoma[34]. Delineation of the pathogenesis of GERD will allow for the development of more effective treatment strategies while making it possible to prevent complications. In

Table 1 The comparison of luminol, lucigenin, MCP-1 and IL-8 levels between endoscopic esophagitis and normal groups (mean ± SD)

	Esophagitis	Normal	*P* value[1]
Luminol (rlu/mg)	175.2 ± 98.5	152.7 ± 71.3	0.349
Lucigenin (rlu/mg)	154.1 ± 74.9	155.7 ± 79.9	0.939
MCP-1 (pg/mg)	39.8 ± 1.7	47.8 ± 1.7	0.248
IL-8 (pg/mg)	331.1 ± 2.9	323.6 ± 2.3	0.898

[1]Student-*t* test.

Table 2 The comparison of luminol and lucigenin levels according to the severity of histopathological esophagitis (mean ± SD)

Vandenplas classification	Normal (*n* = 12)	Mild esophagitis (*n* = 43)	Moderate esophagitis (*n* = 4)	*P* value[1]
Luminol (rlu/mg)	129.9 ± 80.9	164.4 ± 76.3	236.8 ± 142.8	0.049
Lucigenin (rlu/mg)	156.8 ± 91.7	145.4 ± 65.4	244.8 ± 94.6	0.044

[1]Kruskal Wallis test (normal *vs* moderate esophagitis).

Table 3 The comparison of MCP-1 and IL-8 levels according to the severity of histopathological esophagitis (mean ± SD)

Vandenplas classification	Normal (*n* = 12)	Mild esophagitis (*n* = 43)	Moderate esophagitis (*n* = 4)	*P* value[1]
MCP-1 (pg/mg)	38.9 ± 1.7	44.5 ± 1.7	53.7 ± 2.7	0.804
IL-8 (pg/mg)	262.4 ± 2.3	337.3 ± 2.5	630.9 ± 5.6	0.614

[1]Kruskal Wallis test.

recent years, the role of ROS and chemokines in the pathogenesis of GERD and reflux esophagitis are being investigated both in experimental esophagitis models and in humans[12,17,19,27,29,35].

The relationship between reflux esophagitis and free oxygen radicals was elaborated in this study, no statistical difference could be identified between normal cases and those having different degrees of endoscopic and histopathologic esophagitis. However, although it was statistically not significant, ROS levels were found to be increased in patient groups compared to the normal group.

Free oxygen radicals in general and superoxide radical in particular were shown to increase in animals with esophagitis and it was claimed that free radical scavengers like SOD and DA-9601 as well as anti-inflammatory agents like ketotifen could prevent the tissue damage[12,13,15,17,35]. However, in the study by Soteras *et al*[36] free oxygen radicals were found to play a role mostly in severe esophagitis and it was concluded that free oxygen radicals did not increase in the mild histopathological esophagitis model and thus ROS might not be influential on the pathogenesis of mild esophagitis. Studies performed in adults with reflux esophagitis are in support of the experimental esophagitis models showing that free oxygen radicals do play a role in the pathogenesis of reflux esophagitis[18,19]. Olyaee *et al*[37] have identified a significant correlation between the degree of esophagitis and the levels of free oxygen radicals in the tissue; under the light of this finding they stipulated that in mucosal epithelial cells affected by the injury, the production of ROS was increased. After examining mucosal biopsies of cases with erosive gastritis and Barrett esophagus; another group of researcher reported that the main oxidant product responsible for the development of reflux esophagitis was superoxide anion[38].

Although there are no studies investigating the role of free oxygen radicals in children with reflux esophagitis, our findings are different than the results obtained from adult patients with reflux esophagitis. In our study, neither lucigenin which showed superoxide radical production nor luminol reflecting the productions of other free radicals were found to be increased in children with esophagitis compared to controls. Despite not reaching the level of statistical significance, free oxygen radical levels were found to be higher in children with esophagitis when compared to the controls. The reason behind not identifying a statistically significant difference between the cases of esophagitis and normal may be attributed to the fact that in most of the cases with histopathological reflux esophagitis was of mild degree.

Likewise, the levels of IL-8 and MCP-1 that are chemokines in relation with neutrophil and mononuclear cell migration were found to be higher in the group with histopathological reflux esophagitis compared to normal, however this difference did not reach a level of statistical significance either. In studies performed in adults, tissue chemokine levels are reported to be higher in cases with esophagitis than normal and the increase is reported to be in parallel with the severity of the histopathological esophagitis concerned. Fitzgerald *et al*[26] have identified 3-10 fold higher levels of tissue cytokines in the esophageal mucosa of adults with esophagitis when compared to patients with Barrett esophagus and normal controls. Furthermore, adult patients with nonerosive esophagitis were reported to have higher levels of tissue cytokines when compared to normal[27-30]. Similarly, in a study comparing 10 children with reflux esophagitis to normal patients, esophageal tissue IL-6 levels were reported to be significantly higher in patients with esophagitis[31]. In our study, the lowest levels were measured in normal cases whereas the highest levels were identified in patients with a moderate degree of esophagitis. The difference between these two groups was not statistically significant, this can be explained by the fact that the number of individuals who were normal and who had mild and moderate esophagitis were quite different from each other.

In *H pylori* gastritis, it has been shown that levels of IL-8, MCP-1 and other cytokines increased in gastric mucosa but we do not know the effect of this infection on esophageal tissue which is not normally colonized by the bacteria[39-41]. In our study, we could not identify any

effects of *H pylori* gastritis on esophageal tissue ROS and chemokine levels.

One drawback of this study is the lack of a true control group. All the study group was selected from the patients who had GER symptoms. The subjects who had either endoscopic or histopathological reflux esophagitis were compared to their nonreflux counterparts. The subjects who were included in the normal group according to the endoscopic findings might have a nonerosive reflux disease. Similarly, the subjects included in the normal group according to the histopathological examination might have an increased tissue level of proinflammatory substances before the establishment of the well-known histopathological changes. This might be another explanation for the lack of a clear cut statistical significance between the groups. However it is not possible to find a true control group for these kind of studies because it is not ethical and possible to perform endoscopy in totally normal children.

In parallel with the results of the studies on adult GERD or esophagitis, the patients in our study had higher levels of tissue ROS and chemokines; however, this increase did not reach a level of statistical significance. The number of studies aiming at identifying the pathogenesis of reflux esophagitis in children is very limited. Studies to be performed with higher numbers of patients with the aim of identifying the pathogenesis will allow for the development of further diagnostic and therapeutic opportunities for GERD which is a significant cause of morbidity.

COMMENTS

Background

Gastroesophageal reflux disease (GERD) is one of the most frequently encountered gastrointestinal pathology not only in adults but also in children. Since it may be related to some serious complications such as esophagitis, failure to thrive, esophageal strictures, Barrett esophagus and adenocarcinoma, early diagnosis and treatment of reflux disease is crucial. In recent years, it has been reported that reactive oxygen species (ROS) and inflammatory chemokines play an important role in the pathogenesis of GERD in adults but there is scarce data regarding children with reflux esophagitis.

Research frontiers

Inflammatory chemokine levels were found to be significantly high in the esophageal tissues of the patients with esophagitis and a significant correlation was found between the severity of the reflux esophagitis and chemokines levels in adults. The aim was to investigate the role of chemokines and ROS in children with reflux esophagitis. Since the duration of reflux disease might be shorter in children compared to adults, the potential role of chemokines and ROS was interrogated in early or mild cases of reflux esophagitis.

Innovations and breakthroughs

In this study we showed that the level of ROS and chemokines in esophageal tissue were higher in children with esophagitis compared to the subjects without esophagitis. However the difference was not statistically significant. Hence, factors apart from ROS, IL-8 and MCP-1 might be important in the pathogenesis of reflux esophagitis in children.

Applications

It was found that ROS and chemokines increased in children with reflux esophagitis. Opposite to the adults, the exposure of the noxious agents to the esophagus is not long enough in children, endoscopic or histopathological esophagitis might be obscure. Hence tissue level of ROS or/and chemokines might be an important finding in early diagnosis of childhood esophagitis. Furthermore, the use of antioxidants or antiinflammatory agents might be alternative treatment modalities to the established treatment regimens.

Peer review

This manuscript showed that ROS and chemokines are increased in esophageal tissue in children with reflux esophagitis, though not statistically significant. This finding might be important for the delineation of the pathogenesis of reflux esophagitis in children, and therapeutic alternatives targeting these chemokines or ROS could be an option in the future.

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S- Editor Zhong XY **L- Editor** Alpini GD **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3224

World J Gastroenterol 2008 May 28; 14(20): 3224-3230
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Prognostic factors in patients with advanced cholangiocarcinoma: Role of surgery, chemotherapy and body mass index

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Received: January 22, 2008 Revised: March 11, 2008
Accepted: March 18, 2008
Published online: May 28, 2008

Abstract

AIM: To study the factors that may affect survival of cholangiocarcinoma in Lebanon.
METHODS: A retrospective review of the medical records of 55 patients diagnosed with cholangiocarcinoma at the American University of Beirut between 1990 and 2005 was conducted. Univariate and multivariate analyses were performed to determine the impact of surgery, chemotherapy, body mass index, bilirubin level and other factors on survival.
RESULTS: The median survival of all patients was 8.57 mo (0.03-105.2). Univariate analysis showed that low bilirubin level (< 10 mg/dL), radical surgery and chemotherapy administration were significantly associated with better survival (P = 0.012, 0.038 and 0.038, respectively). In subgroup analysis on patients who had no surgery, chemotherapy administration prolonged median survival significantly (17.0 mo *vs* 3.5 mo, P = 0.001). Multivariate analysis identified only low bilirubin level < 10 mg/dL and chemotherapy administration as independent predictors associated with better survival (P < 0.05).
CONCLUSION: Our data show that palliative and postoperative chemotherapy as well as a bilirubin level < 10 mg/dL are independent predictors of a significant increase in survival in patients with cholangiocarcinoma.

Key words: Cholangiocarcinoma; Biliary tract cancer; Chemotherapy; Bilirubin; Prognosis

Peer reviewer: Gustav Paumgartner, Professor, University of Munich, Klinikum Grosshadern, Marchionini str. 15, Munich D-81377, Germany

Farhat MH, Shamseddine AI, Tawil AN, Berjawi G, Sidani C, Shamseddeen W, Barada KA. Prognostic factors in patients with advanced cholangiocarcinoma: Role of surgery, chemotherapy and body mass index. *World J Gastroenterol* 2008; 14(20): 3224-3230 Available from: URL: http://www.wjgnet.com/1007-9327/14/3224.asp DOI: http://dx.doi.org/10.3748/wjg.14.3224

INTRODUCTION

Cholangiocarcinoma is a rare and highly fatal neoplasm that arises from biliary epithelium. It constitutes approximately 2% of all reported cancer[1], and accounts for about 3 percent of all gastrointestinal malignancies[2]. Up to date, radical surgery remains the optimal therapy for cholangiocarcinoma offering a potential for cure[1,3,4]. In surgical patients with negative margins, five-year survival rates approach 20%-35% as compared to zero in those with positive margins[5]. However, most patients present with advanced disease precluding surgery[6-8]. Overall prognosis in these patients is poor and survival is limited to a few months[9]. Thus, it is crucial to identify factors that would improve survival in such patients.

The role of chemotherapy in cholangiocarcinoma is yet to be determined, with conflicting data regarding its effect on survival. This is due to lack of randomized clinical trials, and absence of a standard chemotherapeutic

regimen[10]. While some authors believe that chemotherapy prolongs survival in cholangiocarcinoma[11,12], others deny this survival benefit[13-15].

The impact of excess body weight on survival in patients with different cancers is variable. While it is associated with improved survival in patients with cancers of the gastric cardia[16], less aggressive disease in renal cell cancers[17], and lower malignant potential in ovarian tumors[18], it was found to increase mortality in early stage breast cancers[19], cancer of the esophagus, colon and rectum, liver, gallbladder, pancreas, and kidney[20]. However, the relationship of increased body mass index (BMI) and survival in cholangiocarcinoma has not been thoroughly investigated.

Many factors are well known to increase the risk of cholangiocarcinoma; these include age, primary sclerosing cholangitis (PSC), hepatolithiasis, and liver flukes[6].

Several studies have provided evidence that excess body weight and obesity increase the risk of overall cancers. In a study by Lee *et al*, overweight people had a one and a half times increased risk of cancer compared to those with normal weight in both sexes[21]. Similarly, obese people had a 33% increase in overall cancer incidence[22]. In cancers of the biliary tract, cancer of the gall bladder was linked previously with increased BMI in women[23,24]; whether increased BMI is a risk factor for cholangiocarcinoma is not known yet. In our study, the effect of increased BMI was given special emphasis to investigate its role as a risk factor and as a prognostic indicator.

In this study, we sought to determine the clinicopathologic characteristics of patients with cholangiocarcinoma. We also tried to identify the determinants of prognosis and survival in those patients with special emphasis on the role of surgery, chemotherapy and BMI.

MATERIALS AND METHODS

Patients diagnosed with cholangiocarcinoma at the American University of Beirut-Medical Center during the 15 year period between 1990 and 2005 were identified. Patients' demographics, clinical data, radiological and histopathologic findings, surgical intervention, chemotherapy administration, and survival data were obtained retrospectively from hospital medical charts and by contacting patients or their family members. All histopathology slides and radiographic studies were reevaluated by a pathologist and a radiologist to obtain data about tumor location, grade, stage, lymphatic spread, vascular invasion and metastasis.

Tumors were classified as intrahepatic if originating from intrahepatic ductules (proximal to the bifurcation of the right and left hepatic ducts), and extrahepatic if perihilar (involving the confluence of the right and left bile ducts) or distal (if originating distal to the confluence of hepatic ducts).

AJCC 2003 criteria were used for TNM staging of the tumor[25].

The impact of high bilirubin level at presentation, tumor location, size, grade, metastasis, presence of vascular or perineural invasion, positive surgical margins, type of treatment including palliative stenting, surgery and chemotherapy on survival was examined.

Parameters examined as possible risk factors for cholangiocarcinoma were age, gender, diabetes, BMI, history of cholelithaisis, Hepatitis B and C infection, smoking, alcohol consumption, presence of cirrhosis, inflammatory bowel disease (IBD), PSC, and parasitic infestations.

To determine if increasing BMI is a risk factor for cholangiocarcinoma, patients were compared to controls of the same age groups that were selected from a large study about obesity in the Lebanese population[26]. According to WHO standards[27], subjects were categorized according to their body mass index (normal: < 25, overweight: 25-30, and obese: $\geqslant 30$ kg/m^2).

All data was coded and entered using SPSS 14.0 computer program. The Kaplan-Meier method was used to estimate survival which was measured from time of presentation to AUB-MC to the date of death or date of last follow up. Differences in survival between subgroups were compared using the log-rank test. Univariate analysis was performed using the chi-squared testing. Multivariate analysis was performed with the Cox proportional hazards model. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Demographics and clinical data

During the 15-year period, a total of 55 patients diagnosed with cholangiocarcinoma were studied. The demographic and clinical data of all patients are listed in Table 1. There were 34 males (60.7%) and 22 (39.3%) females. The mean age for all patients was 62.6 ± 13.0 years and ranged from 28 years to 91 years. Seventeen patients were older than 70 years (11 females and 8 males). In males, the incidence of the tumor was highest in the age group of 50-59 years, while in females it was in the older than 70 years age group.

The most common presenting symptoms were jaundice (72.7%), dark urine (61.8%), weight loss (43.6%), abdominal pain (43.6%), pruritus (36.4%), and fever (10.9 %).

Risk factors

None of the patients had primary sclerosing cholangitis or any evidence of parasitic infestation on histological examination. One patient had inflammatory bowel disease (1.8%). One patient had a history of hepatitis B infection and two had liver cirrhosis of unknown etiology (3.6%). Ten patients had a history of cholecystectomy (17.8%) and 17 had a history of cholelithiasis (30.9%). Fifteen patients had a history of diabetes mellitus (25.5%) and one third (33%) were obese (BMI $\geqslant 30$ kg/m^2). One patient had a family history of cholangiocarcinoma (1.8%).

Microscopic and macroscopic appearance

Tissue diagnosis was obtained on 30 patients (54.5%). Histopathologic findings are listed in Table 2. Tumors

Table 1 Demographic and clinical data of 55 patients with cholangiocarcinoma

	Number of patients (%)	
Age (mean ± SD)	62.6 ± 13	
Range (yr)	28-91	61/39
Male/Female	34/22	
Diabetes (Yes/No)	14/41	25/75
BMI (kg/m^2)		
< 25	18	35
25-30	16	31
⩾ 30	18	35
Not available	3	
History of cholelithiasis (Yes /No)	17/38	31/69
Clinical manifestations		
Jaundice	40	72.7
Dark urine	34	61.8
Weight loss	24	43.6
Abdominal pain	24	43.6
Total bilirubin		
< 10 mg/dL	27	49
⩾ 10 mg/dL	17	31
Missing	11	20
AJCC staging		
Ⅰ	1	
Ⅱ	1	
Ⅲ	0	
Ⅳ	49	89
Missing	4	
Surgery		
Yes	21	38
No	34	62
Adjuvant chemotherapy		
Yes	14	25
No	41	75

larger than 3 cm, as measured in resected specimens and by radiology, comprised 34.5%, compared to 23.6% for those less than 3 cm. The most common morphological type was intraductal growing (n = 34, 61.8%) followed by mass forming (n = 21, 38%). Mass forming morphology was present in 63% and 35% of intrahepatic and extrahepatic tumors, respectively, with periductal infiltrating morphology comprising the rest of the patients.

Tumor grade was available on 27 patients. They were mostly moderately (55.6%) and poorly differentiated (29.6%). Vascular involvement on histology was evident in 12 patients (21.8%), while perineural invasion was found in 10 patients (18.2%).

Tumors presented at stage Ⅳ in 49 out of 55 patients (89%).

Distribution of tumor could be obtained on 48 patients (Table 2). Thirty-seven tumors (67.2%) were extrahepatic *versus* 11 intrahepatic (20%).

Of the extrahepatic tumors, 19 were distal (34.5%) and 18 were perihilar (32.7%). Nineteen patients had metastatic disease. The most common sites of metastases were the liver (25.4%, n = 14/55), followed by the peritoneum (10.9%, n = 6/55). Two patients had lymph node metastasis. One patient had brain metastasis and another had bone metastasis.

Treatment

The resection rate of the tumor was low (21/55, 31.8%).

Table 2 Tumor location, size, grade, nodal and margin status and relation to survival in patients with cholangiocarcinoma

	n	Median survival (95% CI)	P
Location			
Intrahepatic	10	6.23 (0.76-11.7)	0.68
Perihilar	18	11.47 (6.73-16.2)	
Distal	21	9.17 (0.52-17.8)	
Not available	6		
Size			
< 3 cm	19	3.47 (1.9-5.0)	0.31
⩾ 3 cm	13	11 (4.13-17.87)	
Not available	23	13	
Grade			
Poor	4	16.96 (8.9-25.0)	0.31
Moderate	15	11 (2.77-19.2)	
Well	8	13 (0-31)	
Missing	28		
Margin status			
Positive	7	9.9 (4.6-15.19)	0.90
Negative	14	14.3 (5.5-23.1)	
Vascular invasion			
Yes	7	22 (4.9-39.2)	0.78
No	14	10.2 (4.04-16.42)	
Perineural involvement			
Yes	10	6.2 (4.9-7.5)	0.66
No	13	11 (5.8-16.2)	

Rate of radical operation was only 11% (6/55). Extrahepatic tumors were more resectable (n = 13, 23.6%) as compared to intrahepatic tumors (n = 6, 10.9%). In 2 surgical patients, location of tumor could not be ascertained.

Of the twenty one patients who underwent surgery: 6 had Whipple procedure, 6 hepatic resection, and 9 enbloc resection of bile ducts and gall bladder. Two patients had positive lymph nodes. Surgical margins were positive in 7 patients (n = 7/21, 33%). Thirty-four patients had unresectable disease because of gross vascular involvement, locally advanced disease, or peritoneal metastasis discovered by imaging or during surgical exploration.

Twenty-four patients underwent palliative stenting, 9 had endoscopic stenting (37.5%), 14 had percutaneous radiological stenting (58.3%), and only one patient underwent surgical stenting (4.2%).

Fourteen patients received chemotherapy in the form of postoperative chemotherapy (n = 6) or as palliative in the setting of non-resectable disease (n = 8). Chemotherapy regimens consisted of gemcitabine or 5-Fu. Gemcitabine was given mainly as a single agent. As part of combination therapy, it was co-administered with other drugs such as oxaliplatin, 5-Fu, or CPT-11. 5-Fu was given as part of combination therapy at all times.

Factors influencing survival in cholangiocarcinoma

The median survival for all patients was 8.57 mo (0.03-105.2), with 1-year, 3-year and 5-year survival rates of 10.8%, 5.4%, and 5.4%, respectively.

The longest survival time among all patients was 103 mo.

Multiple clinical, tumor-related and treatment parameters were evaluated by univariate analysis to determine their impact on survival in cholangiocarcinoma (Table 3).

Table 3 Association between clinical variables and survival in patients with cholangiocarcinoma

Variable (*n*)	Median survival (mo)	*P* (Univariate)
Age		
< 50 (11)	10.23 (1.87-18.6)	0.410
⩾ 50 (44)	9.17 (3.9-14.4)	
Gender		
Male (33)	9.17 (3.8-14.5)	0.386
Female (22)	9.9 (0.4-19.4)	
Bilirubin		
< 10 (27)	9.9 (3.1-16.7)	0.012
⩾ 10 (17)	2.87 (1.2-4.5)	
BMI		
< 25 (18)	13.0 (8.5-17.6)	0.412
25-30 (16)	7.0 (3.2-10.8)	
⩾ 30 (18)	4.0 (0.5-7.6)	
Surgery		
Yes (21)	10.23 (4.82-15.64)	0.038
No (34)	8.7 (1.8-15.6)	
Type of surgery		
Whipple (6)	16.6 (0.0-39.4)	0.988
Hepatic lobectomy (6)	10.2 (3.9-16.6)	
Bile duct excision (9)	14.3 (8.5-20.1)	
Metastasis		
Yes (19)	7.07 (0.0-15.83)	0.256
No (36)	9.09 (6.05-13.75)	
Stenting		
Yes (24)	9.9 (0.45-19.35)	0.930
No (32)	9.16 (4.6-13.7)	
Chemotherapy		
Yes (14)	16.96 (11.5-22.4)	0.038
No (41)	6.2 (0-12.9)	
Chemotherapy in unresected patients		
Yes (8)	17 (12.76-21.18)	0.001
No (25)	3.5 (1.12-5.8)	

Parameters that did not influence survival were age, gender, diabetes, history of cholelithiasis, type of operation, resection margin status, presence of metastasis, and stenting. Tumor size, grade, location, vascular and perineural invasion also did not impact survival (Table 2). Increasing BMI was associated with a non-significant decrease in survival.

On univariate analysis, parameters that did influence survival included bilirubin level less than 10 mg/dL at presentation, surgical resection, and chemotherapy administration (Table 3). Since 49 out of 55 patients were stage Ⅳ, only these patients were included in Kaplan Meier survival (Figure 1) and multivariate analysis. Using Cox regression, a multivariate analysis was performed and only two were identified as independent predictors of increased survival: bilirubin level less than 10 mg/dL (Figure 1A) and chemotherapy administration (Figure 1B). Compared to patients with bilirubin levels less than 10 mg/dL, patients with higher bilirubin levels had a more than 2-fold increase in death risk from cholangiocarcinoma ($P < 0.05$). Although the risk of dying was less in patients who underwent surgery, results did not attain statistical significance. On the contrary, patients who received chemotherapy had better survival ($P < 0.05$, Table 4).

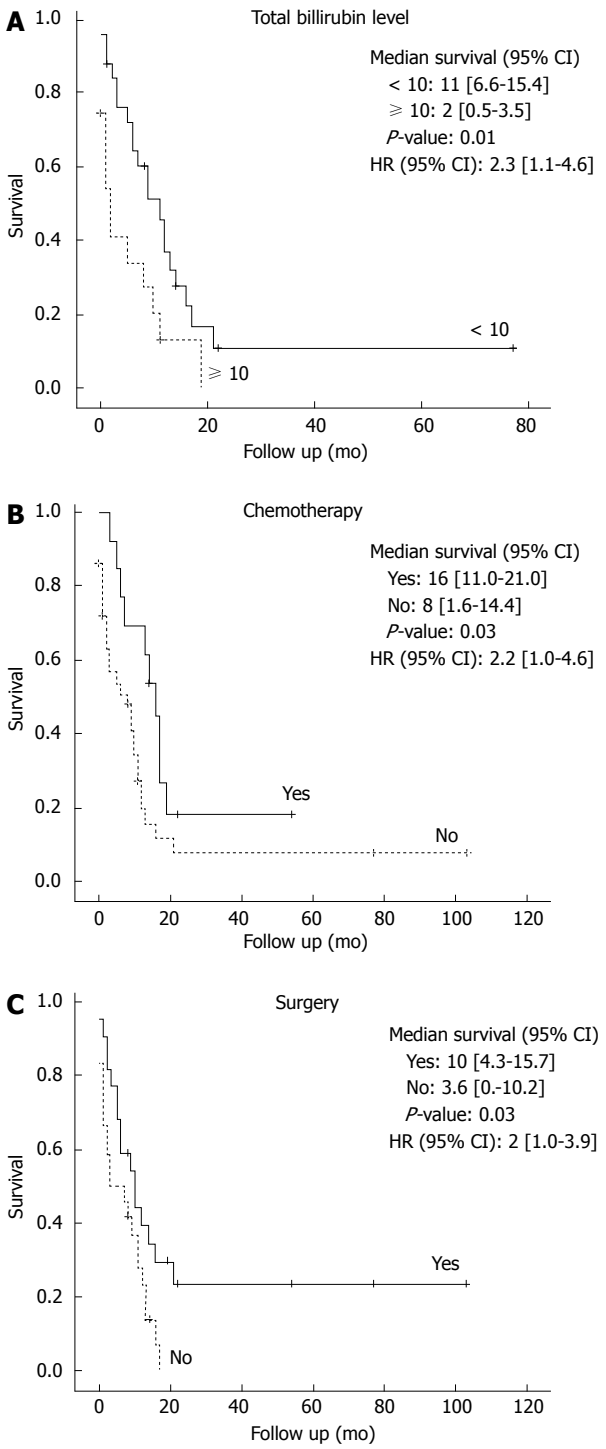

Figure 1 **A:** Bilirubin level ⩾ 10 mg/dL at presentation was associated with decreased survival as compared to lower levels; **B**: Chemotherapy administration prolonged survival in cholangiocarcinoma patients; **C**: Surgery added survival benefit in patients with cholangiocarcinoma.

DISCUSSION

This is the first report of cholangiocarcinoma from Lebanon, a small country in the Middle East, with a population of 3.4 million people. Our study shows the positive impact of surgery, chemotherapy, and low bilirubin level on survival in patients with advanced cholangiocarcinoma.

In previous studies, variables such as low preoperative bilirubin[4,14,28,29], radical resection[28,29], negative resection margin[11,30] and well-differentiated tumor histology[1,31]

Table 4 Multivariate analysis of prognostic factors in patients with advanced cholangiocarcinoma

	Hazard's ratio	95% CI	*P*
Bilirubin			
< 10	1	1.13-0.52	0.023
⩾ 10	2.421		
Surgery			
No	1	0.27-1.38	0.238
Yes	0.611		
Chemotherapy			
No	1	0.16-0.92	0.038
Yes	0.383		

were found to be predictors of improved outcome. On the other hand, less-differentiated histology[32], perineural involvement, positive surgical margins, vascular or lymphatic invasion were associated with worse prognosis[30,32-37].

The findings of our study emphasize the importance of increased bilirubin level upon presentation as an independent predictor of decreased survival in cholangiocarcinoma. Due to the small number of patients, we could not confirm the impact of the previously proposed variables on survival.

Radical surgery is considered as the most effective therapy for cholangiocarcinoma[5]. Only 20% of patients present with resectable disease[1]; yet surgery remains the only potential chance of cure. Without surgery, cholangiocarcinoma is a rapidly fatal disease with 5-year survival rates of less than 5%[9], while in curative resections 5-year survival approaches 20%-35% with negative surgical margins[5]. In our findings, we could not document an improved survival after surgical resection which can be explained by the advanced stage at which all patients presented and the small number of the study group.

The significance of chemotherapy in cholangiocarcinoma is still not clear especially with the low response rates[5], disappointing efficacy results and lack of a superior standard chemotherapeutic regimen[7,38]. Conflicting data exist regarding the role of chemotherapy in cholangiocarcinoma. Some studies suggest that chemotherapy, whether given in a setting of non-resectable disease or postoperatively, has little or no impact on the course of the disease or on survival outcome[13-15,29,39-41] and is therefore considered palliative more than curative[42]. Other studies report survival benefit from chemotherapy[11,12,43]. Recently, a pooled analysis of all clinical trials from 1985 to 2007 concluded that the combination of gemcitabine with oxaliplatin or cisplatin may improve survival in cholangiocarcinoma[10].

Our study adds further evidence to the previously published reports showing that chemotherapy improves survival in cholangiocarcinoma. We found that chemotherapy markedly improves survival in patients with either resected or unresected cholangiocarcinoma (17.0 mo *vs* 6.0 mo; $P < 0.01$). Additionally, chemotherapy prolonged survival significantly in patients with unresectable tumors (17.0 mo *vs* 3.5 mo; $P = 0.001$). Thus, in patients with advanced cholangiocarcinoma who are not surgical candidates, Gemcitabine and/or 5-Fu based chemotherapy might offer a survival advantage.

Our results also show a non-significant decrease in survival with increasing BMI. The median survival for patients with BMI < 25 was 17.0 mo (8.5-17.6), 7.0 mo (3.2-10.8) for patients with BMI 25-29.99, and 4 mo (0.5-7.6) for patients with BMI ⩾ 30 ($P = 0.412$). The fact that these results did not attain statistical significance may be attributed to the small sample size. Further prospective studies are needed to determine the effect of increased body mass index on prognosis in cholangiocarcinoma.

In line with other reports, cholangiocarcinoma in Lebanon affected older patients[44,45] and more males than females[44-46], except in the above 70-year-old group where females were more commonly affected. However, the mean age of our patients was higher than that of patients in the USA[47]. The clinical symptoms and signs observed in Lebanese patients with cholangiocarcinoma were mostly of biliary obstruction and abdominal pain, as was previously reported[28].

Most of the tumors in our study were distal extrahepatic lesions, whereas perihilar lesions are the most common type usually reported[5,6,48]. A moderate degree of differentiation was noted in the majority of tumors in our patients, while well-differentiated histology is more commonly reported[5,6].

Similar to other reports[4], this series did not identify any risk factors associated with cholangiocarcinoma, which is different from reports of biliary tract cancers elsewhere. In Asian countries, well-established risk factors are hepatolithiasis and liver fluke infestations[49], while in western countries hepatitis B and C infection, HIV, cirrhosis, diabetes, alcohol consumption, and IBD were recently implicated as potential risk factors for cholangiocarcinoma[44,47,50].

Our patients might have had some of the known risk factors for cholangiocarcinoma that might have been missed at the time of patient presentation. Therefore, absence of those risk factors can not be ascertained due to the retrospective design of our study.

The prevalence of diabetes in our patients was 33% with the highest incidence being observed in patients over the age of 65, which was very close to the 29% prevalence rate of diabetes in the general Lebanese population older than 65 years[51]. Therefore, diabetes can not be considered a risk factor for cholangiocarcinoma in our population, unlike other populations where diabetes increased the risk 2-3 folds[44,50,52].

Cholelithiasis was present in 30% of our patients. Prevalence of cholelithaisis in cholangiocarcinoma patients was previously reported to fall in the 30% to 48% range[53]. It was described as a risk factor for cholangiocarcinoma in a number of studies[50,53,54]. However, a definitive cause-effect relationship has not been established yet.

Few reports addressed the association between BMI and bile duct cancer; Samonic *et al* showed that obese black men are at a significant risk of extrahepatic bile duct cancers[55]. On the other hand, Welzel *et al* showed

that obesity was not a risk factor for intrahepatic cholangiocarcinoma[50]. Others suggested that increased body mass index was associated only with cancer of the extrahepatic duct[54]. Furthermore, in a large Korean cohort, a significant positive linear relationship was found between increasing BMI and risk of cholangiocarcinoma[56]. The risk of cholangiocarcinoma increased approximately 1.6 folds in patients with BMI > 30 kg/m^2[56]. In our study, 68% of all patients with cholangiocarcinoma had excess BMI (median BMI was 26.9 kg/m^2). In the Lebanese adult population, 53% are overweight (BMI $\geqslant$ 25), 17% are obese (BMI $\geqslant$ 30) and the mean BMI is estimated to be 25.9 kg/m^2[26], which is comparable to the mean BMI of our study group.

There are several limitations in our study. The first is the small sample size, which is due to the rarity of the disease under investigation. Second, the study represents cases seen at a single tertiary care center, which may limit its utility in patients with cholangiocarcinoma in general. Third, our study is limited by its retrospective design; a key limitation resulting from such a design is the missing data, which may result in fewer patients included in multivariable models, generally increasing the risk for both type one and type two errors. Fourth, our study is non-randomized and lacks a control group. Despite the limitations of retrospective studies, absence of prospective and controlled data in the current literature makes the results of our study of more interest.

In conclusion, bilirubin levels less than 10 mg/dL at presentation and chemotherapy administration both in advanced disease and in postoperative adjuvant settings are associated with better prognosis and prolonged survival in patients with cholangiocarcinoma. None of the well-established or the potential risk factors for cholangiocarcinoma could be identified in the Lebanese population due to the above mentioned limitations. High body mass index was not found to be a risk factor for cholangiocarcinoma; however, increments were associated with a trend towards a decrease in median survival.

COMMENTS

Background

Cholangiocarcinoma is an infrequent malignancy that involves the biliary epithelium. It has a poor prognosis with a survival less than 5% at five years. Radical surgery is the only potentially curative treatment modality, while the impact of chemotherapy on survival remains controversial. Due to small number of patients, determinants of prognosis in cholangiocarcinoma are not well characterized.

Research frontiers

A retrospective review of the medical records of 55 patients diagnosed with cholangiocarcinoma at the American University of Beirut between 1990 and 2005 was conducted. Univariate and multivariate analysis were performed to determine the impact of surgery, chemotherapy, body mass index, bilirubin level and other factors on survival.

Innovations and breakthroughs

Bilirubin levels less than 10 mg/dL at presentation and chemotherapy administration both in advanced disease and in postoperative adjuvant settings are associated with better prognosis and prolonged survival in patients with cholangiocarcinoma. High body mass index was not found to be a risk factor for cholangiocarcinoma; however, increments were associated with a trend towards a decrease in median survival.

Applications

Palliative and postoperative chemotherapy as well as a bilirubin level < 10 mg/dL are independent predictors of a significant increase in survival in patients with cholangiocarcinoma. Large prospective controlled studies are needed to verify these results.

Peer review

This article reports interesting epidemiological data on cholangiocarcinoma in Lebanon and the effects of surgical resection and chemotherapy on survival. Multivariate analysis identified only a bilirubin level < 10 mg/dL and chemotherapy as independent predictors of better survival.

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Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3231

World J Gastroenterol 2008 May 28; 14(20): 3231-3235
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Prevention and treatment of gastrointestinal dysfunction following severe burns: A summary of recent 30-year clinical experience

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Received: February 25, 2008 Revised: April 9, 2008
Accepted: April 16, 2008
Published online: May 28, 2008

Abstract

AIM: To sum up the recent 30-year experience in the prevention and treatment of gastrointestinal dysfunction in severe burn patients, and propose practicable guidelines for the prevention and treatment of gastrointestinal (GI) dysfunction.

METHODS: From 1980 to 2007, a total of 219 patients with large area and extraordinarily large area burns (LAB) were admitted, who were classified into three stages according the therapeutic protocols used at the time: Stage 1 from 1980 to 1989, stage 2 from 1990 to 1995, and stage 3 from 1996 to 2007. The occurrence and mortality of GI dysfunction in patients of the three stages were calculated and the main causes were analyzed.

RESULTS: The occurrence of stress ulcer in patients with LAB was 8.6% in stage 1, which was significantly lower than that in stage 1 ($P < 0.05$). No massive hemorrhage from severe stress ulcer and enterogenic infections occurred in stages 2 and 3. The occurrence of abdominal distension and stress ulcer and the mortality in stage 3 patients with extraordinarily LAB was 7.1%, 21.4% and 28.5%, respectively, which were significantly lower than those in stage 1 patients ($P < 0.05$ or $P < 0.01$), and the occurrence of stress ulcer was also significantly lower than that in stage 2 patients ($P < 0.05$).

CONCLUSION: Comprehensive fluid resuscitation, early excision of necrotic tissue, staged food ingestion, and administration of specific nutrients are essential strategies for preventing gastrointestinal complications and lowering mortality in severely burned patients.

Key words: Severe burn; Gastrointestinal function; Fluid resuscitation; Staged food ingestion

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Xiao SC, Zhu SH, Xia ZF, Lu W, Wang GQ, Ben DF, Wang GY, Cheng DS. Prevention and treatment of gastrointestinal dysfunction following severe burns: A summary of recent 30-year clinical experience. *World J Gastroenterol* 2008; 14(20): 3231-3235 Available from: URL: http://www.wjgnet.com/1007-9327/14/3231.asp DOI: http://dx.doi.org/10.3748/wjg.14.3231

INTRODUCTION

Gastrointestinal dysfunction is a common complication of severe burns. Injury to GI function, especially to GI barrier function, is an important initiator as well as a stimulator for occurrence of systemic inflammatory response syndrome (SIRS), sepsis and multiple organ dysfunction syndrome (MODS) following severe burns[1]. With the deeper understanding of GI function and changes in the stereotype of clinical treatment in recent 30 years, a series of new therapies including fluid resuscitation, early escharectomy, continuous renal replacement therapy, and use of glutamine and growth factor has been adopted in the treatment of severe burns[2,3]. Although animal experiments have shown that these new therapies do play a positive role in the prevention and treatment of GI dysfunction following severe burns, there has been a lack of convincing clinical

data to confirm the outcome[4-6]. The present study reviewed the clinical data of 219 patients with large area burns (LAB) in recent more than 20 years, who were classified into different stages according to the therapeutic protocols used at the time. Based on the review, the outcomes of GI function protection and treatment were compared, analyzed and summarized in an attempt to propose some practicable guidelines for the effective prevention and treatment of GI dysfunction.

MATERIALS AND METHODS

Clinical data

This study included 219 patients with severe burns who were admitted to this burn center from January 1980 to August 2007. They were classified as LAB patients (50%-79% TBSA, or degree Ⅲ burn area > 20%) and extraordinarily large area burn (ELAB) patients (80%-100% TBSA, or degree Ⅲ burn area > 50%). According to the therapeutic protocols used at the time, they were assigned to three stages: stage 1 from 1980 to 1989, stage 2 from 1990 to 1995, and stage 3 from 1996 to 2007. The occurrence of GI dysfunction and mortality were analyzed statistically.

Stage 1 (1980-1989): Limited fluid resuscitation was advocated during the shock phase of burn patients. In other words, the total fluid input was minimized as long as the vital signs were stably maintained, and the urine output was controlled at a level of 0.5 mL/h per kg body weight. The first escharectomy was usually done 4-7 d after burn injury, and the operation area was 20%-30% TBSA in most cases. Patients were mostly starved in the early stage of burn and relied on intravenous nutrition. To prevent stress ulcer, gastric mucosal protection agents and anti-acid drugs were administered routinely.

Stage 2 (1990-1995): The major therapeutic changes were advancing the first escharectomy to 3-4 d after burn injury, expanding the operation area as much as possible, and excising the necrotic tissue as early as possible. When bowel sounds recovered 2-4 d after burn injury, food intake was started gradually through the gastric tube and patients were encouraged to take food orally, with administration of appropriate amounts of gastrokinetic drugs such as domperidone to promote gastrointestinal peristalsis. Oral norfloxacin and nystatin were administered routinely within 2 wk after burn. Selective decontamination of the digestive tract (SDD) was also recommended.

Stage 3 (1996-2007): The comprehensive resuscitation strategy was advocated for shock burn, which includes sufficient resuscitation and maintenance of urine output at 1-1.5 mL/h per kg BW; routine intravenous instillation of vasoactive drugs such as small doses of dopamine; adjustment of the gastrointestinal tract and renal blood perfusion; and use of antioxidants such as large doses of Vitamin C and E to eliminate free oxygen radicals. In addition, antibiotics were used prudently, including shortening the duration of antibiotic administration and reducing the variety of antibiotics. So far as gastrointestinal nutrition is concerned, staged food ingestion was advocated, where small amounts of light fluids (20-40 mL/h) were instilled through the gastric tube 2 h after burn to stimulate gastrointestinal peristalsis. Once bowel sounds recovered, the amount of food was increased gradually. Usually the amount of enteral nutrition fed through the gastric tube was increased to 2000-2500 Kcal/d 3-6 d after burn. Specific nutrients were used such as oral glutamine, L-arginine, dietary fiber and subcutaneous growth factor.

Since 2003, early administration of continuous renal replacement therapy (CRRT) for 5-8 consecutive days has been advocated in patients with GI failure accompanied with sepsis. The content of endotoxin, IL-1β, IL-6 and IL-8 in plasma were analyzed before and after CRRT treatment. TNF-α content was measured by radioimmunoassay. The activity of diamine oxidase (DAO) in plasma was tested according to the previous report[7].

Indexes for assessing GI function

There was no uniformed criterion for assessing GI dysfunction[8]. Based on the diagnostic criteria for MODS and GI symptoms commonly seen in burned patients, GI dysfunction is summarized as follows: (1) abdominal distension: bowel sound was reduced and food intolerance exceeded more than 5 d; (2) stress ulcer: gastric fluid aspirated from the gastric tube appeared bloody macroscopically and gastric mucosa was erosive and ulcerative gastroendoscopically; (3) severe stress ulcer: blood loss exceeded 800 mL within 24 h; (4) alteration of intestinal microbiota: Gram-negative *E.coli* was amplified, and the bacillus/coccus ratio was greater than 10:1; and (5) enterogenic infection: highly suspected systemic infection occurred after ruling out wound surface, pulmonary and indwelling catheter infections[9,10].

Statistical analysis

Data were testified by Pearson's Chi-square test, and Fisher's two-tail exact test.

RESULTS

Of the 219 severe burn cases analyzed (Table 1), 89 cases were LAB and 130 cases were ELAB. There was no significant difference in age distribution and burn area between the three stages of patients.

Table 2 shows that the occurrence of stress ulcer in LAB patients of stage 3 was 8.6%, which was significantly lower than 30.3% of stage 1 patients ($P < 0.05$). No hemorrhage from severe stress ulcer and enterogenic infection occurred in the patients of stage 2 and 3.

Compared with LAB patients, the occurrence of gastrointestinal complications and mortality in ELAB patients were significantly higher, indicating that occurrence of gastrointestinal complications was closely

Table 1 General clinical data of 219 burned patients

	LAB			ELAB		
Stage	*n*	Age (yr)	TBSA (%)	*n*	Age (yr)	TBSA (%)
1	33	26.5 ± 18.7	65.1 ± 12.4	45	28.5 ± 13.7	91.4 ± 10.2
2	21	27.7 ± 13.8	74.6 ± 10.3	29	26.9 ± 14.6	89.3 ± 8.2
3	35	25.9 ± 16.3	69.5 ± 12.2	56	26.4 ± 13.3	93.1 ± 9.4

TBSA: Total body surface area.

Table 2 GI complications and mortality in LAB patients (%)

Stage	*n*	AE	SU	SSU	FA	EI	Mortality
1	33	12.1	30.3	3.0	15.1	3.0	12.1
2	21	4.8	19.0	-	4.8	-	4.8
3	35	2.9	8.6[a]	-	2.9	-	2.9

AE: Abdominal extension; SU: Stress ulcer; SSU: Severe stress ulcer; FA: Flora alteration; EI: Enterogenic infection. Compared with the stage 1, [a]$P < 0.05$.

Table 3 GI complications and mortality in ELAB patients (%)

Stage	*n*	AE	SU	SSU	FA	EI	Mortality
1	45	24.4	60.0	11.1	20.0	15.5	55.6
2	29	13.8	48.3	6.9	13.8	10.3	41.3
3	56	7.1[a]	21.4[b,c]	3.6	8.9	7.1	28.5[b]

Compared with stage 1, [a]$P < 0.05$, [b]$P < 0.01$; compared with stage 2, [c]$P < 0.05$.

Table 4 Endotoxin, IL-1β, IL-6, IL-8 and TNF-α levels and plasma DAO activity before and after CRRT

	Endotoxin (Eu/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	DAO (U/mL)
Before CRRT	0.76 ± 0.13	272 ± 28	518 ± 64	583 ± 51	2.98 ± 0.94
After CRRT	0.045 ± 0.017[b]	57 ± 15[b]	98 ± 25[b]	105 ± 31[b]	1.27 ± 0.54[b]

Compared with before CRRT, [b]$P < 0.01$.

associated with the severity of burn. Table 3 shows that the occurrence of abdominal extension and stress ulcer and mortality in the stage 3 ELAB patients were 7.1%, 21.4% and 28.5%, respectively, which were significantly lower than those of stage 1 ($P < 0.05$ or $P < 0.01$), and the occurrence of stress ulcer in the stage 3 ELAB patients was also significantly lower than that of stage 2 patients ($P < 0.05$).

In the 5 patients with GI failure accompanied with severe sepsis, endotoxin, IL-1β, IL-6, IL-8 and TNF-α levels and plasma DAO activity were decreased significantly after CRRT (Table 4) ($P < 0.01$).

DISCUSSION

Timely and effective fluid resuscitation is the basis and guarantee of curing severely burned patients[11]. Before the 1990s, the therapeutic concepts were limited to such that excessive fluid infusion would aggravate edema so that limited resuscitation was addressed. Under the bunker of stable vital signs lies the problems of GI hypoxia and ischemia, or occult GI shock[12,13]. Since the mid and late 1990s, comprehensive resuscitation strategies for maintaining the stability of vital signs and splanchnic resuscitation to restore GI blood supply as early as possible and reduce hypoxic and ischemic injuries as mush as possible have been recommended[14]. It is suggested that small doses of dopamine should be administered to dilate the renal and GI vessels[15], and free oxygen radical clearing agents to attenuate ischemia/reperfusion injury in the process of resuscitation[16,17]. These comprehensive resuscitation measures played an important role in protecting GI function, helping resume bowel sound earlier and digestive function[18]. No stress ulcer occurred during the shock phase, which laid a sound foundation for future treatment.

It has been generally accepted that early enteric nourishment plays an essential role in preventing GI dysfunction following severe burns[19,20]. But as LAB often causes serious edema of GI mucosa, there is a concern that early food intake would give additional burden to the GI tract or even cause acute gastric dilation, resulting in vomiting and aspiration. For this reason, there is controversy over when and how to take food. Before the 1990s, food intake was usually started when patients resumed bowel sound. After the mid and late 1990s, the idea of staged food intake was advanced: a small amount of light fluid is started several hours after burn so as to not only supplement nutrition but stimulate GI peristalsis and improve GI blood supply. Once bowel sound resumes, the amount of food can be increased. Using immunoregulatory nutrients such as oral glutamine, L-arginine and dietary fiber promoted post-burn repair of GI mucosa, maintained GI barrier function, and reduced translocation of enterogenic bacteria and endotoxins[21,22].

The microenvironment formed by GI resident bacteria forms an ecologic barrier in the intestinal lumen, preventing intestinal pathologic bacteria from colonization and substantial proliferation[23]. To maintain normal intestinal microbiota, we paid special attention to the followings: prudent use of antibiotics and routine use of SDD. In earlier treatment of severe burns, a variety of broad-spectrum antibiotics were often used concomitantly. But our clinical experiences showed that mere use of antibiotics failed to control infections effectively in severely burned patients; instead it often caused alteration of bacterial flora, resulting in superinfection. Since the mid and late 1990s, the principle of "bold use of antibiotics and bold discontinuation of them" has been advocated. In other words, the duration and variety of antibiotics should be minimized, and the use of antibiotics should be enhanced properly during the edema reabsorption phase and the perioperative period. By doing so, the incidence of systemic infection was lowered, and furthermore it avoided alteration of intestinal microbiota effectively. The use of SDD within two weeks of burn injury inhibited the growth of Gram bacteria and fungi and maintained the stability of intestinal microbiota, which may also be beneficial to reducing superinfection

of intestinal bacteria[24].

Early excision of necrotic tissue and closure of the wound surface are essential in the treatment of severe burns[25]. Positive surgical treatment has become a generally accepted idea. Our practice is that surgery is started 3-4 d after burn injury and the area of escharectomy at a time is much larger than before, usually reaching 60%-75% TBSA. The wound surface is covered with heterogeneous skin, which plays an important role in preventing systemic inflammatory reaction and protecting organ functions[26].

In some patients in whom fluid resuscitation was not implemented effectively for various reasons, wound surface infection often caused severe injury to the GI function, or even toxic paralytic ileus palsy, greatly increasing toxin absorption and bacterial superinfection. Toxins absorbed in the blood act on the GI tract, which in turn lowers the GI kinetics, resulting in a vicious cycle[27]. Treatment of this kind of critically severely burned patients is a real challenge, in whom the mortality rate is usually high. Apart from the above mentioned routine treatments, we also used CRRT to filtrate inflammatory mediators and toxins in the body, which significantly lowered the content of endotoxins and inflammatory factors and DAO activity. As the vicious cycle was broken off, the therapeutic outcome was usually good[28].

In summary, post-burn GI dysfunction is caused by multiple factors, and therefore maintaining GI function is a systematic engineering project. The therapeutic strategy should not rely on a single treatment or a single drug[29,30]. Furthermore, as severe burn itself may cause serious injury to various functions of the body, prevention of multi-organ functions should be addressed. Clinical experiences in recent 30 years have demonstrated that comprehensive fluid resuscitation, early excision of necrotic tissue, staged food ingestion, and administration of specific nutrients are essential strategies for preventing gastrointestinal complications in severely burned patients. Once severe GI dysfunction and sepsis occur, individualized comprehensive treatment should be implemented without delay. CRRT developed in recent years appears to be a promising strategy in the treatment of severe burns[31].

COMMENTS

Background

Gastrointestinal dysfunction is a common complication of severe burns. Injury to GI function, especially to GI barrier function, is an important initiator as well as a stimulator for occurrence of systemic inflammatory response syndrome (SIRS), sepsis and multiple organ dysfunction syndrome (MODS) following severe burns.

Research frontiers

The study analyzed and summarized the authors' clinical experiences in recent 30 years in the prevention and treatment of gastrointestinal dysfunction in severely burned patients in an attempt to propose some practicable guidelines for the effective prevention and treatment of GI dysfunction following severe burns.

Innovations and breakthroughs

Comprehensive fluid resuscitation, early excision of necrotic tissue, staged food ingestion, and administration of specific nutrients are essential strategies for preventing gastrointestinal complications and lowering the mortality in severely burned patients.

Applications

The study provided some practicable guidelines for the effective prevention and treatment of GI dysfunction following severe burns.

Peer review

The paper analyzed and summarized some practicable experience for the effective prevention of GI dysfunction. It is valuable to see the actual results from the therapies by the authors over the last 30 years.

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S- Editor Li DL **L- Editor** Ma JY **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3236

World J Gastroenterol 2008 May 28; 14(20): 3236-3241
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Venous diethylene glycol poisoning in patients with preexisting severe liver disease in China

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Received: January 9, 2008 Revised: March 26, 2008
Accepted: April 2, 2008
Published online: May 28, 2008

Abstract

AIM: To analyze the clinical presentation of venous diethylene glycol (DEG) poisoning in patients with preexisting severe liver disease and factors that correlate with DEG poisoning.

METHODS: Retrospective chart review was performed to analyze the epidemiology, clinical presentation, hepatorenal functions, hemodynamics and pathological characteristics of 64 patients with severe liver disease who received intravenous armillarisin-A, the solvent of which was DEG. Comparative analyses of correlating factors and causes for poisoning were based on the presence or absence of poisoning.

RESULTS: Of the 64 patients who received armillarisin-A, 15 were found to have DEG poisoning. Twelve poisoned patients died. After a mean of 5 d, the poisoned patients displayed acute renal failure. Metabolic acidosis occurred in 13 cases. BUN, Cr, and CO_2 values were significantly elevated and exacerbation of digestive tract symptoms and/or symptom was noted in 11 cases. Neurological system impairment was observed in 10 cases after 2 wk. Compared to the 49 non-poisoned patients, the poisoned patients exhibited significantly lower RBC and Hb values and higher WBC count. Renal biopsy from the poisoned patients revealed acute tubular necrosis and interstitial nephritis. Significant differences in preexisting severe hepatitis, ascites, renal disease, and diuretic therapy were found between groups. Prior to diethylene glycol injections, the mean values for neutral granular cells, BUN, Cr, calcium and phosphorous ions differed significantly between groups.

CONCLUSION: Venous diethylene glycol poisoning is characterized by oliguric acute renal failure, metabolic acidosis, digestive symptoms, nervous system impairment, and a high probability of anemia and WBC proliferation. Mortality is high. Correlative factors include preexisting severe liver disease, renal disease, and infection.

Key words: Diethylene glycol; Poisoning; Liver disease; Clinical feature

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Lin BL, Zhao ZX, Chong YT, Li JG, Zuo X, Tao Y, Lou TQ, Gao ZL. Venous diethylene glycol poisoning in patients with preexisting severe liver disease in China. *World J Gastroenterol* 2008; 14(20): 3236-3241 Available from: URL: http://www.wjgnet.com/1007-9327/14/3236.asp DOI: http://dx.doi.org/10.3748/wjg.14.3236

INTRODUCTION

Diethylene glycol (DEG) is a chemical substance used primarily for industrial purposes. Tested in animals, DEG induces liver impairment and kidney toxicity presenting as acute renal failure (ARF)[1,2]. In 1937, 358 human cases of ARF resulting in 107 deaths were described following ingestion of sulfanilamide dissolved in DEG in America[3]. Similar reports of DEG poisoning appeared subsequently in the other countries[4-10], with most cases involving pediatric poisoning through oral ingestion and with fundamentally milder complications.

On April 22th 2006 and April 24th 2006, two patients in the Department of Infectious Diseases, Third Affiliated Hospital of Sun Yat-Sen University, with severe liver disease developed ARF. On April 29th

2006 and April 30th 2006, another six patients in this department with severe liver disease also developed ARF. Upon further investigation, armillarisin-A[11], a drug produced by the Qiqihar No. 2 Pharmaceutical Co. Ltd, for treatment of gall-bladder disease, was found to have been administered to all patients who developed ARF. Administration of the drug was immediately suspended. Subsequently, the situation was reported to the relevant pharmacy. All preparations of armillarisin-A were sealed and forwarded to the Guangdong Drug Examination Center for investigation. Findings revealed that DEG was present in these preparations at a concentration of 30%. Subsequent judiciary investigation disclosed that the Qiqihar No. 2 Pharmaceutical Co. Ltd, selected DEG to serve as an economic substitute for trimethylene glycol in armillarisin-A preparations.

Review of 64 patients who received armillarisin-A in the hospital during the relevant time period was therefore undertaken, and findings were described in the present report. Of the 15 patients subsequently died, 12 were diagnosed with DEG poisoning. No other patients with similar complications have been reported since May 2nd 2006. The investigation described in the present report has the following features: (1) all subjects were adults who received armillarisin-A with DEG intravenously; (2) clinical presentation was recorded before and after DEG poisoning, and the exact injection volumes and DEG concentrations in the preparations were recorded; (3) the majority of patients presented with concurrent severe liver disease. In the present report, the clinical presentation of venous diethylene glycol poisoning and the pathological characteristics of renal tissue of poisoned patients were described and factors that correlate with this form of poisoning were identified.

MATERIALS AND METHODS

Subjects

The 64 patients enrolled in the present study were treated with armillarisin-A in the Third Affiliated Hospital of Sun Yat-Sen University in Guangzhou between April 19th 2006 and May 1st 2006. All the patients, including 49 (76.6%) males, were diagnosed with severe liver diseases. Of these 64 patients, 14 had severe hepatitis, 16 had liver cirrhosis caused by hepatitis B virus, 21 had chronic active hepatitis, 6 had primary hepatocellular carcinoma, 2 underwent liver transplantation, 2 had biliary cirrhosis, and 1 had hepatolenticular degeneration and liver impairment due to malignant lymphoma and cholangiocarcinoma.

Diagnostic methods

Based on the published findings long before and the consensus of experts, the Department of Health of Guangdong Province established the criteria for clinical diagnosis of DEG poisoning. The following three criteria are considered essential: (1) a history of DEG prescription (oral/venous injection), (2) acute renal impairment or renal failure characterized by oliguria or anuria occurring within 2 wk of the last ingestion/injection), and (3) elimination of all other causes of acute renal impairment or renal failure.

Diagnosis of viral hepatitis was based on the standardized "viral hepatitis prevention study" performed in 2000 by the Society of Infections Diseases and Society of Liver Diseases of the Chinese Medical Association[12].

Research methods

Retrospective chart review was applied to all 64 patients who received DEG intravenously. These patients were assigned to either the poisoned group or the non-poisoned group. For each poisoned patient, analyses of epidemiology, clinical symptoms, prognosis, hepatorenal functions, hemodynamics and pathology of renal tissue were performed before and after poisoning for comparison purposes. Analyses were also performed for the poisoned group as compared to the non-poisoned group prior to receipt of DEG to identify factors predisposing to DEG poisoning.

Renal tissues from poisoned patients were examined with several methods. Ten or more renal corpuscles were extracted and subjected to HE and PASH staining followed by microscopic observation. The nature and degree of corpuscular and tubular-interstitial pathologies were evaluated. Immunofluorescence staining of frozen sections was performed to observe the deposition sites and degree of deposition of immune-complex compounds. Electron microscopy was performed to identify the ultrastructural changes in renal tissue.

Liver function and biochemical parameters were detected using an automatic chemistry analyzer. The concentration of DEG in armillarisin-A was determined by spectrophotometry.

Statistical analysis

Normality distribution was analyzed for the continuous variables. The *t*-test was performed to detect significant differences between groups with normality. The data are presented as mean ± SD. Group comparison for data without a normal distribution involved evaluation by independent nonparametric testing. Findings were presented as the medians. The Chi-square test was performed to examine numerical data. $P < 0.05$ was considered statistically significant. SPSS13.0 for windows was used for all statistical analyses.

RESULTS

Basic information concerning patients who received DEG intravenously

Sixty-four patients who received intravenous injections of armillarisin-A were observed. On June 30th 2006, DEG poisoning was present in 15 patients and absent in 49 patients. Comparative statistics was performed based on the presence or absence of DEG poisoning, and findings are listed in Table 1. The DEG concentration in the patients ranged from 1.2% to 6%, with a cumulative dosage volume of 2.4-114 mL, but no statistical differences in these values were observed between the poisoned and non-poisoned groups. Liver impairment was more severe in the DEG-poisoned group than in

Table 1 Basic information concerning patients receiving venous diethylene glycol injections (n = 64)

Item	DEG-poisoned group (n = 15)	Non-DEG-poisoned group (n = 49)	Statistical value	P value
Male sex (%)	14 (93.3)	35 (71.4)	3.071	0.080
Age (yr)				
Median	50	48	1.11	0.267
Range	33-76	5-72		
DEG intake-ml				
Median	24	36	0.27	0.787
Range	9-72	2.4-114		
DEG concentration (%)				
Median	6	6	0.713	0.476
Range	3-6	1.2-6		
Alcoholics (%)	7 (46.7)	19 (38.8)	0.296	0.586
Diagnosis			11.691	0.039
TLD (%)	12 (80.0)	21 (42.9)	6.344	0.012
CH (%)	2 (13.3)	19 (38.8)		0.112[2]
Other (%)	1 (6.7)	9 (18.4)		0.258[2]
Diuretics (%)	12 (80.0)	16 (32.7)		0.020[2]
Complication				
Ascites (%)	10 (66.7)	9 (18.4)		0.000
Renal disease[2] (%)	5 (33.3)	3 (6.1)		0.014[1]
Serum checking				
ALT (U/L)	180.9 ± 269.9	201.4 ± 284.3	0.251	0.804
TB (μmol/L)	359.2 ± 245	239.3 ± 221.5	1.767	0.082
BUN (mmol/L)	7.9 ± 3.8	4.3 ± 2.9	3.372	0.003
Creatinine (μmol/L)	94.2 ± 24.6	58.7 ± 22.6	5.141	0.000
Ca^{2+} (mmol/L)	2.37 ± 0.17	2.25 ± 0.21	2.157	0.035
Phosphonium (mmol/L)	0.72 ± 0.43	1.00 ± 0.33	2.574	0.013
WBC (10^9/L)	6.47 ± 2.08	6.00 ± 5.34	0.326	0.746
NEUT	0.716 ± 0.114	0.587 ± 0.153	3.003	0.004
RBC (10^{12}/L)	3.03 ± 0.92	3.33 ± 0.79	1.208	0.232
Hemoglobin (g/L)	99.9 ± 25.6	106.6 ± 18.8	1.035	0.305
Platelet count (10^9/L)	106.9 ± 50.6	125.9 ± 73.3	0.293	0.354

TLD: Terminal liver disease, including severe hepatitis, liver cirrhosis, recurrence of post liver transplantation; ALT: alanine aminotransferase; CH: Chronic hepatitis; TB: total bilirubin; WBC: White blood cells; RBC: Red blood cells; NEUT: Ratio of neutral leucocyte; BUN: Blood urea nitrogen. [1]Fisher's exact test; [2]Pre-existing renal diseases, including kidney stones, proliferative renal cysts and urinary tract infections. One case of renal carcinoma was observed in the non-poisoned group.

the non-DEG-poisoned group. Of the 15 poisoned patients, 12 had terminal liver disease. Data analyses revealed significant differences between the poisoned and non-poisoned groups with respect to the severity of pre-injection liver conditions, presence of ascites and renal diseases, use of diuretics, pre-injection neutral granular cell count, serum BUN, serum Cr, calcium and phosphate ion (IP) concentrations. Death occurred in 12 patients of the poisoned group and 8 patients of the non-poisoned group. Hepatic failure and multiple organ dysfunction syndromes (MODS) were identified as the main causes of death.

Clinical presentation of patients with DEG poisoning

Oliguric ARF was present for a mean of 5 d in 15 patients with intravenous DEG poisoning. The clinical characteristics of these 15 patients are presented in Table 2. The urine volume decreased rapidly. The majority of poisoned patients developed digestive tract symptoms, such as nausea, vomiting and bloating, or exhibited an increase in the severity of these symptoms. Half of the patients exhibited concomitant mild pyrexia. Ten patients displayed nervous system impairment involving the cranial nerves, including the facial, optic, oculomotor and glossopharyngeal nerves, at an average of 14 d after the initial injection. A few patients exhibited peripheral nerve involvement presenting as limb tremor and paralysis. Respiratory muscle paralysis might have been present in some patients, leading inevitably to respiratory failure. DEG poisoning was also associated with an increase in the severity of hepatic encephalopathy among patients previously exhibiting this complication. Retrospective analyses of 13 patients before and after DEG poisoning revealed that all patients experienced metabolic acidosis at an average of 9 d after injection and 4 d following development of ARF. The most severe manifestations of metabolic acidosis occurred on d 10 after initial ingestion of DEG. Twelve of the 15 patients diagnosed with DEG poisoning died. Death generally occurred 1 wk following the initial signs of renal failure. Among the 3 patients who survived the poisoning, however, urine volume was observed to recover 3 wk after poisoning and urine volume was normal 1 mo after poisoning. One of the three patients who survived underwent combined liver-kidney transplantation 16 d after exhibiting DEG poisoning.

Table 2 Clinical characteristics of 15 DEG-poisoned patients

Characteristics	Data
Age (yr)	50 (33-76)
Male sex (%)	14 (93.6)
Injected DEG volume (mL)	24 (9-72)
ARF (%)	15 (100)
Incubation period of ARF (d)	5 (2-12)
Incubation periods of anuria (d)	6 (3-13)
Fever (%)	7 (46.7)
Incubation periods (d)	6 (1-13)
Dig. tract symptoms (%)	11 (73.3)
Incubation period (d)	9 (3-19)
Nerv. syst. impair (%)	10 (66.7)
Incubation periods (d)	14 (7-24)
Cranial nerves (%)	10 (64.7)
Peripheral nerves (%)	5 (33.3)
Central nerv. syst. (%)	6 (40.0)
Metab. acidosis (n = 13) (%)	13 (100)
Incubation periods of abnormal Cr and/or BUN (d)	5 (2-12)
Time of peak Cr (d)	11 (6-19)
Time of peak BUN (d)	14 (6-23)
Incubation periods of abnormal CO_2 (d)	9 (2-14)
Time of peak CO_2 (d)	10 (5-16)
Death (%)	12 (80.0)
Death time after injection (d)	12.5 (8-65)
Causes of death (n = 12)	
MODS (%)	7 (58.3)
Infection (%)	4 (33.3)
Dig. tract bleed (%)	1 (8.3)

Hepatorenal functions and peripheral blood cell count before and after DEG poisoning

When the liver function, renal function and peripheral blood cell counts before DEG poisoning were compared

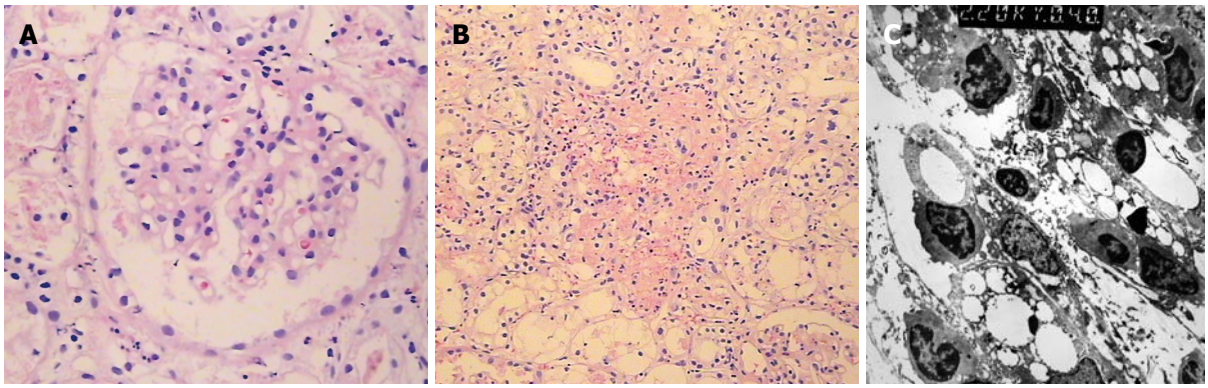

Figure 1 Pathological changes in renal tissue of patients with DEG poisoning. **A**: Glomerulus of a patient poisoned by intravenously administered DEG revealing no remarkable changes (HE, × 200); **B**: Tubular necrosis and interstitial inflammatory response in renal tissue following poisoning by intravenously administered DEG (HE, × 100); **C**: Microscopic observation of tubular vacuolation and interstitial inflammatory response in renal tissue following poisoning by intravenously administered DEG (× 6000).

Table 3 Liver-renal function measurements and peripheral blood cell counts before and after DEG poisoning

Item	*n*	BP	AP	*t*-value	*P*	CI
TB (μmol/L)	15	376.7 ± 244.6	354.7 ± 257.1	0.945	0.362	-28.36-72.44
PT (s)	15	24.4 ± 13.1	22.4 ± 8.8	1.33	0.210	-1.34-5.41
GGT (U/L)	14	163.2 ± 225.5	109.4 ± 115.8	1.451	0.170	-26.3-133.8
ALP (U/L)	14	217.0 ± 265.4	146.7 ± 148.8	1.888	0.082	10.2-150.7
BUN (mmol/L)	15	7.4 ± 3.9	31.2 ± 9.68	8.373	0.000	17.61-30.00
Cr (μmol/L)	15	94.2 ± 24.1	691.6 ± 197.8	10.659	0.000	475.28-719.51
CO_2 (mmol/L)	13	24.4 ± 3.9	13.1 ± 2.6	11.75	0.000	9.20-13.39
Ca^{2+} (mmol/L)	14	2.38 ± 0.18	2.41 ± 0.22	-0.737	0.474	-0.13-0.07
Phosphonium (mmol/L)	14	0.73 ± 0.45	1.31 ± 0.50	-4.088	0.001	-0.90-(-0.28)
WBC (10^9/L)	15	6.59 ± 2.33	9.78 ± 3.75	3.325	0.008	1.05-5.33
RBC (10^{12}/L)	15	2.99 ± 0.94	2.32 ± 0.76	2.968	0.014	0.16-1.17
Hb (g/L)	15	99.6 ± 25.1	79.5 ± 23.6	2.823	0.018	4.25-36.11
PLT (10^9/L)	15	119.6 ± 50.1	94.6 ± 72.6	1.336	0.211	-16.75-66.93

BP: The last values before DEG poisoning; AP: The peak values after DEG poisoning; GGT: Gamma-glutamyl transpeptidase; ALP: Alkaline phosphatase.

with the peak value after DEG poisoning: (1) the patients' blood urea nitrogen (BUN), creatinine (Cr), and phosphate (P) concentrations increased significantly after DEG poisoning, while serum CO_2 concentration dropped significantly, but serum calcium had no remarkable change; (2) DEG did not cause aggravation of liver function, while serum total bilirubin level, GGT, ALP and prothrombin time did not change significantly; (3) the peripheral blood cell counts increased significantly after DEG poisoning, while the red blood cell counts and hemoglobin value dropped significantly, but platelet counts did not change obviously (Table 3).

Pathological changes in renal tissue from patients with DEG poisoning

Renal tissues were taken from two patients with DEG poisoning on the third and forth days after ARF, respectively. Biopsies of renal tissue indicated significant tubular pathological changes, partial dissolution and necrosis of epithelial cells, and interstitial inflammatory cell infiltration (Figure 1). No pathological changes in the glomerular basement membrane of these patients were observed.

DISCUSSION

In 1937, the Massengill Company (USA) developed an "elixir of sulfanilamide", a preparation of 9-10 g of sulfanilamide dissolved in 100 mL of DEG. Other cases of DEG poisoning have been largely associated with foul play or deliberate consumption of alcoholic mixtures containing DEG[3-10,13]. In this study, injection of armillarisin-A produced by the Qiqihar No. 2 Pharmaceutical Co. Ltd. resulted in events similar to those described previously in response to DEG poisoning. Sixty-four patients with severe liver disease received venous armillarisin-A injections containing high concentrations of DEG (325.9 mg/mL and 30% concentration as reported by the Heilongjiang Province Drug Inspection Center and the Guangdong Province Drug Inspection Center, respectively). Fifteen patients were diagnosed with DEG poisoning. The rate of poisoning was 23.4%.

Liver impairment was more severe in the DEG-poisoned group than in the non-DEG-poisoned group. Metabolism of DEG involves the actions of alcohol dehydrogenase and aldehyde dehydrogenase[1]. Alcohol dehydrogenase ordinarily converts DEG to an aldehyde and aldehyde dehydrogenase ordinarily converts this

aldehyde to certain acids. As alcohol dehydrogenase and aldehyde dehydrogenase are mainly restricted to the liver, loss of these enzymes as a consequence of severe liver disease may significantly impair DEG metabolism. Furthermore, secondary infection is a common complication in patients with terminal liver disease. Peritonitis caused by Gram-negative bacilli and hepatobiliary infection are the most prevalent complications. Data also indicate that the pre-injection rate of neutral granular cells in the poisoned group was significantly higher than that in the non-poisoned group. Infection-induced endotoxemia increases alcohol dehydrogenase activity[14], and accumulation of the aldehyde intermediate can provoke DEG poisoning. Concurrently, serious liver diseases often produce massive ascites requiring diuretic therapy. Resultant renal hemodynamic changes occurring in response to such therapy may inevitably lead to exacerbation of renal damage. Therefore, lower dosages and concentrations of DEG can provoke poisoning *via* the intravenous route in patients with severe liver disease as compared to the oral route in patients without severe liver disease.

In the present study, the poisoned patients had a significantly higher incidence of renal disease and significantly higher serum BUN and Cr concentrations than the non-poisoned patients, suggesting that patients with renal disease are more susceptible to DEG poisoning than those without renal disease.

Currently available information about DEG indicates that this glycol induces acute poisoning, but no chronic poisoning. This apparent discrepancy can be explained by the short half-life of DEG (approximately 3 h)[15]. DEG poisoning was previously considered similar to ethylene glycol poisoning, which is associated with renal impairment attributable to renal accumulation of calcium ions and to the final product, oxalic acid, with resultant accumulation of calcium oxalates. Recent findings show that the final product of DEG metabolism is a 2-hydroxy-ethoxyacetic acid rather than an oxalic acid. DEG-induced pathological changes and necrosis of tubular epithelial cells are attributable to a metabolic intermediate that poisons tubular epithelial cells rather than to deposition of calcium oxalates[16-19]. Renal impairment is observed at early stages of poisoning and is prominent in all cases of poisoning, as was observed in the present study.

The clinical characteristics of patients poisoned by intravenous DEG were similar to those of patients poisoned following oral ingestion of DEG in the present study. It was reported that renal impairment occurs at early times following ingestion, with metabolic acidosis and delayed neurological impairment mainly involving the cranial and peripheral nerves commonly observed[20-24]. Poisoning *via* the intravenous route differs notably from poisoning *via* the oral route in that exhibition of mild fever and an increase in severity of digestive tract symptoms before occurrence of renal failure, along with a later occurrence of organ impairment, is specific for intravenous poisoning. This finding may be attributable to the age of patients in the present study and to their preexisting severe liver disease which could have limited the actions of alcohol and aldehyde dehydrogenases. Prospective research is warranted for further clarification. Due to the scarcity of DEG poisoning survivors, it is difficult to evaluate the process of systematic recovery. While previous reports indicate that recovery of the nervous system after oral DEG poisoning requires 4-6 mo[25], the present findings disclose that nervous system recovery occurs 1 mo following intravenous poisoning.

In the present study, 80% mortality was observed in the poisoned patients. Seven died of MODS, 4 died of severe infection, and 1 died of severe digestive tract bleeding. The lethal dose of DEG varies with species[26]. It was reported that DEG at a cumulative dosage of 0.22-4 mL/kg with a concentration of 17.5%-72% in humans can lead to death[6,7]. The DEG concentration in the present study ranged from 3% to 6%, with a cumulative dosage volume of 9-72 mL, but no statistical differences in these values were observed between the poisoned and non-poisoned groups, indicating that the severity of preexisting liver disease leading to loss of alcohol and aldehyde dehydrogenase activities constitutes a primary important predisposing factor for poisoning.

In the present study, the patients with DEG poisoning had higher serum calcium values and lower serum IP values than the non-poisoned patients, the serum IP concentrations were significantly increased after intravenous DEG poisoning. The importance of calcium and phosphates in DEG poisoning remains to be determined.

Although the DEG-poisoned patients described in the present study presented with concomitant severe liver diseases, no exacerbating degenerative features of general liver function (no changes in bilirubin, aldehyde dehydrogenase, albumin, and hemostatic function) were found. Furthermore, no significant increase in gamma-glutamyl transpeptidase or alkaline phosphatase was noted, indicating that venous injections of DEG do not directly affect the hepatobiliary system and drug-induced liver damage is absent. These observations may be attributable to the fact that, in contrast to oral ingestion of DEG[27], venous injection of this glycol does not participate in liver metabolism.

Additional analyses indicated that DEG-poisoned patients might present with anemia characterized by decreased red blood cells and hemoglobin. A similar form of anemia was observed in ethylene glycol poisoning. The DEG-poisoned patients described in the present study also presented with an increase in white blood cell count, with a significant increase in neutral granular cells but no remarkable changes in eosinophils. This phenomenon can be attributed to an increase in the severity of infection in patients with severe liver disease and is indicative of acute renal failure as a result of DEG poisoning rather than allergen induction.

Renal biopsy findings revealed that DEG induced tubular epithelial cell dissolution and necrosis and renal interstitial inflammatory cell infiltration, but no pathological changes in the glomerulus. These alterations differ from those associated with the hepatorenal complications and glomerulonephritis induced by severe

liver disease and are therefore important in differential diagnosis.

In conclusion, venous diethylene glycol poisoning is characterized by oliguric acute renal failure, metabolic acidosis, digestive symptoms, nervous system impairment, and a high probability of anemia and WBC proliferation. Mortality is high, and correlative factors include preexisting severe liver disease, renal disease, and infection.

COMMENTS

Background

Diethylene glycol (DEG) is a chemical substance used primarily for industrial purposes. DEG induces kidney toxicity presenting as acute renal failure (ARF) and has been used as a chemical substance for industrial purposes in many countries since 1937. In 2006, 64 patients with severe liver disease received venous armillarisin-A injections containing high concentrations of DEG, and 15 were diagnosed with DEG poisoning in Guangzhou, China. In the present report, the clinical presentation of venous diethylene glycol poisoning and the pathological characteristics of renal tissue from poisoned patients were described and factors that correlate with this form of poisoning were identified.

Research frontiers

All the clinical researches about DEG poisoning based on events of herbal toxicity, have been limited in oral DEG intake and normal persons.

Innovations and breakthroughs

The investigation described in the present report was characterized by the following features: (1) all subjects were adults who received armillarisin-A with DEG intravenously, (2) clinical presentation was recorded before and after DEG poisoning and the exact injection volumes and DEG concentrations in the preparations were recorded, (3) the majority of patients presented with concurrent severe liver diseases.

Applications

This work may help to know the clinical presentation of venous diethylene glycol (DEG) poisoning in patients with preexisting severe liver disease and factors that correlate with this form of poisoning.

Peer review

This is a nice report on an outbreak of Ⅳ DEG poisoning. Authors analyzed the features of venous DEG poisoning and serious consequences to remind government of paying attentions to drug safety and supervising.

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doi: 10.3748/wjg.14.3242

World J Gastroenterol 2008 May 28; 14(20): 3242-3248
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Effects of quercetin on hyper-proliferation of gastric mucosal cells in rats treated with chronic oral ethanol through the reactive oxygen species-nitric oxide pathway

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Supported by State Education Ministry Scientific Research Foundation for the Returned Overseas Chinese Scholars, No. 1999747
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Received: January 26, 2008 Revised: April 1, 2008
Accepted: April 8, 2008
Published online: May 28, 2008

Abstract

AIM: To investigate the effect of quercetin (3,3′,4′,5,7-pentahydroxy flavone), a major flavonoid in human diet, on hyper-proliferation of gastric mucosal cells in rats treated with chronic oral ethanol.

METHODS: Forty male Sprague-Dawley rats, weighing 200-250 g, were randomly divided into control group (tap water *ad libitum*), ethanol treatment group (6 mL/L ethanol), quercetin treatment group (intragastric gavage with 100 mg/kg of quercetin per day), and ethanol plus quercetin treatment group (quercetin and 6 mL/L ethanol). Expression levels of proliferating cell nuclear antigen (PCNA) and Cyclin D1 were detected by Western blot to assay gastric mucosal cell proliferation in rats. To demonstrate the influence of quercetin on the production of extra-cellular reactive oxygen species/nitrogen species (ROS/RNS) in rats, changes in levels of thiobarbituric acid reactive substance (TBARS), protein carbonyl, nitrite and nitrate (NOx) and nitrotyrosine (NT) were determined. The activity of inducible nitric oxide synthase (NOS) including iNOS and nNOS was also detected by Western blot.

RESULTS: Compared to control animals, cell proliferation in the gastric mucosa of animals subjected to ethanol treatment for 7 days was significant increased (increased to 290% for PCNA density $P < 0.05$, increased to 150 for Cyclin D1 density $P < 0.05$ and 21.6 ± 0.8 *vs* 42.3 ± 0.7 for PCNA positive cells per view field), accompanied by an increase in ROS generation (1.298 ± 0.135 μmol *vs* 1.772 ± 0.078 μmol for TBARS $P < 0.05$; 4.36 ± 0.39 mmol *vs* 7.48 ± 0.40 mmol for carbonyl contents $P < 0.05$) and decrease in NO generation (11.334 ± 0.467 μmol *vs* 7.978 ± 0.334 μmol $P < 0.01$ for NOx; 8.986 ± 1.351 μmol *vs* 6.854 ± 0.460 μmol for nitrotyrosine $P < 0.01$) and nNOS activity (decreased to 43% $P < 0.05$). This function was abolished by the co-administration of quercetin.

CONCLUSION: The antioxidant action of quercetin relies, in part, on its ability to stimulate nNOS and enhance production of NO that would interact with endogenously produced reactive oxygen to inhibit hyper-proliferation of gastric mucosal cells in rats treated with chronic oral ethanol.

Key words: Quercetin; Cell proliferation; Reactive oxygen species; Nitric oxide; Gastric mucosa; Ethanol

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Liu JL, Du J, Fan LL, Liu XY, Gu L, Ge YB. Effects of quercetin on hyper-proliferation of gastric mucosal cells in rats treated with chronic oral ethanol through the reactive oxygen species-nitric oxide pathway. *World J Gastroenterol* 2008; 14(20): 3242-3248 Available from: URL: http://www.wjgnet.com/1007-9327/14/3242.asp DOI: http://dx.doi.org/10.3748/wjg.14.3242

INTRODUCTION

Chronic ethanol consumption is a major risk factor for oropharyngeal, esophageal, and rectal cancer[1]. Chronic ethanol consumption resulting in gastric mucosal lesions might thus be expected to influence the kinetic balance between cell proliferation and cell death. Because hyper-

regenerative gastrointestinal mucosa has an increased susceptibility to chemical carcinogens and thus influences carcinogenesis. Various studies have been performed to evaluate the effect of chronic ethanol consumption on gastric mucosal cell turnover[2,3]. However, the role of ethanol in the altered cell proliferation in rat stomach remains poorly understood. There is evidence that alcohol is involved in gastric mucosa oxidant injury as studies showed that ethanol-induced damage can be prevented if antioxidant treatment or therapy is given concurrently or prior to alcohol exposure[4-6]. Previous studies in our laboratory found that cell proliferation is enhanced in gastric mucosa of rats treated with ethanol in a dose- and time-dependent manner[7]. These findings indicate that ethanol-associated gastric cell proliferation may involve oxidative stress[8].

Oxidative stress occurs when there is a significant imbalance between generation of reactive oxygen species (ROS) and nitrogen species (RNS) and its clearance by antioxidant defenses[9]. 3, 3', 4', 5, 7-pentahydroxy flavone (quercetin) is a potent bioflavonoid widely distributed throughout vegetables and fruits. It was reported that quercetin has many beneficial effects on human health, including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti-inflammatory effects[10,11]. These effects of quercetin due to its antioxidant properties of potent anti-oxidant, scavenge free radicals directly[12], inhibit xanthine oxidase and lipid peroxidation[13,14], and alter the anti-oxidant defense pathway *in vivo* and *in vitro*[15]. It was recently reported that quercetin inhibits oxidative damage in ethanol-induced gastric lesions of rats[16].

In light of these findings, we hypothesized that quercetin has an effect on gastric mucosa cell proliferation in rats that chronically administer ethanol involving inhibition of the ROS-nitric oxide (ROS-NO) pathway. To establish the potential antiproliferative mechanism of quercetin, we detected the expression levels of proliferating cell nuclear antigen (PCNA) and Cyclin D1, which are significantly associated with gastric mucosal cell proliferation in rats. To demonstrate the influence of quercetin on the production of extra-cellular ROS/RNS, changes in thiobarbituric acid reactive substance levels (TBARS) as an index of lipid peroxidation, protein carbonyl content as a marker of free radical-mediated modification of proteins, nitrite and nitrate (NOx) and nitrotyrosine (NT) levels as the marker of NO production, were also determined.

MATERIALS AND METHODS

Animals and treatment protocol

Male Sprague-Dawley rats, weighing 200-250 g, were used in this study. Twenty-four rats were housed in plastic cages in an air-conditioned and light controlled room at 24 ± 2℃ and 60% ± 5% humidity. The study protocol was approved by the Nanjing Medical University Animal Care and Use Committee. After a 3 d adaptation period, the rats were randomly divided into four groups (6 in each group). Group 1 had free access to tap water, group 2 had drinking water containing 6 mL/L ethanol as previously described[7], group 3 was given 50 mg/kg quercetin (Sigma, St Louis, MO, USA) by intragastric gavage twice a day, group 4 was given 50 mg/kg quercetin by intragastric gavage twice a day and 6 mL/L ethanol. Quercetin was dissolved using DMSO as the vehicle and diluted in PBS to 2 mL, with the maximum concentration of DMSO being 0.1%. As controls, animals in groups 1 and 2 were also treated with 2 mL 0.1% DMSO, twice a day. The time and doses of ethanol and quercetin treatment were determined on the basis of results from our preliminary experiment. The mean ethanol consumption was 6.52 g/kg body weight per day, the mean plasma ethanol concentration at the time of stomach excision was 18.47 mmol/L in animals of groups 3 and 4. The rats were anesthetized with urethane and sacrificed after 7 d. Their stomachs were dissected and used for this study.

Cell proliferation assay

Nuclear extracts from gastric mucosa were prepared using a nuclear extract kit (Active Motif Japan, Japan) following the instructions of its manufacturer. PCNA and Cyclin D1 detected by Western blot were applied to determination of gastric mucosal cell proliferation in rats.

Lipid peroxidation

To evaluate the extent of lipid peroxidation, the amount of thiobarbituric acid reactive substances (TBARS) in gastric tissue, a measurement of the extent of lipid peroxidation, was detected with the modified thiobarbituric acid (TBA) method[17,18]. Each sample was homogenized in a 1.15% KCl solution containing 10 mmol/L deferoxamine, 0.04% butylated hydroxytoluene (BHT), and 2% ethanol. Each homogenate was incubated for 60 min at 95℃ in an oil bath with a stock TCA-TBA-HCl reagent consisting of 15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid, 0.25 mol/L hydrochloric acid and 2% BHT. After cooling, the precipitate was removed by centrifugation, and the extinction coefficient of the supernatant at 535 nm was determined spectrophotometrically and compared with a known TBARS standard.

Protein oxidation

Protein carbonyls in gastric tissues were determined by spectrometric DNPH assay according to Fagan *et al* with minor modifications[19]. Briefly, gastric tissues were homogenized by sonication in a lysis buffer containing PBS (pH 7.2), 1% Triton X-100, 1 mmol/L EDTA and 1X protease inhibitor cocktail and removal of insoluble cellular debris was performed by centrifugation. Aliquots in protein samples were precipitated with 10 volumes of HCl-acetone (3:100) and washed with 5 mL of 10% TCA solution. Pellets were re-suspended in 500 μL buffer solution and reacted with 500 μL of 10 mmol/L DNPH (in 2 mol/L HCl) by vortexing for 15 min. To remove the un-reacted DNPH, the centrifuged pellets were washed with 5 mL of 20% TCA and 5 mL of ethanol: ethylacetate mixture (v/v = 1:1). The

final precipitate was resolved in 1 mL of 6 mol/L guanidine HCl, and the absorbance at 380 nm was determined for the sample treated with DNPH and HCl, which was subtracted as a background and compared with a known protein carbonyl standard.

Nitric oxide (NO) assay

The amount of stable nitrite (nitrite and nitrate), the end product of NO in gastric mucosa, was determined by colorimetric assay as described previously[20]. Briefly, 50 μL of gastric mucosa homogenate was mixed with an equal volume of Griess reagent consisting of 1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride and 2.5% H_3PO_4, and incubated at room temperature for 10 min. The absorbance was read at 540 nm on a microplate reader (Elx800, Bio-TEK Ins, USA). The amount of nitrite was calculated from a $NaNO_2$ standard curve.

Measurement of nitrotyrosine (NT) levels

Gastric mucosa was homogenized on ice in the prepared solution (20 mmol/L Tris-HCl containing 1% NP-40, 100 mmol/L NaF, 137 mmol/L NaCl, 5 mmol/L EDTA, 0.1 mol/L PMSF, 1% proteinase inhibitor, and 10% glycerol, pH 7.5) for 30 min at 4℃. The homogenate was centrifuged at 12000 r/min for 20 min to remove cellular debris. Protein concentration was determined using a BCA protein assay reagent kit.

Nitrotyrosine levels were quantified as previously described[21]. In short, assay was performed in 96-well plates coated with 5 mg/L of nitrotyrosine-BTG conjugate, which was blocked with gelatin to prevent nonspecific binding. A standard curve was plotted by incubating serial dilutions of NT with biotin labeled anti-nitrotyrosine Fab' in PBS containing 0.1% gelatin for 1 h. Subsequently, plates were incubated with a strepavidin peroxidase conjugate followed by o-phenylenediamine (OPD). The reaction was terminated after 20-30 min by addition of 4 mol/L H_2SO_4. Data on the standard curve were fitted to a logistic plot and the levels of NT were measured. All samples and standards were assayed in triplicate.

Anti-nitrotyrosine monoclonal antibody used in ELISA was a kind gift from Dr. Yang TB (Institute of Space Medico-Engineering, Beijing, China). The study of cross-reaction with nitrotyrosine-like compounds showed that the antibodies have a high specificity for NT[21].

Measurement of neuronal and inducible NO synthase (nNOS and iNOS) levels

The stomach was homogenized on ice in a buffer containing 50 mmol/L Tris-Cl, 150 mmol/L NaCl, 0.02% NaN_2, 100 mg/L phenylmethanesulfonyl fluoride, 1 mg /L aprotitin, and 1% Triton X-100. Lysates were centrifuged at 12000 r/min for 25 min at 4℃. The supernatant was used for nNOS and inducible NOS (iNOS) determined by Western blot analysis.

Western blot analysis

Proteins were detected by the Bradford method using bovine serum albumin as a standard. An equal amount of 40 μg protein from each sample was run per lane on 10% sodium dodecyl sulphate polyarcylantide gel electrophoresis (SDS-PAGE) and electroblotted to nitrocellulose membranes. The membranes were blocked by overnight incubation in 5% dry milk at 4℃, and thereafter incubated with primary antibodies (1:200-1000 dilution) for 3 h at room temperature. Each blot was probed with monoclonal anti-PCNA, anti-Cyclin D1 (Santa Cruz Biotech, USA), polyclonal anti-β-actin (Upstate, USA), anti-nNOS, and anti-iNOS (Santa Cruz Biotech, USA). The membranes were washed and incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbbit IgG (1:2000 dilution) (Upstate, USA) for 1 h. Immune complexes were visualized with an ECL kit (Pierce; Rockford, IL, USA) according to the manufacturer's protocol. Signal intensity was quantified using a Bio-Rad image analysis system and the results were normalized to the signal intensity of β-actin for each blot.

Immunohistochemical analysis

Stomachs were excised from three rats in each group and fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned at 5 μm for immunohistochemical staining. Staining was performed according to the routine standard procedures. Briefly, the sections were deparaffinized in xylene, cleared in graded ethanol to PBS, then quenched in 3% hydrogen peroxide (H_2O_2) containing 0.1% sodium azide to suppress the endogenous peroxidase activity and placed in 10 mmol/L citrate buffer (pH 6.0) for 15 min at 100℃ for antigen retrieval. A routine streptavidin-biotin protocol using the DAKO LSAB + kits (Dako Japan, Kyoto, Japan) was applied. The tissue sections mounted on glass slides were incubated in PBS containing 0.5% BSA to reduce nonspecific protein binding, and sequentially incubated to react with monoclonal anti-PCNA primary antibody overnight at 4℃. The antibody was then linked with streptavidin conjugated to horseradish peroxidase (HRP). HRP sites were visualized with 3,3'-diaminobenzidine (DAB) and H_2O_2, counterstained with hematoxylin. The presence of PCNA was detected by light field microscopy as a dark brown reaction product in cell nuclei. Some sections were reacted with normal mouse IgG instead of the specific antibody as a negative control. An image analysis system (NYD100) was used for quantitative analysis of cell density (cell number/view field) of the PCNA-positive cells in the rat stomach. Four sections from four rats were used. PCNA-positive cells per section were counted in five randomly selected view fields at a magnification of × 400. At least, 20 fields in each group were analyzed.

Statistical analysis

All experiments were done in triplicate and stomach tissues were excised from three rats in each group. One-way analysis of variance was used to estimate the overall significance followed by *post hoc* Tukey's test corrected for multiple comparisons. Data are presented as mean ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

Quercetin treatment could partially prevent ethanol-induced cell proliferation in gastric mucosa

PCNA is a polypeptide that specifically increases in nuclei during G1 and S phases of the cell cycle. It is considered to be an essential cofactor for the activation of DNA polymerase during DNA replication. Therefore, PCNA-positive nuclei indicate that cells replicate DNA and undergo proliferation. It is well known that Cyclin D1 promotes G1 phase progression. The levels of PCNA and Cyclin D1 were higher in gastric mucosa exposed to 6% ethanol for 7 d than in normal control rats, while the expression of PCNA and Cyclin D1 was reduced after treatment with quercetin in this study (Figure 1). PCNA immunohistochemistry and computer image analysis showed, a significantly increased number of PCNA positive cells in the fundic gland of rats treated with ethanol for 7 d. The number of PCNA positive cells in ethanol + quercetin and quercetin treated rats was very analogous to that in the control rats (Figure 2, Table 1).

Quercetin treatment could prevent ethanol-induced lipid peroxidation and protein oxidation in gastric mucosa

As TBASR shown in Figure 3, ethanol-induced ROS may increase lipid peroxidation. Quantitative measurement of TBASR in gastric mucosa revealed a significant effect of ethanol treatment on ethanol-induced lipid peroxidation and protein oxidation in gastric mucosa (1.772 μmol/g protein) compared to the normal control rats (1.298 μmol/g protein), which was reduced to 1.500 μmol/g protein ($P < 0.05$). TBARS was slightly decreased in the rats treated with quercetin (Figure 3A), suggesting that quercetin can decrease lipid peroxidation in gastric mucosa. The mean values of carbonyl contents in gastric tissue are shown in Figure 3B, revealing a similar pattern of TBARS in each group of rats.

Quercetin treatment could prevent ethanol-induced decrease in nitrite/nitrate content in gastric mucosa

The nitrite/nitrate content in gastric mucosa was determined using the Griess method. As shown in Table 1, the nitrite/nitrate content in the group treated with 6% ethanol for 7 d was significantly lower than that in the control group ($P < 0.01$) and significantly higher in rats treated with combined ethanol and quercetin than that in rats treated with ethanol only ($P < 0.01$). The gastric nNOS level was slightly increased in rats treated with quercetin, suggesting that quercetin treatment can prevent ethanol-induced decrease of nitrite/nitrate content in rat gastric mucosa.

Quercetin treatment could prevent ethanol-induced decrease in nNOS levels

NO produced by nNOS was detected by Western blot in gastric mucosa (Figure 4). Quantitative analysis revealed a significant effect of ethanol treatment on ethanol-induced decrease in nNOS levels. The gastric nNOS level in rats treated with combined ethanol and quercetin was significantly higher than that in rats treated with ethanol only. The gastric nNOS level was slightly increased in rats treated with quercetin, suggesting that quercetin can prevent ethanol-induced decrease in nNOS, which is in agreement with the data on nitrite/nitrate (Table 1) in rat gastric mucosa. No iNOS expression was detected in each group.

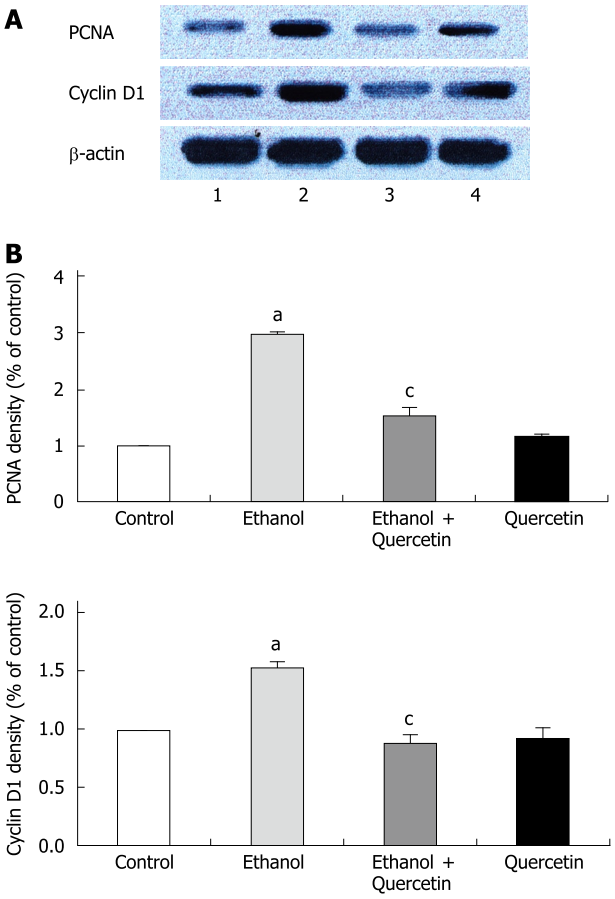

Figure 1 Immunoblotting of nuclear extracts from gastric mucosa with antibodies to PCNA and Cyclin D1 in the 4 groups as indicated in lanes 1-4 (**A**) and values normalized by arbitrarily setting the densitometry of control to 1.0 (**B**). β-actin staining was performed to ensure an equal loading. The results indicated are in percentage above the control value and are representative of four independent experiments. $^{a}P < 0.05$ *vs* control animals, $^{c}P < 0.05$ *vs* ethanol-treated animals.

Quercetin treatment could prevent ethanol-induced decrease in protein-bound 3-NT

Ultimately, increased NO, nitrite/nitrate, and peroxynitrite resulted in production of protein-bound 3-NT in gastric mucosa (Table 1). Quantitative analysis revealed a significant effect of ethanol on ethanol-induced decrease in protein-bound 3-NT. Gastric 3-NT levels in rats treated with combined ethanol and quercetin were significantly higher than those in rats treated with ethanol only. The level of 3-NT was slightly increased in rats not treated with ethanol, suggesting that quercetin treatment can prevent ethanol-induced decrease in 3-NT, which is in agreement with the data on nitrite/nitrate (Table 1) and nNOS level in rat gastric mucosa (Figure 4).

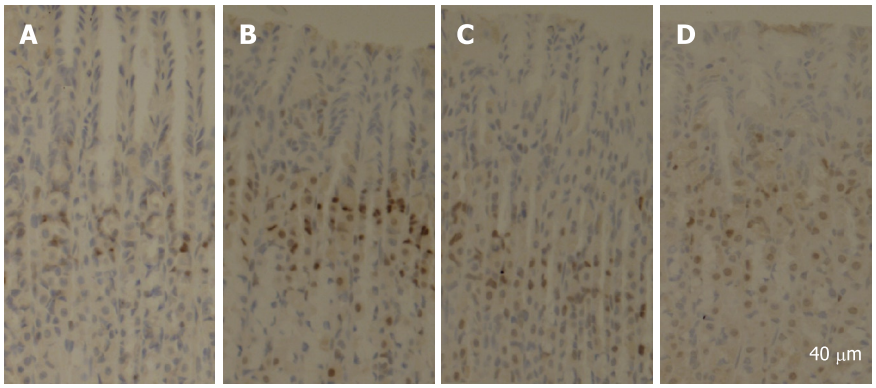

Figure 2 Staining of PCNA from rats in the 4 groups, respectively (**A-D**). Stem cells at the neck position were positively stained, while other cells were negatively stained. A significantly increased number of PCNA positive cells were observed in the fundic gland of rats treated with ethanol for 7 d. **A**: Control; **B**: Ethanol; **C**: Ethanol + Quercetin; **D**: Quercetin.

Table 1 Number of PCNA positive cells and levels of NO and NT in rat gastric mucosa (mean ± SD)

	Control	Ethanol	Ethanol + Quercetin	Quercetin
PCNA	21.6 ± 0.8	42.3 ± 0.7[b]	37.1 ± 0.4[a]	18.6 ± 0.6
NOx (μmol/L)	11.334 ± 0.467	7.978 ± 0.334[b]	9.889 ± 0.620[a]	12.098 ± 0.516
Nitrotyrosine (μmol/L)	8.986 ± 1.351	6.854 ± 0.460[b]	8.071 ± 1.208[a]	10.875 ± 1.034

[a]$P < 0.05$ *vs* ethanol group, [b]$P < 0.01$ *vs* control group.

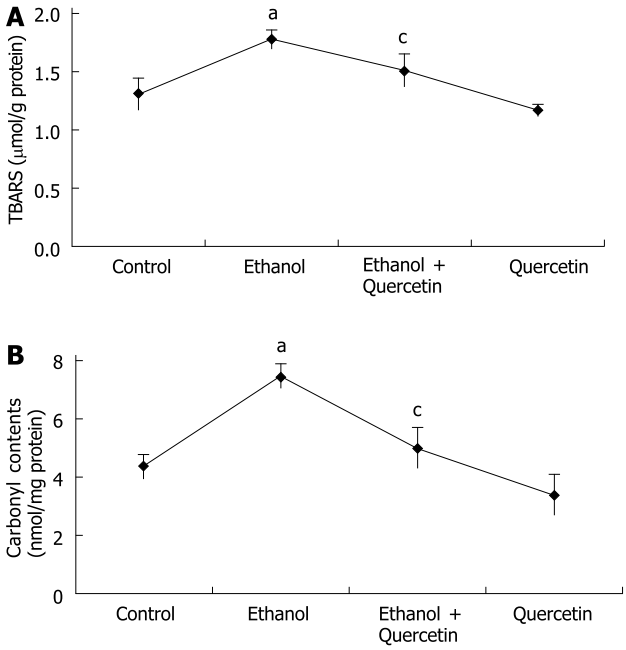

Figure 3 Lipid peroxidation (**A**) and protein oxidation (**B**) determined in gastric mucosa of rats after treatment with different agents. The data are expressed as mean ± SD of four independent experiments. [a]$P < 0.05$ *vs* control animals, [c]$P < 0.05$ *vs* ethanol-treated animals.

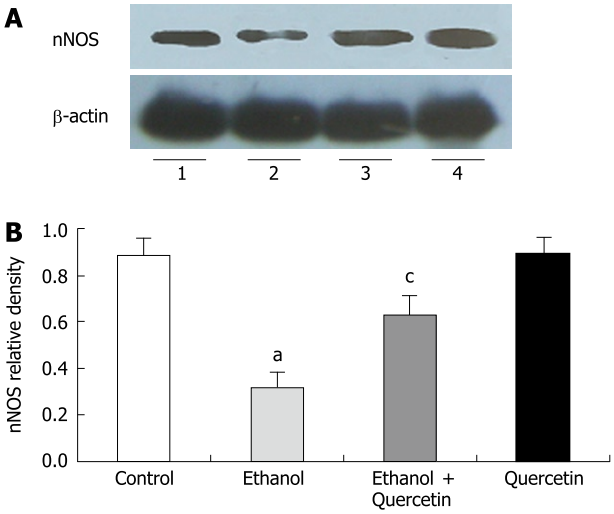

Figure 4 Immunoblotting of gastric homogenate with the antibody to nNOS in different treatment groups as indicated in lanes 1-4 (**A**) and values normalized by arbitrarily setting the densitometry of actin (**B**). The results indicated are in percentage above the control value and are representative of the four independent experiments. [a]$P < 0.05$ *vs* control animals, [c]$P < 0.05$ *vs* ethanol-treated animals.

DISCUSSION

ROS, such as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (OH), lipid peroxidation and nitric oxide (NO), are involved both in the regulation of cell proliferation and apoptosis and in macromolecular damage to gastric cells, leading to increased oxidative stress and stress-induced senescence[22,23]. ROS are oxygen-containing molecules having either unpaired electrons or ability to abstract electrons from other molecules. Lipids are modified by ROS and visualized as a thiobarbituric acid-reactive substance (TBARS). Oxidative damage to proteins generates increased carbonyl groups due to oxidation of sensitive amino acids, such as histidine, proline, arginine and lysine[24]. We measured the TBARS and protein carbonyls to serve as an indicator for intracellular oxidation in gastric mucosa. NO formation in cells is rapidly converted to nitrite. After reducing nitrate to nitrite with bacterial nitrate reductase, nitrite levels can be determined as an indicator for NO synthesis based on the Griess reaction[20]. O_2^- reacts with NO to produce peroxynitrite ($ONOO^-$), which is considered a more powerful oxidant than $O_2^{\cdot-}$[25], entering the cells rapidly. A variety of nitrate macromolecules are chiefly at the aromatic rings[26]. The nitration of tyrosyl residues on proteins is considered the stable "foot print" of RNS stress both *in vitro* and *in vivo*[27]. In this study, we also measured the levels of NO_X and 3-NT to provide an index of NO in gastric mucosa. Using these indicators, the effect of quercetin on chronic ethanol-induced generation of ROS and NO was detected.

The present study demonstrated a clear enhancement of cell proliferation in gastric mucosa of animals subjected to ethanol treatment for 7 d, which is similar to that in our previous studies[7]. Since PCNA and Cyclin D1 were strongly up-regulated (Figure 1), and the number of PCNA positive cells was increased in gastric mucosa (Figure 2 and Table 1). This enhancement function was accompanied with an increase in ROS generation and abolished by co-administration of quercetin and ethanol, which was accompanied with a decrease in ROS level. Quercetin has the ability to directly block the cell cycle at the G1/S transition in colon and gastric cancer cells[28] as well as in human leukemic T cells[29]. However, the protein levels of PCNA and Cyclin D1 were similar to the control values irrespective of quercetin administration alone in our study. These results show that an excessive amount of ROS can induce enhanced cell proliferation in gastric mucosa of rats *in vivo*.

In addition to ROS, RNS in the form of NO has also been implicated in regulation of cellular proliferation, but its role as a proliferative signal is not well defined, because it appears to depend on the cell type responsible for its release and the NOS isoforms within cells, as well as on the concentration of released NO and the composition of intracellular milieu[30,31]. The neuronal and endothelial isoforms are thought to be responsible for production of low levels of NO[32] and both isoforms have been identified in gastric mucosa[33,34]. NO is a lipophilic radical, which can exert beneficial effects by reacting with O_2^- when produced in a small amount and, in this manner, behaves as an antioxidant. A low level of NO could protect against ROS and inhibit gastric cancer cell proliferation[23]. NO donors retard gastric wound healing by inhibiting cell migration and proliferation and inducing cell apoptosis in a dose- and time-dependent manner[35]. However, excess NO produced by inducible NOS (iNOS) plays a potent role as a cytotoxic agent during infection and inflammation, with essential involvement of chronic inflammation, especially increased rates of cell proliferation, in *H pylori*-associated glandular stomach carcinogenesis[36]. Suppression of NO generation by iNOS inhibitors (aminoguanidine, AG) could also suppress cancer cell proliferation in gastric cancer xenografts[37].

In the present study, iNOS expression was not detectable in gastric mucosa. After treatment with 6% ethanol for 7 d, the expression of nNOS and the levels of NOx (nitrite/nitrate) and NT in gastric homogenates were decreased, suggesting that the nNOS activity is decreased. These results are consistent with the reported data[38]. Surprisingly, the decreased nNOS activity could be abolished by co-administration of quercetin and ethanol. Without further examination, we cannot rule out the mechanism of quercetin-enhanced activity of nNOS. It was reported that resveratrol, another kind of flavonoids, can inhibit gastric cancer cell proliferation by stimulating the activity of NOS *in vitro*[23], suggesting that quercetin may play a role as resveratrol in the inhibition of gastric cell proliferation *in vivo*.

In conclusion, our findings indicate that the antioxidant action of quercetin resides depends in part, on its ability to stimulate nNOS and increase production of NO that would interact with endogenously produced reactive oxygen to inhibit hyper-proliferation of gastric mucosal cells in rats that have chronic ethanol consumption.

COMMENTS

Background

Chronic ethanol consumption resulting in hyper-regenerative gastrointestinal mucosa has an increased susceptibility to chemical carcinogens and thus influences carcinogenesis. Some studies indicate that ethanol-associated gastric cell proliferation may involve oxidative stress. It was reported that quercetin, a 3,3',4',5,7-pentahydroxy flavone, has effects on oxidative damage to ethanol-induced gastric lesions due to its antioxidant properties of potent anti-oxidant. We hypothesized that quercetin has an effect on gastric mucosal cell proliferation in rats that have chronic ethanol consumption, thus inhibiting gastric cancer.

Research frontiers

In this study, we developed an animal model by continuous ethanol ingestion for 7 d. By using this model, we investigated the relationship between chronic ethanol intake and gastric mucosal cell proliferation, which is related to reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Innovations and breakthroughs

Our findings indicate that the antioxidant action of quercetin resides, depends in part, on its ability to stimulate nNOS and increase production of NO that would interact with endogenously produced reactive oxygen to inhibit gastric mucosal cell roliferation in rats that have chronic ethanol consumption.

Applications

This animal model established by continuous ethanol ingestion for 7 d is a useful tool for studying the mechanism of gastric mucosal cell proliferation *in vivo*. We will investigate the signal transduction of ROS in gastric mucosal cell proliferation.

Terminology

ROS are oxygen-containing molecules having either unpaired electrons or ability to abstract electrons from other molecules. Reactive RNS are forms of NO.

Peer review

This is an interesting paper, in which the authors showed that ethanol could induce gastric mucosal cell proliferation in their animal model. The ROS/RNS pathway may be involved. Further study is needed to show signal transduction of ROS in gastric mucosal cell proliferation.

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S- Editor Yang RH **L- Editor** Wang XL **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3249

World J Gastroenterol 2008 May 28; 14(20): 3249-3253
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Proteasome inhibitor ameliorates severe acute pancreatitis and associated lung injury of rats

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Received: November 18, 2007 Revised: February 27, 2008
Accepted: March 5, 2008
Published online: May 28, 2008

Key words: Severe acute pancreatitis; Proteasome inhibitor; MG-132; Lung injury

Peer reviewers: Parimal Chowdhury, Professor, Department of Physiology and Biophysics, College of Medicine, University of Arkansas for Medical Sciences, 4301 W Markham Street Little Rock, Arkansas 72205, United States; Yoshiharu Motoo, MD, PhD, FACP, FACG, Professor and Chairman, Department of Medical Oncology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

Chen X, Li SL, Wu T, Liu JD. Proteasome inhibitor ameliorates severe acute pancreatitis and associated lung injury of rats. *World J Gastroenterol* 2008; 14(20): 3249-3253 Available from: URL: http://www.wjgnet.com/1007-9327/14/3249.asp DOI: http://dx.doi.org/10.3748/wjg.14.3249

Abstract

AIM: To observe the effect of proteasome inhibitor MG-132 on severe acute pancreatitis (SAP) and associated lung injury of rats.

METHODS: Male adult SD rats were randomly divided into SAP group, sham-operation group, and MG-132 treatment group. A model of SAP was established by injection of 5% sodium taurocholate into the biliary-pancreatic duct of rats. The MG-132 group was pretreated with 10 mg/kg MG-132 intraperitoneally (ip) 30 min before the induction of pancreatitis. The changes in serum amylase, myeloperoxidase (MPO) activity of pancreatic and pulmonary tissue were measured. The TNF-α level in pancreatic cytosolic fractions was assayed with an enzyme-linked immunosorbent assay (ELISA) kit. Meanwhile, the pathological changes in both pancreatic and pulmonary tissues were also observed.

RESULTS: MG-132 significantly decreased serum amylase, pancreatic weight/body ratio, pancreatic TNF-α level, pancreatic and pulmonary MPO activity ($P < 0.05$). Histopathological examinations revealed that pancreatic and pulmonary samples from rats pretreated with MG-132 demonstrated milder edema, cellular damage, and inflammatory activity ($P < 0.05$).

CONCLUSION: The proteasome inhibitor MG-132 shows a protective effect on severe acute pancreatitis and associated lung injury of rats.

INTRODUCTION

Acute pancreatitis (AP) is a common clinical disease and its incidence has been increasing in recent years. Although most patients experience mild and self-limited AP, some patients have severe acute pancreatitis (SAP)[1-3]. Abnormal activation of digestive enzymes within pancreatic acinar cells is thought to be a critical initiating event[4]. Pancreatic injury leads to a localized and a subsequent systemic inflammatory response which determines the severity of pancreatitis. It may lead to the development of multiple organ dysfunction syndrome (MODS), which is responsible for the mortality rate associated with this disease. The major component of MODS is lung injury, clinically manifested as acute respiratory distress syndrome[5]. So how to ameliorate the injury of pancreatic acinar cells and downstream events is the way to influence the severity of pancreatitis.

MG-132 (Z-Leu-Leu-Leu-aldehyde) is a member of the peptide aldehyde proteasome inhibitors, including calpains, cathepsins and the proteasome[6,7]. Both calpain Ⅰ and proteasome can degrade Iκb and enhance the activity of NF-κb[8]. Cathepsins , especially cathepsin B, has been confirmed to be a key agent for the abnormal activation of digest enzymes within pancreatic acinar cells[9]. MG-132 can also inhibit the activation of NF-κb by blocking Iκb degradation and enhance the expression of heat shock proteins (HSP) that suppress the inflammatory response[10]. In the present study, we used a model of severe acute pancreatitis (SAP) established

by retrograde injection of 5% sodium taurocholate (1 mL/kg) into the pancreatic duct to observe the effect of the proteasome inhibitor MG-132 on severe acute pancreatitis and associated lung injury of rats.

MATERIALS AND METHODS

Animals and materials

Male Sprague-Dawley rats, weighing 250-300 g, were obtained from Experimental Animals Center of Medical College of Xi'an Jiaotong University. The animals were fasted overnight with free access to water and standard rat chow diet before the experiment. Five percent sodium taurocholate was purchased from Sigma, USA. MG-132 was purchased from Calbiochem, Germany. TNF-α ELISA kit was obtained from Genzyme, USA. Serum amylase kit and MPO kit were purchased from Nanjing Jiancheng Company, China.

Induction of acute pancreatitis

The rats were randomly divided into control group, SAP group and MG-132 treatment group (SAP + MG-132), 10 in each group. The animals were anesthetized with ketamine and subjected to a midline laparotomy. A blunt needle was inserted transduodenally into the common biliary-pancreatic duct as previously described[11]. The hepatic duct was closed at the hilum of the liver with a bulldog clamp to prevent backflow. Sodium taurocholate (5% in saline) was infused using a fine needle inserted into 5 mm of the common biliary-pancreatic duct and each rat received a total volume of 1 mL/kg body weight for 1 min. After 5 min, the needle and clamp were removed, and the laparotomy incision was closed. Animals in the treatment group were injected intraperitoneally (ip) with 10 mg/kg MG-132 dissolved in 0.25 mL dimethyl sulfoxide (DMSO). Thirty minutes later, pancreatitis was induced as above. Animals in the control group underwent a sham operation consisting of laparotomy and puncture of the duodenum, under an identical anesthesia. Six hours after duct infusion or sham operation, the animals were killed by depletion and samples were taken for study. Blood was collected by cardiac puncture using heparinized syringes, centrifuged at 4000 r/min for 10 min, and stored at 4℃ for further analysis. The pancreas and lungs were carefully isolated and weighed for subsequent experiments. Tissues for histological examination were isolated, fixed in 10% formalin and embedded in paraffin for sectioning.

Measurement of the ratio of pancreas weight to body weight

Changes in pancreatic weight were assessed for pancreatic interstitial edema. The whole pancreas was removed and weighed. Weight of each pancreatic sample was used to estimate the water content in pancreas as previously described[12], which was presented as a ratio of pancreas/body weight for evaluating the consequence of pancreatic edema.

Water content in lung

For quantification of lung edema, the left lung was resected, blotted dry, and weighed (wet weight). Thereafter, the left lung was desiccated for 24 h at 80℃ and weighed again (dry weight). Water content in the lung was determined by calculating the wet weight/dry weight ratio as previously described[13].

Serum amylase and MPO activity

Serum amylase was assayed with an amylase kit with a kinetic spectrophotometric method according to the manufacturer's instructions. Briefly, the methodology is based on the enzymatic degradation of ethylidene-*p*-nitrophenol-G7 by amylase coupled with glucosidase, thus producing *p*-nitrophenol which exhibits strong absorbance at 405 nm. Enzymatic activity was expressed as units/liter.

Pancreatic and lung myeloperoxidase (MPO) activity, a quantitative indicator of neutrophil infiltration, was assessed according to the instructions from Nanjing Jiancheng Company. Briefly, tissues were thawed, homogenized in a 20 mmol/L phosphate buffer (pH 7.4), centrifuged at 13000 r/min for 10 min at 4℃. The resulting pellet was re-suspended in 50 mmol/L phosphate buffer (pH 6.0) containing 0.5% hexadecyl trimethylammonium bromide. The suspension was subjected to four cycles of freezing and thawing. The sample was then centrifuged at 10000 r/min for 5 min at 4℃. The supernatant was used for MPO assay. The absorbance at 450 nm of the resulting mixture was determined. The MPO activity was expressed as U/g tissue.

TNF-α level in pancreas

TNF-α level in pancreatic cytosolic fractions was measured with a commercial ELISA kit according to the instructions of its manufacturer. The absorbance was read on a microplate reader and concentrations were calculated according to the standard curve.

Histological examination

Paraffin-embedded tissues ware sectioned, stained with hematoxylin and eosin, and assessed by two different attending physicians unaware of the experimental protocol, at Department of Pathology of Xi'an Jiaotong University. The pancreas damage evaluation system was used as previously described[14], the sections were examined and scored on a scale of 0-3 with 0 being normal and 3 being severe. Six characteristics were included, namely the presence of edema, acinar necrosis, inflammatory infiltrate, hemorrhage, fat necrosis, and perivascular inflammation. Five parameters were used as criteria for lung injury, manifested as alveolar thickening, vascular congestion, hemorrhage, edema, and leukocyte infiltration. Each observer was required to give a score from 0 to 2 for each slide according to the criteria mentioned above. A score of 0 indicates that there was no histological damage. A score of 1 indicates only mild or intermediate histological damage in the slides, and a score of 2 was given to the tissue sections with severe morphological deterioration in most of the areas observed.

Statistical analysis

All results are expressed as mean ± SE. Statistical

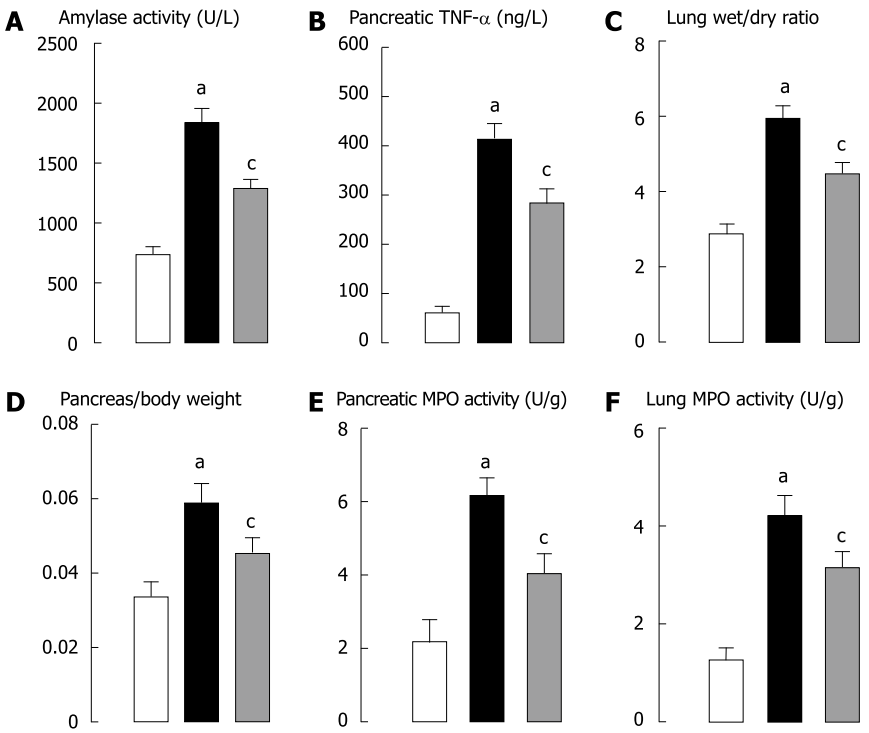

Figure 1 Effect of MG-132 on serum amylase level (**A**), pancreatic TNF-α level (**B**), lung water content (**C**), pancreas/body weight ratio (**D**), and MPO activity in pancreas (**E**) and lung (**F**). Write bars represent the control group (a sham operation consisting of laparotomy and punctrue of the duodenum), black bars represent the SAP group (with retrograde injection of sodium taurocholate into pancreatic duct) and grey bars represent the MG-132 group (10 mg/kg MG-132 ip 30 min before the induction of pancreatitis). $^{a}P < 0.05$ *vs* sham group, $^{c}P < 0.05$ *vs* SAP group.

analysis of data was accomplished by analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Effect of MG-132 on serum amylase activity

Serum amylase activity, a most common indicator for assessing pancreatitis, was markedly increased in the SAP animals (Figure 1A). The effect of MG-132 on pancreatitis was statistically significant.

Effect of MG-132 on pancreatic TNF-α level

In the SAP animals, the concentration of TNF-α was increased (Figure 1B), which could be ameliorated in rats treated with MG-132, showing that TNF-α could improve pancreatitis.

Effect of MG-132 on lung wet/dry weight ratio

The ratio of lung wet/dry weight, a commonly used indicator for estimating the water content in acute lung injury was significantly increased in the SAP group compared with the sham group (Figure 1C). Treatment with MG-132 could reduce the water content lung.

Effect of MG-132 on the ratio of pancreas to body weight

Pancreatic edema, one of the major criteria for assessing pancreatitis was found in our experiment. Injection of 5% sodium taurocholate into the biliary-pancreatic duct of rats could significantly increase the ratio of pancreas to body weight (Figure 1D). Treatment with MG-132 showed a beneficial effect on pancreatic edema.

Effect of MG-132 on pancreatic and lung MPO activity

SAP is associated with a rise in both pancreatic and lung MPO activity, indicating the presence of sequestered neutrophils[15]. Pre-treatment of the animals with MG-132 significantly reduced the MPO activity both in pancreas and in lung (Figure 1E and F).

Effect of MG-132 on pancreatic and lung histology

To assess the effects of MG-132 on local pancreatic injury, the morphology of pancreas was examined and compared with the treatment group. The results showed that the SAP group exhibited severe edema and a high degree of destruction of histoarchitecture of the acini cells. The architecture and integrity of acini cells were improved in the MG-132 group.

Normal lung tissue morphology (Figure 2A) was observed in the sham group. Histological examination of the sections confirmed lung injury with significant alveolate thickening, vasocongestion and infiltration with leukocytes observed in the SAP group (Figure 2B). In contrast, the lung injury was significantly ameliorated in the animals treated with MG-132 (Figure 2C). The scores of histological evaluation of pancreatitis and lung injury are summarized in Table 1.

DISCUSSION

Acute pancreatitis is a life-threatening disease with a high mortality rate, especially in the setting of systemic inflammatory response and multiple organ failure when severe infection of necrosis occurs[16]. Under physiological conditions, digestive enzymes are synthesized by and stored in pancreatic acinar cells as inactive proenzymes known as zymogens which are secreted into the duodenum where enterokinase initiates their activation. The pathogenesis of acute pancreatitis remains obscure. However, it is believed that the premature activation of zymogens within acinar cells is a critical initiating event,

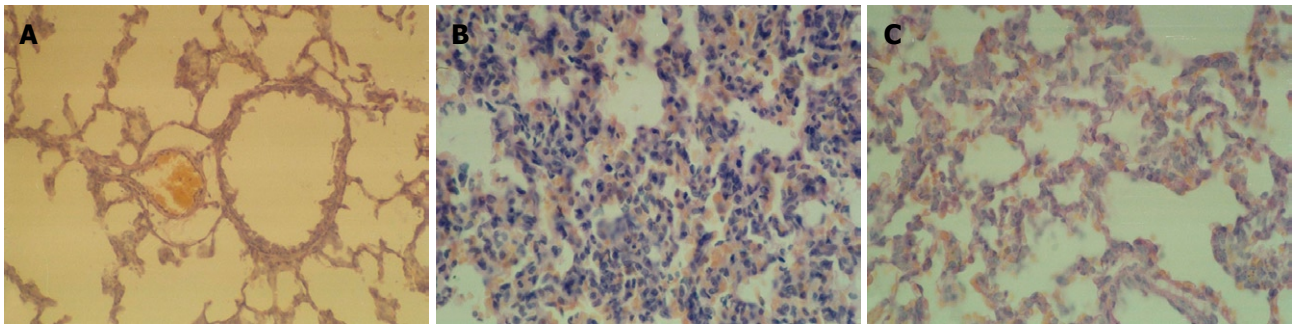

Figure 2 Representative images illustrating histologically observed morphology of pancreas in pulmonary sections (HE, × 200).

Table 1 Effect of MG-132 on histological damage to pancreas and lung

	Sham	SAP	MG-132
Pancreas	0.36 ± 0.04	13.82 ± 1.12[a]	8.33 ± 0.52[c]
Lung	0.23 ± 0.05	7.63 ± 0.65[a]	4.46 ± 0.31[c]

[a]$P < 0.05$ *vs* sham group, [c]$P < 0.05$ *vs* SAP group.

thus leading to auto-digestion of the gland. Afflicted acinar cells release factors that lead to recruitment of inflammatory cells and generation of multiple mediators, such as reactive oxygen species and cytokines[17]. Two potential key elements involved in this process are cathepsin B and NF-κb.

Cathepsin B is a lysosomal hydrolase, which activates human trypsinogen *in vitro* and is redistributed in a zymogen-granule enriched subcellular compartment during the early course of experimental pancreatitis[18,19]. It was reported that inhibition of lysosomal protease cathepsin B can suppress pancreatic inflammation[20,21]. Studies in cathepsin B gene knocked-out mice showed that the premature and intracellular activation of trypsinogen largely depends on the presence of cathepsin B[22].

NF-κb is a member of the transcription factors which, under normal conditions, are coupled to an inhibitor (Iκb) in cytoplasm[23,24]. In response to stress, a cascade of phosphorylation events results in Iκb phosphorylation and degradation by proteasome. NF-κb with subsequent up-regulation of the expression of genes coding for a variety inflammatory factors including cytokines and chemokines such as TNF-α, IL-1, IL-6, IL-8. In acute pancreatitis[25], NF-κb activation can be inhibited by blocking the degradation of Iκb, which has been shown to ameliorate the severity of pancreatitis[26].

During acute pancreatitis, lung injury is associated with the accumulation of neutrophils within the interstitial and alveolar spaces. Leukocyte sequestration within an inflamed area is a multistep process that begins with leukocyte activation[27], followed by the rolling of inflammatory cells and the adhesion of circulating activated inflammatory cells to the endothelium via adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1)[28]. Cytokines, such as IL-1 and TNF-α, can also increase lung injury and spread to other distant organs, indicating that NF-κb plays key role in this proinflammatory process[29-31].

MG-132 is a cell-permeable aldehyde proteasome inhibitor, which has been shown to suppress the inflammatory cascade by decreasing NF-κb activity through blocking Iκb degradation and increasing cellular HSP level[32]. MG-132 can also suppress pancreatic inflammation by inhibiting calpains and cathepsin B.

Since the SAP model established by injecting sodium taurocholate is most similar to the situation in human severe acute pancreatitis[33], we chose it to examine the effect of proteasome inhibitor MG-132 on pancreatitis and lung injury in order to simulate the situation in humans.

In the present study, administration of MG-132 significantly suppressed the elevation of serum amylase, TNF-α and pancreatic MPO activity. Significant improvements were also observed in pancreatic histology after treatment with MG-132. Edema, acinar cell and fat necrosis, perivascular inflammation occurring in almost all inflammatory processes in any organ, were resolved in pancreatic tissues from the animals treated with MG-132.

The group pre-treated with MG-132, as compared with the sham and SAP groups, showed a significant reduction in the lung water content and MPO activity. These results are in close agreement with the histological analysis, suggesting that alveolar thickening, vasocongestion and recruitment of leukocytes in lung tissue can be suppressed with MG-132.

In conclusion, MG-132, a proteasome inhibitor, can ameliorate sodium taurocholate-induced SAP and its associated lung injury.

COMMENTS

Background

Severe acute pancreatitis (SAP) is still a fatal disease and its pathogenesis has not been fully understood. Pathological activation of digestive zymogens within pancreatic acinar cells is considered the key initiator of AP. The effect of MG-132 was investigated in experimental severe acute pancreatitis in the present study.

Research frontiers

Proteasome inhibitors have a broad inhibitory action on pancreatic enzymes and production of proinflammatory cytokines. Therefore, they are expected to prevent necrotic changes in the pancreas and reduce the mortality rate.

Innovations and breakthroughs

The SAP model established by retrograde injection of 5% sodium taurocholate (1 mL/kg) into the pancreatic duct was used to observe the effect of the

proteasome inhibitor MG-132 on severe acute pancreatitis and its associated lung injury of rats. Taurocholate-induced pancreatitis is a reliable model of severe acute pancreatitis rats with significantly greater pancreatic damage and systemic inflammatory response in comparison with cerulein-induced pancreatitis. Pancreatic and lung injury was a distant organ injury which is the key determinant of mortality in AP patients.

Applications

MG-132 shows its protective effect on SAP induced by sodium taurocholate and its associated lung injury of rats. Moreover, proteasome inhibitors may promote further studies on the treatment of AP.

Peer review

This paper describes the effect of MG-132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal), a proteosome inhibitor, on SAP and its associated lung injury of rats. The model selected is appropriate and induces SAP and lung injury. Further researches are needed to explore its mechanism.

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S- Editor Zhong XY **L- Editor** Wang XL **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3254

World J Gastroenterol 2008 May 28; 14(20): 3254-3258
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Change of intestinal mucosa barrier function in the progress of non-alcoholic steatohepatitis in rats

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Received: December 21, 2007 Revised: April 8, 2008
Accepted: April 15, 2008
Published online: May 28, 2008

Abstract

AIM: To explore the change of intestinal mucosa barrier function in the progress of non-alcoholic steatohepatitis (NASH) in rats.

METHODS: Thirty-two Sprague-Dawley (SD) rats were randomly divided into control group and model group. Rats in the control group were given normal diet, and rats in the model group were given fat-rich diet. Eight rats in each group were killed at end of the 8th and 12th wk, respectively. The levels of endotoxin, D-xylose, TG, TC, ALT and AST, intestinal tissue SOD and MDA as well as intestinal mucus secretory IgA (sIgA) were measured. The pathology of liver was observed by HE staining.

RESULTS: At end of the 8th wk, there was no marked difference in the levels of endotoxin, D-xylose and sIgA between the two groups. At end of the 12th wk, rats in the model group developed steatohepatitis and had a higher serum level of endotoxin (P = 0.01) and D-xylose (P = 0.00) and a lower serum level of sIgA (P = 0.007).

CONCLUSION: Intestinal mucosa barrier malfunction may exist in NASH rats and may be an important promoter of NASH in rats.

Key words: Non-alcoholic steatohepatitis; Intestinal mucosa barrier; Endotoxin; Secretory IgA

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Li S, Wu WC, He CY, Han Z, Jin DY, Wang L. Change of intestinal mucosa barrier function in the progress of non-alcoholic steatohepatitis in rats. *World J Gastroenterol* 2008; 14(20): 3254-3258 Available from: URL: http://www.wjgnet.com/1007-9327/14/3254.asp DOI: http://dx.doi.org/10.3748/wjg.14.3254

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a type of non-alcoholic fatty liver disease (NAFLD), and may progress to hepatic fibrosis and cirrhosis[1-3]. The pathogenesis of NASH remains unclear. Nowadays, lipid metabolism abnormality, insulin resistance and oxidative stress and lipid peroxidation reaction[4-8] are thought to play an important role in the pathogenesis of NASH[9,10]. It was reported that the change of intestinal environment may play a role in NASH, which may be a cause of enterogenous endotoxemia[11,12]. Since the relationship between intestinal mucosa barrier function and NASH is uncertain, we established an animal model of NASH by giving fat-rich diet to explore the change of intestinal mucosa barrier function in the progress of NASH.

MATERIALS AND METHODS

Materials

Thirty-two healthy female mice, provided by Nanjing Qinglongshan Experimental Animal Center, were used in this study. D-xylose, SOD and MDA kit were purchased from Nanjing Jiancheng Bioengineering Institute (NJBI). Quantitative chromogenic end-point tachypleus amebocyte lysate kit was purchased from Xiamen Houshiji, Ltd. Secretory IgA (sIgA) kit was purchased from Beijing North Institute of Biological Technology (BNIBT).

Methods

Establishment of animal mode: 32 female Sprague-Dawley (SD) rats, weighing 130-150 g, after a week of adaptive feeding, were randomly divided into model group and control group (16 in each group). Rats in the control group were given normal diet and rats in the model group (n = 16) were given fat-rich diet containing 88% normal diet, 10% lard and 2% cholesterol. All rats were maintained at controlled room temperature in a

12-h light/dark cycle with free access to laboratory feed and water. Eight rats in each group were killed at wk 8, 12 respectively during the study. All rats had no access to food and water for 12 h, but received intra-gastric 5% D-xylose (0.5 mL/100 g, BW) and 0.3% pentobarbital (0.15-0.2 mL/kg) *via* abdominal cavity, 25 min and a short wile, respectively, before they were killed.

Histological evaluation: Liver specimen was obtained from the central part of liver, observed by HE staining, and evaluated according to the guidelines for diagnosis and treatment of nonalcoholic fatty liver diseases revised by Fatty Liver and Alcoholic Liver Study Group of the Chinese Liver Disease Association[13].

Measurement of ALT, AST, TG and TC: Two milliliters blood was taken from abdominal aorta and serum was taken after centrifugation at 4000 r/min for 10 min. The levels of ALT, AST, TG and TC were measured with an automatic biochemical analyzer.

Measurement of D-xylose: Two milliliters blood was taken from abdominal aorta, and collected into a tube (containing heparin) immediately and plasma was taken after the blood was centrifuged at 4000 r/min for 10 min. The Level of D-xylose in plasma was measured with a D-xylose kit.

Measurement of endotoxin: One milliliter blood was taken from portal vein and collected into an apyrogenic tube (containing heparin) immediately. Plasma was taken after the blood was centrifuged at 3000 r/min for 10 min (environmental temperature: 0℃). The levels of endotoxin were measured by limulus amebocyte lysate test.

Detection of sIgA: sIgA was detected as previously described[14]. A 10 cm long tissue was obtained from the small intestine, dissected and washed with normal saline carefully. Intestine mucus was collected into an Eppendorf tube, and centrifuged at 3000 r/min for 10 min (environmental temperature: 0℃) after 1 mL 0.01 mol/L PBS was added. The supernatant was harvested. The level of sIgA was measured by double antibody sandwich immunoradiometric assay. The total protein of intestine mucus was assayed by Bradford brilliant blue method simultaneously. The sIgA content in total protein of one milligram small intestine mucus was detected.

Detection of SOD and MDA in small intestine tissue: The small intestine tissue was weighed to prepare 10% tissue homogenate by adding normal saline according to weighing ratio The homogenate was centrifuged at 3000 r/min for 10 min (environmental temperature: 0℃). The supernatant was harvested to make 1% tissue homogenate by adding normal saline. The levels of SOD and MDA in tissue homogenate were measured.

Statistical analysis

All statistical analyses were performed using SPSS 11.5 software package. All data were expressed as mean ± SD. Group comparison was done by one-factor analysis of variance. $P < 0.05$ was considered statistically significant.

Table 1 Level of serum TG,TC, ALT and AST (mean ± SD, *n* = 8)

Group	Time (wk)	TG (mmol/L)	TC (mmol/L)	ALT (U/L)	AST (U/L)
Control	8	0.72 ± 0.17	1.21 ± 0.29	39.00 ± 7.46	134.88 ± 35.11
Model		0.79 ± 0.20	1.78 ± 0.35[a]	61.75 ± 15.85[a]	96.63 ± 52.80[a]
Control	12	0.76 ± 0.17	1.26 ± 0.25	41.88 ± 6.27	138.00 ± 36.70
Model		0.85 ± 0.18	1.99 ± 0.26[a]	87.75 ± 26.89[a]	248.88 ± 53.09

[a]$P < 0.05$ *vs* control group.

RESULTS

Liver histology

The structure of hepatic lobules and the morphology of liver cells were normal in the control group. Lipid droplets were observed in 50%-75% of hepatic cells in the model group after 8 wk of fat-rich diet, predominantly bullules, consistent with the diagnostic criteria for simple fatty liver disease. Fatty degeneration in hepatic cells exceeded 75% and patch necrosis, mild to moderate chronic inflammation could be seen after 12 wk of fat-rich diet, consistent with the diagnostic criteria for steatohepatitis.

Contents of serum TG, TC, ALT and AST

Serum TC, ALT and AST levels were higher in the model group than in the control group in the 8th, and 12th wk. There was a statistical difference between the two groups ($P < 0.05$). The serum TG level was slightly higher in the model group than in the control group, but there was no statistical difference between the two groups (Table 1).

Level of serum D-xylose, endotoxin and intestine mucus sIgA

At end of the 8th wk, there was no significant difference in the levels of endotoxin, D-xylose and sIgA between the two groups. At end of the 12th wk, rats in the model group developed steatohepatitis and had a higher serum level of endotoxin and D-xylose ($P < 0.05$), but a lower level of sIgA ($P < 0.05$) (Table 2).

Level of SOD and MDA in small intestine tissue

The level of SOD in small intestine tissue was lower in the model group than in the control group in the 8th and 12th wk. There was a statistical difference between the two groups ($P < 0.05$). The level of MDA in small intestine tissue was higher in the model group than in control group in the 8th and 12th wk. There was a statistical difference between the two groups ($P < 0.05$) (Table 3).

Correlation analysis of serum D-xylose, endotoxin and intestine mucus sIgA

A line tendency could be observed in scatter plots bellow. The serum level of endotoxin in portal vein was positively

Table 2 Level of serum D-xylose, endotoxin and intestine mucus sIgA (mean ± SD, n = 8)

Group	Time (wk)	D-xylose (mmol/L)	sIgA (μg/mg)	Endotoxin (EU/mL)
Control	8	0.65 ± 0.21	1.72 ± 0.67	0.267 ± 0.022
Model		0.71 ± 0.17	1.55 ± 0.58	0.272 ± 0.021
Control	12	0.72 ± 0.23	1.64 ± 0.60	0.270 ± 0.023
Model		1.33 ± 0.37[a]	0.78 ± 0.27[a]	0.302 ± 0.020[a]

[a]P < 0.05 *vs* control group.

Table 3 Level of SOD and MDA in small intestine tissue SOD and MDA (mean ± SD, n = 8)

Group	Time (wk)	SOD (U/mgprot)	MDA (nmol/mgprot)
Control	8	83.29 ± 10.56	0.55 ± 0.06
Model		71.61 ± 9.28[a]	0.72 ± 0.05[a]
Control	12	80.79 ± 7.76	0.59 ± 0.04
Model		61.26 ± 7.01[c]	0.93 ± 0.08[c]

[a]P < 0.05, [c]P < 0.05 *vs* control group.

correlated with that of D-xylose in abdominal aorta (r = 0.846, n = 8, P < 0.01) and negatively correlated that of sIgA in intestine mucus (r = -0.873, n = 8, P < 0.01) (Figure 1A and B).

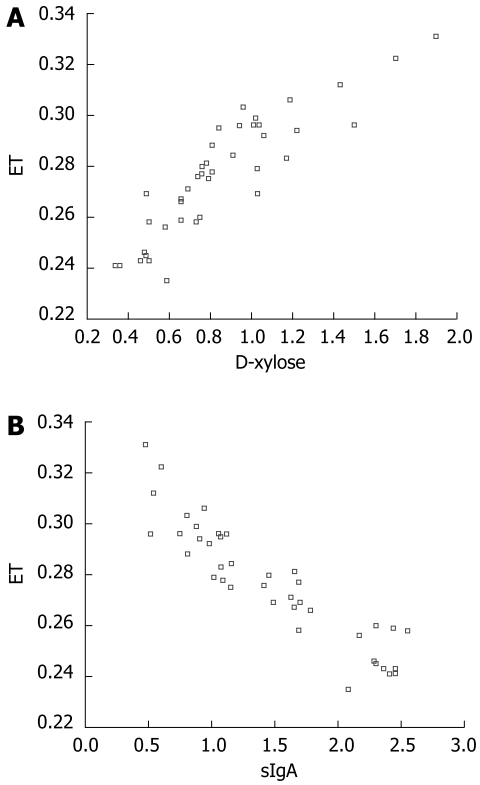

Figure 1 Relationships scatter plot. **A**: ET and D-xylose; **B**: ET and sIgA.

DISCUSSION

At present, the specific pathogenesis and progress of NASH remain unclear. It was reported that there is enterogenous endotoxemia in NASH, suggesting that NASH is closely related to endotoxin[15-17]. Wigg *et al*[11] reported that small intestinal bacterial overgrowth is present in 50% of patients with non-alcoholic steatosis not accompanying increased intestinal permeability or elevated endotoxin levels. Brun P *et al*[12] showed that obese mice with NASH have higher intestinal mucosa permeability and circulating level of endotoxemia in portal vein than the control mice, suggesting that genetically obese mice display an enhanced intestinal permeability, leading to severe endotoxemia in portal vein. Therefore, whether there is an increased intestinal permeability and a change in intestinal mucosa barrier function in NASH patients needs to be further explored.

Intestinal mucosa barriers include mechanical barrier, chemical barrier, immunologic barrier and biology barrier[18-21], any damage of these barriers will damage intestinal mucosa barrier function. In this study, we used plasma D-xylose, endotoxin and intestine mucus sIgA to evaluate the intestinal mucosa barrier function in rats with NASH and to observe its change in NASH rats. Intestine mucus sIgA is a major ingredient of intestinal immunologic barrier, mostly secreted by plasmocytes of intestinal mucosa, and may restrain intestinal bacteria to adhere to intestinal mucosa surface, to counteract toxin, enzyme and virus in the intestinal tract, thus playing an important role in intestinal immunity[22-24]. SOD is the most important anti-oxidation enzyme in anti-oxidation defense system and MDA is the end product of lipid peroxidation, which can cause tissue injury. SOD activity and MDA level can reflect the degree of lipid peroxidation and oxidative stress. It was reported that NASH is closely related to lipid peroxidation and oxidative stress[25-27]. In the present study, we successfully established the NASH model by giving fat-rich diet, and observed the change of intestinal mucosa barrier function in simple fatty liver disease and NASH. The results showed that there was no statistical difference in serum D-xylose, endotoxin and intestine mucus sIgA between the two groups at the 8th wk, suggesting that there might be no damage to the intestinal mucosa barrier at the stage of simple fatty liver disease. However, the SOD activity was decreased in intestinal tissue, while the level of MDA was increased, suggesting that lipid peroxidation and anti-oxidation are imbalanced. There was a significant difference in serum D-xylose, endotoxin and intestine mucus sIgA between the two groups (P < 0.05) at the 12th wk. Serum D-xylose and endotoxin levels were higher in the model group than in the control group, while the intestine mucus sIgA levels were lower in the model group, suggesting that the intestinal mucosa barrier is damaged at the stage of NASH and that the SOD activity is further decreased in the intestinal tissue while MDA level is further elevated and lipid peroxidation reaction is further aggravated. The fact that serum endotoxin level in portal vein was positively correlated with that of serum D-xylose in abdominal aorta, but negatively correlated with that of sIgA in intestine mucus, suggesting that the damage to intestinal mucosa barrier may cause enterogenous endotoxemia.

In our study, no damage to intestinal mucosa barrier occurred at the early stage of nonalcoholic fatty liver disease. With the progress from simple fatty liver disease to NASH, severe damage to intestinal mucosa barrier occurred. The pathogenesis of intestinal mucosa barrier damage is unclear. It may be due to the increased lipid peroxidation reaction in intestinal tissue and intestinal mucosa damage caused by endotoxin[28]. It needs to be further studied. Increased sIgA levels in intestine mucus would lead to the ability of intestinal bacterium to inhibit adherence to intestinal mucosa surface and decrease counteracting toxin, so bacteria and endotoxin are increased in the intestinal tract. Wigg *et al*[11] reported that bacteria grow in small intestine of patients with non-alcoholic steatosis, suggesting that and decreased sIgA promotes overgrowth of bacteria in small intestine. We suppose that small intestinal bacterial overgrowth in small intestine can produce more endotoxin in enteric cavity, thus damaging intestinal mucosa barrier and absorbing more endotoxin, finally leading to enterogenous endotoxemia. It was reported that endotoxin can not only injure hepatic cells but also activate Kupffer cells by combing receptor CD14 and signal receptor TLR4. The activated Kupffer cells release a series of bioactive substances, such as TNF-α, causing hepatic injury, thus aggravating the effect of endotoxin and promoting development of NASH[29-31].

COMMENTS

Background

The pathogenesis of non-alcoholic steatohepatitis (NASH) remains unclear. Insulin resistance, obesity-related inflammation, oxidative stress, microcirculation disturbance, and malnutrition are thought to play a key role in the pathogenesis of NASH. Studies have also demonstrated that change in intestinal environment may also play a role in the pathogenesis of NASH, and may be a cause of enterogenous endotoxemia. It has been found that a higher intestinal permeability may also play a role in the process of NASH. However, it is not accepted that there exists enterogenous endotoxemia in NASH.

Research frontiers

Great effort has been made to clarify the pathogenesis of NASH. The source and pathogenesis of endotoxin in the process are two hot spots.

Innovations and breakthroughs

In this study, the relationship between intestinal mucosa barrier function and NASH was studied.

Applications

The intestinal mucosa barrier malfunction may lead to NASH. There might be a vicious circle between intestinal mucosa barrier malfunction and NASH.

Terminology

NASH is a kind of liver disease which resembles alcoholic liver disease accompanying steatosis, inflammation, necrosis, and fibrosis. Intestinal mucosa barriers include mechanical barrier, chemical barrier, immunologic barrier and biology barrier.

Peer review

This paper explores the change of intestinal mucosa barrier function in the progress of NASH in rats. The well designed study demonstrated that the intestinal mucosa barrier malfunction may exist in NASH rats, and may be an important promoter of NASH in rats.

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S- Editor Li DL **L- Editor** Wang XL **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3259

World J Gastroenterol 2008 May 28; 14(20): 3259-3261
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Multiple giant diverticula of the foregut causing upper gastrointestinal obstruction

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Received: November 8, 2007 Revised: March 31, 2008
Accepted: April 7, 2008
Published online: May 28, 2008

Abstract

Small bowel diverticulosis represents an uncommon disorder (except for Meckel diverticulum) often misdiagnosed since it causes non-specific gastrointestinal symptoms. Most of times the diagnosis is carried out in case of related complications, such as diverticulitis, hemorrhage, perforation or obstruction. Intestinal obstruction can be caused by inflammatory stenosis due to repeated episodes of diverticulitis, volvulus, intussusception or jejunal stones. Herein we report a case of multiple jejunal diverticula causing chronic gastrointestinal obstruction.

Key words: Jejunal diverticula; Chronic symptoms; Gastrointestinal obstruction; Jejunal resection

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Balducci G, Dente M, Cosenza G, Mercantini P, Salvi PF. Multiple giant diverticula of the foregut causing upper gastrointestinal obstruction. *World J Gastroenterol* 2008; 14(20): 3259-3261 Available from: URL: http://www.wjgnet.com/1007-9327/14/3259.asp DOI: http://dx.doi.org/10.3748/wjg.14.3259

INTRODUCTION

Multiple diverticulosis of the foregut is uncommon[1]. Although it is often asymptomatic, it can lead to severe complications, such as obstruction, hemorrhage, diverticulitis and perforation. Obstruction can be caused by inflammatory stenosis due to repeated episodes of diverticulitis, volvulus or intussusception, voluminous jejunal stones or dyskinesia of the small bowel[2-4]. We herein report a case of chronic gastrointestinal obstruction in a patient with a previous diagnosis of jejunal multiple giant diverticula.

CASE REPORT

A 49-year-old woman was admitted to our department, because of abdominal pain and vomiting together with a history of repeated episodes of obstructive gastrointestinal symptoms in the last two years.

In a previous diagnostic work out, she underwent contrast barium that showed multiple giant diverticula in the proximal small bowel tract (Figure 1).

At physical examination, she was dehydrated and her abdomen was distended but soft. A plain X-ray abdominal film showed distended small bowel loops and multiple gas-fluid levels

The actual clinical condition, the long duration of symptoms and the previous diagnosis were all considered indications for surgery.

At the operation, diffuse giant diverticula were observed in the duodenum (Figure 2) and proximal jejunum so that duodenal diverticulectomy and jejunal resection were performed.

No postoperative complication was observed. During the 4-mo follow-up, the patient remained free of GI symptoms.

DISCUSSION

Jejunal diverticulosis is a rare entity with an incidence rate ranging from 0.3%-1.3% in autopsy series to 2.3% of radiographic findings[1].

Like colonic diverticula, small bowel diverticula other than Meckel's, are false diverticula resulting from mucosal herniation at the point where blood vessels penetrate the intestinal wall. This also explains their

Figure 1 Contrast barium study showing multiple giant jejunal diverticula.

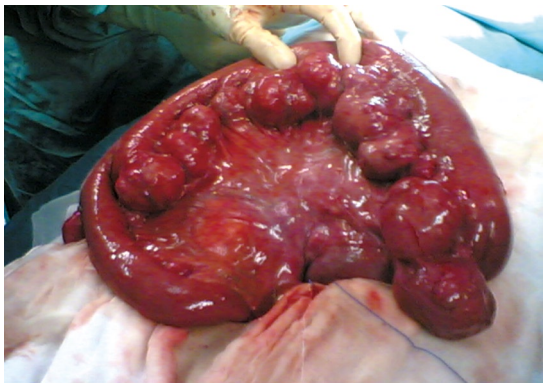

Figure 2 All diverticula arising at the mesenteric border.

typical location at the mesenteric side[2].

Aetiology of jejunal diverticula is still unclear since an anatomic wall defect seems not to be the only factor. Increased intraluminal pressure can play a main role as happens in case of colonic diverticulosis. Small bowel diverticula in fact can be seen in patients older than 50 years with peristaltic disorders, such as progressive systemic sclerosis, visceral myopathy and visceral neuropathies leading to increase of intraluminal pressure[3].

This condition is often misdiagnosed as it is often asymptomatic or causes minor, non-specific gastrointestinal symptoms. Nevertheless, it could lead to severe complications, such as hemorrhage, diverticulitis, perforation and obstruction.

The small number of symptomatic patients may explain chronic post prandial abdominal pain, nausea and vomiting, borborygmi, alternating diarrhea and constipation, and weight loss, and present with anemia, steatorrhea, tenderness, and fever[1,4]. All these symptoms reflect inflammation, malabsorption, hemorrhage or mechanical obstruction.

Hemorrhage and perforation are the consequence of progressive ulceration in case of acute diverticulitis. While perforation is the result of progressive ulceration in case of acute diverticulitis, hemorrhage could be caused by diverticulitis with ulceration, or diverticulosis associated with trauma and irritation, or congenital arteriovenous malformations[5].

Obstruction can be caused by inflammatory stenosis due to repeated episodes of diverticulitis, volvulus or intussusception and voluminous jejunal stones. The latter seem to be caused by the intradiverticular milieu including alteration of the chemical environment and malabsorption, both of which are related to intestinal stasis and stagnant diverticula. Dyskinesia of the small bowel, in fact, causes an intraluminal stasis with bacterial overgrowth leading to deconjugation of bile acids. Deconjugated bile acid, together with choleic acid formed from bile salts and precipitate in aggregates, starts enterolith formation[6,7].

In our patient, intermittent occlusive symptoms were probably caused by hyperdistension of the voluminous diverticula (Figure 2), resulting in external obstruction of jejunal loops.

Asymptomatic diverticula are found only in case of radiography or surgery performed for unrelated causes.

The diagnostic work up in symptomatic patients can start with plain abdominal X-ray film that could show distension of jejunal loops and gas-fluid levels into voluminous diverticula. Upper gastrointestinal X-ray study by barium contrast clearly shows the presence of multiple diverticula.

In case of acute abdomen due to diverticular perforation or intestinal obstruction, X-ray studies show typical signs of these conditions giving no information about the cause that will be recognized at surgery.

The treatment of choice for jejunal diverticulosis, often performed emergently, is resection of all the affected jejunum even in case of perforation or peridiverticular stenosis, in order to avoid further complications[4,8].

In case of obstruction due to an enterolith, some authors suggest conservative management by performing the manual breakage of all stones, intradiverticular and blocking ones, pushing their fragments to the colon[1,4,8,9].

This treatment is to be discouraged because of the persisting risk of stone formation and diverticular complications.

In contrast to jejunal diverticulosis, duodenal diverticula have been treated with simple diverticulectomy. The decision to operate a duodenal diverticulum, however, should be made with great caution because postoperative complications such as fistula formation and pancreatitis are not rare given the periampullary location of many of these diverticula.

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S- Editor Zhong XY **L- Editor** Wang XL **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3262

World J Gastroenterol 2008 May 28; 14(20): 3262-3265
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Acute ischemic colitis during scuba diving: Report of a unique case

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Received: November 7, 2007 Revised: March 19, 2008
Accepted: March 26, 2008
Published online: May 28, 2008

Abstract

The presentation of clinical symptoms due to decompression during diving, varies significantly, as mainly minor disturbances for the gastrointestinal tract in particular have been reported. The following case debates whether diving can cause severe symptoms from the gastrointestinal system. We describe a clinical case of ischemic colitis presented in a 27-year-old male, who manifested abdominal pain while in the process of scuba diving 20 meters undersea, followed by bloody diarrhoea as soon as he ascended to sea level. Taking into account his past medical history, the thorough, impeccable clinical and laboratory examinations and presence of no other factors predisposing to ischemia of the colon, we assume that a possible relationship between diving conditions and the pathogenesis of ischemic colitis may exist. This unusual case might represent a hematologic manifestation of decompression sickness, due to increased coagulability and/or transient air emboli, occurring during a routine scuba diving ascent to sea level.

Key words: Air emboli; Barotraumas; Coagulability; Decompression sickness; Ischemic colitis; Scuba diving

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Goumas K, Poulou A, Tyrmpas I, Dandakis D, Bartzokis S, Tsamouri M, Barbati K, Soutos D. Acute ischemic colitis during scuba diving: Report of a unique case. *World J Gastroenterol* 2008; 14(20): 3262-3265 Available from: URL: http://www.wjgnet.com/1007-9327/14/3262.asp DOI: http://dx.doi.org/10.3748/wjg.14.3262

INTRODUCTION

Diving is a difficult underwater activity in which environmental conditions can affect body structure and function. Barotrauma is caused by compression during descent or expansion during ascent, of the gas filled spaces of the body and it may be associated with pneumothorax, pneumomediastinum and embolism. Decompression sickness may occur when gas, which has dissolved in tissues at depth, eventually produces bubbles which occasionally result in severe cardiorespiratory and neurological emergencies[1].

Approximately 13% of divers complain of gastrointestinal disturbances upon ascent, while most of them present as aerophagy[2]. Rarer, more severe diving-associated gastrointestinal manifestations have been reported in the literature, including a few cases of gastrointestinal barotraumas, mainly gastric rupture[3-6] and a case of small bowel infarction due to thrombosis of mesenteric veins[7]. We describe a clinical case of ischemic colitis in a 27-year-old male admitted to our emergency department, who manifested abdominal pain while he was in the process of scuba diving 20 meters undersea, followed by bloody diarrhoea as soon as he ascended to sea level. Taking into account his past medical history, the thorough impeccable clinical and laboratory examinations and presence of no other factors predisposing to ischemia of the colon, we assume that a possible relationship between the diving conditions and the pathogenesis of ischemic colitis may exist. This case, is as far as we know, the first report relating scuba diving with acute ischemic colitis.

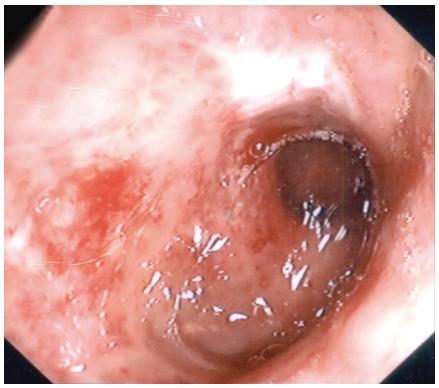

Figure 1 Endoscopic image of patient's ischemic colitis.

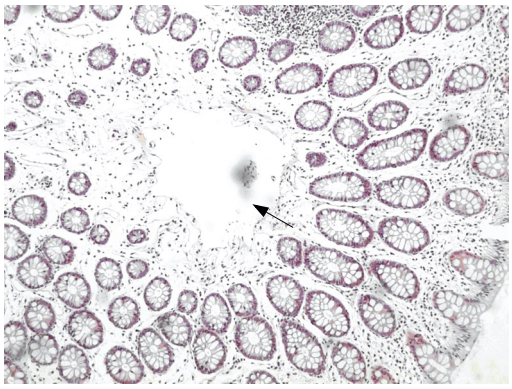

Figure 2 HE staining of colonic mucosa with an air bubble in the lamina propria (arrow).

CASE REPORT

A 27-year-old male novice scuba diver came to the emergency department of our hospital, accompanied by his father, suffering from lower abdominal pain with concurrent bloody diarrhoea. He reported that 2 h earlier, he was submerged for approximately 5 min at a depth of 20 meters when he experienced an acute and intense lower abdominal pain with a simultaneous urge to defecate. Further, he insisted that although under stress, his ascent had been completely normal, under control and in accordance with a scheduled dive plan. Immediately after reaching the surface, he had two loose bowel movements followed by bloody diarrhoea and tenesmus. Our patient was a non-smoker, was taking no medication, and his past medical history was limited to a known Gilbert's syndrome. When admitted to the accident and emergency department his vital signs were: blood pressure of 120/80 mmHg, pulse rate of 72 bpm and respiratory rate of approximately 12 breaths/min. Physical examination revealed a moderate abdominal tenderness at the left lower quadrant and a mild abdominal distention, while per rectum examination confirmed the presence of fresh blood in the lumen with no other apparent physical signs of clinical importance detected.

Laboratory results were: Hematocrit (HCT): 47.1%, white cell counts (WCC): 7.800/μL with 63% neutrophils, 27% lymphocytes, 7% monocytes and 3% eosinophils, platelet (PLT): 236.000/μL, total-bilirubin: 2.73 mg/dL, Direct-bilirubin: 0.25 mg/dL and serum lactic dehydrogenase: 223 U/L. Coagulation tests including prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen, protein S, protein C, antithrombin Ⅲ and V leiden factor were in the normal range. Urea and electrolytes, antiphospholipid antibodies, serum amylase, blood gases and all other tests including urine analysis were also normal. Chest and abdominal X-rays plus ultrasound examination of both upper and lower abdomen did not reveal any pathological findings. Colonoscopy, with inspection of the terminal ileum, performed 24 h later, revealed an edematous mucosa of the sigmoid colon in extent of 20 cm, with redness, superficial ulcerations and submucosal haemorrhages (Figure 1). The histological study of specimens taken from the affected area showed findings compatible with ischemic colitis, while the pathologist noticed presence of air in the lamina propria but not intravascularly (Figures 2 and 3). No air was detected in the colon wall by magnetic resonance tomography performed 2 d after admission.

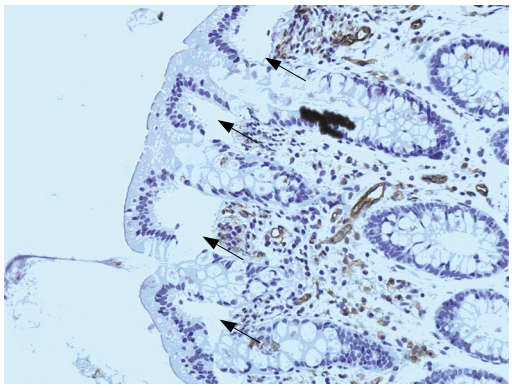

Figure 3 Immunohistochemistry for CD31 revealing lack of staining in the areas of air bubbles in the lamina propria (arrows).

The patient recovered uneventfully with bloody diarrhoea ceasing a few hours after admission and abdominal pain progressively diminishing till disappearance in 48 h. The patient was discharged with complete comeback 4 d after admission. The follow-up colonoscopy two mo later showed complete endoscopic and histologic healing of the mucosa (Figure 4) with the patient free of any clinical symptoms.

DISCUSSION

Abdominal discomfort and belching due to air swallowing are quite frequent manifestations during diving, with more severe gastrointestinal complications scarcely described. Gastric rupture due to expansion of intra-gastric air during a quick ascent to sea level has been described in a few case reports[3-6]. Massive pneumoperitoneum without rupture of an abdominal hollow viscous organ, possibly caused by lung

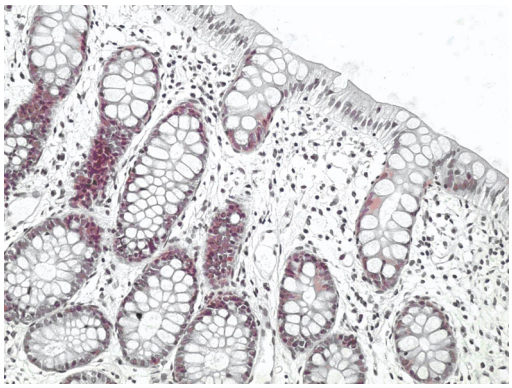

Figure 4 HE staining of normal histologic appearance of our patient's colonic mucosa, 2 mo after the ischemic colitis episode.

barotrauma has also been reported[8]. Gertler *et al* have presented a case of mesenteric vein thrombosis as a unique complication of decompression sickness[7]. Finally, we must emphasize that a confirmed case of acute ischemic colitis in association with scuba diving has never been reported.

In our case, the patient's clinical manifestations of ischemic colitis initiated with acute abdominal pain while at a depth of 20 meters under sea, progressing to bloody diarrhoea on the surface. Several environmental parameters during diving can alter body function and structure[1], as for approximately every 10 meters of descent in sea water ambient pressure increases by 100 kPa (1 bar). The air trapped in body cavities including the gastrointestinal tract, is therefore subjected to compression during descent and expansion during ascent to sea level, which may lead to tissue damage such as gastric rupture[3-6]. Decompression sickness occurs when the partial pressure of gas trapped in the hollow cavities of the body raises in direct proportion to the increase in ambient pressure. This leads to large volumes of inert gas, mainly nitrogen, which dissolve in tissues while at depth transforming to bubbles during ascent to sea level. In addition breathing workload increases due to a combination of increased gas density, increased hydrostatic pressure and altered respiratory mechanisms. Finally, a number of unpredictable events such as malfunction of a diving equipment or technical issues, as well as panic attacks or hypothermia of the diver, may increase the existing physical and/or psychological stress. In our case, except for the arduous and stress inducing conditions during underwater activity, other factors predisposing to ischemic colitis were not identified.

Our patient's past medical history was free of thrombotic events whilst complete examination and evaluation of the patient, including specific blood tests, did not reveal any type of coagulopathy. On the other hand, the pathophysiologic mechanism of decompression sickness could predispose to vascular obstruction and venous infarction[9]. The partial pressure of body gases increases during a scuba dive, resulting in a time-dependent concentration of mainly nitrogen in body fluids and tissues. Bubbles can be formed during ascent due to rapid decrease of barometric pressure. Bubbles are formed predominately in the venous circulation, although in overwhelming decompression sickness they can be found in the arterial circulation also[10], causing vascular obstruction due to coalition. Although bubble formation is considered as the causative mechanism of decompression sickness, a series of heamatological disorders leading to a hypercoagulable state have been described[9,11]. *In vitro* experiments concluded that increased vascular permeability, vascular obstruction due to fat emboli and interaction of the bubble surface with the cellular elements of the blood, may contribute to the pathogenesis of decompression sickness[9,12]. Mesenteric vein thrombosis[7], retinal artery occlusion[13] and vascular obstruction due to fat emboli[14], have previously been reported as unique complications of decompression sickness. The presence of trapped air in the lamina propria, likely of intravascular origin, demonstrated in biopsies taken from the colonic area with ischemic lesions, supports the thesis that decompression sickness was the main cause of our patient's colonic ischemia. Vascular bubbles and bowel wall congestion are the visceral changes described in decompression sickness[15]. It has recently been reported that magnetic resonance imaging (MRI) may be superior to autopsy in the demonstration of gas in intraparenchymal blood vessels of internal organs[16]. Our patient's MRI scan didn't reveal any intravascular air bubbles, which can be attributed to the 48 h delay between the scan and the onset of the acute episode. Finally, a procedure that may lead to rapid alleviation of decompression sickness symptoms[3,6,8] is recompression, which in our case was not implemented due to the rapid clinical improvement and complete recovery of the patient.

In conclusion, this unusual case emphasizes the probability that scuba diving can cause colonic ischemia, even in young patients with no known coagulation disorders or other factors predisposing to colonic ischemia.

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S- Editor Sun YL **L- Editor** Lalor PF **E- Editor** Lu W

Online Submissions: wjg.wjgnet.com
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doi: 10.3748/wjg.14.3266

World J Gastroenterol 2008 May 28; 14(20): 3266-3268
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

A symptomatic cyst of the ligamentum teres of the liver: A case report

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Received: January 30, 2008 Revised: April 16, 2008
Accepted: April 23, 2008
Published online: May 28, 2008

Abstract

Cysts of the liver ligaments are extremely rare and cysts of the ligamentum teres of the liver have been sporadically reported in the literature during the last century. The present report describes a case of a symptomatic patient with a cyst of the ligamentum teres of the liver. The patient presented with right upper quadrant pain and indigestion during the last 2 years. Ultrasound and computed tomography scans revealed a water-density mass attached to the anterior abdominal wall, but definite diagnosis could not be reached. The cyst was completely excised during laparotomy. Cysts of the ligamentum teres of the liver, although infrequent, may produce clinical symptoms and require excision. Ultrasound and computed tomography scan preoperatively cannot rule out malignancy, thus exploratory laparotomy and total resection of these lesions are necessary.

Key words: Ligamentum teres mass; Liver cyst; Right upper quadrant mass; Congenital liver cyst

Peer reviewer: Gianluigi Giannelli, MD, Dipartimento di Clinica Medica, Immunologia e Malattie Infettive, Sezione di Medicina Interna, Policlinico, Piazza G. Cesare 11, Bari 70124, Italy

Lagoudianakis EE, Michalopoulos N, Markogiannakis H, Papadima A, Filis K, Kekis P, Katergiannakis V, Manouras A. A symptomatic cyst of the ligamentum teres of the liver: A case report. *World J Gastroenterol* 2008; 14(20): 3266-3268 Available from: URL: http://www.wjgnet.com/1007-9327/14/3266.asp DOI: http://dx.doi.org/10.3748/wjg.14.3266

INTRODUCTION

Ligamentum teres (or round ligament) of the liver is a cord-like ligament found within the falciform ligament on the inner surface of the anterior abdominal wall and represents a remnant of the umbilical vein, which is a connecting venous structure between the placenta and the umbilical portion of the left portal vein. It is located at the dorsal free margin of the falciform ligament.

Lesions of the liver ligaments are extremely rare and cysts of the falciform ligament have been previously reported[1-3].

The aetiology of these cysts is not well understood yet and their clinical manifestations vary a lot. They may appear completely asymptomatic or may produce palpable masses and pain. The differential diagnosis in such cases includes benign or malignant tumors arising from the liver ligaments or the abdominal wall, fatty masses, disseminated cancer and hepatic lesions.

CT scan, although necessary, may not well define the nature and origin of these masses. Definite diagnosis is made based on laparotomy and pathologic examination of the surgical specimen.

We report a case of a symptomatic patient with a cyst of the ligamentum teres of the liver treated with total excision. The pathologic features of this cyst are also presented.

CASE REPORT

A 57-year old woman was referred to our department due to right upper quadrant pain and episodes of vomiting during the last 2 years. The pain was mild, experienced at irregular periods of time and had no relation to meals. The patient did not have any previous medical history. She was a non-smoker and did not receive any medication in a regular basis. Physical examination was unremarkable. Complete blood cell count, electrolytes,

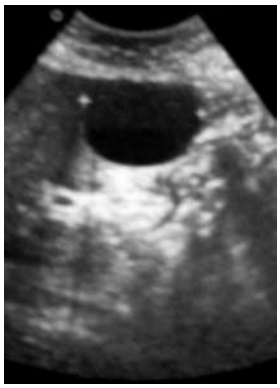

Figure 1 Ultrasound appearance of the cystic abdominal mass.

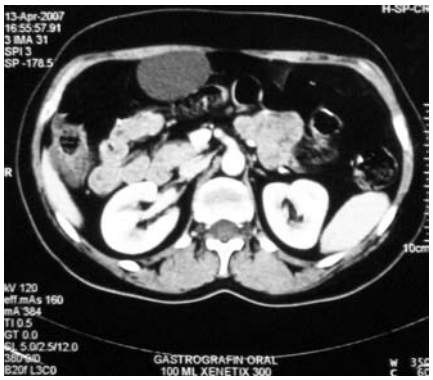

Figure 2 Abdominal CT scan with intravenous contrast media used showing a water-density mass attached to the anterior abdominal wall. A well circumscribed area of low attenuation in contact with the abdominal wall is identified.

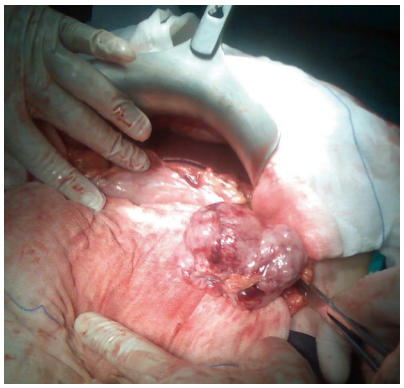

Figure 3 The cyst is shown originating from the ligamentum teres.

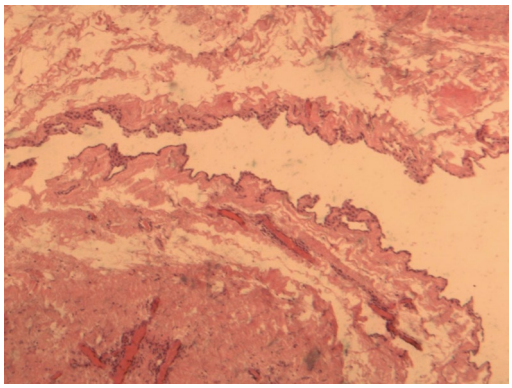

Figure 4 Hematoxylin-eosin staining of the cyst wall shows the single layered cuboidal epithelium (× 40).

eosinophil count, serum biochemistry and urinalysis were within normal limits. Levels of serous neoplasmatic markers, such as carbohydrate antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA 125), and α-fetoprotein (AFP) were normal as well. Anti-echinococcal IgM and IgG antibodies and viral markers for hepatitis B and C were negative. Chest X-ray was normal. Plain radiographs of the abdomen did not reveal any pathological entity. The patient underwent ultrasound examination (US) that showed a 4.7 cm × 3.5 cm cystic mass on the anterior surface of the liver (Figure 1). CT scanning demonstrated the presence of a 5 cm × 4 cm × 7 cm solitary water-density mass, in contact with the right rectus abdominis muscle, showing no enhancement after intravenous contrast media injection (Figure 2). No ascites, lymphnodes or other intra-abdominal masses were found either in the liver or in the peritoneum.

The cyst was removed without rupture by a midline abdominal incision (Figure 3). The cyst's origin was at the attachment between the ligamentum teres and the anterior abdominal wall. It was neither drained nor aspirated during the procedure. An appropriate dissection plane between the cyst and the ligament could easily be found as it was not hard and adhesive. The exploration of the rest of the peritoneal cavity did not reveal any other lesions. Macroscopic examination showed a circumscribed serous cyst, 5 cm in diameter, with a thick fibrous wall of 1 mm. Microscopically, the wall of the lesion was composed of a single layered cuboidal epithelium. No signs of malignancy were identified (Figure 4). The patient's postoperative course was uneventful. The patient was discharged on the second postoperative day. After 6 mo, the patient was well and in good condition with no further symptoms.

DISCUSSION

Unusual lesions reported in the falciform ligament and ligamentum teres of the liver are lipoma[4-6], paraganglioma[7], lymphangioma[8] and leiomyosarcoma[9-14].

Moreover, simple serous cysts of the falciform ligament[1-3,15] and the ligamentum teres of the liver have been sporadically reported in the literature[16-20].

The etiology of liver ligament cysts is diverse and the causes have been classified into primary and secondary[1]. Primary cysts arise during development from congenital defects of mesenteric origin[21]. Secondary cysts are the result of infections (echinococcus, abscesses), trauma (hematomas and biliary leaks) and neoplasms with cystic degeneration. Partial obliteration of the umbilical veins has also been reported to cause falciform ligament cysts[20]. Pathology report suggested the congenital origin of the cyst in our case.

The main symptoms of these patients are unclear and have been reported to include vague abdominal pain and indigestion. Physical examination may sometimes demonstrate a palpable right upper quadrant mass as well.

Our patient was treated with laparotomy to reach a definite diagnosis. Intraoperative findings included a solitary cyst, 5 cm × 5 cm in size, originating from the round ligament of the liver, which was attached to the right rectus abdominis muscle. It was easily dissected since it contained no hard adhesions to the abdominal wall and totally removed. Laparotomy allowed complete inspection of the abdomen to exclude other intra-abdominal masses or lymphnodes.

Ultrasound and computed tomography scans are essential to identify the solid or cystic nature of such lesions but cannot provide a definite diagnosis. In a similar previous report, a falciform ligament cyst has been suggested at CT by a water-density mass at the caudal aspect of the left intersegmental fissure[3]. Nevertheless, a significant number of other benign or malignant lesions have been previously presented as mentioned above.

As a result, the differential diagnosis of a right upper quadrant mass includes several clinical entities and radiological findings can only imply but not ensure a definite diagnosis.

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S- Editor Zhong XY **L- Editor** Wang XL **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3269

World J Gastroenterol 2008 May 28; 14(20): 3269-3272
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Combined choriocarcinoma, neuroendocrine cell carcinoma and tubular adenocarcinoma in the stomach

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Received: October 24, 2007 Revised: April 4, 2008
Accepted: April 11, 2008
Published online: May 28, 2008

Abstract

We described a patient with adenocarcinoma of the stomach combined with choriocarcinoma and neuroendocrine cell carcinoma. An 85-year-old man visited our hospital because of appetite loss. Gastric fiberscopy revealed a large tumor occupying the cardial region and anterior wall of the gastric body. The patient underwent total gastrectomy with lymphnode dissection and partial resection of the liver. Choriocarcinoma, small cell carcinoma and tubular adenocarcinoma existed in the gastric tumor. The choriocarcinomatous foci contained cells positive for beta-subunit of human chorionic gonadotropin (B-hCG) and human placental lactogen mainly in syncytiotrophoblastic cells. The small cell carcinomatous foci contained cells positive for synaptophysin, neuron-specific enolase (NSE), and chromogranin A. The prognosis for gastric adenocarcinoma with choriocarcinoma and neuroendocrine cell carcinoma is exceedingly poor. This patient died about 2 mo after the first complaint from hepatic failure. This is the first reported case of gastric cancer with these three pathological features.

Key words: Gastric cancer; Choriocarcinoma; Neuroendocrine cell carcinoma; Tubular adenocarcinoma; Combined carcinoma

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Hirano Y, Hara T, Nozawa H, Oyama K, Ohta N, Omura K, Watanabe G, Niwa H. Combined choriocarcinoma, neuroendocrine cell carcinoma and tubular adenocarcinoma in the stomach. *World J Gastroenterol* 2008; 14(20): 3269-3272 Available from: URL: http://www.wjgnet.com/1007-9327/14/3269.asp DOI: http://dx.doi.org/10.3748/wjg.14.3269

INTRODUCTION

Primary carcinoma of the stomach is almost always adenocarcinoma or signet ring cell carcinoma and there have been few reports of choriocarcinoma[1-5] or neuroendocrine cell carcinoma[6-9]. We report a patient with adenocarcinoma of the stomach combined with choriocarcinoma and neuroendocrine cell carcinoma. This is the first reported case of gastric cancer with these three pathological features.

CASE REPORT

An 85-year-old man was admitted to Kouseiren Takaoka Hospital because of appetite loss in March 2004. He had been treated for hypertension and gout in another hospital. His family history was negative for family and hereditary disease. On examination, the patient was pale because of severe anemia, and had an ill-defined mobile left hypochondrial mass, approximately 10 cm in size. Findings for the chest and heart were normal. Lymphadenopathy, hepatomegaly, and splenomegaly were not observed and the testes and breasts were normal. Blood examination showed severe anemia, leukocytosis, and platelet count was increased. The level of serum carcinoembryonic antigen (CEA) was slightly elevated (Table 1). Radiographic examination of the

Table 1 Laboratory data of the patient on admission

	Data
CBC	
WBC	12100/μL
RBC	234 × 10^4/μL
Hb	6.3 g/dL
Ht	20.8%
Plts	51.9 × 10^4/μL
Blood chemistry	
T-Bil	0.4 mg/dL
D-Bil	0.4 mg/dL
AST	22 IU/L
ALT	13 IU/L
LDH	260 IU/L
ALP	365 IU/L
ZTT	10.9 K-U
TTT	3.6 M-U
Ch-E	61 IU/L
γ-GTP	27 IU/L
T-AMY	189 IU/L
CPK	41 IU/L
Na	135 mEq/L
K	4.2 mEq/L
Cl	102 mEq/L
Ca	8.4 mg/dL
Fe	16 μg/dL
BUN	20.3 mg/dL
Cr	1.3 mg/dL
UA	4.2 mg/dL
Tch	141 mg/dL
TG	92 mg/dL
FBS	123 mg/dL
TP	6.2 g/dL
Alb	3.1 g/dL
Tumor marker	
AFP	7.5 ng/mL
CEA	5.4 ng/mL
CA19-9	< 2.0 ng/mL

upper gastrointestinal tract demonstrated a Borrmann type 1 tumor in the cardia. Gastric fiberscopy revealed a large tumor occupying the cardial region and anterior wall of the gastric body accompanied by areas of hemorrhage. Tumor invasion to the esophagus was highly suspected. Biopsy specimens were interpreted as showing adenocarcinoma without features of choriocarcinoma or neuroendocrine cell carcinoma. Contrast-enhanced computed tomography (CT) of the abdomen showed a 7-cm low-density tumor suspected to be regional lymph node metastasis. Liver and lung metastases and abnormality with his testes and breast were not detected radiologically. The patient underwent total gastrectomy on March 15, 2004, with the preoperative diagnosis of primary gastric carcinoma. Liver metastasis, peritoneal dissemination, and ascites were not investigated, and distant metastasis to other organs was not present. There was an invasive tumor encircling the gastric body and cardia and this tumor was invading the liver. Total gastrectomy with lymphnode dissection and partial resection of the liver were performed. The Roux-en-Y method of reconstruction was performed after resection.

Gross findings

The resected specimen included an elevated tumor with ulcer measuring 12.0 cm × 11.5 cm in the cardia, the body of the stomach, and abdominal esophagus, and the tumor had invaded the liver. Cut surfaces of the tumor showed two different features, a hemorrhagic brown area and a whitish-yellow area. Most of the tumor was composed of the hemorrhagic brown area (Figure 1).

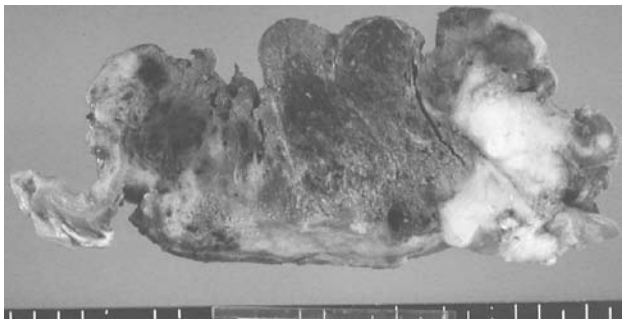

Figure 1 Cut surfaces of the tumor demonstrate two different features, a hemorrhagic brown area and a whitish-yellow area.

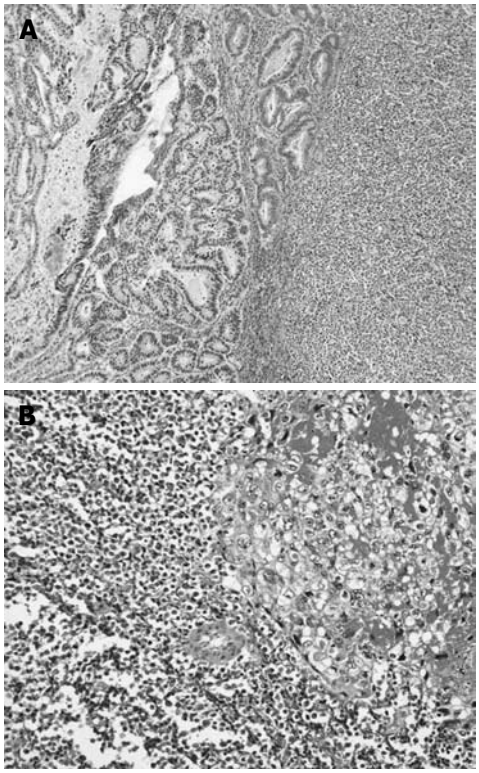

Figure 2 **A:** The hemorrhagic brown area is composed of choriocarcinoma; **B:** The whitish-yellow area contains small cell carcinoma.

Histopathological findings

The hemorrhagic brown area was composed of choriocarcinoma, and consisted mostly of clusters of cytotrophoblastic cells separated by steaming masses of syncytiotrophoblasts (Figure 2A). Cytotrophoblastic cells were small cells with large, oval nuclei, and syncytiotrophoblasts were large cells with bizzare nuclei. The whitish-yellow area contained small cell carcinoma, consisting of small amounts of cytoplasm with large nuclei (Figure 2B) and tubular adenocarcinoma.

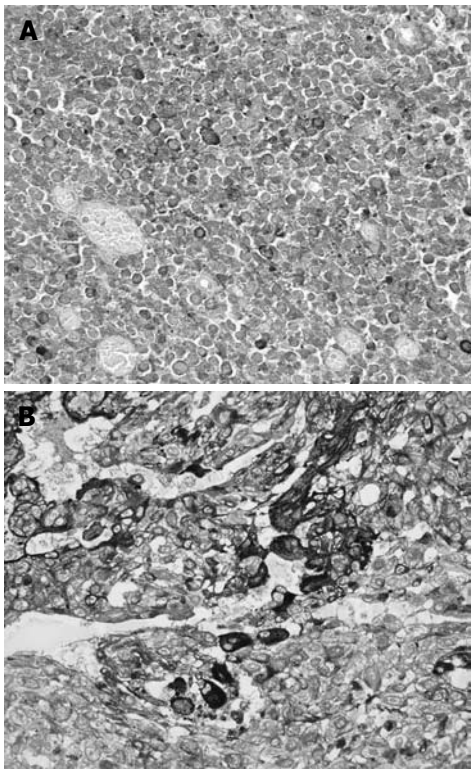

Figure 3 **A:** The choriocarcinomatous foci contain cells positive for B-hCG; **B:** The small cell carcinomatous foci contain cells positive for chromogranin A.

Immunohistochemical findings

The choriocarcinomatous foci contained cells positive for beta-subunit of human chorionic gonadotropin (B-hCG) and human placental lactogen mainly in syncytiotrophoblastic cells (Figure 3A). These findings enabled us to diagnose these cells as choriocarcinoma. The small cell carcinomatous foci contained cells positive for synaptophysin, neuron-specific enolase (NSE), and chromogranin A (Figure 3B). From these results, we diagnosed them as neuroendocrine cell carcinoma.

Outcome

The patient was discharged uneventfully 3 wk after surgery. He presented to our hospital with general malaise 2 wk after discharge. CT revealed multiple liver tumors, and his serum hCG level was 67 000 IU/mL. The liver tumor progressed, the patient died eventually from hepatic failure 6 wk after operation.

DISCUSSION

Choriocarcinoma can be gonadal or extragonadal in origin, and most often arises in the uterus in association with pregnancy[10]. The most common sites for extragonadal tumors are the mediastinum, ovary and testis[11]. There are many reported cases with metastatic choriocarcinoma to the stomach[12], but primary choriocarcinomas of the stomach are extremely rare. Primary neuroendocrine carcinomas are known to arise in the stomach, although they are also rare. Motoyama *et al*[13] reported a case of combined choriocarcinoma, hepatoid carcinoma, small cell carcinoma, and tubular adenocarcinoma in the esophagus in 1995, but there has been no reported case of combined choriocarcinoma, neuroendocrine carcinoma and tubular adenocarcinoma in the stomach in the English-language literature.

There are several theories of the histopathogenesis of primary choriocarcinoma of the stomach. These hypotheses include origin from a gonadal angle displaced in the abdomen[14], histological resemblance to choriocarcinoma[10], origin from an underlying gastric teratoma[15], and the retrodifferentiation or opisthoplatia of carcinoma cells to the level of the embryonal ectoderm with the ability to form trophoblasts[16]. The finding that gastric choriocarcinomas are frequently accompanied by adenocarcinoma is supported by this retrodifferentiation theory. In the present case, choriocarcinomas, neuroendocrine carcinomas and tubular adenocarcinoma existed in the same tumor of the stomach, and this finding suggests that choriocarcinoma and neuroendocrine carcinoma represent aberrant differentiation in common adenocarcinoma.

In the present case, we failed to diagnose adenocarcinoma combined with choriocarcinoma and neuroendocrine carcinoma before operation based on pathological examination of biopsy specimens. Therefore, larger biopsy specimens from the whole tumor should be taken when encountering large and hemorrhagic tumors so that pathologic components are not missed.

It is well known that choriocarcinomas and neuroendocrine carcinomas readily metastasize to distant organs and carry a poor prognosis because effective regimens have not been established. Further studies to establish new regimens are required.

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S- Editor Li DL **L- Editor** Ma JY **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi: 10.3748/wjg.14.3273

World J Gastroenterol 2008 May 28; 14(20): 3273-3275
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Pneumatosis cystoides intestinalis after fluorouracil chemotherapy for rectal cancer

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Received: February 20, 2008 Revised: April 30, 2008
Accepted: May 6, 2008
Published online: May 28, 2008

Abstract

Pneumatosis cystoides intestinalis (PCI) is a relatively rare condition characterized by intraluminal gas in the gastrointestinal tract. Several chemotherapeutic agents have been reported to be associated with PCI, although fluorouracil-related PCI is extremely rare. We report a case of a 76-year old man who received adjuvant chemotherapy for rectal cancer with fluorouracil (FU) and leucovorin (LV). After 1 cycle of the treatment, he presented with diarrhea and abdominal pain. Abdominal radiogram revealed the presence of free air under the diaphragm and intramural gas in the intestine. Laparotomy was performed, showing a suspected diagnosis of perforation in the gastrointestinal tract. Intraoperative findings revealed pneumatosis of the intestine without evidence of perforation. He was treated supportively and his symptoms improved. In conclusion, we should consider the possibility of PCI occurring in patients with malignancies during chemotherapy treatment.

Key words: Pneumatosis cystoides intestinalis; Chemotherapy; Fluorouracil; Colorectal cancer

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Mimatsu K, Oida T, Kawasaki A, Kano H, Kuboi Y, Aramaki O, Amano S. Pneumatosis cystoides intestinalis after fluorouracil chemotherapy for rectal cancer. *World J Gastroenterol* 2008; 14(20): 3273-3275 Available from: URL: http://www.wjgnet.com/1007-9327/14/3273.asp DOI: http://dx.doi.org/10.3748/wjg.14.3273

INTRODUCTION

Fluorouracil (FU) is one of the most commonly used chemotherapeutic agents in clinical oncology regimens. With regard to colorectal cancer, treatment involving FU with leucovorin (LV) can improve the survival, tumor response and quality of life[1] of patients. We report a case of pneumatosis cystoides intestinalis (PCI) in a patient who received adjuvant chemotherapy with 5-FU and l-LV[2]. To our knowledge, FU-related or FU-induced PCI has not been reported previously. This case will add to the reported series of patients with FU-induced small bowel toxicity[3,4] and chemotherapy-related PCI[5-9].

CASE REPORT

A 76-year-old male underwent anterior resection for stage Ⅲ rectal cancer. He received an adjuvant chemotherapy protocol comprising intravenous bolus injection of 600 mg/m^2 5-FU at 1 h after the initiation of 2 h-long 250 mg/m^2 l-LV infusion, once a week for 6 wk, followed by 2 wk of rest[2]. After 1 cycle of this treatment, the patient presented with diarrhea and abdominal pain. Although his abdomen was distended, he did not exhibit any peritoneal signs. He was afebrile and had no neutropenia. His stool culture was negative. An abdominal radiogram revealed the presence of free air under the diaphragm and intramural gas in the entire intestine (Figure 1). Abdominal computed tomography (CT) revealed the presence of free air in the intestinal wall, retroperitoneal space (Figure 2A), and falciform ligament (Figure 2B). Since bowel perforation was strongly suspected, an emergency operation was performed. Laparotomy revealed pneumatosis of the intestine (Figure 3) and colon, and pneumoretroperitoneum without evidence of perforation. Therefore, gastrostomy was performed to reduce the pressure in the bowel. PCI was

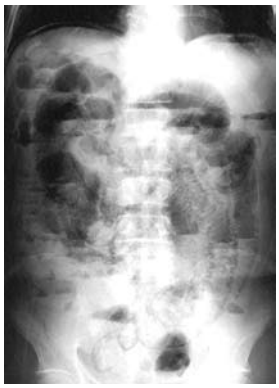

Figure 1 Abdominal radiogram showing intraluminal gases in the entire small intestine and free air under the diaphragm.

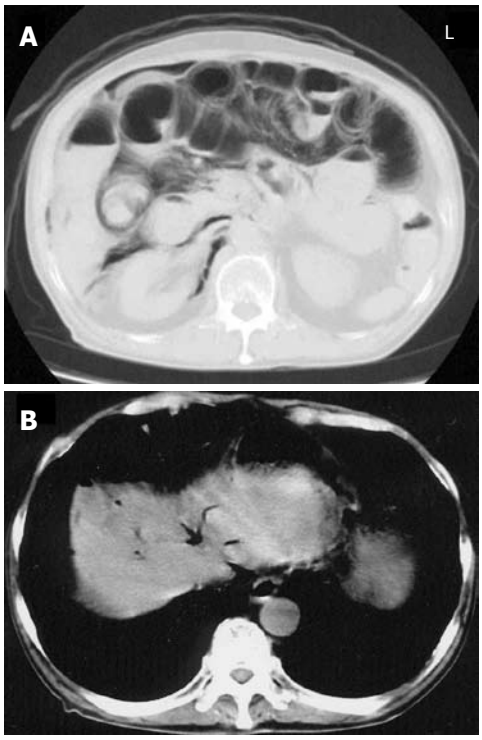

Figure 2 Abdominal CT scan showing excessive intraluminal gases in the entire small intestine and free air in the retroperitoneal space (**A**), and free air in the falciform ligament (**B**).

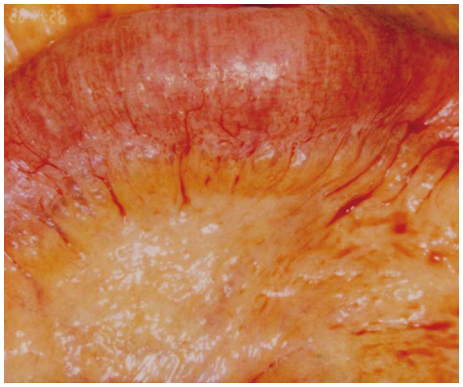

Figure 3 Expanded intraluminal air spaces in the small intestine and mesenterium during intra-operation.

disappeared within 2 wk after parenteral nutrition, antibiotic treatment and oxygen therapy. Enema showed no incidence of anastomotic stenosis and he administered oral uracil-ftorafur, and no recurrence of PCI was observed during the 1-year follow-up.

DISCUSSION

PCI is relatively rare condition characterized by multiple intraluminal gases existing in any part of the gastrointestinal tract. The mechanism and etiology of PCI are not fully understood. According to most hypotheses, mechanical and bacterial factors are the predominant causes for PCI[10-12]. However, in this present case, no mechanical or bacterial factors, including bowel ischemia, bowel obstruction[13,14], inflammatory bowel disease and infectious colitis, for the gas production in the intestinal wall were observed.

Several chemotherapeutic agents have been reported to be associated with PCI, including cyclophosphamide, cytarabine, vincristine, doxorubicin, daunorubicin, etoposide, docetaxel, irinotecan and cisplatin[5-9]. Although fluorouracil-related PCI has not been previously described, the cytotoxic effect of chemotherapy on the epithelial bowel can also play a role in the pathogenesis of PCI[7]. Because the intestinal mucosa is highly proliferative, mucosal damage occurs easily during chemotherapy[6]. Moreover, the chemotherapeutic agent might interfere with the mucosal integrity of the intestinal tract, resulting in extensive intramural air[8]. Tamura *et al*[15] reported that PCI following chemotherapy might be due to depletion of submucosal lymphoid tissue or leukemic infiltrates, such as denuded Peyer's patches producing mucosal defects, thereby permitting entry of gas into the bowel wall. It was reported that chemotherapy-related PCI occurs due to immunosuppressive treatment for hematological malignancies[5,6]. Neutropenia is an important factor for the development of PCI[5-9]. However, the current patient did not suffer from neutropenia before or when PCI was diagnosed.

Several studies have reported severe erosion and superficial ulceration in the ileum after chemotherapy comprising 5-FU and LV in colon cancer patients[3,4]. The mechanisms are thought to be multifactorial, including alteration in the local mucosal blood flow and thrombogenic and vasospastic effects of 5-FU on the vascular epithelium[5]. The mechanism underlying 5-FU-induced PCI is thought to be multifactorial, including bowel toxicity caused by 5-FU itself.

In conclusion, although PCI is a rare complication of chemotherapy, the possibility of PCI occurring in patients undergoing chemotherapy should be kept in mind.

ACKNOWLEDGMENTS

The written consent was obtained from the patient for publication of this case report.

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World J Gastroenterol 2008 May 28; 14(20): 3276
World Journal of Gastroenterology ISSN 1007-9327

ACKNOWLEDGMENTS

Acknowledgments to Reviewers of *World Journal of Gastroenterology*

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

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World J Gastroenterol 2008 May 28; 14(20): 3277
World Journal of Gastroenterology ISSN 1007-9327

Meetings

Events Calendar 2008-2009

FALK SYMPOSIA 2008
January 24-25, Frankfurt, Germany
Falk Workshop: Perspectives in Liver
Transplantation

International Gastroenterological Congresses 2008
February 14-16, Paris, France
EASL-AASLD-APASL-ALEH-IASL Conference Hepatitis B and C virus resistance to antiviral therapies
www.easl.ch/hepatitis-conference

February 14-17, Berlin, Germany
8th International Conference on New Trends in Immunosuppression and Immunotherapy
www.kenes.com/immuno

February 28, Lyon, France
3rd Congress of ECCO - the European Crohn's and Colitis Organisation
Inflammatory Bowel Diseases 2008
www.ecco-ibd.eu

February 29, Québec, Canada
Canadian Association of Gastroenterology
E-mail: general@cag-acg.org

March 10-13, Birmingham, UK
British Society of Gastroenterology Annual Meeting
E-mail: BSG@mailbox.ulcc.ac.uk

March 14-15, HangZhou, China
Falk Symposium 163: Chronic Inflammation of Liver and Gut

March 23-26, Seoul, Korea
Asian Pacific Association for the Study of the Liver
18th Conference of APASL: New Horizons in Hepatology
www.apaslseoul2008.org

March 29-April 1, Shanghai, China
Shanghai-Hong Kong International Liver Congress
www.livercongress.org

April 05-09, Monte-Carlo (Grimaldi Forum), Monaco
OESO 9th World Congress, The Gastro-esophageal Reflux Disease: from Reflux to Mucosal Inflammation-Management of Adeno-carcinomas
E-mail: robert.giuli@oeso.org

April 9-12, Los Angeles, USA
SAGES 2008 Annual Meeting - part of Surgical Spring Week
www.sages.org/08program/html/

April 18-22, Buenos Aires, Argentina
9th World Congress of the International Hepato-Pancreato Biliary Association
Association for the Study of the Liver
www.ca-ihpba.com.ar

April 23-27, Milan, Italy
43rd Annual Meeting of the European Association for the Study of the Liver
www.easl.ch

May 2-3, Budapest, Hungary
Falk Symposium 164: Intestinal Disorders

May 18-21, San Diego, California, USA
Digestive Disease Week 2008

May 21-22, California, USA
ASGE Annual Postgraduate Course
Endoscopic Practice 2008: At the Interface of Evidence and Expert Opinion
E-mail: education@asge.org

June 4-7, Helsinki, Finland
The 39th Nordic Meeting of Gastroenterology
www.congrex.com/ngc2008

June 5-8, Sitges (Barcelona), Spain
Semana de las Enfermedades Digestivas
E-mail: sepd@sepd.es

June 6-8, Prague, Czech Republic
3rd Annual European Meeting: Perspectives in Inflammatory Bowel Diseases
E-mail: meetings@imedex.com

June 10-13, Istanbul, Turkey
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June 11-13, Stockholm, Sweden
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June 13-14, Amsterdam, Netherlands
Falk Symposium 165: XX International Bile Acid Meeting. B ile Acid Biology and Therapeutic Actions

June 13-14, Prague, Czech Republic
Central and Eastern European Conference on Colorectal "Cancer" Screening, Prevention and Management
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June 25-28, Barcelona, Spain
10th World Congress on Gastrointestinal Cancer
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June 25-28, Lodz, Poland
Joint Meeting of the European Pancreatic Club (EPC)
and the International Association of Pancreatology (IAP)
E-mail: office@epc-iap2008.org
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June 26-28, Bratislava, Slovakia
5th Central European Gastroenterology Meeting
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ILTS 14th Annual International Congress
www.ilts.org

September 10-13, Budapest, Hungary
11th World Congress of the International Society for Diseases of the Esophagus
E-mail: isde@isde.net

September 13-16, New Delhi, India
Asia Pacific Digestive Week
E-mail: apdw@apdw2008.net

III FALK GASTRO-CONFERENCE
September 17, Mainz, Germany
Falk Workshop: Strategies of Cancer Prevention in Gastroenterology

September 18-19, Mainz, Germany
Falk Symposium 166:
GI Endoscopy - Standards & Innovations

September 18-20, Prague, Czech Republic
Prague Hepatology Meeting 2008
www.czech-hepatology.cz/phm2008

September 20-21, Mainz, Germany
Falk Symposium 167:
Liver Under Constant Attack - From Fat to Viruses

September 24-27, Nantes, France
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www.escp.eu.com

October 8-11, Istanbul, Turkey
18th World Congress of the International Association of Surgeons,
Gastroenterologists and Oncologists
E-mail: orkun.sahin@serenas.com.tr

October 18-22, Vienna, Austria
16th United European Gastroenterology Week
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October 31-November 4, Moscone West Convention Center, San Francisco, CA
59th AASLD Annual Meeting and Postgraduate Course
The Liver Meeting
Information: www.aasld.org

November 6-9, Lucerne, Switzerland
Neurogastroenterology & Motility
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E-mail: ngm2008@mci-group.com
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November 12, Santiago de Chile, Chile
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November 28-29, Cairo, Egypt
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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462]

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

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

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of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325]

Issue with no volume

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