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[1]Passed away on October 20, 2007
[2]Passed away on June 14, 2008

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Abstract

Idiopathic achalasia is a rare primary motility disorder of the esophagus. The classical features are incomplete relaxation of a frequently hypertensive lower esophageal sphincter (LES) and a lack of peristalsis in the tubular esophagus. These motor abnormalities lead to dysphagia, stasis, regurgitation, weight loss, or secondary respiratory complications. Although major strides have been made in understanding the pathogenesis of this rare disorder, including a probable autoimmune mediated destruction of inhibitory neurons in response to an unknown insult in genetically susceptible individuals, a definite trigger has not been identified. The diagnosis of achalasia is suggested by clinical features and confirmed by further diagnostic tests, such as esophagogastroduodenoscopy (EGD), manometry or barium swallow. These studies are not only used to exclude pseudoachalasia, but also might help to categorize the disease by severity or clinical subtype. Recent advances in diagnostic methods, including high resolution manometry (HRM), might allow prediction of treatment responses. The primary treatments for achieving long-term symptom relief are surgery and endoscopic methods. Although limited high-quality data exist, it appears that laparoscopic Heller myotomy with partial fundoplication is superior to endoscopic methods in achieving long-term relief of symptoms in the majority of patients. However, the current clinical approach to achalasia will depend not only on patients' characteristics and clinical subtypes of the disease, but also on local expertise and patient preferences.

Key words: Achalasia; Esophageal motility disorder; Dysphagia; Esophagus; Lower esophageal sphincter; Pneumatic dilation; Botulinum toxin; Heller myotomy

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INTRODUCTION

Idiopathic achalasia is a rare primary motility disorder of the esophagus. The classical features are incomplete relaxation of a frequently hypertensive lower esophageal sphincter (LES) and a lack of peristalsis in the tubular esophagus. Although major strides have been made in understanding the pathogenesis of this rare disorder, including a probable autoimmune mediated destruction of inhibitory neurons in response to an unknown insult in genetically susceptible individuals, a definite trigger has not been identified. The motor abnormalities of achalasia are responsible for a number of clinical symptoms with variable response to current treatment options. Current therapies should be based on the results of clinical findings and further diagnostic tests, such as imaging studies, esophagogastroduodenoscopy (EGD), manometry, and possibly high resolution manometry (HRM). This editorial will review the clinical presentation, the latest diagnostic tools and the treatment options for this rare disorder and an individualized therapeutic approach, based on the current evidence, will be suggested.

CLINICAL PRESENTATION

The hallmark of achalasia (Greek: failure to relax) is dysphagia for solids and liquids in up to 100% and 97% of patients, respectively[1-3]. As a result of stasis and retention of food and liquids in the esophagus, patients frequently experience weight loss (30%-91%), chest pain (17%-95%), regurgitation (59%-64%), and nocturnal cough (11%-46%)[1]. Difficulty with belching might result from alteration of the upper esophageal belch reflex[4]. Patients might frequently complain of heartburn. Although heartburn is the cardinal symptom

of gastroesophageal reflux, which is the antithesis of achalasia, it occurred with a frequency of 72% in one study, even after the onset of dysphagia[5]. The sensation of heartburn in patients with achalasia might be explained by retention of acidic or noxious food contents or by lactate production from bacterial fermentation within the esophagus[6]. Hiccups can also occur, and probably result from esophageal distention and stimulation of afferent vagal fibers[7]. The distribution of symptoms can differ in the population studied. Chest pain occurs predominantly in younger patients (mean age 40 years) and appears to improve over time[8]. However, neither manometric, nor radiographic findings predict the occurrence of retrosternal pain. Most symptoms do not appear to have a specific gender distribution, although in Iranian patients, chest pain appeared to be more common among females[9]. Physicians need to be aware of the spectrum of symptoms of achalasia, because diagnostic delays for years after the onset of symptoms appear to be due to misinterpretation of typical findings, rather than atypical presentations[10].

The most common extraesophageal manifestations of achalasia are pulmonary complications. Structural or functional pulmonary abnormalities occur in more than half of patients and might be due to recurrent aspiration or tracheal compression from a dilated esophagus[11]. In cases of extreme dilation and distortion of the cervical esophagus, a "bull frog neck" appearance can develop, leading to tracheal obstruction above the larynx and associated stridor[2]. Although some investigators have observed delayed gastric emptying or gallbladder dysfunction in patients with achalasia[12-14], others were unable to confirm these observations[15], and it still remains elusive whether a selective defect of vagal ganglionic neurons might affect other parts of the gastrointestinal tract as well.

DIAGNOSIS AND CLINICAL VARIANTS

A number of tests are available to confirm the diagnosis of achalasia, once the clinical suspicion arises. Radiographic studies, EGD and esophageal manometry are the primary tools of investigation. Although EGD appears normal in 44% of patients with achalasia[1], it might show esophageal dilatation and retention of food or secretions. During inversion of the endoscope in the stomach, tight adherence of the distal esophagus with downward motion of the gastroesophageal junction upon endoscope advancement can sometimes be visualized. However, despite the commonly elevated LES pressure, the esophagogastric junction can usually be traversed easily, and firm resistance should raise the suspicion of neoplastic infiltration or other causes of pseudoachalasia. Pseudoachalasia can mimic all endoscopic, radiographic and manometric findings of achalasia and has a broad differential diagnosis of neoplastic and non-neoplastic causes, which have been recently reviewed[16,17]. Infiltration of the esophageal myenteric plexus by neoplastic cells or paraneoplastic processes have been suggested in patients with a malignant etiology[16]. A shorter duration of symptoms and older age at presentation should raise a suspicion of pseudoachalasia, which often requires further testing with endoscopic ultrasound or CT scan to rule out malignancy[17]. In our view, a simple and non-invasive initial test to differentiate between primary and secondary achalasia is transabdominal ultrasonography, which often allows a clear visualization of the gastric cardia and its surrounding structures[18]. However, a negative ultrasound does not always exclude pseudoachalasia, and CT or other cross-sectional imaging should be added if clinical suspicion remains strong.

Table 1 Manometric variants of achalasia

Standard Manometry
Vigorous achalasia (high amplitude esophageal body contractions)
A short segment of esophageal body aperistalsis
Retained complete deglutitive LES relaxation with aperistalsis
Intact transient LES relaxation with aperistalsis
High resolution manometry (patients with impaired EGJ relaxation)
Type Ⅰ: Minimal esophageal pressurization
Type Ⅱ: Esophageal pressurization > 30 mmHg
Type Ⅲ: Esophageal spasm

LES: Lower esophageal sphincter; EGJ: Esophagogastroduodenoscopy.

A barium esophagogram (barium swallow) is the most commonly used initial diagnostic study. It classically shows a typical smooth tapering of the distal esophagus ("bird's beak") with proximal dilation of the esophagus and lack of peristalsis during fluoroscopy. The value of obtaining a timed barium esophagogram in patients with achalasia lies in its potential to monitor the success of therapeutic interventions and to detect disease recurrence prior to the development of symptoms[19]. The timed barium swallow is performed by having the patient drink 100-250 mL of barium in an upright position and by taking radiographs one, two and five minutes after the last swallow. The distance from the distal oesophagus to the top of the barium column, as well as the maximal esophageal width, are measured for comparison before and after treatment.

Manometry remains the diagnostic modality with the highest sensitivity and should be part of the diagnostic evaluation in all patients with achalasia. Three cardinal features support the diagnosis of classic achalasia: Aperistalsis of the smooth muscle portion of the esophagus, incomplete LES relaxation and elevated LES resting pressure. As mentioned above, manometric variants of achalasia have been described (Table 1). Vigorous achalasia is a variant characterized by aperistaltic, simultaneous esophageal contractions with higher average amplitudes (> 37 mmHg)[20]. It has been suggested that vigorous achalasia might present an earlier form of achalasia, in which esophageal contractions against the outflow obstruction at the LES are still maintained. However, vigorous achalasia appears to be independent of age of onset and symptom duration, and is not associated with return to normal peristalsis after surgical myotomy[21,22]. Although it has been suggested that patients with vigorous achalasia might show better success with botulinum toxin injection than patients with classic

achalasia[23], this has minor clinical relevance, because other treatment options are superior in most cases. Therefore, it remains unclear whether dividing patients into those with "vigorous achalasia" and "classic achalasia" has any clinical implications. Despite such reservations, physicians need to be aware that achalasia might present with a spectrum of manometric findings that might not meet all of the criteria specified above (Table 1)[24]. Their significance lies in the recognition that these sometimes confusing manometric findings are consistent with achalasia when combined with additional clinical data supportive of the diagnosis. As mentioned for the timed barium swallow, manometry also plays a role in monitoring treatment response and predicting treatment success of pneumatic dilatation, as discussed below.

The recent introduction of HRM with pressure topography plotting into the diagnostic armory has brought a renaissance to the classification of idiopathic achalasia into variants with possible clinical implications. A retrospective study by Pandolfino *et al*[25] described three distinct variants, with type Ⅰ exhibiting minimal esophageal contractility without pressurization, type Ⅱ with absent peristalsis but compartmentalized, pan-esophageal pressurization, and type Ⅲ with lumen obliterating spasm. The authors showed that pan-esophageal pressurization (type Ⅱ) had the best overall treatment response, whereas type Ⅲ predicted a poor treatment response to all types of therapy. Further prospective studies are needed to confirm these interesting early results.

TREATMENT

Treatment of idiopathic achalasia remains strictly palliative. In view of the suspected autoimmune mechanism of the disease, it appears surprising that no study has systematically addressed the use of immunosuppressive therapy in an attempt to prevent disease progression[26]. Therefore, current treatment modalities are primarily directed at relieving distal esophageal obstruction and consist of pharmacologic therapy, endoscopic treatment with pneumatic dilation or botulinum toxin injection, and surgery. The appropriate choice of therapeutic options depends on multiple factors, such as the patient's characteristics, clinical presentation, local expertise and patient preferences, but should be based on the best available evidence.

Pharmacological therapy is directed at achieving a reduction of LES pressure by the use of smooth muscle relaxants, such as calcium channel blockers (e.g. nifedipine 10-30 mg sublingually 30-45 min before meals), nitrates (e.g. isosorbide dinitrate 5 mg sublingually 10-15 min before a meal) or phosphodiesterase 5 inhibitors[27,28]. The main limitations of these agents are their short duration of action, limited improvement of dysphagia despite documented LES relaxation, or the frequent occurrence of side effects, such as peripheral edema, headaches or hypotension, which especially occur with calcium channel blockers and nitrates. Their use is, therefore, limited to symptomatic relief in patients who have very early disease, or as a temporary measure for patients who are awaiting a more definite treatment option, or are high risk for more invasive options[29].

Endoscopic options of treatment include disruption of the LES by pneumatic dilation or botulinum toxin injection. Botulinum toxin is a potent neurotoxin that leads to a blockade of the release of acetylcholine from excitatory motor neurons. In a landmark study, Pasricha *et al*[30] showed that endoscopic injection of botulinum toxin into the area of the LES lead to symptomatic improvement in patients with achalasia, which was accompanied by reduced esophageal retention over a period of 6 mo. One randomized controlled trial (RCT) has shown that the two commercially available formulations of botulinum toxin are equally effective, but need to be given in different dosages because of variable potency[31]. The treatment effect of one of these formulations (Botox® Allergan Inc, Irvine, California, USA) might be maximized when a repeated injection of 100 IU is given one month after the first injection[32]. In contrast, a lack of an initial symptomatic response and residual LES pressure ⩾ 18 mmHg after botulinum toxin are associated with a poor overall response[33].The best results of botulinum toxin have been achieved in patients with vigorous achalasia, older patients and patients whose LES pressures do not exceed ⩾ 50% of the upper limit of normal[23,34]. However, the use of botulinum toxin is limited by its lack of long-term efficacy with recurrence rates of approximately 50% after one year and universal symptomatic relapse at two years[35,36]. Two recent meta-analyses concluded that although botulinum toxin has an excellent safety profile, it seems slightly less effective than pneumatic dilatation in the short-term and is clearly inferior in the long-term for the treatment of achalasia[37,38].

Pneumatic dilatation has been used for the treatment of patients with achalasia for more than half a century and is currently considered the most effective non-surgical treatment for achalasia[39,40]. A number of different pneumatic dilators with variable balloon compliance have been used in clinical trials. Currently, the low-compliance polyethylene pneumatic dilator (Rigiflex®, Boston Scientific, Boston, MA, USA) appears to be the most widely used. Although pneumatic dilators from other manufacturers are available (e.g. Cook Medical, Bloomington, IN; USA or Hobbs Medical, Stafford Springs CT, USA; HCDD, latex balloon, Rüsch Inc, Germany), only limited comparative data exist, which have not shown a difference in efficacy or safety[41,42]. Using a graded approach with the polyethylene balloon dilator, with increasing diameters from 3.0 to 4.0 cm, a 93% response rate was achieved over a mean follow up period of four years with a relatively low complication risk[43]. The most feared complication of pneumatic dilation is perforation, which occurred in 1.6% of patients in a meta-analysis with 1065 patients in experienced hands[39,40]. Studies assessing the long-term efficacy of pneumatic dilation have shown that a permanent treatment success can only be achieved in 40%-60% of patients after a follow up of ⩾ 15 years[44-46]. Although one study showed that "on demand" repeat dilations may again lead to remission in the majority of patients[47], others have shown that longer lasting treatment effects cannot be expected

from such therapy[48].

Predictors of treatment failure with balloon dilation appear to be younger age (< 40 years), male gender, pulmonary symptoms and failed response to one or two initial dilations[49-52]. In contrast older age appears to be associated with favorable outcomes of pneumatic dilation. Manometric findings that predict poor outcome are high initial LES pressures (e.g. > 15-30 mmHg) or a reduction of LES pressure < 50% after the first dilation[50,52,53]. Manometry should, therefore, be routinely performed pre- and post-interventionally.

With the advent of minimally invasive laparoscopic approaches, surgery has evolved from an ancillary procedure, used when pneumatic dilation failed, to the favored primary approach by many surgeons and gastroenterologists in a majority of patients with achalasia[54]. The goal of surgery is to alleviate the esophageal obstruction by myotomy of the LES. To prevent secondary gastroesophageal reflux, the procedure has usually been combined with some type of fundoplication procedure. The superiority of surgical myotomy over pneumatic dilation was suggested by three recent meta-analyses in the English and Chinese literature that mostly considered retrospective cohort studies[38,39,55]. Although both pneumatic dilation and surgical myotomy have a substantial risk of subsequent need of interventions (repeated pneumatic dilation, surgical myotomy or esophagectomy) over a period of 10 years, the probability was significantly smaller in the latter group (56% *vs* 26%, respectively) in one study[56]. To date, only one RCT with data on long-term follow-up has been published by Csendes *et al*[57] comparing myotomy followed by 180° Dor fundoplication to pneumatic dilation with a Mosher bag. Although this study has been criticized because of potentially technique-related suboptimal results in the pneumatic dilation group, it still remains the best available evidence to date. The authors showed good response after a five year follow up period for 95% of surgically treated patients *vs* 65% of patients in the pneumatic dilation group. However, very late results in the surgical group showed that clinical deterioration occurs, reducing the surgical success rate to 75% after a mean follow up of 15.8 years[58]. Of the patients with poor surgical results, 92% resulted from complications of severe reflux disease and not from incomplete myotomy. A number of trials have, therefore, investigated the benefit of anti-reflux procedures in addition to myotomy. In a prospective RCT, reflux symptoms were reduced from 47.6% with laparoscopic Heller myotomy alone to 9.1% when a Dor fundoplication was added[59]. In another RCT, laparoscopic myotomy with Dor fundoplication was equally effective as a myotomy with "floppy" Nissen fundoplication in controlling reflux, but dysphagia rates were significantly higher in the latter group (2.8% *vs* 15%, respectively; $P < 0.001$)[60]. With success rates of 47%-82% at 10 years, laparoscopic Heller myotomy with partial fundoplication appears to have evolved into the surgical procedure of choice[39,61,62]. A recent single center RCT compared laparoscopic cardiomyotomy with partial toupet fundoplication to pneumatic dilation in patients with newly diagnosed achalasia. Similar to the Csendes study, it also showed significantly fewer treatment failures in the surgical arm after a period of 12 mo[63]. Another head to head multicenter RCT has been ongoing for a number of years, but publication is still pending[64].

Predictors of a negative outcome with surgical myotomy were severe preoperative dysphagia, lower preoperative LES pressures of < 30-35 mmHg, progressive esophageal body dilation with flask type or sigmoid esophagus, and preoperative endoscopic treatment in some studies[61,65-68]. However, other studies showed treatment responses even in (selected) patients with dilated esophageal bodies or sigmoid esophagus, and in patients who previously failed pneumatic dilation[48,69,70]. The effect of surgical myotomy on chest pain remains controversial, and patients should be aware that this symptom might not reliably improve after either pneumatic dilation or surgery[8]. Occasionally, a temporary placement of self-expanding metal stents (SEMS) has been suggested as a possible means of dilation or as a bridge to surgery[71,72]. However, because no information with regard to its long-term effectiveness exist and complications might be frequent and potentially severe[73], stent treatment for achalasia cannot be recommended at the present time.

Finally, it should not be forgotten that for patients not responding to any one of the above mentioned therapies, subtotal esophageal resection with gastric pull-up remains as a viable treatment option. Although such therapy is extremely invasive and associated with a high post-operative morbidity, favorable long-term results with significant improvement of symptoms can be achieved, even if endoscopic therapy or surgical myotomy have persistently remained unsuccessful[74].

COMPLICATIONS AND PROGNOSIS

Complications in patients with achalasia might occur from the natural course of the disease (e.g. aspiration, squamous cell carcinoma, and megaesophagus), from iatrogenic interventions (e.g. perforation after balloon dilation, or postoperative complications after myotomy), or from the late consequences of a successful intervention (e.g. reflux related complications, such as strictures or adenocarcinoma).

As a result of the natural course of the disease, structural parenchymal pulmonary disease occurs in 33% of patients with achalasia, probably from chronic microaspiration. Furthermore, delayed diagnosis, or ineffective intervention, might lead to progressive dilation of the esophagus and the development of a megaesophagus. This complication occurs in 10% of patients at a median of 18-21 years after the onset of symptoms and might require esophagectomy in the most severe cases[75,76].

In addition, treatment modalities carry their own inherent risks. As previously mentioned, the main risk of pneumatic dilation is perforation, which occurs at a mean of 1.6% (range from 0%-8%), even in experienced hands[39]. The risk of perforation appears to be highest during initial dilation, as opposed to subsequent

Table 2 Predictors of treatment response in achalasia

Treatment option	Positive predictors	Negative predictors
Botulinum toxin injection	Vigorous achalasia	Initial LES pressure ⩾ 50% of the upper limit of normal
	Older patients	Lack of clinical response or residual LES pressure ⩾ 18 mmHg after initial botulinum toxin treatment
Pneumatic dilatation	Older patients	Male Gender
		Pulmonary symptoms
		Failed response to 1-2 initial dilations
		High initial LES pressure (> 15-30 mmHg)[1]
		Reduction of LES pressure < 50% after the first dilation
Myotomy	Younger patients (< 40 yr)	Severe preoperative dysphagia
		Lower preoperative LES pressures of < 30-35 mmHg[1]
		Esophageal body dilation (flask type or sigmoid esophagus)
		Preoperative endoscopic treatment (in some studies)

[1]Pressure values show considerable inter-study variability, depending on techniques used by different authors. Therefore, the pressure values shown in the table only reflect estimates based on the available literature.

dilations[77]. Although the perforation risk of laparoscopic myotomy is smaller with 0.7% (range 0%-8%), the overall rate of postoperative complications is 6.3%, with a periprocedural mortality of 0.1%[39]. As a result of endoscopic or surgical treatment, reflux esophagitis occurred in approximately 10% of patients in our own prospective cohort, even though 43% of patients received acid suppressing medications[76]. Reflux esophagitis was more commonly observed after surgical myotomy with Dor fundoplication (14%) than after pneumatic dilation (5%), possibly indicating more effective disruption of the LES. Late reflux complications, such as esophageal stricture occurred in half of these patients.

The most feared complication of achalasia is esophageal cancer. A recent review of the available literature reported a mean prevalence of esophageal cancer of 3% in patients with achalasia, indicating a fifty-fold increased risk over the general population[78]. Squamous cell carcinoma appears to occur most commonly, and probably results from stasis, causing bacterial overgrowth and production of nitrosamines, which in turn lead to chronic inflammation, dysplasia and cancer[79]. In addition, adenocarcinoma may result from long-standing reflux after successful treatment[80,81]. Although insufficient data are available to make evidence-based surveillance recommendations, many experts support a strategy of surveillance for cancer or reflux complications. Accordingly, the latest ASGE guideline suggests that it would be reasonable to consider such a strategy after 15 years of symptoms[82]. Annual follow up surveillance intervals have been suggested at least by one author[77]. Patients should be kept on a liquid diet three to four days before the surveillance endoscopy and an esophageal lavage should be considered immediately before the procedure to optimize visualization. Despite the described cancer risk and frequent long-term complications, patients with achalasia do not appear to experience a significant compromise of their overall life expectancy[76].

CONCLUSIONS AND FUTURE PERSPECTIVES

Achalasia is an idiopathic disorder, likely caused by autoimmune mediated destruction of inhibitory neurons in response to an unknown, possibly viral, insult in genetically susceptible individuals. Physicians should be aware of typical and atypical presentations of achalasia to avoid diagnostic delays. Standard diagnostic work-up should include an EGD, timed barium swallow and manometry. Additional testing may become necessary if pseudoachalasia is suspected. The appropriate choice of therapy depends on multiple factors, including local expertise, patient preferences, and known predictors of treatment failures (Table 2). Based on the current evidence, we prefer laparoscopic myotomy in combination with partial fundoplication in young patients (< 40 years) with low surgical risk as the primary treatment option. In older patients, or those who want to avoid surgery, pneumatic dilation produces good long-term results, unless the first one to two dilations are unsuccessful, or LES pressure is not adequately decreased. Botulinum toxin might be especially useful in very old patients, or those with major comorbidities, because of its excellent safety profile. Subsequent treatments should be based on symptom recurrence. Pharmacological therapy should be reserved for patients awaiting a more definite treatment option. For patients not responding to any one of the above mentioned therapies, or patients with megaesophagus, esophageal resection remains a viable option.

In the future, well designed prospective studies are needed to identify optimal treatment options for different subgroups of patients with idiopathic achalasia. The advent of new exciting diagnostic methods, such as HRM, may aid in predicting treatment responses and warrants further investigation. Finally, with growing insight into the pathophysiology of this disease, novel treatment options that aim at preventing the late stages of the disease might evolve.

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EDITORIAL

Effect of perioperative blood transfusion on clinical outcomes in hepatic surgery for cancer

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Abstract

Allogeneic blood transfusion during liver resection for malignancies has been associated with an increased incidence of different types of complications: infectious complications, tumor recurrence, decreased survival. Even if there is clear evidence of transfusion-induced immunosuppression, it is difficult to demonstrate that transfusion is the only determinant factor that decisively affects the outcome. In any case there are several motivations to reduce the practice of blood transfusion. The advantages and drawbacks of different transfusion alternatives are reviewed here, emphasizing that surgeons and anesthetists who practice in centers with a high volume of liver resections, should be familiar with all the possible alternatives.

Key words: Blood transfusion; Blood products; Allogeneic blood transfusion; Intraoperative autotransfusion; Preoperative autologous blood donation; Intraoperative isovolemic hemodilution; Infectious complications; Liver resection; Hepatocellular carcinoma

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INTRODUCTION

Improvements in surgical techniques, in pre- and post-operative care, and increased experience have improved the safety of liver resections for hepatocellular carcinoma (HCC), and these procedures frequently can be carried out without blood transfusions[1-5]. By contrast, riskier hepatectomies, including posterior resections with reconstruction of the vena cava or resection of the caudate lobe, represent complex procedures which could require perioperative blood transfusion. Transfusion of allogenic blood has been reported to be associated with potentially devastating complications such as transmission of human immunodeficiency virus and hepatitis, transfusion reactions, increased postoperative infection rate, and increased incidence of recurrences for certain cancers[6]. Moreover, pulmonary oedemas occurring during or after a blood transfusion appear as the most frequent serious immediate incidents: they include transfusion-associated circulatory overload and transfusion-related acute lung injury (TRALI)[6]. Transfusion of allogenic whole blood products has been shown to induce variations in certain immune functions[7,8], such as reduced NK cell activity, T lymphocyte blastogenesis, and increased suppressor T lymphocyte activity, which may be of great relevance for host resistance to infection and the spread of neoplastic cells. But, the adverse effects of allogeneic whole blood transfusion on cancer recurrence and survival rates[9-12], regardless of innumerable published studies, continue to be debatable, since as many studies can be found that invalidate[13-18] as those that substantiate[19-27] this hypothesis.

Recent advances in surgical techniques to control blood loss and transfusion need[28-32], and the growing vast experience with hepatic resections, have been

responsible for a remarkable reduction in the use of blood and blood products during surgery. Despite these efforts, allogeneic blood transfusion rates during hepatic resections have been reported at 40% to 80% depending upon the magnitude of the resection[3]. Furthermore, even though the introduction of the hepatic inflow occlusion technique introduced by Pringle[33] and selective and/or intermittent inflow occlusion have been very effective at reducing blood loss during hepatic resection, back bleeding from the hepatic veins and their tributaries during the Pringle manoeuvre can still be unpredictable, severe, and unexpected[34].

This paper outlines the current perspectives on blood transfusion in hepatic resection, focusing on allogeneic blood transfusion, intraoperative autotransfusion, preoperative autologous blood donation, and intraoperative isovolemic hemodilution.

ALLOGENEIC BLOOD TRANSFUSION

New measures to reduce transfusion errors have recently been defined by Regan *et al*[35]. The incidence of allogeneic blood transfusion is high in patients with cirrhotic livers undergoing liver resections for HCC, and for that reason it is vital to determine whether these transfusions stimulate tumor recurrence. The postoperative recurrence of HCC associated with perioperative blood transfusion has been supported[36] and disputed[37]. Furthermore the relationship between perioperative allogeneic blood transfusions, recurrence free survival, and the immunologic profiles of patients with HCC who have undergone curative liver resections has been investigated[38]. These studies have shown that in transfused patients, the CD4 levels are decreased by 90 postoperative days, whereas the CD8 levels are elevated during 14-90 d after surgery, as compared with nontransfused patients. Postoperative levels of the CD57+NK-cell subset and PHA responses in the transfused group are elevated as compared with the nontransfused group, and the PHA response of the transfused patients is significantly increased at seven postoperative days. Recurrence free survival seems not to be affected by perioperative blood transfusions.

All these studies suggest the significance of perioperative blood transfusion as an independent prognostic variable in terms of recurrence, survival, complications, and death. Patients who need preoperative, intraoperative, or postoperative transfusions are generally those with large lesions that either require a tri-segmentectomy, or are too close to the vena cava. On the other side, patients who do not need blood transfusions tend to have smaller, more peripheral lesions that can be resected under close hemostatic control. This suggests that patients with large HCC (with poor prognosis) are more likely to receive blood, and possible other factors should be taken into consideration for a more accurate evaluation. In regard to survival, for instance, the margin of resection, evidence of metastatic disease, liver failure or other perioperative complications should always be reviewed.

INTRAOPERATIVE AUTOTRANSFUSION

Intraoperative autotransfusion [also known as autologous blood salvage or intraoperative blood salvage (IBS)] is a medical procedure involving recovering blood lost during surgery and re-infusing it into the patient. Different medical devices have been developed to assist in salvaging the patient's own blood in the perioperative setting. IBS is widely used in a variety of surgical procedures, including cardiovascular, orthopedic, and gynecologic procedures, and emergency medical situations[39-41], but IBS in oncologic patients has not been widely studied. IBS has been cited as a contraindication[42] because of the potential risk of disseminating metastasis. This concept was introduced firstly by Yaw *et al*[43] who demonstrated tumor cells in processed blood that passed through filters in the Bentley autotransfusion device. Other studies support that IBS can be safely used in patients with cancer[44-46]. Because the Haemonetics cell saver processes blood by centrifuge-based washing after filtration, the risk of reinfusion of malignant cells seems to be lower than by the Bentley system. Clinical evidence of dissemination of cancer cells caused by IBS has not been reported, and several studies show no correlation between the presence of malignant cells and their subsequent dissemination[47,48]. The haemonetics cell saver was employed by Fujimoto *et al*[49] as an intraoperative scavenger of blood in patients undergoing hepatectomy for HCC. In this study autotransfusion was shown to be safe and effective, and the pattern and frequency of recurrence suggest that autotransfusion is not responsible for recurrence or metastasis. Hashimoto *et al*[50] showed that IBS in living liver donors undergoing liver resection for graft procurement offered the advantage of reduced blood loss during parenchymal transection.

At the present time, the processes used to assist in salvaging the patient's own whole blood in the perioperative setting can be categorized into three general types: (1) Cell processors and salvage devices that wash and save red blood cells (RBCs), i.e. "cell washers" or RBC-savers; (2) Direct transfusion; (3) Ultrafiltration of whole blood. Cell processors are red cell washing devices that collect anticoagulated shed or recovered blood, wash and separate the RBCs by centrifugation, and reinfuse the RBCs. RBC washing devices can help remove byproducts in salvaged blood such as activated cytokines, anaphylatoxins, and other waste substances that may have been collected in the reservoir suctioned from the surgical field. However, they also remove viable platelets, clotting factors, and other plasma proteins essential for homeostasis. Direct transfusion is a blood salvaging method associated with cardiopulmonary bypass circuits or other extracorporeal circuits that are used in surgery such as coronary artery bypass grafts, valve replacement, or surgical repair of the great vessels. Hemofiltration or ultrafiltration devices constitute the third major type of blood salvage appearing in operating rooms. In general, ultrafiltration devices filter the patient's anticoagulated whole blood. The filtration process

removes unwanted, excess non-cellular plasma water, low molecular weight solutes, platelet inhibitors and some particulate matter through hemoconcentration, including activated cytokines, anaphylatoxins, and other waste substances making concentrated whole blood available for reinfusion. Hemofiltration devices return the patient's whole blood with all the blood elements and fractions including platelets, clotting factors, and plasma proteins with a substantial Hb level. Presently, the only whole blood ultrafiltration device in clinical use is the *Hemobag*.

Concerns about possible contamination of autologous RBC with cancer cells responsible for metastasis still continues to limit the use of IBS in cancer patients. This is despite the fact that no evidence has been reported showing an increase in metastasis or a decrease in patient survival, regardless of the obvious demonstration that salvaged blood is contaminated with viable tumor cells which are not washed out of the RBC layer during IBS. Total elimination of the risk of reinfusion of cancer cells by irradiation has been proposed by Hansen[51], who has been able to show that IBS with blood irradiation is safe as it provides efficient elimination of contaminating cancer cells, does not compromise the quality of RBC, and is very effective in saving blood resources. The effectiveness of this procedure has been shown on a large number of oncologic patients[52].

PREOPERATIVE AUTOLOGOUS BLOOD DONATION

Evidence that allogeneic transfusion may lead to a potential risk of postoperative infections, and the increased demand for blood with a declining population of qualified, willing, and healthy donors, give reason for the current support for preoperative autologous transfusion (PAD)[53,54]. The overall benefits of PAD have been assessed in both randomized trials and cohort studies[55]. Assuming that the donor is not bacteremic at the time of donation and/or there are no clerical errors resulting in the accidental transfusion of the wrong unit of blood, the patient is also protected against hemolytic, febrile or allergic transfusion reactions; alloimmunization to erythrocyte, leukocyte, platelet or protein antigens; and graft-versus-host disease (GVHD). An additional benefit is that erythropoiesis may be stimulated by repeated phlebotomies, thereby enabling the patient to regenerate hemoglobin at an accelerated rate after surgery.

PAD programs are not without some disadvantages. Perhaps the most important is that autologous blood is considerably more expensive than allogeneic blood. This problem is compounded by the fact that current reimbursement programs of most of the National Health systems around the world either deny the medical necessity of PAD or ignore the well-documented increase in cost[56]. Moreover, the blood that is not transfused to the intended recipient (approximately 50% of donated blood) is generally wasted rather than being transfused to other patients[57]. This wastage of blood and the costs of administering autologous programmes result in collection expenses that are higher than those for allogeneic transfusion.

Patients undergoing PAD may donate a unit (450 ± 45 mL) of blood as often as twice weekly, until 72 h before surgery. Under normal conditions, patients conventionally donate once weekly. Oral iron supplements are routinely prescribed. This iatrogenic blood loss is accompanied by a response in endogenous erythropoietin (EPO) levels that, although increased significantly over basal levels, remain within the normal range. The erythropoietic response that occurs under these conditions is therefore modest[58]. With routine PAD, erythropoiesis of 220-351 mL (11%-19% RBC expansion)[59,60] or the equivalent of 1-1.75 blood units, occurs in excess of basal erythropoiesis, which indicates the efficacy of this blood conservation practice.

The use of autologous blood deposits for cancer patients undergoing elective surgical procedures has been studied by Lichtiger[61], who was able to show that the majority (132/182) of his patients (with head and neck, neurosurgical, gastrointestinal and colorectal, adrenal, gynecologic, soft tissue and bone, breast, and genitourinary tumors) underwent surgery using only autologous transfusions. Kajikawa *et al*[62] evaluated the benefit of autologous blood transfusion and the effect of recombinant human erythropoietin (rh-EPO) on preoperative autologous blood donation for hepatectomy in patients with cirrhosis. Their study shows that autologous blood transfusion yields clinically superior results for hepatectomy in patients with cirrhosis when compared with homologous transfusion. In addition preoperative rh-EPO administration minimizes presurgical decreases in hematocrit (HCT) caused by autologous blood donation[62]. Likewise preoperative autologous blood donation in combination with rh-EPO therapy markedly reduces the requirements for homologous blood transfusion during hepatic resections[63].

Other studies on patients undergoing hepatic resection have shown that the predeposition of autologous blood decreased the need for homologous transfusions from 56% to 38%. A further reduction in the transfusion rate of 25% could have been possible if all patients had donated 2 U of autologous blood[64].

To determine if predonation of autologous blood impacts upon transfusion practice and clinical outcome following liver resection, clinical records of 379 consecutive patients undergoing hepatic resection for metastases of colorectal cancer were identified from the prospective hepatobiliary database and reviewed by Chan *et al*[65]. No conclusion could be drawn from their data concerning the influence of allogeneic transfusion on tumor recurrence, since their study was not a randomized trial comparing allogeneic blood transfusion with autologous transfusion. Data from their study however demonstrated that PAD alone is insufficient to alter the rate of tumor recurrence or disease-specific survival. Furthermore major hepatic resections using current surgical techniques can be performed safely with low blood loss so that transfusion is required for only a minority

of patients. PAD may further reduce the need for allogeneic blood. Autologous blood transfusion is safe after storage and it has advantages if compared with homologous blood transfusion with regard to postoperative liver function and survival rate after hepatectomy for HCC[66].

In a recent study, Hirano *et al*[67] have shown that their autologous blood program, with IBS and preoperative blood donation, reduces the volume of banked blood needed and improves the prognosis of patients undergoing hepatectomy for HCC.

INTRAOPERATIVE ISOVOLEMIC HEMODILUTION

Acute isovolemic hemodilution (ANH) is another possible alternative to allogeneic blood transfusions, which was introduced in the early 1970s[68]. The procedure implies the removal of blood from the patient immediately before operation and the simultaneous replacement with appropriate volume of crystalloid or colloid fluids. ANH will reduce the HCT so that blood shed during the operative procedure will result in less RBC mass loss. The amount of blood removed varies between one and three units (450-500 mL constitutes 1 U), although larger volumes may be withdrawn safely in certain circumstances. The removed blood is then reinfused as autologous whole blood after the major blood loss portion of the procedure is completed. The blood withdrawn is anticoagulated and maintained at room temperature, in the operating room, for up to 8 h. It is reinfused into the patient as needed during, or after, the surgical procedure. ANH can be used as the only blood preservation technique, or it can be combined with preoperative autologous donation, blood salvage, or both.

Hemodilution could be classified according to the target HCT as mild (HCT $\geqslant$ 30%), moderate (30% < HCT $\geqslant$ 20%), or severe (HCT < 20%)[16]. The target HCT with ANH is variable but is often around 25%-30%. Severe hemodilution (e.g. 20%) is likely to be more efficacious with regards to blood conservation, but the risks are greater, particularly for patients with preexisting medical conditions such as coronary heart disease[69].

ANH should be taken into consideration for patients with good initial HCTs who are assumed to be deprived of more than two units of blood (900-1000 mL) during surgery. This technique works better in healthy, young adults, but it has been successfully employed in children and elderly patients. ANH has been used in vascular, orthopedic, and in some general surgical procedures. In addition, Jehovah's Witnesses patients accept this technique with the modification that we keep the blood moving and in direct contact with the patient's vascular system. Some Jehovah's Witnesses will agree to ANH if the blood is maintained in a closed circuit continuous flow system[70].

ANH is contraindicated in cardiac disease, since the main compensatory mechanism for the induced anemia is an increase in the cardiac output, when renal function is impaired, since large amounts of infused fluids need to be excreted, and when baseline hemoglobin is below 110 mg/L (11 g/dL). Furthermore low concentrations of coagulation proteins, inadequate vascular access, and the absence of appropriate monitoring capability indicate that ANH should not be used[71].

In the last 20 years several groups reported the use of ANH during major hepatic resections[72-76], and the overall conclusion is that ANH, in selected patients, is a safe and effective technique that appears to reduce the number of patients requiring homologous blood transfusion as well as the number of units transfused per patient. Furthermore, Jehova's Witnesses with hepatic tumors represent a major problem for liver surgeons to achieve good outcome, in fact these patients, because of their religious beliefs, refuse transfusion of blood and blood products. In order to avoid transfusion Barakat *et al*[75] have recently described the use of ANH in a Jehova's Witness who underwent a combined left trisegmentectomy and caudate lobectomy to treat a large intrahepatic cholangiocarcinoma.

ANH is considered a simple and inexpensive procedure, and has the advantage that fresh autologous blood is readily available. Numerous studies of its efficacy, however, have produced conflicting results, perhaps because of the heterogeneity of the surgeries in which it was used, differences in study protocol, and differences in the definition of outcome variables[77,78].

DISCUSSION

Liver resection is still the mainstay of treatment for patient with HCC. Even though improved surgical techniques and anesthesia have remarkably decreased the mortality rates of liver resections, morbidity rates, remain high. One of the major risks of hepatectomy is large-volume blood loss, which necessitates perioperative blood transfusion. The possible consequences of homologous blood transfusion are well known and include noninfectious risks such as transfusion reactions, transient immunodeficiency, transfusion-associated GVHD, and TRALI[79-84]. Thus there are conclusive motivations to reduce blood loss during surgery and, as a consequence to lessen blood transfusion. It has been clearly shown that transfusion has a significant negative effect on perioperative mortality, complications, and length of hospital stay, even if it is difficult to demonstrate that transfusion is the only factor that decisively affects the outcome. The magnitude of the surgical procedure has always to be considered the most critical factor. It is intuitive that anterior, small, marginal atypical resections are quite different to complicated posterior large resections which include reconstruction of resected vena cava.

An association between transfusion and postoperative complications has been shown in preclinical models[85,86] and in clinical studies[87-91]. The review of 378 consecutive elective liver resections performed in our institution shows that 62% of the patients were not transfused, and the remaining 38% received blood products delivered with different procedures (Figure 1).

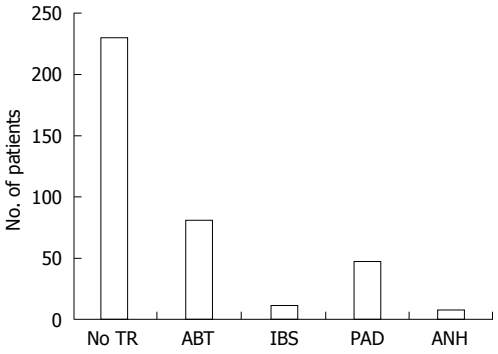

Figure 1 Transfusion procedures in 378 patients undergoing liver resection. No TR: Not transfused (62%); ABT: Autologous blood transfusion (21%); IBS: Intraoperative blood salvage (3%); PAD: Preoperative autologous blood donation (12%); ANH: Acute normovolemic hemodilution (2%, seven of the eight pts were Jehowa's Witnesses). Data from the Department of Surgical Sciences, University of Insubria, Varese, Italy.

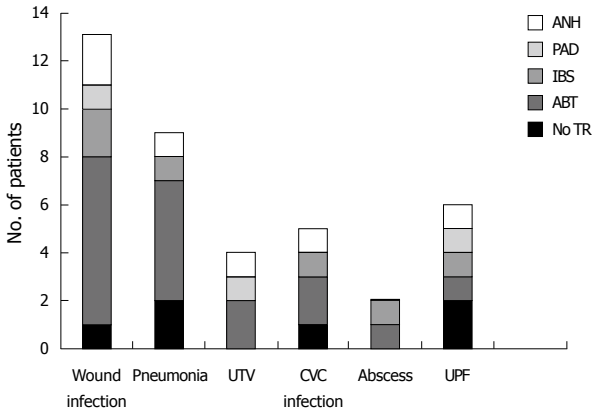

Figure 2 Details of postoperative infectious complications (44 pts, 11.6%) occurred in 378 patients undergoing liver resections and correlated to transfusion procedures. UTV: Urinary tract infection; CVC: Central venous catheter; UPF: Undiagnosed post-operative fever. Data from the Department of Surgical Sciences, University of Insubria, Varese, Italy.

Infectious complications (wound infections, pneumonia, urinary tract infections, central venous catheter infections, abscesses, and undiagnosed postoperative fever) have been more frequent in the transfused group of patients (33 *vs* 7). Most of the infections complications (18) have been recorded in the patients receiving autologous blood transfusions, the most frequent being wound infections (7) and pneumonia (5). Our results confirm the observation of Alfieri *et al*[92] who in a series of 254 liver resections found a significant association between blood transfusions and development of complications. More recently, Kooby *et al*[93] have been able to show that perioperative blood transfusion is a prognostic factor for the development of complications in univariate and multivariate analysis. Transfusion predicted development of both minor and major complications. Transfused patients had twice as high a chance of developing major complications and four times the risk of perioperative death. Transfused patients also had a higher incidence of infectious complications (17% *vs* 13%, $P = 0.03$)[93].

Despite these results and studies, it is still debatable whether transfusion is the only and independent factor related to short term outcome, and specifically the only determinant of postoperative infectious complications. Is the transfusion itself and not the reason for the transfusion the cause of postoperative morbidity? Intraoperative hypotension, complexity of operation (extended hepatectomies *vs* lesser resections), duration of anesthesia, age, stage of the neoplastic lesion, degree of liver dysfunction, nutritional status, and possible neoadjuvant treatment, are all factors which could interfere with some aspects of the complex immunologic response. Furthermore, timing of the transfusion and the circumstances necessitating transfusions have been proposed as the real determinants of prognosis[94]. Today we are not able to conclude that transfusion is the factor producing the infectious complication, and the correlation we found of transfusion with complications should not be interpreted as a direct cause and effect relationship. The infectious complications are different in the transfused and non-transfused patients, but we cannot say for sure that immunologic irregularities are what produces the difference.

In recent years, we have had the occasion to carry out seven major liver resections on Jehova's Witnesses with large tumors. The management of Jehova's Witnesses with HCC, or any other type of liver tumor, entails a multidisciplinary, adapted plan in harmony with their religious beliefs to achieve good outcome[95]. This approach enabled us to perform the surgical procedure respecting their religious conviction, and authorized us to anticipate that ANH could be considered a safe alternative for use in selected cases in which allogeneic blood transfusion is considered of high risk. This approach, in our series, has been associated with a relative high incidence of infectious complications, if compared with other autologous blood transfusion procedures (Figure 2).

CONCLUSION

A substantial discrepancy is apparent in transfusion practice for elective surgery, and even more so for liver resections[96]. Reducing unneeded exposure to blood components by blood saving measures is essential in patients undergoing elective surgery. A publication for anesthesists reviews good transfusion practices in surgical patients[97].

Perioperative blood transfusion has been described as one of the risk factors for poor outcome after liver resection. This seems particularly verifiable for infectious complications. The postoperative recurrence of HCC associated with perioperative blood transfusion has been the subject of controversy due to conflicting results. Although allogeneic blood transfusion may have immunosuppressive effects, perioperative blood transfusions seem not to influence the cancer free survival rate in patients with HCC. Even if there is no evidence of one transfusion procedure which prevails over the others, surgeons who practice in Centers with high volume of liver resections should be familiar with

all the possible alternatives (ABT, IBS, PAD, ANH), since each of them, when blood products are needed, have a place depending upon the different clinical pattern.

Finally, maintaining a low central venous pressure has been shown recently to be effective in reducing blood loss during partial liver resections. Moreover antifibrinolytic drugs have proved to be effective in reducing blood loss during liver transplantation[98].

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REVIEW

Comparative genomics of *Helicobacter pylori*

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Abstract

Genomic sequences have been determined for a number of strains of *Helicobacter pylori* (*H pylori*) and related bacteria. With the development of microarray analysis and the wide use of subtractive hybridization techniques, comparative studies have been carried out with respect to the interstrain differences between *H pylori* and inter-species differences in the genome of related bacteria. It was found that the core genome of *H pylori* constitutes 1111 genes that are determinants of the species properties. A great pool of auxillary genes are mainly from the categories of *cag* pathogenicity islands, outer membrane proteins, restriction-modification system and hypothetical proteins of unknown function. Persistence of *H pylori* in the human stomach leads to the diversification of the genome. Comparative genomics suggest that a host jump has occurs from humans to felines. Candidate genes specific for the development of the gastric diseases were identified. With the aid of proteomics, population genetics and other molecular methods, future comparative genomic studies would dramatically promote our understanding of the evolution, pathogenesis and microbiology of *H pylori*.

Key words: *Helicobacter pylori*; Genomics; Pathogenesis; Cancer

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INTRODUCTION

The gastric pathogen, *Helicobacter pylori* (*H pylori*), is a member of the epsilon-bacteria. This microaerophilic, Gram-negative bacterium colonizes the human stomach[1]. It is estimated that over half of the human population are infected by *H pylori*[2]. The infection causes mucosal inflammation, atrophy, ulceration and cancer[3,4]. Five strains of *H pylori* and a number of related bacteria have been sequenced. Genomics, evolutionary studies and population genetics have advanced our understanding of this bacterium.

GENOMIC FEATURES

In 1997, *H pylori* strain 26 695 was firstly sequenced[5]. It was isolated from an English patient with chronic gastritis. The chromosome of strain 26 695 is circular and composed of 1 667 867 base pairs. The average GC content is approximately 39%. In the initial annotation, it has 1590 open reading frames that are possibly protein-coding[5], in addition to the RNA coding genes (2 copies of 16S rRNA and 23S rRNA genes, 36 tRNA genes). Later analysis of the genome sequence suggested a smaller number of ORFs in strain 26 695[6]. The ongoing studies have found genes that were neglected in the initial analysis. A general secretion machinery is widely present in bacteria, which functions in secretion of outer membrane proteins from the inner membrane and delivery of proteins to extracellular environments[7]. The initial annotation revealed a partial general secretion machinery because it lacked SecE in 26 695[5]. A further analysis of the genome sequences with GeneMark, Glimmer and BlastX found a small open reading frame between nusG and rmpG (HP1203-HP1204)[8]. It has a high homology and structural similarity to the SecE protein in related bacteria. Therefore, strain 26 695 has a complete general secretion machinery, which is consistent with the fact that the bacterium is capable of protein secretion. In addition, small RNA genes are universally present in bacteria[9]. The tmRNA gene (*ssrA*) has been found in *H pylori*, which encodes a functional RNA molecule and a small peptide that is involved in

quality control of translation[10]. In addition, *H pylori* also possesses a sRNA gene encoding the RNA component of Rnase P and the 4.5S RNA gene which is involved in secretion[11,12].

In 1999, strain J99 was sequenced which was isolated from an American patient with a duodenal ulcer[6]. Compared to strain 26 695, it has a slightly smaller circular chromosome (1 643 831). The overall genomic organization, gene order and predicted proteomes of the sequenced strains are very similar. The predicted open reading frames are less in strain J99, amounting to 1495. There are 1406 genes shared by both strains, but 86 open reading frames are absent from strain 26 695. Both strains contain a complete *cag* pathogenicity island that codes for a type Ⅳ secretion system which delivers the CagA cytotoxin protein into gastric epithelial cells[13]. Comparison of the two genomes reveals the occurrence of translocation and inversion events. A 83 kb inversion contains most of the strain specific genes. This region was named a plasticity zone since it has a much lower GC content (35%) than the rest of the genome.

In 2006, a chronic atrophic gastritis *H pylori* strain, HPAG1, was sequenced[14]. It was isolated from an 80 year-old female patient who was enrolled in a Swedish case-control study of gastric cancer[15]. Similar to the sequenced strains 26 695 and J99, HPAG1 is a type 1 strain that contains *cagA* and a virulent allele of *vacA*[15]. The genome of HPAG1 (1 596 366 bp) is the smallest in the three sequenced strains. A total of 1536 open reading frames were predicted. Of these, 43 genes are only present in HPAG1. Analysis revealed that 29 genes that are found in both J99 and 26 695 were missing from HPAG1. If genes in a strain are absent from other strains, they are called strain-specific genes. The comparison of three sequenced *H pylori* strains shows that the majority of strain-specific genes are functionally unknown. Another group of strain-specific genes is composed of genes of the R-M system (restriction-modification). They encode proteins involved in DNA restriction or modification. Other strain-specific genes include those encoding outer membrane proteins and *cag* proteins.

H pylori strain G27 was sequenced recently[16]. It was originally isolated from an Italian patient[17] and has been used widely in *H pylori* research. This strain is naturally transformable[18], capable of delivering CagA into epithelial cells in culture[19,20], and capable of adapting to variable environments[21].

The G27 genome has a similar size to the other three sequenced strains. It is 1 652 983 bp long and has a GC content of 38.9%. 1515 open reading frames was predicted. In addition, G27 also contains one 10 032 bp AT rich (65.2%) plasmid resembling that found in strain HPAG1[14]. The plasmid encodes 11 genes. In agreement with the previous report[6], the *cag* pathogenicity island of G27 is disrupted by a transposon. This, however, does not seem to interfere with any of the open reading frames or to the delivery of CagA into host cells. Unlike strain 26 695, there is a single plasticity region in G27 which contains a large number of *H pylori* specific genes. It is predicted that strain G27 has 58 genes that are not found in 26 695, J99, or HPAG1.

The *H pylori Shi470* genome has also been sequenced by Washington University Medical School. It is 1.61 Million bp long and contains approximately 1609 predicted genes. The sequences are available on the university website (http://hpylori.ucsc.edu).

The finding of strain-specific genes from the comparison of the sequenced strains is in agreement with the earlier studies which demonstrated the high diversity of the *H pylori* genome[22-24]. No identical strains of *H pylori* have been found in their genetic types unless they are isolated from a family[25-27]. *H pylori* has great mutation and recombination capacities. Analysis of the genomic sequences failed to identify a complete mismatch repair system controlling the confidentiality of replication, despite the presence of a homology of MutS[28,29]. This results in a high mutation rate of *H pylori*. Examination of 29 clinical isolates revealed that approximately 1/4 of them had mutator phenotype with higher mutation frequencies than Enterobacteriaceae mismatch-repair defective mutants[30]. In another study, examination of paired strains isolated from a patient at different times suggested a mutation rate of 4.1×10^{-5}, which is comparable to that of *E. coli* mutator[31]. *H pylori* is naturally competent for transformation[32]. Nonrandomly distributed repetitive sequences are found in the genome, which leads to frequent recombination events[33]. The recombination rate (recombination events starting at any particular nucleotide) is estimated to be 6.9×10^{-5}[34,35]. High levels of recombination and mutation could explain the observed genomic diversity in *H pylori*.

DETERMINATION OF THE CORE GENOME OF *H PYLORI*

Inter-strain diversity, represented by variations in number and contents of genes, chromosomal rearrangements and allelic diversities, is not unique to *H pylori*[36]. This has been noted in a number of other bacterial species. For *H pylori*, each strain contains many strain-specific genes[7,14]. It has been proposed that a particular bacterial species contains a core set of genes and the auxiliary genes[37]. The core genome contains genes that are present in all or nearly all of the strains. It determines the properties that are characteristic of the species. The auxiliary genes are present in some of strains. They are determinants of the biological properties unique to some of the strains. Salama *et al*[38] firstly explored the core set of genes in *H pylori*. A total of 15 strains of *H pylori* mainly isolated from Western countries were examined using a microarray method[38]. It was found that 1281 genes were common to all the examined strains, therefore constituting the core genome of *H pylori*. Considering the limited number of strains examined and the fact that *H pylori* is highly prevalent in human, could these genes represent the actual core set of the *H pylori* species? Additionally, these strains were only isolated from Western individuals. In fact, molecular typing of global strains has found that the modern population of *H pylori* divides into five major

groups, hpEurope, HpAfrica1, hpafrica2, hpEastAsia and hpAsia2[39,40]. They are possibly derived from different ancestral groups. Gressmann *et al*[41] further examined 56 globally representative strains of *H pylori*. The number of the genes in the core set of *H pylori* was diminished to 1150. The author concluded through a calculation that the core genome of *H pylori* only consists of 1111 genes.

The auxiliary set of genes in *H pylori* amounts to 22%-27% of the genome[14,41]. In agreement with the findings from the whole genome sequencing of *H pylori* strains, the auxiliary genes consist of those coding for functionally unknown proteins, cag protein, outer membrane proteins and proteins of DNA metabolism[7].

Candidate genes specific for development of gastric diseases.

The long term clinical outcomes of the *H pylori* infection are diverse. The infected gastric mucosa may develop inflammation, atrophy, intestinal metaplasia, ulceration, cancer and MALT lymphoma[1,3,4]. Genes in the auxiliary set are specific only for some strains. Do they play roles in the determination of the final outcome of an infected individual? *H pylori* broth culture filtrates cause the formation of intracellular vacuoles in mammalian cells[42]. The protein which has the vacuolation activity was purified and named VacA (Vacuolating cytotoxin). Despite of the universal presence of the *vacA* gene in *H pylori*, some strains do not cause the vacuolation of epithelial cells. This is attributed to the mosaic structure of *vacA*[42]. A signal region in the N-terminal and a mid region of *vacA* are polymorphic. The signal region affects the vacuolating activity of the cytotoxin: a 12 amino acid extension on the s2 form blocks the activity, although not all s1 forms have the cytotoxic activity[43]. The mid region is a determinant of the cell specificity of VacA[44]. There are three vacA genotypes, s1/m1, s1/m2 and s2/m2 in *H pylori*. The association of s1/m1 strains with severe diseases has been observed in some studies. A recent study found an intermediate region (i-region) of *vacA* between the signal region and the mid region that also contributes to the levels of vacuolating activity[45]. The genotype i1 was more frequently found in gastric cancer associated *H pylori* than the i2[45]. Strains possessing *vacA* i1 are strongly associated with peptic ulcer[46]. Another protein has been found to be co-present in almost all of the strains possessing the vacuolating activity[47]. The protein was named as cytotoxin-associated gene A (*cagA*) protein. The gene is present in the majority of strains. Those possessing the vacuolating activity and the CagA expression are called type I strains, or virulent strains[48]. The presence of *cagA* is generally the marker for a large DNA region called *cag* pathogenicity island[49]. Proteins produced by the *cag* island make up a type Ⅳ secretion system which delivers CagA into the epithelial cells[50,51]. The type Ⅳ secretion system locates across the inner and outer membrane and forms a pilus-like structure at the surface[51,52].The CagL protein is a specialized adhesin that is targeted to the pilus surface[53]. Through an arginine-glycine-aspartate motif, it binds to and activates integrin $\alpha5\beta1$ receptor on gastric epithelial cells. This interaction triggers CagA delivery into target cells[54] and activation of Src of gastric epithelial cells[55]. Translocated CagA remains associated with the host membrane and becomes tyrosine phosphorylated on carboxyl-terminal repeat motifs (Glu-Pro-Ile-Tyr-Ala, or EPIYA motifs)[56,57] by members of the Src family of protein tyrosine kinases such as c-Src, Fyn, Lyn, and Yes[58]. Phosphorylated CagA interacts with SHP-2[59], which thereafter activates a number of phosphorylases inducing alteration of signaling pathways. This alters the spreading, migration, adhesion, polarity and cytoskeletal structures of epithelial cells[60-63] . A large European study, demonstrated that *cagA* positive strains are significantly associated with the development of gastric cancer[64]. The *cag* island is thus an important determinant of the clinical outcomes of the *H pylori* infection. Most *H pylori* strains, and almost all in certain geographical locations, however, are virulent (that is they expressing CagA and VacA). Are there any other genomic differences associated with the clinical outcomes?

Comparison of the genomic contents of different strains has found genes that are potentially disease-specific. Peptic ulcer disease frequently occurs in humans with severe, or even lethal complications. The disease may also affect children. Oleastro *et al*[65] reported the study of the genomic comparison of a *H pylori* strain isolated from a child presenting with duodenal ulcer and a strain from a sex and age matched child with gastritis. It was found that genes jhp0562 and jhp0870 are more frequently seen in children with peptic ulcer than in those with gastritis. Both genes are located in the plasticity zone. Jhp0562 encodes a putative LPS glycosyltransferase involved in LPS biosynthesis[66], whereas jhp0870 codes for an outer membrane protein. LPS and outer membrane proteins play roles in the induction of an inflammatory response from the gastric mucosa[66,67]. Whether jhp0562 and jhp0870 contribute to the development of ulceration in children deserves further study. Other genomic comparison studies have found that the *cag* island and a 670 bp-long DNA fragment that is partially homologous to the hymidylate kinase gene are potentially associated with peptic ulcer diseases[68]. Gastric mucosa infected by *H pylori* develops inflammation, and gradually become atrophic. Mucosal atrophy is an important stage in stomach carcinogenesis. A thorough examination of the genome of 6 strains from atrophic gastritis found a set of 121 "ChAG-associated" (ChAG, chronic atrophic gastritis) genes[14,69]. They are universally present in these 6 strains but absent from 56 globally derived strains of *H pylori*[69]. Their putative roles in the development of atrophy and promotion of carcinogenesis are yet to be studied. Intestinal metaplasia of gastric mucosa is a precancerous lesion. *H pylori* in the patient with intestinal metaplasia is likely associated with progression into gastric cancer. Comparison of a cancer strain and a duodenal ulcer strain of *H pylori* found a novel sequence named Clone P32 with homology to GepA in *Dichelobacter nodosus*[70]. Examination of strains from diverse gastric diseases demonstrated that Clone P32 is inversely associated with intestinal metaplasia. Gastric B cell lymphoma of mucosa associated lymphoid tissue is highly associated with the *H pylori* infection[1]. Eradication of the bacterium leads to the alleviation of the disease. Comparing

the genome of a strain from gastric B cell lymphoma with that from gastritis revealed that jhp0950 encoding a *H pylori* specific protein of unknown function was potentially associated with the development of the disease[71]. It was present in about 3 quarters of strains from gastric lymphoma, but only present in about half of strains from gastritis, duodenal ulcer or gastric adenocarcinoma. If other virulent factors were taken into account, the odds of having gastric MZBL among patients harbouring JHP950, *iceA1* (coding for a restriction enzyme), and *sabA* (coding for a major adhesin) "on" strains were 10 times higher than for the others[72]. Although these genes are specific for strains from a specified disease, it is uncertain whether they are pathogenic for a particular disease. Actually, different gastric diseases greatly differ in a variety of environmental factors that have potential impacts on the biological behaviors and genetics of the bacterium. For example, secretion of gastric acid is varied in different diseases[73]. Therefore, further studies are required to say that a gene is specific for the pathogenesis of a particular disease.

INTRA-HOST EVOLUTION OF THE *H PYLORI* GENOME

It is believed that the *H pylori* infection is usually acquired in childhood[74]. The bacterium is transmitted from parents to their children with a bias of mother to children transmission[75,76]. Once the infection is established, the bacterium persists in the host for decades unless eradicated with antibiotics. Transmission of bacteria to a new host is a major barrier for bacterial spreading. It may affect the bacterial genome contents. Four healthy adults were experimentally infected with *H pylori*. Examination of isolates form 15 d or 90 d postinfection demonstrated that their genomic contents were identical to the challenging strain[77]. A similar result was found in a study of experimental infected mice[78]. These findings suggest that for *H pylori*, transmission does not cause any alteration of the gene components of the genome, or, in the other words, the establishment of the *H pylori* infection does not require the involvement of novel genes. Further evidence supporting this conclusion comes from a study of the analysis of the strains from a mother and her three children[75]. Microarray analysis demonstrated that the genomic contents of isolates from the mother were identical to those from the children. *H pylori* persists in the human stomach for decades, probably from childhood. It may experience a variety of ecological alterations, which may in turn have large impacts on the genome of the organism. The output of gastric acid alters with aging and with infection by *H pylori*. Alterations of the constituents and the quantity of the gastric mucus underneath which the bacterium resides are observed during the course of the *H pylori* infection. The gastric epithelial cells may undergo metaplasia and changes in surface proteins, which affect adhesion and the supply of nutrients. The gastric mucosa may produce immune and inflammatory products against the bacterium. The co-infection with other microbes is also frequent seen in the stomach. These alterations may affect genomic contents of *H pylori*. Kraft *et al*[79] examined paired strains of *H pylori* with respect to their genomic contents using the microarray method. Paired strains were isolated from the same patients at an interval from 3 to 36 mo. Of 21 pairs of strains examined, 4 pairs showed differences in their genomic contents, suggesting the occurrence of evolutionary events. These included a complete deletion and a partial loss of the *cag* pathogenicity island, a replacement of an open reading frame of unknown function with the restriction-modification system HpyAIV, an acquisition of 14 genes in the plasticity zone, a duplication of the *ceuE* genes (HP1561/HP1562) and a truncation of tandem arranged *ackA* and *pta* genes resulting in the formation of pseudogenes. A study has compared 2 pairs of strains obtained from the same patients at an interval of 4 years[69]. The patients had progressed from atrophic gastritis to cancer. Six genes were absent, including 3 genes involved in DNA repair, an outer membrane protein and two hypothetical proteins. Nine genes were gained, including a ligase, a metalloprotease, a tRNA formyltransferase, a putative ribonuclease, a restriction enzyme and four hypothetical genes. The comparison suggests that with the progression of the atrophy to cancer, the bacterium may have a propensity for losing its diversifying capacity. Findings from these studies demonstrate that *H pylori* may acquire or lose genes during the intra-host colonization[80]. The genes involved fall into the same categories as the strain specific genes. This was further supported by the results from the comparison of the sequenced strain J99 with isolates obtained 6 years later[81]. These comparative studies of the *H pylori* genome draw a picture of the genomic changes during the cycle of invasion, colonization and transmission to a new host. It appears that invasion into a new host has little effect on the gene composition of the genome. This indicates that the current genome of *H pylori* has sufficient capacities for permitting bacterial invasion into a human host or even into a host of different species under experimental conditions. Once the infection is established, the bacterium has to cope with the dynamic changes of the ecology during the long-term coexistence with the host. Genomic diversifications, or gain and/or loss of genes, occur in response to these changes. The diversifications involve genes that are mainly those strain specific genes observed from comparative studies of unrelated strains of *H pylori*. Intra-host evolution of *H pylori*, thus, results in the creation of a pool of genes that are generally needed by some strains. This pool of genes can be considered as the auxiliary set of genes of *H pylori*.

COMPARATIVE STUDIES OF *H PYLORI* AND ITS RELATED BACTERIA

Since the isolation of *H pylori*, a number of closely related bacteria have been identified, constituting a

Table 1 General genomic features of *helicobacters* and *campylobacters*

Species	*Helicobacter pylori*			*H. acinonychis*	*H. hepaticus*	*W. succinogenes*	*C. jejuni*	*C. coli*	*C. lari*	*C. upsaliensis*
Strain	26695	j99	HPAG1	Sheeba	ATCC51449	DSM1740	NCTC11168	RM2228	RM2100	RM3195
Origin	Clinical	Clinical	Clinical	Felines	Rodents	Cattle	Clinical	Chicken	Clinical	Clinical
Genome size (bp)	1667867	1643831	1596366	1553928	1799146	2110355	1641481			
GC content (%)	39.0	39.0	-	38.2	35.9	48.5	30.5	31.3	29.6	34.5
Coding sequences										
Predicted number	1590	1459	1536	1611	1875	2046	1634	1764	1554	1782
Coding area (%)	91.0	90.8	-	89.7	93.0	93.0	94.3	-	-	-
Average length (bp)	945	998	-	865	1082	964	948	-	-	-
Flexible genome pool										
Plasmids	None	None	pHPAG1	pHac1	None	None	None	pCC178	pCL46	pCU3, pCu110
Insertion elements	IS605, IS606	IS606	None	ISHa1675, ISHa1942, ISHa1152	None	ISWsu1302, ISWsu1203	None	IS605	-	-
Genomic islands	cag PAI	cag PAI	cag PAI	HacGI	HHGI1	WSUGI I and II	None	-	-	-

new bacterial genus named Helicobacter genus[82,83]. Bacteria within this genus have been shown to colonise the gastrointestinal tract of mammals. Many of these Helicobacter species are involved in the pathogenesis of gastrointestinal diseases[82,83]. Phylogenetic analysis has shown that the *helicobacters* can be separated into two clusters[84]. Gastric species that colonise the stomach of mammals form a cluster. Species that inhabit the intestine and biliary tracts cluster together to form the enterohepatic cluster. In addition to *H pylori*, the genome sequences have been determined for several other *helicobacters*, including *H. mustelae* from ferret, *H. acinonychis* from large felines (cheetahs, lions and tigers)[85], *H. hepaticus* from mice which causes hepatoma[86], and Wolinella succinogenes from cattle[87].

General genomic features of these *helicobacters* are listed in Table 1, which also includes information for several species of *campylobacter*[88-91]. Of these related bacteria, the size and GC content of *H. acinonychis* are most similar to those of *H pylori*[85]. Comparison of 612 orthologues that are present in both *H. acinonychis* and *H pylori* found that they differ at only few of their amino acids. The Blast scores against *H pylori* of most coding sequences in *H. acinonychis* are very high. These findings lead to the conclusion that a host jump has occurred from human to felines[85]. This event probably occurred 100000 years ago. More studies are required to confirm this conclusion considering that universally accepted criteria to identify a host jump event are currently unavailable. The study also found a set of fragmented genes and newly acquired genes in *H. acinonychis*. These genes include a set of genes encoding outer membrane proteins and a cluster of genes encoding proteins for sialylation of bacterial surface carbohydrates. It has been suggested that these genes are probably beneficial for the bacterium to evade host immune defenses[92].

Information from comparative genomics has greatly enhanced our understanding of the microbiology, physiology, evolution and pathogenesis of *H pylori*. Candidate genes specific for the development of the gastric disease, particularly gastric cancer have been identified. Considering the striking diversities in the *H pylori* genome which are intensified by intra-host evolution, further studies exploring these genes must take account of them.

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ORIGINAL ARTICLES

MRI of gastric carcinoma: Results of T and N-staging in an *in vitro* study

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Abstract

AIM: To determine the accuracy of 1.5-T magnetic resonance imaging (MRI) in the evaluation of gastric wall invasion and perigastric lymph node metastasis in gastric adenocarcinoma.

METHODS: Twenty resected gastric specimens containing 20 tumors were studied with a 1.5-T MR system using a commercial head surface coil. MR scanning was performed with a T1 weighted image (TR/TE = 500/20), and a T2 weighted image (TR/TE = 2500/90). MR findings were compared with pathologic findings.

RESULTS: A T1-weighted image demonstrated three layers in the normal gastric wall. All of the gastric tumors were well demonstrated by lesions and location. In a MRI findings of gastric wall invasion, there was 1 case of T1, 7 of T2, 11 of T3. Pathologic results of resected specimens included 3 cases of pT1, 4 of pT2, and 12 of pT3. The accuracy of T staging with MRI was 74% (14 of 19). MRI findings of lymph node metastasis included 6 cases of N0, 13 cases of N1. The accuracy of the N staging with MRI was 47% (9 of 19).

CONCLUSION: MRI has a high diagnostic accuracy in the evaluation of the T staging of gastric cancer *in vitro* and thus potentially enables preoperative histopathologic staging.

Key words: Gastric cancer; Magnetic resonance imaging; Neoplasm; Staging; Stomach; Depiction of wall layer

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INTRODUCTION

The preoperative staging workup of gastric carcinoma is performed mainly with computed tomography (CT). CT has been a favored method for preoperative evaluation and staging in patients with gastric carcinoma[1-3]. Parallel advances in CT equipment and scanning techniques have reduced scanning time and decreased motion artifacts. Simultaneously, rapid IV contrast administration with an automatic power injector has improved contrast enhancement of the gastric wall and gastric cancer. Helical CT has advantages over conventional CT, including faster scanning time and fewer respiratory misregistration artifacts in a single breath-hold[4]. However, CT is limited, particularly in the diagnosis of lymph node metastasis, peritoneal metastasis, and small hematogenous metastasis[5,6].

Endoscopic sonography has been reported to be the most accurate technique for the T staging of gastric carcinoma because it can define five layers of the gastric

wall. But this technique cannot evaluate other factors such as liver metastasis and peritoneal seeding[7]. In addition, endoscopic sonography is an invasive technique dependent on the operator.

Magnetic resonance imaging (MRI) has not become popular for staging because of a number of limitations, including motion artifacts, lack of a stable contrast medium, and the high cost[8,9]. However, continuous technical improvements have been made in MRI of the abdomen, thereby reducing motion artifacts and improving image quality. These improvements include breath-hold fast imaging techniques, placement of abdominal binders, administration of antiperistaltic agents, and the use of phased array coils[10]. *In vitro* studies using 1-4.7- T MR systems have shown that MRI allows the depiction of gastric wall layers and therefore, technically permits the evaluation of the local tumor stage of gastric carcinomas[11-13].

The purpose of this study was to assess the accuracy of the evaluation of gastric carcinomas and lymph node metastasis *in vitro* by using gastrectomy specimens that were studied with 1.5-T MRI.

MATERIALS AND METHODS

Subjects

Over a period of 13 mo, a total number of 20 consecutive patients with histopathologically proven gastric carcinoma underwent subtotal or total gastrectomy. There were twenty resected gastric specimens that were obtained from the patients who were diagnosed with gastric carcinoma histologically by fiberoptic biopsy. The patients underwent subtotal or total gastrectomy. They consisted of fourteen men and six women [mean age, 53 years: 34-77 years (14 men, 6 women)]. Nineteen subtotal gastrectomy specimens and one total gastrectomy specimen was obtained and used in this study.

Specimen preparation

We needed to distend the gastric specimens for MRI. During their operations, all the stomachs were not opened for the purpose of this study. To distend the stomach of the specimens, the duodenal resection border was sealed with a continuous suture before the specimen was filled with saline solution. The specimens were then placed in a plastic box that had been filled with 5-6 L of saline solution.

The proximal portion of the gastrectomy specimen was hanged at the cap by strings. The box was capped and placed in the head coil of a 1.5-T MRI (SMT 1.5 scanner, Shimazu Co., Tokyo, Japan). Then the MR examination was performed. The study protocol was approved by the Institutional Review Board, and agreement was obtained from each patient before surgery.

MRI protocol

The MRI obtained in this study was based on the following multisection spin echo sequences for T1-weighted images, repetition time (TR) ms/echo time (TE) ms = 500/20, and for T2-weighted images, 2500/90. Two numbers of excitation were applied in this scanning. The matrix size was 256 × 256. Slice thickness was 5 mm and the intersection gap was 1 mm. Field of view was 20 cm. MR scans of the gastrectomy specimen were taken along the axial and sagittal planes. A head coil was used for scanning.

Image analysis

The MR images of 20 resected gastric specimens were analyzed by two radiologists (H.S and S.K with 3 and 10 years of abdominal CT and MRI experience, respectively) in consensus before the results from the pathologic examination were available. The number of visible wall layers and their specific signal intensity (SI) characteristics were examined. Wall-layer correlation was made on the basis of the layer thickness of the visible layers in MRI compared with the ones visible in histology. The presence of a tumor, defined as destruction of the normal gastric wall layers, was noted. The tumors were examined for variations in SI. The depth of infiltration was evaluated according to earlier publications[12,13]. A normal gastric wall was identified as having 3 layers. In terms of scanning direction and degree of distention of the wall, a gastric wall that was more than 1 cm thick or that showed an abrupt change of pattern from normal to pathologic was considered abnormal. The location, gross appearance, size and degree of serosal invasion of tumors were evaluated. Location was classified according to four areas: antrum, body, body and antrum, and fundus. Gross appearance was classified into four categories by Bormann's classification for advanced gastric carcinoma[14]. T and N staging were based on the TNM system developed by the American Joint Committee on Cancer (AJCC)[15]. Early gastric cancer was evaluated according to the Japanese Research Society for Gastric Cancer[16]. The degree of tumor invasion in the gastric wall according to the T stage was measured as follows: T1 meant that MR showed obliteration of SI within the thickened mucosal layer and second submucosal layer, T2 meant that thickening of the gastric wall and obliteration of the third layer of muscularis propria, and T3 meant irregular SI in the outer margin of the third layer.

We counted the total number of lymph nodes which were located in the perigastric area. A lymph node of > 8 mm at the short axis was considered to be pathologic[17]. N staging of lymph nodes was performed. N0 is defined as no regional lymph node metastasis, N1 as metastasis in one to six regional lymph nodes, N2 as metastasis in seven to 15 regional lymph nodes, N3 as metastasis in more than 15 regional lymph nodes. Results of MR images were compared with findings in pathologic specimens and a report made by pathologists.

Histologic preparation

Immediately after the MR examination of resected gastric specimens, the specimens were transferred to the department of pathology. The time interval between resection and fixation of the specimens was 2-3.5 h. The pathologist was not informed of the findings of

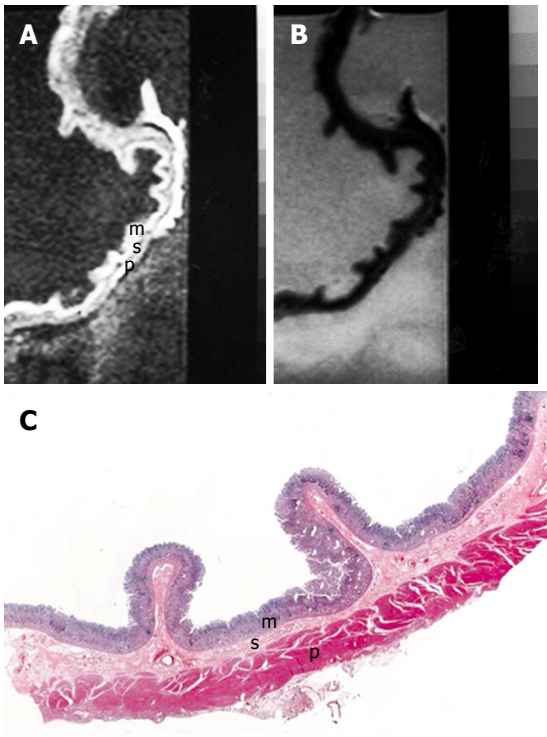

Figure 1 MRI and histology of normal gastric wall. A: T1-weighted (500/20) sagittal image of resected gastric wall showed three layers. The inner layer corresponds to the mucosa (m) and the middle layer to the submucosa (s). The outer layer basically consists of the muscularis propria (p) from which the serosa cannot be differentiated; B: T2-weighted (2500/90) MRI showed low SI on mucosa and muscularis propria and relatively high SI on submucosa; C: Light microscopic section of normal gastric wall obtained from the greater curvature site of stomach body showed three layers which are compatible with inner mucosal layer (m), middle submucosa layer (s) and outer muscularis propria and serosal layer (p) (HE stain; original magnification, × 1).

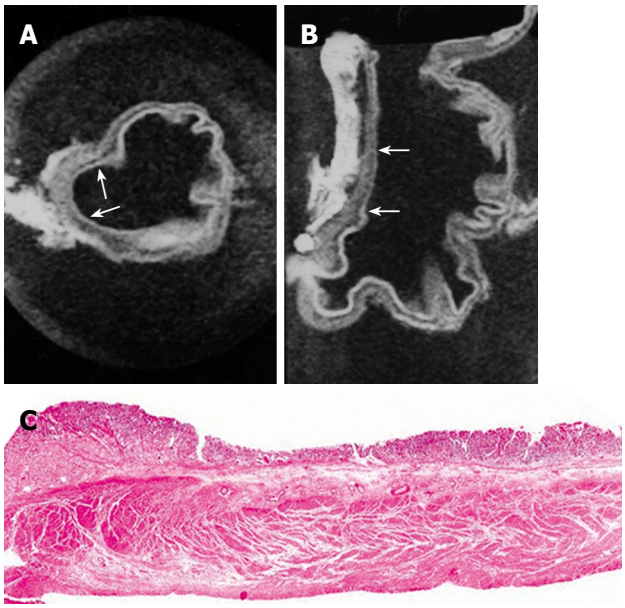

Figure 2 MRI and histology of early gastric cancer. A: T1-weighted (500/20) axial image showed depression of gastric wall and obliteration of submucosal low SI (arrows); B: T1-weighted sagittal MRI showed depressed mucosa with tumor invasion to submucosa layer (arrows); C: Light microscopic section showed depressed mucosa with tumor invasion to submucosa (HE stain; original magnification, × 1).

the MRI. The gastrectomy specimens were cut in planes corresponding to MRI imaging planes. The location, gross appearance, tumor size, and the histologic depth of invasion were determined for each specimen. The gastric carcinomas were staged according to the American Joint Committee on Cancer[15]. The diagnosed early gastric carcinomas (EGCs) were evaluated[16]. Finally, the histopathological staging of the specimens were compared with the staging by MRI. The depth of tumor invasion was decided according to the T factor of the TNM classification. Invasion of the mucosa or submucosa is classified as pT1, invasion of the muscularis propria as pT2, and tumor penetration of the serosa as pT3.

All the lymph nodes in the perigastric area of the specimens were counted and examined. Lymph nodes were stained with hematoxylin-eosin and examined by a light microscope for metastasis. Correlation between MR staging of lymph node metastasis and pathologic staging were performed by AJCC protocol[15].

RESULTS

SI characteristics of normal gastric wall

On MRI, two to three layers with different SI in the normal gastric wall can be depicted. However, there was a mainly three-layered structure (multilayered pattern) of the gastric wall by MRI. The inner layer showed an increase of SI and was 1-3 mm thick on the T1-weighted images. The second had a lower SI with thickness that varied at different sites in the same individual. The outer layer showed a slightly higher SI.

On T2-weighted images, the inner and outer layers regularly had a low SI, and the middle layer a high SI. On the basis of the comparison, the inner layer corresponds to the mucosa, the middle to the submucosa and the outer to the muscularis propria and serosal layers (Figure 1).

Detection of primary tumor

MRI of gastric carcinoma on resected specimens showed as follows: two cases of Bormann's type 1 carcinoma (polypoid type), seven cases of Bormann's type 2 (ulcerative type), six cases of Bormann's type 3 (ulcerative type with infiltration), and four cases of Bormann's type 4 (infiltrating type). One case of early gastric carcinoma with type IIc was observed, whose lesion was seen as a depression of the mucosa with thinning of the gastric wall on axial and sagittal scannings (Figure 2). Gross pathologic findings showed tumor lesions as follows; two cases of Bormann's type 1, four of Bormann's type 2, nine of Bormann's type 3, four of Bormann's type 4. One case of early gastric carcinoma with type IIc was proved upon histologic examination.

In terms of the classification of gross appearance in the nineteen lesions detected as advanced gastric carcinoma, the accuracy of MRI in the Bormann's type classification was 89% (16 of 19). Differentiation between Bormann's type 2 and type 3 lesions was

Table 1 Magnetic resonance imaging (MRI) and pathologic correlation of tumor invasion in gastric wall

	Diagnosis at histologic examination				
Diagnosis' at MR	pT0	pT1	pT2	pT3	Total
T0					
T1		1			1
T2		2	3	2	7
T3			1	10	11
		3	4	12	19

Table 2 MRI and pathologic correlation of lymph node metastasis

	Diagnosis at histologic examination			
Diagnosis at MR	pN0	pN1	pN2	Total
N0	2	3	1	6
N1	4	7	2	13
N2				
	6	10	3	19

erroneous in three lesions.

The location of gastric carcinoma was also identified on the MR images. There were nine cases of gastric carcinoma involvement in the gastric antrum, three cases in the stomach body, seven cases in the antrum and the body, one case involving the entire stomach. Upon gross specimen examination, there was no difference between them and the MRIs.

Depth of tumor invasion

Degree of invasion was evaluated in the nineteen cases of advanced gastric carcinoma (Table 1). MRIs of gastric carcinoma in resected specimens showed various findings, including thickening of the gastric wall with irregularity in the mucosal SI obliteration, thickening of the gastric wall with first and second layer SI obliteration, diffuse thickening of the gastric wall with third layer SI obliteration and irregularity with ulceration as well.

T1-weighted images showed intermediate SI in regions affected by gastric carcinoma compared to the surrounding normal mucosa and muscularis propria SI. T2-weighted images showed low SI in the gastric carcinoma. Most tumors had a homogenous SI. However, in some cases necrosis and calcification caused an inhomogeneous SI. It is not possible to differentiate between the muscularis propria, subserosa, and serosa. The reason for this inability was that we considered the subserosa and serosa as being located on the outer border of the joint layer representing the muscularis propria, subserosa, and serosa. If an infiltration was visible, the tumor was classified as T2 as long as it did not reach the outer border. Penetration of the external margin meant at once infiltration of the serosa, and the tumor was staged as a T3 carcinoma. A tumor infiltrating the subserosa without penetrating the serosa was still considered T2, according to the AJCC[5]. The MRI findings of gastric wall invasion included 1 case of T1, 7 of T2 (Figure 3), and 11 of T3 (Figure 4). Pathologic results of resected specimens included 3 cases of pT1, 4 of pT2, and 12 of pT3. Differentiation between T1 and T2 classifications was not difficult in cases displaying a distinction between three layers. However, two cases of pT1 were over staged as T2. One case of pT2 was over staged as T3. Two cases of pT3 were understaged as T2. Differentiation between T2 and T3 lesions was difficult due to the outer muscularis propria and serosal layer's thinness and could not always be demonstrated by MRIs. The level of accuracy in determining the T factor according to the TNM classification was 74% (14 of 19 lesions).

Regional lymph node metastasis

The lymph nodes presented with intermediate SI on T1-weighted images, intermediate SI on T2-weighted images. The sizes were measured as being from 0.35 cm to 3.5 cm. We counted 34 lymph nodes in the MRIs. Only 1 lymph node was measured as less than 0.8 cm on its short axis and the other 33 lymph nodes were measured as more than 0.8 cm on their short axis.

One hundred forty lymph nodes were removed from 19 cases of resected gastric specimens. The short axis of resected nodes proved malignant upon pathologic examination ranging from 0.3 to 3.5 cm. Overall metastasis was found in 60 lymph nodes. Degree of lymph node metastasis was also evaluated in 19 cases of gastric carcinoma (Table 2). MRI findings of lymph node metastasis included 6 cases of N0, and 13 of N1 (Figure 5). Pathologic findings of lymph node metastasis included 6 cases of pN0, 10 of pN1 and 3 of pN2 (Figure 6). Four cases of pN0 were over staged as N1 on the MR images. Three cases of pN1 were understaged as N0. Three cases of pN2 were understaged as N1 and N0. The accuracy of N staging by MRI was 47% (9 of 19).

DISCUSSION

Imaging modalities, such as CT and endoscopic sonography are performed for the staging of gastric carcinoma. However, the accuracy of staging gastric carcinoma is still controversial in any diagnostic modality and a definitive diagnostic modality has not been established yet. Preoperative staging of gastric carcinoma is limited by the fact that available imaging modalities do not enable accurate evaluation of depth of infiltration of the gastric wall[18-21]. CT imaging is still evaluated for gastric wall invasion and staging of gastric carcinoma. However, the results of CT in local tumor staging are also insufficient, particularly because no reliable anatomic layer differentiation of the gastric wall can be achieved[20]. Without depiction of the wall layers, a secure distinction between T1, T2 and T3 tumors cannot be achieved. Results of early studies concerning CT diagnosis of gastric carcinoma were encouraging; however, findings in later articles were pessimistic about the ability of CT to enable staging of gastric carcinoma[7,9]. Recently, spiral CT has been founded to be more accurate than previous CT studies[22,23].

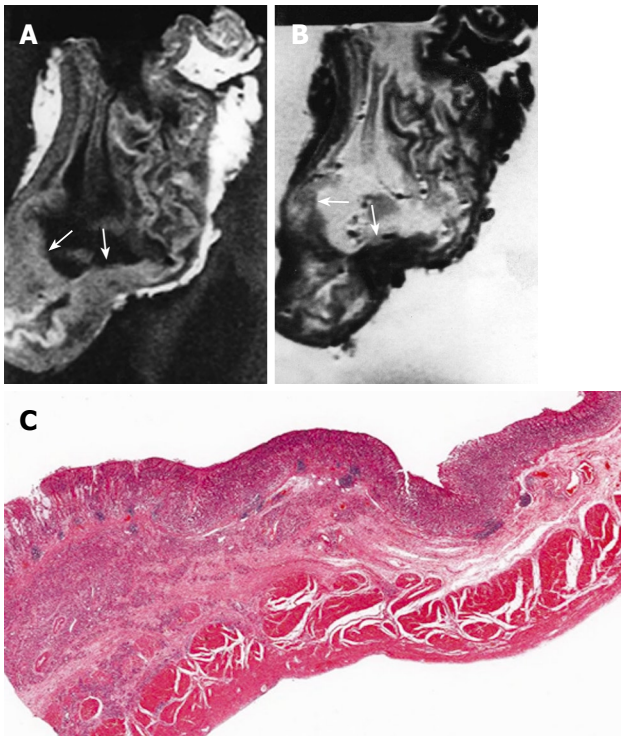

Figure 3 MRI and histology of T2 gastric cancer. A: T1-weighted (500/20) sagittal image showed diffuse thickening of gastric wall with obliteration of mucosa, submucosa and muscularis propria SI in antrum and lower body, while preserved outer marginal SI (arrows); B: T2-weighted (2000/90) sagittal MRI showed ill defined lesion with minimal increased and same SI compared to surrounding normal gastric wall (arrows); C: Light microscopic section demonstrate proper muscle invasion of gastric cancer (HE stain; original magnification, × 1).

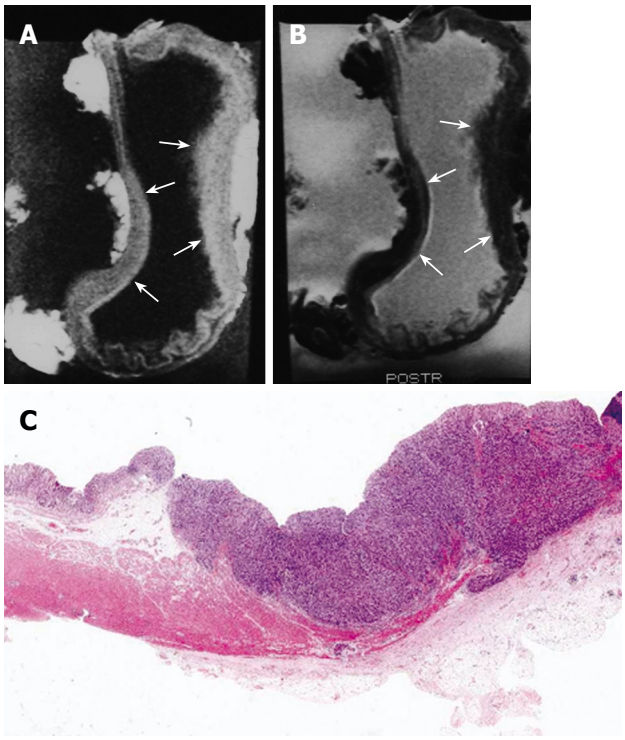

Figure 4 MRI and histology of T3 gastric adenocarcinoma. A: T1-weighted (500/20) sagittal image showed thickening of gastric wall with all three layer SI change in lesser and greater curvature site of stomach body (arrows); B: T2-weighted (2000/90) sagittal MRI showed minimal increase of SI on lesion site and poor delineation of gastric wall SI at out layer margin compared to normal gastric wall (arrows); C: Light microscopic section showed extension of tumor invasion to serosal layer (HE stain; original magnification, × 1).

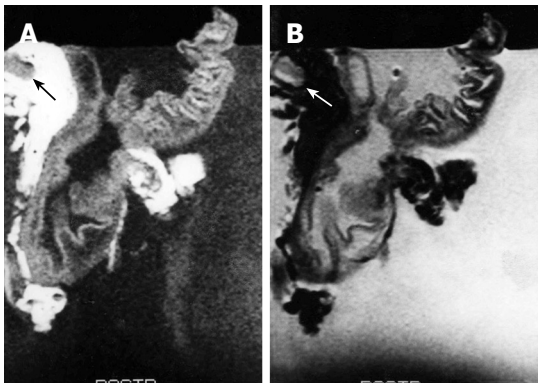

Figure 5 MRI of N1 gastric adenocarcinoma. A: T1-weighted (500/20) MR image showed single lymph node on lesser curvature site of stomach body (arrow); B: T2-weighted (2000/90) MRI showed intermediated signal SI of lymph node (arrow).

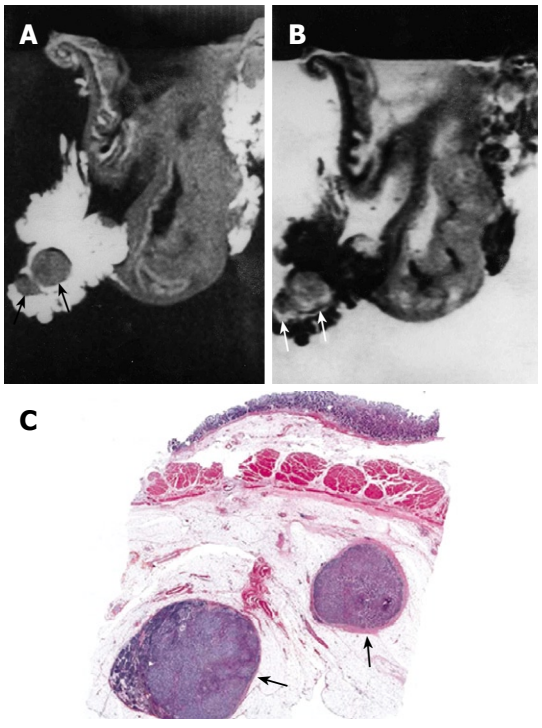

Figure 6 MRI and histology of N2 gastric adenocarcinoma. A: T1-weighted (500/20) MRI showed two lymph nodes in lesser curvature site of stomach antrum (arrows). Eight lymph nodes are detected in total in perigastric area (not shown); B: T2-weighted (2000/90) MRI showed intermediate SI in the lymph nodes (arrows); C: Light microscopic section showed two lymph nodes in lesser curvature site of gastric antrum (arrows) (HE stain; original magnification, × 1).

Endoscopic sonography is effective for detection of lymph node involvement in the perigastric area[24]. Moreover, Botet *et al*[7] reported the accuracy of the N factor and overall staging with endoscopic sonography to be 78% (39 of 50), which is significantly higher to that examined with the conventional dynamic CT technique. However, there are limitations to endoscopic sonography in the evaluation of distant perigastric lymph node metastasis. Another problem is that endoscopic sonography is invasive and the results are

highly operator-dependent.

Interest in the use of MRI for the staging of gastric carcinoma is increasing, but most clinical studies stage the local tumor situation without the differentiation of gastric wall layers[4,8-10]. Studies that use depiction of gastric wall layers as a basis for local tumor staging and lymph node metastasis are rare[13,25].

The high quality of soft-tissue imaging of MR systems enables the depiction of anatomic wall layers. Auh *et al*[11] studied the gastric wall using an experimental 4.7-T system whereas Lubienski *et al*[12] used an experimental 2.4-T system. Both groups proved that the depiction of gastric-wall layers is technically possible. Auh *et al*[11] depicted 3 layers whereas Lubienski *et al*[12] was able to differentiate 4 layers and correlated them to the mucosa, lamina muscularis mucosa, submucosa and muscularis propria. Typically 3 gastric wall layers are visible. The inner layer corresponds to the mucosa and lamina muscularis mucosa and the middle layer to the submucosa. The outer layer showed the same SI as the muscularis propria in the study of Lubinski's *et al*[12] study and therefore mainly consisted of muscle tissue and serosal layers. Palmowski *et al*[13] demonstrated that a reliable depiction of gastric-wall layers can be achieved by a conventional 1-T MRI. As no subserosa and serosa could be depicted, it must be presumed that they were located on the outer side of the third layer. So the third layer represented the muscularis propria, subserosa, and serosa together[13]. We could demonstrate that the inner and outer layers as hyperintense and the middle layer as hypointense at 1.5-MRI. When the three layers were depicted in the gastric wall, the mucosa and the muscularis propria were clearly different from the intervening submucosal layer on T1-weighted images. The distinction among the layers is based mainly on the lower SI of the submucosa compared with that of the mucosa or muscularis propria. The difference between the three layers was also depicted in the T2-weighted images.

In this study, gastric carcinomas appeared as masses with destruction of the normal structure of the gastric wall or diffuse thickening of the gastric wall and showed intermediate SI compared to surrounding normal gastric walls on T1-weighted images and low SI on T2-weighted images. Both sequences were useful for tumor localization and complement each other because some carcinomas in the study could only be recognized by deviating signal behavior in one of the 2 sequences. In our study, signal characteristics of the carcinoma depending on the MR sequence were not analyzed. Palmowski *et al*[13] reported that carcinomas show an intermediate SI on T1-weighted images, a low SI on T2-weighted images and a high SI on opposed phase images. Opposed phase images were not obtained in our study, but Dux *et al*[25] demonstrated that opposed phase images show a very high SI in gastric tumors and insisted that this was useful for the staging of gastric carcinoma. In this study, the infiltration of gastric carcinoma was correctly defined in 74% of the cases. This was not different from that of CT images that had an accuracy rate of 50%-85% and that of MR images that had an accuracy rate of 73%[4,7,26]. Yamada *et al*[27] reported that gastric specimens that were imaged after fixation in formalin and then MR imaged could also depict early gastric carcinoma. In this study, one case of early gastric carcinoma was depicted on MRI with a shallow depressed wall. This was made possible by adequate distention of the resected stomach with saline. To our knowledge, this is the first MRI depiction of early gastric carcinoma using gastric specimens without fixation in formalin. In this study, unfortunately, cases with pathologic T4 were not included because most patients who were diagnosed as T4 on preoperative imaging studies did not undergo surgery.

The evaluation of lymph node metastasis on MRIs had some limitation in this study, since the size criteria was used only on MRIs and there was no trial of contrast enhancement because of *in vitro* study of gastric carcinoma. Lymph node borders and signal intensity were not also evaluated for diagnosis of lymph node metastasis. But some cases of lymph nodes showed intermediate SI on T1 and T2-weighted images in the tumor infiltration region and this was correctly correlated with the histology. One-to-one pathologic-to-radiologic correlation on each lymph node was not performed in our study. According to Dux *et al*[25] study, lymph nodes showed a high SI on opposed phased images. MRI had low rate in depicting lymph node metastasis, with an accuracy of 47%. However, the result was similar to the other reports[25,27]. Further study is needed to increase accuracy in the finding of lymph node metastasis in gastric carcinoma.

In conclusion, the present study demonstrated that MRI can reliably depict several anatomical layers of the gastric wall and also MRI of gastric carcinoma could enable accurate diagnosis of location, gross appearance, degree of gastric wall invasion of the tumors and delineation of regional lymph node metastasis. A clear image of the tumor can be achieved. Therefore, an evaluation of the local tumor stage of gastric carcinoma and perigastric lymph node metastasis based on morphologic criteria is technically possible. This study, using a conventional 1.5-T MRI in combination with standard sequences, demonstrated the potential of MRI in the staging of gastric carcinomas. Although the result obtained in N-staging was not acceptable, it should be explored further. However, we were able to show not only MR findings of gastric wall invasion but also perigastric lymph node involvement in the gastric carcinoma. The results of our study cannot, at this time, be transferred to clinical practice, because conventional imaging acquisition techniques do not provide an image quality comparable to that of those taken of *in vitro* specimens. Advances in the variation of sequence techniques, as well as application of ultrafast imaging techniques, may in the future allow preoperative staging of gastric carcinomas by MRI.

COMMENTS

Background

The preoperative staging workup of gastric cancer is performed mainly with

computed tomography (CT). Magnetic resonance imaging (MRI) has not become popular for staging because of a number of limitations, including motion artifacts, lack of a suitable oral contrast medium, and the high cost. However, continuous technical improvements have been made in MRI of the abdomen, thereby reducing motion artifacts and improving image quality.

Research frontiers

MRI has not yet reached clinical importance because of some limitations. However, *in vitro* studies using experimental 2.4-4.7-T MR systems have shown that MRI allows the depiction of gastric wall layers and therefore technically permits the evaluation of the local tumor stage of gastric cancer.

Innovations and breakthroughs

MRI in the staging of gastric cancer is not usually applied in clinical practice. Its results are the same or inferior to CT in accuracy. There are many MRI *in vitro* and clinical imaging gastric cancer studies. *In vitro* MRI systems demonstrate well gastric wall layers and tumor invasion well and MRI has shown tumor invasion and lymph node metastasis in some clinical studies as well. However, normal gastric wall depiction and tumor invasion of the gastric wall are important to tumor staging, as are demonstrations of perigastric lymph node metastasis. The authors studied resected gastric specimens for the accurate depiction of normal gastric walls, tumor invasion and lymph node metastasis using 1.5-T MRI.

Applications

The study results suggest that MRI could be useful in gastric wall invasion in the staging of gastric cancer. However, further study in the staging of lymph node metastasis is still needed.

Peer review

The authors successfully demonstrated normal gastric wall layers and tumor invasion in their *in vitro* study using 1.5-T MRI. The results suggest that MRI is a potential diagnostic tool in the staging of gastric cancer.

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BRIEF ARTICLES

Efficacy of intramuscular diclofenac and fluid replacement in prevention of post-ERCP pancreatitis

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Abstract

AIM: To assess the efficacy of intramuscular diclofenac and fluid replacement for prevention of post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis.

METHODS: A prospective, placebo-controlled study was conducted in 80 patients who underwent ERCP. Patients were randomized to receive parenteral diclofenac at a loading dose of 75 mg followed by the infusion of 5-10 mL/kg per hour isotonic saline over 4 h after the procedure, or the infusion of 500 mL isotonic saline as placebo. Patients were evaluated clinically, and serum amylase levels were measured 4, 8 and 24 h after the procedure.

RESULTS: The two groups were matched for age, sex, underlying disease, ERCP findings, and type of treatment. The overall incidence of pancreatitis was 7.5% in the diclofenac group and 17.5% in the placebo group (12.5% in total). There were no significant differences in the incidence of pancreatitis and other variables between the two groups. In the subgroup analysis, the frequency of pancreatitis in the patients without sphincter of Oddi dysfunction (SOD) was significantly lower in the diclofenac group than in the control group (P = 0.047).

CONCLUSION: Intramuscular diclofenac and fluid replacement lowered the rate of pancreatitis in patients without SOD.

INTRODUCTION

Pancreatitis is the most common complication of endoscopic retrograde cholangiopancreatography (ERCP), with a reported incidence of 1%-10% in most prospective studies[1-9]. The generally accepted criteria for the diagnosis of post-ERCP pancreatitis were proposed in 1991 during a consensus workshop. These criteria include new onset of pancreatic-type abdominal pain associated with at least a threefold increase in serum amylase or lipase occurring within 24 h after ERCP, and the pain symptoms need to be sufficiently severe to require admission to the hospital or to extend the length of stay of patients who are already hospitalized[1,10].

There have been numerous theories about the mechanisms of pancreatitis. The most widely accepted theory is that mechanical trauma to the papilla or pancreatic sphincter, caused during instrumentation, creates transient obstruction of outflow of pancreatic juice[1]. Another theory suggests that the increased hydrostatic pressures in the pancreatic duct caused by injection of contrast or saline could cause injury to the pancreatic duct or parenchyma[1].

Risk factors reported for ERCP-induced pancreatitis include a history of pancreatitis[11], difficult cannulation[2], repeated injection of the pancreatic duct[11], pancreatic acinar opacification[12], sphincter of Oddi dysfunction (SOD)[3,13] and precut or needle-knife endoscopic sphincterotomy[9,13,14].

Cellular events that lead to pancreatitis involve an inflammatory process with premature activation of trypsin in acinar cells[15,16]. Phospholipase A_2 is believed to play a critical role in the initial inflammatory cascade of acute pancreatitis by regulating a number of pro-inflammatory mediators, including arachidonic acid products and platelet-activating factors[17]. Prevention or interruption of this cascade may prevent development of pancreatitis and its consequences. Although drug development has been impressive, the availability of effective drugs in the prevention and management of pancreatitis remains limited[15].

Chemoprevention of pancreatitis still remains a debated question. Pharmacological prevention of pancreatitis after ERCP has been the topic of several investigations in recent years.

Diclofenac, a potent inhibitor of phospholipase A_2 activity, administered immediately after the procedure, is effective at preventing pancreatitis[9,18]. Advantages of this prophylaxis are the low cost and the possibility of "on-demand" treatment. Addition of non-steroidal anti-inflammatory drugs (NSAIDs) has also been shown to have beneficial effects in experimental acute pancreatitis[19].

The aim of this study was to evaluate the efficacy of intramuscular (IM) diclofenac and fluid replacement for the prevention of pancreatitis in all eligible patients who underwent ERCP at our medical center.

MATERIALS AND METHODS

The study described in this report was approved by the ethics committee of Suleyman Demirel University School of Medicine, Isparta, Turkey. Between August 2006 and April 2008, 91 patients fulfilled the inclusion criteria, 80 of whom were included in the final analysis. Patients were excluded from study participation if they had a contraindication for diclofenac, including patients with recently diagnosed peptic ulcer disease, renal failure, those who had taken an NSAID during the preceding week, those who developed acute pancreatitis during the 2 wk before ERCP, those with a history of chronic pancreatitis, and those who did not agree to participate in the study. Entry to the study was restricted to patients advised to have endoscopic retrograde cholangiography with or without pancreatography for extrahepatic cholestasis and/or impaired liver function tests.

A prospective, placebo-controlled trial was conducted in 80 patients who underwent ERCP. The patients received 75 mg IM diclofenac and intravenous (IV) isotonic saline at a rate of 5-10 mL/kg per hour for 4 h or an inert placebo (500 mL IV isotonic saline) immediately after ERCP. At the end of each procedure, the researchers recorded the details of the maneuvers performed, including the total time of the procedure, the number of attempts at cannulation, the number of pancreatic duct cannulations, the final diagnosis, and whether a sphincterotomy, a needle-knife papillotomy, or stent placement were performed. We did not use pancreatic duct stenting for prevention of pancreatitis.

Patients were sedated with IV midazolam. Xylocaine spray was used as a local anesthetic.

Serum amylase was determined 4 h after ERCP. If the 4-h serum amylase level was < 3 times the upper normal limit and there was no clinical evidence of acute pancreatitis at that time, patients were allowed free oral fluids and a diet. If the 4-h serum amylase level was > 3 times the upper normal limit and the patient exhibited pain or nausea and vomiting, then the patient was kept fasting and IV crystalloid fluids with opiate analgesics were prescribed. The following 8 h and 24 h blood tests were repeated for serum amylase and the patients were interviewed and examined for clinical evidence of acute pancreatitis. Acute pancreatitis was defined as serum amylase > 3 times the upper limit of normal associated with epigastric pain, back pain, and epigastric tenderness. Patients with persistent signs and symptoms of pancreatitis after 48 h underwent contrast-enhanced computed tomography.

Pancreatitis was graded as mild, moderate, or severe. Sphincter of Oddi dysfunction (SOD) was defined according to the Milwaukee Biliary Group Classification[20].

The instruments used were cannula, sphincterotome, guidewire, and stone basket (Boston Scientific, Natick, MA, USA).

Statistical analysis

Data were summarized by descriptive statistics. The χ^2 square and Fischer's exact tests were used to compare categorical patient data. The Mann-Whitney *U* test and Student's *t* test were used to compare continuous variables. Two-tailed $P < 0.05$ were considered to indicate significance.

RESULTS

A total of 80 patients were eligible for the study. Forty patients received 75 mg diclofenac and isotonic saline replacement (diclofenac group), and 40 received inert parenteral fluid replacement (control group). No patients discontinued the study medication because of adverse effects. Overall, the baseline characteristics were consistent across all treatment groups (Table 1). The mean ages of patients in the diclofenac and control groups were 60.3 ± 16.1 years and 59.3 ± 14.4 years, respectively. There were 15 women in the diclofenac group and 22 in the control group. Similarly, there were no statistically significant differences between the groups considering the procedures, and factors that might increase the risk of pancreatitis, including single or repeated pancreatic duct injection, SOD, younger age, female sex and precut endoscopic sphincterotomy (Table 2). Although the frequency of pancreatitis in the patients with SOD did not differ between the diclofenac and control groups, it was statistically significant between groups when the patients with SOD was excluded ($P = 0.047$). The most frequent indication for ERCP was bile duct stone in the diclofenac (57.5%) and control (27.5%) group. Post-endoscopic bleeding

Table 1 Characteristics of patients in the diclofenac and control groups[1]

	Diclofenac group (n = 40)	Control group (n = 40)	P
Variables			
Age (yr)[2]	60.3 ± 16.1	59.3 ± 14.5	0.766
Female sex	15 (37.5)	22 (55.0)	0.116
Bile duct canulation time (min)[3]	5.0 (3.0-5.0)	5.0 (3.0-10.0)	0.601
Operation time (min)[3]	15 (15-20)	15 (10-25)	0.904
Sphincterotomy			
Precut	10 (25)	12 (30)	0.617
Pancreatic duct injection	19 (47.5)	18 (45.0)	0.823
Pancreatic duct cannulation, twice or more	8 (20.5)	6 (15.0)	0.556
Post-ERCP pancreatitis	3 (7.5)	7 (17.5)	0.176
Final diagnosis			
BD stone	23 (57.5)	11 (27.5)	
SOD	3 (7.5)	10 (25.0)	0.082
Biliopancreatic tumors	5 (12.5)	9 (22.5)	
Others[4]	9 (22.5)	10 (25.0)	

[1]All data were presented as [n (%)] and comparisons were made with χ^2 or Fisher's exact test unless otherwise stated. [2]Data was presented as (mean ± SD) and comparison was made with Student's t test. [3]Data were presented as [median (interquartile range)] and comparisons were made with Mann-Whitney U test. [4]Primary sclerosing cholangitis, hydatid cysts communicating with the bile ducts, Mirizzi sendrome, and postoperative complications. Post-ERCP: Post-endoscopic retrograde cholangiopancreatography; SOD: Sphincter of Oddi dysfunction.

Table 2 Incidence of post-ERCP pacreatitis in different subgroups[1]

	Diclofenac group (n = 40)	Control group (n = 40)	P
Variables			
Age group (yr)			
> 60	2/23	4/20	0.300
⩽ 60	1/17	3/20	0.420
Sex			
Male	0/25	2/18	0.850
Female	3/15	5/22	0.092
Pre-cut sphincterotomy			
Yes	1/10	4/12	0.210
No	2/30	3/28	0.590
Pancreatic duct injection			
Yes	2/19	4/18	0.350
No	1/21	3/22	0.330
Pancreatic duct cannulation, twice or more	2/8	1/6	0.730
SOD			
Yes	2/3	2/10	0.150
No	1/37	5/30	0.047[1]

[1]All data were comparisons with the χ^2 test. There were no statistically significant differences except in the patients without SOD.

Table 3 Serum amylase level (mean ± SE, IU/L) following ERCP in diclofenac and control groups

Group	Amylase 4 h	Amylase 8 h	Amylase 24 h
Diclofenac	223.95 ± 33.45	218.39 ± 35.44	161.82 ± 31.03
Control	283.15 ± 82.74	308.34 ± 96	231.56 ± 57.73

There were no statistically significant differences.

because of sphincterotomy was observed in three of 75 sphincterotomy patients (3.75%). All the bleeding was seen during the procedure. No case of delayed bleeding occured. Two of three bleeding episodes in the control group and one in the diclofenac group were self-limited and stopped during endoscopy without intervention.

Pancreatitis occurred in 10/80 patients (12.5%), three of whom (7.5%) belonged to the diclofenac group and seven (17.5%) belonged to the control group (Table 1). Four and eight hours after endoscopy, the mean ± SE serum amylase level was 283.15 ± 82.74 IU/L and 308.34 ± 96 IU/L in the control group and 223.95 ± 35.45 IU/L and 218.39 ± 35.44 IU/L in the diclofenac group. Twenty-four hours after endoscopy, the mean ± SE serum amylase level was 231.56 ± 57.73 IU/L in the control group and 161.82 ± 31.03 IU/L in the diclofenac group (Table 3). In the diclofenac group, the mean values of amylase were low but the statistical difference was not significant ($P > 0.01$).

DISCUSSION

The number of ERCP procedures performed annually worldwide has increased dramatically over the past 25 years. Pancreatitis occurs in 1%-10% of patients but may approach ⩾ 25% depending on the presence of other risk factors[15].

Several mechanical and pharmacological interventions have been evaluated in the prevention of pancreatitis. The availability of effective drugs and strategy of chemoprevention are unresolved issues in the pharmacological prophylaxis of pancreatitis. Previous studies on reducing the incidence of pancreatitis have targeted reduction of pancreatic secretion, prevention of intra-acinar trypsinogen activation, interruption of the inflammatory cascades, relaxation of the sphincter of Oddi, and prevention of infection[21].

An ideal agent is highly effective in reducing pancreatitis, is safe for the patient, well tolerated, relatively affordable, and does not have a prolonged administration time[1]. Various pharmacological agents (such as nifedipine, glucagon, calcitonin, lidocaine, nitroglycerine, antibiotics, steroids, allopurinol, interleukin-10, and heparin) have been tried, but have met with disappointing results in preventing pancreatitis in randomized controlled trials[1,22-34].

Only two agents seem to offer any clinical benefit: the protease inhibitor gabexate mesilate[35-37] and the antisecretory agent somatostatin may be efficacious in preventing pancreatitis when given by continuous IV infusion[34,38,39]. Since these agents require continuous and prolonged IV infusion, they are not suited for same-day outpatient ERCP[34].

Several prospective randomized studies have shown that pancreatic stents have a beneficial role for prevention of pancreatitis in high-risk patients, including biliary and pancreatic sphincterotomy for SOD[40-42], biliary balloon dilation for stone[43] and precut biliary sphincterotomy[21]. Although pancreatic stenting is

often beneficial, the down sides include the difficulty of stent insertion in patients with small or tortous ducts and the follow-up required for stent removal. A simple prophylactic medication would be highly desirable[21].

NSAIDs may prevent pancreatitis by inhibiting prostaglandin synthesis and interrupting the inflammatory cascade of pancreatitis[34].

In the report by Sotoudehmanesh *et al*[15], eligible patients undergoing ERCP (n = 490) were randomized to receive a 100-mg indomethacin rectal suppository (n = 245) or placebo (n = 245) just prior to ERCP, and rates of post-procedure pancreatitis were assessed. Pancreatitis occurred in 7/221 (3.2%) patients in the indomethacin group and in 15/221 (6.8%) of those receiving placebo (P = 0.06), with an overall pancreatitis rate of 5% (22/442).

Montaño Loza *et al*[45] have reported a randomized prospective clinical trial that compared indomethacin with placebo in the prevention of pancreatitis. They enrolled patients undergoing ERCP for suspected bile duct obstruction rather than selecting for a high-risk cohort. Rectal indomethacin (100 mg) or placebo was administered prior to ERCP. Seventy-five patients were randomized to each group, a sample size that was calculated a priori to detect a 15% reduction in pancreatitis. The overall incidence of pancreatitis was 10.7%. The incidence of pancreatitis was 16% (12/75) in the placebo group and 5.3% (4/75) in the indomethacin group. This difference was statistically significant, with a P value of 0.034. All pancreatitis cases in both groups were categorized as mild[44,45].

Diclofenac, an NSAID, inhibits phospholipase A_2, which is thought to play a critical role in the early inflammatory cascade. In addition, it strongly inhibits neutrophil/endothelial attachment, thus preventing accumulation of neutrophils at the site of tissue damage[46], and inhibits the expression of nitric oxide synthase, an enzyme associated with inflammation and cell damage[47]. It is a cheap, widely available agent with a short, easy method of administration.

Murray *et al*[9] have conducted a single-center, prospective, randomized, double-blind, placebo-controlled study to determine if a single dose of rectally administered 100 mg diclofenac, given after ERCP, reduced the incidence of pancreatitis. Of 220 patients, 110 received rectal diclofenac, and the others, an inert placebo. Pancreatitis occurred in 6.4% of patients in the diclofenac group and in 15.5% of those receiving placebo (P = 0.049). This difference was statistically significant. Also, the drug was not effective in the subgroup of patients with SOD, the very group at highest risk[9].

Khoshbaten *et al*[18] have reported a randomized controlled study that compared 100 mg rectal diclofenac with placebo in 100 patients who underwent high-risk ERCP. To select high-risk cases, only those undergoing pancreatography (with or without cholangiography) were enrolled. The study drug or placebo was administered on arrival in the recovery area. The overall incidence of pancreatitis was 15%. The incidence of pancreatitis in the placebo group was 26% (13/50), whereas the incidence of pancreatitis in the diclofenac group was 4% (2/50). This difference was statistically significant, with $P < 0.01$. No patients in this clinical trial developed necrotizing pancreatitis or required surgical intervention[18,44].

We showed that, in the diclofenac group, pancreatitis was seen less than in the control group, but this was not statistically significant. The absence of the statistical difference may have been caused by the small number of patients.

A number of risk factors for post-ERCP pancreatitis have been identified by a multitude of studies that have different study designs, have examined different candidate predictor variables, and have taken place in a variety of settings[15]. The impact of some of these associations has been supported by large, multicenter prospective trials, while others have been suggested in smaller series and by clinical experience. Risk factors that have been recognized as independent predictors in more than one study include: younger age, female sex, pancreas divisum, SOD, prior ERCP-induced pancreatitis, difficulty of cannulation, and pancreatic duct injection[15]. None of the patients in our study had pancreas divisium or prior ERCP-induced pancreatitis. Also, there was no significant difference in the incidence of pancreatitis when comparing diclofenac with placebo, in patients with younger age, female sex, SOD, pre-cut sphincterotomy and twice or more pancreatic duct cannulation ($P > 0.01$). In our study, subgroup analysis showed that diclofenac significantly decreased the frequency of pancreatitis only in the patients without SOD. All the cases of pancreatitis were mild and the patients were discharged from the hospital within several days without any complication.

Acute pancreatitis is an unstable disease that causes intravascular fluid loss because of local and systemic inflammation. Clinical improvement can be achieved by fluid infusion. Fluid resuscitation is the most important treatment during the first 72 h after onset of acute pancreatitis. Therefore, the two goals of early phase fluid resuscitation are amelioration of tissue hypoxia and prevention of complications[48].

In our study, IV isotonic saline was given to the diclofenac group in the initial 4 h (5-10 mL/kg per hour) after ERCP. Five hundred milliliters isotonic saline was given to the control group to keep the IV line open. Although the importance of fluid management in acute pancreatitis is known, there have not been so many studies about the prophylactic effects of this approach.

In conclusion, our study showed that parenteral diclofenac and hydratation tended to prevent post-ERCP pancreatitis, but the finding was not statistically significant. In the whole group, diclofenac did not prevent the occurrence of pancreatitis but, according to the subgroup analysis, in patients without SOD, it significantly prevented pancreatitis. For this reason, further studies are required on the efficacy of this treatment with other doses and combinations of diclofenac and hydratation that might prevent pancreatitis.

COMMENTS

Background

Pancreatitis is the most common complication of endoscopic retrograde cholangiopancreatography (ERCP). Pharmacological prevention of pancreatitis after ERCP has been the topic of several investigations in recent years.

Research frontiers

Various pharmacological agents have been tried but have met with disappointing results in preventing pancreatitis in randomized controlled trials. Non-steroidal anti-inflammatory drugs may prevent pancreatitis by inhibiting prostaglandin synthesis and interrupting the inflammatory cascade of pancreatitis. The importance of fluid management in acute pancreatitis is known, but there have not been many studies about the prophylactic effects of this approach.

Innovations and breakthroughs

The overall results of this study showed that parenteral diclofenac and fluid replacement had no beneficial effect on the prevention of pancreatitis. Although diclofenac and fluid replacement did not prevent the occurrence of post-ERCP pancreatitis, the rate of pancreatitis was lower in those patients without sphincter of Oddi dysfunction (SOD) who received diclofenac. To prevent post-ERCP pancreatitis, further studies should be carried with the other doses and combinations of diclofenac and hydratation in a larger group of patients.

Applications

This study was designed to evaluate the efficacy of prophylactic intramuscular diclofenac and fluid replacement for the prevention of post-ERCP pancreatitis.

Peer review

This study aimed to find a pharmacological way for preventing post-ERCP pancreatitis. The results show that prophylactic intramuscular diclofenac and fluid replacement has no benefit except in patients without SOD.

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Reaching proficiency in laparoscopic splenectomy

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Abstract

AIM: To investigate the proficiency level reached in laparoscopic splenectomy using the learning curve method.

METHODS: All patients in need of splenectomy for benign causes in whom laparoscopic splenectomy was attempted by a single surgeon during a time period of 6 years were included in the study (n = 33). Besides demographics, operation-related variables and the response to surgery were recorded. The patients were allocated to groups of five, ranked according to the date of the operation. Operation duration, complications, postoperative length of stay, conversion to laparotomy and splenic weight were then compared between these groups.

RESULTS: There was a significant difference regarding operation times between the groups (P = 0.001). An improvement was observed after the first 5 cases. The learning curve was flat up to the 25th case. Following the 25th case the operation times decreased still further. There was no difference between the groups regarding the other parameters.

CONCLUSION: Unlike the widely accepted "L" shape, the learning curve for laparoscopic splenectomy is a horizontal lazy "S" with two distinct slopes. Privileges may be granted after the first 5 cases. However proficiency seems to require 25 cases.

Key words: Laparoscopic splenectomy; Education; Learning curve; Hematology; Proficiency

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INTRODUCTION

Laparoscopic splenectomy has become the gold standard intervention for the removal of the spleen, especially for benign causes. However, the organ's high anatomic location, fragility and generous blood supply makes the procedure an advanced laparoscopic operation[1,2]. Furthermore, unlike patients with gall bladder stones, patients who need splenectomy for benign disorders are rare. These factors may prohibit the laparoscopic surgeon from becoming proficient in laparoscopic splenectomy. Measuring the expertise and setting a minimum number of procedures to be performed in order to be accepted as proficient in this rather rare operation has proved difficult.

One method for quantifying the level of expertise is to split the study population arbitrarily into two, as early and late experience[3,4]. Several variables are then compared between the two groups. A more sensitive method is to depict the learning curve. The learning curve may be briefly defined as the repetition of the procedure until it is learned[5]. In this study, we aimed to define the learning curve for laparoscopic splenectomy based on the experience of a single surgeon.

MATERIALS AND METHODS

All patients with laparoscopically attempted splenectomy, operated on by a single surgeon (TZN), were included in this study. The time period was from November 2002 to January 2008. During this time span 33 patients (23 female) with a mean ± SD age of 43.4 ± 18.4 years were operated. Operative indications were immune thrombocy-

topenic purpura (ITP) (n = 27), hemolytic anemia (n = 3), thalassemia (n = 2), and hydatid cyst (n = 1). Besides demographics, associated disorders, the size of the spleen (long axis) as measured with preoperative ultrasonography, duration of surgery, the volume of gas used, additional procedures during operation, conversions to laparotomy, amount of intra/postoperative blood loss and blood products transfused, presence of accessory splenic tissue, complications, morsellated splenic weight, length of hospital stay, early and late success in hematological disorders and thrombocytosis in the long term were recorded.

Patients with hematological diseases were referred from the hematology clinic with resistant or persistent disease. The hydatid cyst patient had a type 3, 7-cm cyst in the spleen. He had percutaneous treatment for type 1 and 2 cysts located in the liver.

All patients received vaccination against encapsulated bacteria within 2 wk prior to operation. For ITP cases, the patients received pulse steroid or intravenous immunoglobulin therapy before the operation in order to increase the thrombocyte count to at least 50 000/mm^3. General anesthesia was used. A nasogastric tube was placed routinely. A lateral approach was used for the operation[6-8]. The patient was placed in a 70-80° right lateral position with reverse Trendelenburg for the table. The table was flexed 20-25° at the kidney rest to increase the left flank space. Following skin preparation and draping, the table was tilted to the left in order to obtain an approximately supine position for the initial insufflation. Four trocars, each 5 cm apart, were placed along an imaginary left subcostal incision with the most medial on the midline and most lateral on the left anterior axillary line. The table was again tilted to the original position. The 30° optic was introduced through the middle of the lateral 3 trocars. The stomach was manipulated through the most medial 4th trocar. The dissection was started from the inferior pole using clips and ultrasonic dissector for vascular control. Hilar vessels were controlled in the same manner, sometimes utilizing intracorporeal silk ligatures for the large-sized vessels. A vascular stapler was not used routinely: it was deemed necessary for only one patient with large-sized vessels. Lastly, short gastric vessels were controlled. After separating the lateral connections, the spleen was placed in a retrieval bag and was morsellated and removed through the lateral 10 mm trocar. The piecemeal specimen was weighed and sent for pathological assessment.

Patients were usually started on oral feeding the next day and discharged or transferred to the hematology clinic the following day. Hemoglobin and thrombocyte counts were studied on the first postoperative week and 3rd postoperative month to assess the early and late response to splenectomy for hematological diseases. Response to the surgery was defined as hemoglobin level greater than 100 g/L for hemolytic anemia and thrombocyte count of more than 150 000/mm^3 for ITP. Thrombocytosis is defined as a thrombocyte count of more than 600 000/mm^3.

General descriptive characteristics of the group were expressed as mean ± SD and percentages where necessary. In order to define the learning curve, patients were divided into groups of five ranked according to the date of operation (the last group consisted of 3 patients). Dichotomous variables such as rate of complications and conversion to laparotomy were compared between groups by χ^2 analysis. Continuous variables such as the duration of operation, the amount of gas used, splenic weight and length of hospital stay between ranked groups were compared using the ANOVA technique. $P < 0.05$ was accepted as significant. All analyses were performed using SPSS version 13.0 (SPSS, Chicago, Illionis).

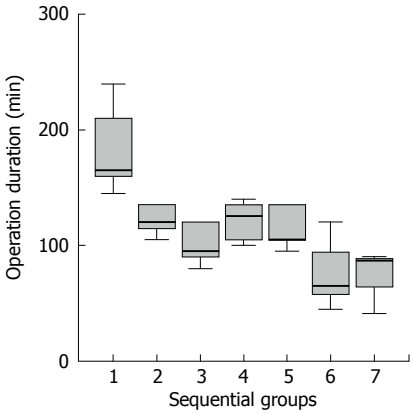

Figure 1 The learning curve for laparoscopic splenectomy based on the operation times. Groups are formed according to the date of the operation.

RESULTS

In 18 (54.5%) patients the size of the spleen was less than 12 cm. The size was in the range of 12-20 cm in 14 (42.4%) patients and over 20 cm in one patient (3.0%). One third of the patients (n = 10) had additional systemic diseases, diabetes mellitus (n = 4) being the most common.

The immediate preoperative thrombocyte count for ITP cases was 60 960 ± 77 440/mm^3. Hemoglobin level for hemolytic anemia and thalassemia cases was 92 ± 38 g/L.

Only one patient (3%) was converted to laparotomy. In this patient (12th case, 3rd group) the spleen was large, over 20 cm and weighed 1 040 g. Hemorrhage could not be controlled and the case was converted at the 105th min. Erythrocyte suspension (5 units) was transfused to 3 hemolytic anemia patients (9.1%) during the perioperative period. Thrombocyte transfusion was not needed.

The mean operation duration for the whole group was 120.2 ± 46.2 min. There was a significant difference between ranked groups regarding operation time (P = 0.001). The last two groups' operation time, i.e. after the 25th case, was less than the first 5 cases according to the Bonferroni post hoc test (Figure 1).

Accessory spleens were detected and removed in 5 patients (15.2%). The average amount of CO_2 used was 137.5 ± 80.7 L. There was no difference between ranked groups regarding CO_2 use (P = 0.119). Postoperative complications were observed in 3 patients (one atelectasis,

one wound infection and one suture reaction). Early and late success rates were 83.9% and 81.0%, respectively, and were not significantly different between the groups. Thrombocytosis was observed in 5 patients (15.2%). Antiaggregant therapy with salicylic acid was instituted for these patients.

DISCUSSION

There has been a rush for minimal access surgery with the introduction of laparoscopic cholecystectomy in the 1980s. However, the surgical community was not technically prepared for this uncontrolled demand. Although weekend courses, seminars and hands-on courses were held worldwide, minimal access surgery education was less than optimal[9-11]. Surgical societies, not wishing to repeat these mistakes, started regular courses for both basic and advanced laparoscopic procedures. Universities and teaching hospitals included these procedures in their curricula. Several societies advocated a minimum number of procedures to be set to grant privileges to perform an operation[12].

It is difficult to define a point when mastery of a procedure is reached. Subjective assessment in this regard is unreliable and several objective methods have been devised for this task. One simple method is to compare the early with late experience. For this, a chronologically-ranked patient population is divided in two[4]. Markers of expertise such as operation time, complication rate, conversion rates (in case of laparoscopy), time to oral feed and cost are then compared between these arbitrarily separated groups. The learning curve, which is more sensitive, usually is a graphic representation of the expertise level reached for a particular procedure/process. The cases are individually ranked on a chronological basis and are placed on one axis of the graph. The above-mentioned variables are depicted on the other axis. The initial slope of the curve is usually steep where the learning process is fast and with each case performance increases. After a while, the slope flattens and no major improvement can be observed following a certain number of the procedures. The point, the nth case, where the curve starts to flatten is accepted as the instance where the procedure is learnt. Even after this point minor improvement in performance is detected, albeit the difference is usually not significant. A variation of this technique is to allocate patients into small groups and compare these groups with regard to determined variables. Similarly there is a point when a significant difference cannot be observed, which is accepted as the number of procedures needed to perform to reach proficiency.

Since the first report of laparoscopic splenectomy by Delaitre, this procedure has become the preferred treatment for the removal of the spleen[13-15]. However, this being one of the advanced laparoscopic procedures and due to the relatively rare occurrence of the conditions requiring splenectomy, the procedure is difficult to master.

Several studies have defined the minimum number of laparoscopic splenectomies to be performed for proficiency. Operation time is the most used variable as it is easier to calculate and validate. In the study by Rege and Joehl, these authors suggested that an improvement occurred with the first 15-25 patients[3]. In a review generally comparing the results of initial and late experience for laparoscopic procedures, it was determined that 20 laparoscopic splenectomies were sufficient for mastery[5]. In the study by Peters *et al*[16], a minimum of 20 laparoscopic splenectomies were deemed necessary for ITP surgery proficiency. Regarding pediatric surgery, 20 cases, similarly, were declared to be the threshold for proficiency[17].

However, there are some design limitations of the above-mentioned studies. In the reports by Rege and Chan the patient populations were split in two; an initial first 15, and those that came later[3,4]. The cut-off point was chosen arbitrarily and compared only two groups, i.e. the initial and late experience, which may decrease the sensitivity as stated previously. Further, in order to document an accurate progress and exclude personal variations, the performance of a single surgeon should be recorded. If other surgeons' experiences are included, the investigated parameters may differ according to the individual's skill. Among the above-mentioned studies, the experience of a single surgeon was assessed in only one report[17]. Among the rest of the reports, either senior residents gradually started to perform the operations or different hospitals/surgeons were included in the analyses[3,16,18]. The concern about inclusion of low-volume surgeons in such assessments has also been previously stated[19,20]. Although Cordera *et al*[20] have attempted some 42 laparoscopic splenectomies, rightfully they have not calculated a learning curve due to inclusion of several surgeons. Another potential source of bias is the introduction of new devices or technology during the study period. For instance, an ultrasonic dissector was introduced during the latter course of the study in one report[4].

In our study the experience of a single surgeon was assessed to exclude personal variations in skill. In order to eliminate the possible confounding effects of the mechanical set-up, standard techniques and instruments were used during the course of the study. Instead of comparing the arbitrary initial and late experience we have assessed the differences between sequential multiple groups. Twenty to twenty-five laparoscopic splenectomies were cited to be the minimum number for mastery of the procedure in the literature. However, it is common knowledge that privileges are granted with far less experience of this relatively rare operation. Usually 3 to 5 laparoscopic splenectomies, arbitrarily defined, are accepted to be sufficient[3]. However, there is no evidence in the literature supporting this figure. We provide the first evidence that 5 laparoscopic splenectomies could be accepted as the minimum number for the general curricula. There is a dual curve in our study of the learning process (Figure 1). Progress is rapid with the first 5 cases and then the curve steadies up to the 25th case. At that point there is again a significant increase in the performance i.e. a decrease in the operation time.

We have shown that the learning curve for laparo-

scopic splenectomy is not a smooth "L" but has two distinct slopes. With the first 5 cases the novice may be granted privileges for performing laparoscopic splenectomy on an individual basis. Past the 25th case the surgeon could be accepted as an expert in the field. Although unlikely, it is yet to be shown that any further improvement could be observed after this level.

COMMENTS

Background

Laparoscopic revolution resulted in some controversies regarding the education in advanced laparoscopic surgery. In order to assess the level of expertise and to grant privileges in surgery, a learning curve of the procedure is used.

Research frontiers

Laparoscopic splenectomy has become the gold standard intervention for the removal of the spleen, especially for benign causes. In this study, the authors have investigated the proficiency level reached in laparoscopic splenectomy using the learning curve method.

Innovations and breakthroughs

Unlike the widely accepted "L" shape, the learning curve for laparoscopic splenectomy is a horizontal lazy "S" with two distinct slopes. Privileges may be granted after the first 5 cases. However, proficiency seems to require 25 cases.

Applications

The authors have shown that the learning curve for laparoscopic splenectomy is not a smooth "L" but has two distinct slopes. With the first 5 cases the novice may be granted privileges for performing laparoscopic splenectomy on an individual basis. Past the 25th case the surgeon could be accepted as an expert in the field.

Terminology

The learning curve is a graphic representation of the expertise level reached for a particular procedure. This is briefly defined as the repetition of the procedure until it is learned. Reaching proficiency is defined by measuring the expertise and setting a minimum number of procedures to be performed in order to be accepted as proficient.

Peer review

It is an interesting topic for the readers. It should be accepted for publication.

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Splenectomy with chemotherapy *vs* surgery alone as initial treatment for splenic marginal zone lymphoma

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Abstract

AIM: To evaluate the clinical characteristics of splenic marginal-zone lymphoma (SMZL) following antigen expression and the influence of therapeutic approaches on clinical outcome and overall survival (OS).

METHODS: A total of 30 patients with typical histological and immunohistochemical SMZL patterns were examined. Splenectomy plus chemotherapy was applied in 20 patients, while splenectomy as a single treatment-option was performed in 10 patients. Prognostic factor and overall survival rate were analyzed.

RESULTS: Complete remission (CR) was achieved in 20 (66.7%), partial remission (PR) in seven (23.3%), and lethal outcome due to disease progression occurred in three (10.0%) patients. Median survival of patients with a splenectomy was 93.0 mo and for patients with splenectomy plus chemotherapy it was 107.5 mo (Log rank = 0.056, $P > 0.05$). Time from onset of first symptoms to the beginning of the treatment (mean 9.4 mo) was influenced by spleen dimensions, as measured by computerized tomography and ultra-sound ($t = 2.558$, $P = 0.018$). Strong positivity (+++) of CD20 antigen expression in splenic tissue had a positive influence on OS (Log rank = 5.244, $P < 0.05$). The analysis of factors interfering with survival (by the Kaplan-Meier method) revealed that gender, general symptoms, clinical stage, and spleen infiltration type (nodular *vs* diffuse) had no significant ($P > 0.05$) effects on the OS. The expression of other antigens (immunohistochemistry) also had no effect on survival-rate, as measured by a χ^2 test ($P > 0.05$).

CONCLUSION: Initial splenectomy combined with chemotherapy has been shown to be beneficial due to its advanced remission rate/duration; however, a larger controlled clinical study is required to confirm our findings.

Key words: Splenic marginal zone lymphoma; Chemotherapy; Splenectomy; Clinical outcome

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INTRODUCTION

Splenic marginal-zone lymphoma (SMZL) is an indolent B-cell lymphoma, generally presented with splenomegaly, and frequent involvement of the bone marrow and

peripheral blood. The typical immunophenotypic profile is: IgM+, IgD+/-, cytoplasmic Ig-/+, pan B antigens+, CD5-, CD10-, CD23-, CD43-/+, and cyclin D1-. It is characterized by micronodular infiltration of the spleen with marginal-zone differentiation[1-4]. An SMZL variant with villous lymphocytes (SMZL + VL) has been previously described as "splenic lymphoma with villous lymphocytes", and included into the FAB classification of chronic B-cell leukemias[5]. Lymphomas of the marginal zone are recognized as separate clinical phenomena amongst B-cell NHL in the REAL classification. Thus, SMZL was divided from other marginal zone lymphomas, such as mucosa associated lymphoid tissue (MALT) lymphoma or nodal B-type marginal zone lymphoma, and accepted by WHO classification in 2001 as a distinct clinical and pathohistological (PH) entity[6-9]. According to more recent data, SMZL and SMZL with or without villous lymphocytes (SMZL ± VL) are two phases (tissue and leukemic) of the same disease[10,11].

Typical genetic abnormalities in SMZL are deletions at 7q22-7q32[8]. The majority of SMZL patients have good long-time survival. Standard prognostic factors cannot differentiate patients into groups with poor or high-quality clinical outcomes. However, several immune-mediated events, such as hemolytic anemia and thrombocytopenia, as well as the presence of the serum monoclonal component, could be predictive factors for survival-rate. Splenectomy is considered the first-line treatment for SMZL patients[12,13]. Even if this therapy results just in partial remission (PR), surgery-response is usually sufficient to correct (pan) cytopenia and also to improve the patient's quality of life and overall survival-rate. The presence of SMZL in peripheral lymph nodes and extranodal locations is uncommon. Thus, the spleen is considered the site of lymphoma origin, even if there were regional enlargements of the lymph nodes[9-11]. The frequency of mutation in the 5' non-coding region of the *bcl-6* gene has been used as a marker of germinal center derivation, which might be used to establish the molecular heterogeneity[14-16]. Therefore, SMZL is a primary disease of the spleen, with subsequent bone marrow (BM) and peripheral blood (PB) involvement[17-19]. The diagnosis is based on the spleen PH, in accordance with clinical data. Incorporation of immunophenotypic profiles and molecular characteristics into BM and PB morphology, improves the diagnostic validity. SMZL is an indolent lymphoma, although there is a small subset of patients with an aggressive clinical course[20-22]. Studies on this entity have been aggravated by the fact that the disease is very rare[23].

The purpose of this pilot study is to show PH features, as well as clinical data and follow-up in splenectomy with chemotherapy *vs* surgery alone treated SMZL ± VL patients.

MATERIALS AND METHODS

Patients

The study group included 30 patients with SMZL ± VL. Splenectomy plus chemotherapy was applied to 20 patients, while splenectomy as a single treatment-option was performed for ten patients. The follow-up time was 12 years (1994-2006). Diagnosis was established and confirmed after initial splenectomy with consecutive PH and immunohistochemical (IHC) analysis, only upon consensus of two independent hemato-pathologists. Criterion of SMZL + VL was more than 20% of such lymphocytes in the peripheral blood[1]. Clinical stage (CS) was determined according to the Ann Arbor staging classification[24]. The following clinical characteristics were analyzed: sex, age, constitutional "B" symptoms, CS, and time from the onset of first symptoms to the beginning of treatment. Thereafter, complete blood count and standard biochemical analyses were done as follows: serum iron (sFe), ferritin, lymphoma activity parameters: lactate dehydrogenase (LDH) and beta-2-microglobulin (β-2M), serum paraprotein presence (M-component), virological analysis (hepatitis B, C and HIV markers). The size of the spleen was determined by ultrasound (US) or computerized tomography (CT). Its weight in grams was also measured after spleen removal.

Histology and immunohistochemical analyses

Diagnosis was based on analysis of tissue samples (spleen, lymph node, and BM) according to criteria of WHO classification system. All tissue samples were fixed in B5, processed by standard methods, embedded in paraffin, cut by a microtome (4 μm sections) and stained by classical staining methods: hematoxylin eosin (HE), Giemsa and reticulin (Gordon-Sweet).

Immunostaining was performed using a labeled streptavidin-biotin procedure with monoclonal antibodies (DAKO, Glostrup, Denmark). The relevant antibodies used in routine diagnosis were: LCA, EMA, IgD, IgM, CD20, CD79-α, CD5, CD23, CD43, CD10, bcl-2, CD3, Cyclin D-1, and Ki-67.

The strength of CD20 antigen (Ag) expression was graduated semi-quantitatively as strong (+++), moderate (++), or weak (+) according to cell positivity, almost in all cells, more than 50%, and in less than 50% of cells, respectively. For other antigens, expression was graded as, positive (+) or negative (-), whilst Ki-67 expression was evaluated numerically as a percentage of positive cells. Splenic marginal zone areas were selected, focusing in all cases on the areas with the highest growth fraction. Preparations of spleen, lymph nodes and BM were analyzed by standard light microscopy.

Drug-treatment

The initial treatment in all (30) patients was splenectomy. The CHOP (cyclophosphamide, hydrodoxorubicine, vincristin, and prednisolone) protocol was applied in nine (45.0%) cases. Fludarabine containing regimens (FMD - fludarabine, mitoxantrone, dexasone; and FMC - fludarabine, mitoxantrone, cyclophosphamide) were used in 11 (55.0%) cases. The decision to give additional chemotherapy was made according to the presence of constitutional "B" symptoms at presentation and lymphadenopathy. Two types of applied treatment (splenectomy alone or splenectomy with chemotherapy) in a 3-year follow-up period were analyzed for every patient.

Statistical analysis

Among descriptive analysis, the arithmetical mean and standard deviation were used for parametric data and the median was used for description of non-parametric data. For the parametric analytical model, we applied Student's *t*-test. For non-parametric analytical models, we used Pearson's χ^2 tests, Fisher's exact test, Kolmogorov-Smirnov's test, and Kaplan-Maier's method for analysis of OS. For significance, α errors of 0.05 were chosen in all methods. SPSS version 6.0 for Windows was used to create a database. Statistical analyses were completed within the statistics package of the Institute of Medical Statistics and Information Technology, School of Medicine, Belgrade.

RESULTS

Patient's characteristics

There were 11/30 (36.7%) males and 19/30 females (63.3%). The mean age was 58 years (range, 33-76). Performance status according to the Eastern Cooperative Oncology Group (ECOG) scale was 0 = 18%, 1 = 56%, and 2 = 26%. SMZL was diagnosed in 18 patients (60.0%), and SMZL + VL was found in 12 (40.0%) cases. The patients' general clinical features and hematological characteristics included in this study are summarized in Table 1.

Furthermore, HIV 1 and 2 antibodies were all negative (100%). Hepatitis B antibodies were also negative in all patients. Hepatitis C antibodies were positive in only one case (3.3%).

The mean sFe level was 7.24 μg/L (range, 2.20-16.2). Decreased values below absolute in relation to gender were recorded in 19 (63.3%) patients, and normal were reported in 11 (36.7%) subjects. Relative iron deficiency might be explained in two ways. Firstly, iron resorption was reduced due to loss of appetite with an increase of passive intestinal hyperemia on account of splenomegaly. Secondly, more intensive loss of the same oligo-element was stimulated by hemorrhage, (most often occult). Ferritin levels were within normal limits in relation to gender in all subjects.

Immunohistochemical findings

A total of 30 (100%) patients with typical histological and immunohistochemical splenic marginal zone lymphoma pattern were examined (Figure 1).

Immunohistochemical analysis revealed that none of the positive expressing antigens had a significant influence on disease outcome, as assessed using a χ^2 test with the following confirmed results: CD79-alfa (χ^2 = 5.074, $P > 0.05$), CD20 (χ^2 = 4.046, $P > 0.05$), CD43 (χ^2 = 0.910, $P > 0.05$). IgD (χ^2 = 2.503, $P > 0.05$), IgM (χ^2 = 1.147, $P > 0.05$), bcl-2 (χ^2 = 3.667, $P > 0.05$), and Ki-67 (χ^2 = 2.503, $P > 0.05$). Moreover, Ki-67 positivity varied from 5%-35%, with following distributions: 5%-15%, 15%-25%, and 25%-35% in nine (30.0%), 14 (46.7%) and seven patients (23.3%), respectively.

Using the long rank test in the Kaplan Mayer method of survival showed that among all antigens, only CD20 antigen had some impact on OS. Namely, among three groups of patients according to CD20 positivity (strong, moderate, weak), median survival was 113 mo, 83 mo, and only 43 mo, respectively (Log rank = 5.244, $P < 0.05$).

Table 1 Clinical and hematological characteristics of SMZL ± VL patients

Clinical stage	*n* (%)	
Ⅰ E A+B	4 (13.3)	
Ⅱ A+B	0	
Ⅲ A+B	0	
Ⅳ A+B	24 (80.0)	
Ⅴ A+B	2 (6.7)	
Spleen-specific data	Range	Mean
US length of spleen (cm)	6-32	22.72
US width of spleen (cm)	7-18	10.00
CT length of spleen (cm)	7-27	22.00
CT width of spleen (cm)	6-17	10.00
Spleen weight after surgery (g)	50-8000	2236
Spleen infiltration: nodular *vs* diffuse	18 (60.0) *vs* 12 (40.0)	
Hb (g/L)	79-131	110
Anemia (Hb < 100 g/L)	27 (90.0)	
Leukocytosis (WBC > 10×10^9/L)	10 (33.3)	
Leukocytopenia (WBC < 4×10^9/L)	9 (30.0)	
Neutrocytopenia (Ne < 1.5×10^9/L)	16 (53.3)	
Lymphocytosis (Ly > 5×10^9/L)	17 (56.7)	
Thrombocytopenia (Plt < 150×10^9/L)	18 (60.0)	
Thrombocytosis (Plt > 400×10^9/L)	5 (16.7)	
Serum paraprotein (determined)	23 (76.7)	
IgG lambda (found)	1 (3.3)	
LDH elevated (> 320 U/L)	8 (26.7)	
β-2M elevated (> 1.8 μg/L)	25 (83.3)	

Hb: Hemoglobin; WBC: White blood cell; Ne: Neutrophil granulocyte; Ly: Lymphocyte; Plt: Platelet; LDH: Lactate dehydrogenase; β-2M: β-2-microglobulin.

Prognostic factor analysis and overall survival

In this study, all the patients were splenectomized at the beginning of treatment. Therapeutic splenectomy, i.e. as the only curative modality in 10 (33.3%) patients was performed. Splenectomy with chemotherapy (different protocols) was done in the remaining 20 (66.7%) patients. After a mean three-year follow-up, the outcome was as follows: 20 (66.7%) patients had complete remission (CR), seven (23.3%) had partial remission (PR), and three (10.0%) died due to disease progression.

Out of 10 patients having undergone splenectomy only, eight (80.0%) had CR and two (20.0%) had significant PR (76 mo). In addition, all CR were stable after five years (60 mo). No patient with spleen rupture, a rare but possible complication, was reported.

Out of those receiving chemotherapy after splenectomy (20 patients), CR was achieved in 11 (55.0%) patients. The CHOP protocol was used in nine (45.0%) cases, and FMD or FMC were used in 11 (55.0%) cases. Ultimately, the mode of treatment was not a factor interfering with OS of our patients. Median survival of patients with splenectomy only was 93.0 mo and in patients with splenectomy and chemotherapy it was 107.5 mo (Log rank = 0.056, $P > 0.05$).

The cumulative survival of patients included in this study is presented in Figure 2.

As shown, after 120 mo of follow-up, the incidence of survival was constant (about 77%). Time from onset

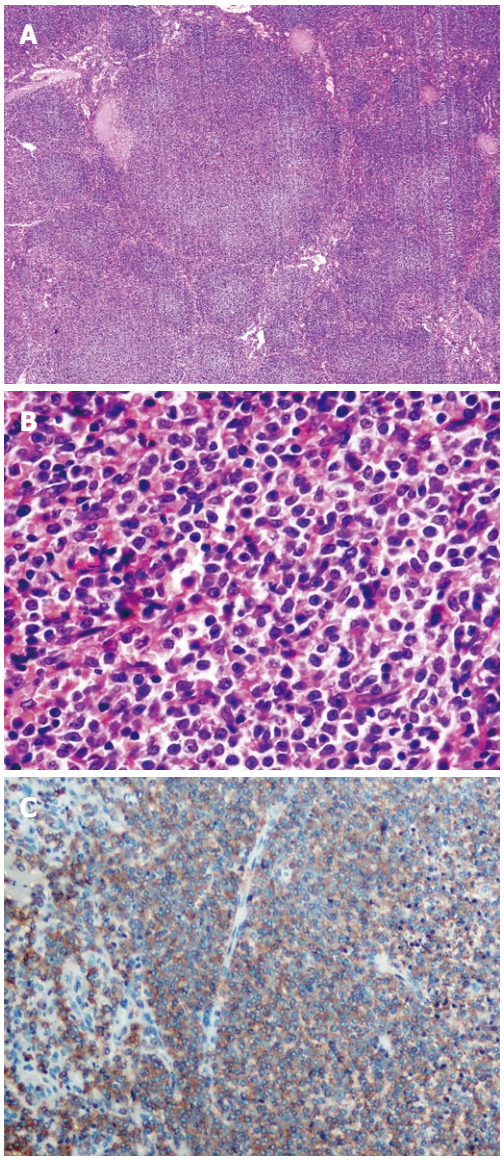

Figure 1 SMZL-splenic tissue. A: Nodular infiltration (HE, × 40); B: Neoplastic cells with monocytoid morphology (HE, × 400); C: Immunohistochemistry for CD20$^+$, brown staining in lymphoma cells (streptavidin-biotin, × 200).

of first symptoms to the beginning of treatment (mean 9.4 mo, range 1-84) was influenced by spleen dimension, as measured by CT and US, and was significantly shorter in patients with higher spleen dimensions (t = 2.558, P = 0.018). The highest mean values of segmented neutrophil percentage were found in subjects who reached CR, and conversely, the lymphocyte percentage mean values were lowest in those who achieved CR (P = 0.026).

Spleen dimensions measured by CT also correlated with clinical stage (P = 0.034).

The analysis of factors interfering with survival, as measured by the Kaplan-Meier method, revealed that gender was not a factor affecting the length of survival (Log rank = 0.643, P > 0.05). Further analysis of such factors showed that the presence of "B" symptoms also had no effect on survival (Log rank = 0.141, P > 0.05). Moreover, CS was not a factor affecting patient survival. (Log rank = 0.560, P > 0.05). Similarly, the type of spleen infiltration (nodular *vs* diffuse) was not a factor

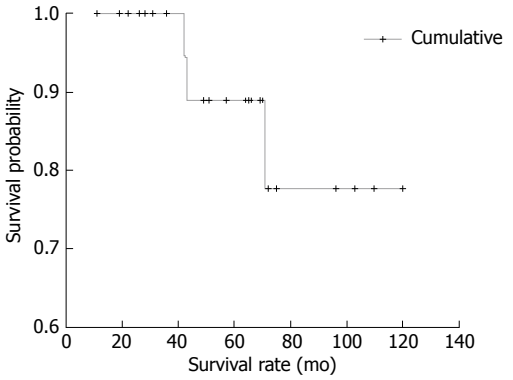

Figure 2 Cumulative survival of patients investigated (with constant incidence of survival after 120 mo).

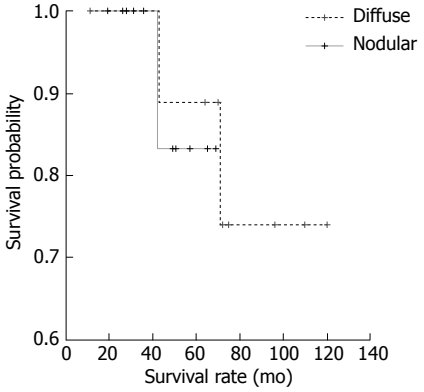

Figure 3 Patients' survival with different type of spleen infiltration (nodular *vs* diffuse).

Table 2 Results of Cox regression method and significance of compared factors of treatment outcome in our patients

Variables	Score	df	*P*
Gender	0.244	1	0.621
Age	0.657	1	0.418
Time from the onset of B symptoms to treatment	13.906	10	0.177
CS	0.557	2	0.757
Spleen infiltration (nodular *vs* diffuse)	0.009	1	0.923
Mode of treatment	0.089	1	0.900

affecting the survival of patients (Log rank = 0.021, P > 0.05), as shown in Figure 3.

Finally, the analysis of lethal outcome predictors by the Cox regression model included the role of factors that could have any effect on such an outcome (Table 2). As illustrated, none of the studied factors appeared to be significant for predicting treatment outcome in our group of patients.

DISCUSSION

Splenic marginal zone lymphoma is a relatively rare entity with a slight male predominance. In our group of patients there was higher incidence of female - 19 (63.3%), in relation to male - 11 (36.7%), as compared to other studies[25,26]. The mean patients' age was 58.2 years, which agreed with the fact that splenic lymphoma is a

disease of older age[1,7].

A majority of the SMZL cases studied carried a non-mutated bcl-6 gene[14]. The frequency of these mutations in normal spleen confirms previous findings on the hyper-mutation IgVH process in normal B-cell populations[27,28]. This data supported the existence of molecular heterogeneity in this entity. It also favored the hypothesis that, in spite of initial morphological observations, a significant proportion of SMZL cases could derive from an non-mutated naive precursor, which is different from those of the marginal zone, and possibly located in the mantle zone of splenic lymphoid follicles. Thus, the marginal zone differentiation of these tumors could be related more to the splenic microenvironment than to the histogenetic characteristics of the tumor[20].

Considering PH findings, in our group there were 18 patients (60.0%) with SMZL, and 12 (40.0%) with SMZL + VL. The largest number of patients had advanced disease in clinical stage Ⅳ + Ⅴ (86.7%). The shortest time of the onset of first symptoms to the beginning of treatment was one month, and the longest was 84 mo. The average was 9.4 mo it was correlated with spleen dimension, as measured by CT and US. It was significantly shorter in patients with higher spleen dimensions. SMZL is a slow-course disease and is detected in progressive phase in the highest percentage[7,29]. In accordance with this, Thieblemont found BM infiltration in 95% of patients[1]. Almost all (97%) patients had clinical stage Ⅲ and Ⅳ, as reported in the large cohort of patients published by Arcaini[25], with a similar proportion of about 90% of BM infiltration in a series of 129 patients[30]. It was apparent that the majority of patients in our study were in clinical stage Ⅳ.

Splenomegaly lasted approximately 9.4 mo before diagnosis (interval of 1-36 mo), referring to a study in 1999[26], which is similar with our results. Another study on 18 patients established an average pre-treatment presence of symptoms to be four months (two-six month interval)[31]. Similarly, a series of 17 patients reported pre-treatment duration of symptoms varying from several days to four months with a mean time of 2.1 mo[9].

The finding that spleen dimension correlated with clinical stage, as well as with the time from onset of first symptoms to the beginning of treatment, is rational since the dominant tumor mass is in the spleen. The maximal weight of the spleen measured intraoperatively was 8000 g and minimal weight was 450 g (mean = 2235.7 g). Such a finding suggested that there was at least one a case with a huge splenomegaly, rarely seen in foreign literature[23,26]. The pattern of spleen infiltration (nodular *vs* diffuse) was not significant for OS in our group of patients. According to the literature, 85% of patients with relapsed or progressive disease have nodal involvement, with a relatively low frequency of nodal involvement at initial diagnosis[23]. This emphasizes the care necessary in sorting nodal involvement of SMZL from primary nodal MZL, and the necessity of differential diagnosis with additional molecular markers.

We found that 27 (90.0%) patients manifested a lower or higher degree of anemia. Anemia is strongly characteristic of spleen lymphoma. It occurs due to hypersplenism rather than BM infiltration[1,7]. In a series of 81 patients reported by Thieblemont, 44 with spleen lymphoma had anemia and of these, 13 had Coombs-positive hemolytic anemia[1]. Similar results, with about half of the patients being anemic, were published for a large group of 309 patients[25].

Despite the presence of anemia (Hb ⩽ 110 g/L) in about 30% of patients with SMZL ± VL with mean value of hemoglobin 118 g/L[30], the percentage of anemic cases in our patients was significant higher, due to the predominance of advanced clinical stage. Leukocytosis and leukocytopenia were found in about a third of the patients, whilst half of the patients had neutrocytopenia and lymphocytosis. Such percentage of cells, rather than platelet count, in our patients was of no prognostic value regarding the survival period. A higher rate of achieving CR was found in patients with higher initial segmented neutrophil count and with a lower level of lymphocytes at presentation.

The distribution of serum paraprotein has varied from 8%[25], which was comparable to our results, to as much as 46%[1]. In a third large series of 129 patients with spleen lymphoma, paraprotein was present in 22% of patients, with the highest concentration of 25 g/L, but without any prognostic value[30]. Contrastingly, Arcaini reported that the presence of paraprotein had prognostic value in terms of shorter time to disease progression[32].

The established positivity of hepatitis C virus antibodies varied from 1%[1], which was similar to our results, to 19%, while hepatitis B virus antibodies were detected in 5% of cases[25].

Serum LDH is an important activity parameter of aggressive disease, while in low-aggressive ones, its increase can designate the transformation of disease to more severe form[18]. In our study group, this parameter was within referential limits in 72% of patients, which suggested low-grade disease status. On the other hand, Chacon reported about 60% patients with significantly elevated LDH[23].

In our patients, β-2M was predominantly elevated (83.3%), ranging from 2.38 to 7.4 μg/L. The results are in accordance with the established fact that this parameter is increased in SMZL as a negative prognostic marker[33,34].

Regarding the type and outcome of treatment, our results indicated that the use of adjuvant chemotherapy following the splenectomy had no influence on OS rate, as reported in published series so far[1,7,23]. This finding is in accordance with the fact that the use of alkylating drug therapy yields a rate of response of about 44%, but complete remissions are rare, probably due to the existing BM infiltration[1,7]. Until now, the treatment of SMZL has been controversial. In all large series, a significant group of patients received no therapy. These patients do not seem to have worst outcome than those initially treated. For these reasons, and assuming that SMZL is an indolent disease, some authors recommend a "watch and wait" approach[23]. Although, in general SMZL behaves as an indolent disease, there is a significant group of patients who died from the disease in a relatively short time period. The role of chemotherapy is still a matter of debate. One

of the striking findings is the relatively low percentage of patients who attain CR after chemotherapy, because of the presence of BM involvement after chemotherapy[23]. The literature presents diverse chemotherapy regimens for SMZL. Generally, splenectomy leads to somatic compensation of patients, rendering it impossible for local relapse in the spleen, prevents continuous dissemination from the primary tumor site, and mostly corrects cytopenias, creating better conditions for chemotherapy[35].

SMZL is a relatively indolent disease, but in some cases it displays more aggressive behavior, which should stimulate the search for predictive biologic factors and alternative therapies. Early splenectomy combined with chemotherapy in properly stratified patients at presentation has been shown to be beneficial because of improvement in remission rate/duration and superior OS. However, there was no statistical significance, probably due to the limited number of patients. Thus, the evaluation of these therapeutic approaches requires larger, randomized, and controlled clinical studies.

COMMENTS

Background

Splenic marginal-zone lymphoma (SMZL) is a relative uncommon low-grade lymphoma and primary disease of the spleen, with bone marrow and peripheral blood involvement. The existence of molecular heterogeneity in this entity gave additional results in favor of the hypothesis that, in spite of initial morphological observations, most SMZL cases could derive from a non-mutated naive precursor, different from those of the marginal zone, and possibly located in the mantle zone of splenic lymphoid follicles. Thus the marginal zone differentiation could be related more to the splenic microenvironment than it is to the histogenetic characteristics of the tumor. Until now, standard prognostic factors could not differentiate patients into groups with poor or favorable clinical outcome.

Research frontiers

Despite the evaluation of different prognostic factors, the treatment of SMZL is still controversial. However, several immune-mediated events, such as hemolytic anemia and thrombocytopenia, as well as the presence of the serum monoclonal component, could be predictive factors for survival-rate. Splenectomy is considered the first-line treatment. Even when this therapy results in partial remission, the response to the surgery is usually sufficient to correct cytopenia and also to improve the patient's quality of life, as well as overall survival-rate.

Innovations and breakthroughs

SMZL is an indolent lymphoma, although there is a small subset of patients with an aggressive clinical course. Despite this fact, there have been significant groups of patients who have received no therapy, with only a "watch and wait" approach being adopted to their indolent clinical course. Early splenectomy combined with consecutive chemotherapy leads to somatic compensation of patients, renders it impossible for local relapse in the spleen, prevents continuous dissemination from the primary tumor site, commonly corrects cytopenias, and improves the survival-rate.

Applications

This data shows that early splenectomy combined with chemotherapy at presentation leads to some improvements in duration of overall survival, although with no statistical significance, probably due to the limited number of patients. Therefore, future approaches will need well controlled and larger clinical studies.

Peer review

In this manuscript, the authors delivered some interesting data on clinical-pathological features and clinical outcomes of 30 SMZL patients. They confirm that early splenectomy, at the beginning of treatment, combined with chemotherapy is helpful to prevent disease recurrence. The data presented might be beneficial for clinical treatment of patients with SMZL.

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BRIEF ARTICLES

Dysregulation of gastric H,K-ATPase by cigarette smoke extract

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Abstract

AIM: To test whether the expression and activity of H,K-ATPase in parietal cells would be affected by cigarette smoke extract.

METHODS: Extracts of cigarette smoke were administered into mice by gastric gavage (5 mg/kg body weight/day) for 3 d or in drinking water for 7 or 14 d. For the latter, each day a mouse consumed 5 mL water containing extracts of two cigarettes, on average. Control littermate mice received only vehicle. To compare the amount of H,K-ATPase in control and smoke-treated mice, the stomach was processed for Western blotting and immunohistochemical analysis using monoclonal antibodies specific for α- or β-subunits of H,K-ATPase. The *p*-nitrophenylphospatase activity assay was used as a measurement for K-dependent H,K-ATPase activity.

RESULTS: Probed transblots showed an increase in the amount of H,K-ATPase in smoke-treated mice which was confirmed by immunohistochemistry and was found to be due to increased amounts of protein per parietal cell rather than an increased parietal cell number. The increase in the amount of H,K-ATPase was associated with an enhancement of its enzymatic activity. K-dependent activity in control and smoke-treated mice was significantly different (respectively, 0.12 μmol/mg *vs* 0.27 μmol/mg per minute, $P < 0.05$).

CONCLUSION: Administration of cigarette smoke extract is associated with an increase in the amount and activity of H,K-ATPase and hence, smokers are susceptible to development of peptic ulcer.

Key words: Proton pump; H,K-ATPase; Parietal cell; Gastric gland; Oxyntic mucosa; Cigarette smoke extract; Smoking

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INTRODUCTION

Cigarette smoking is a major worldwide health problem. According to the World Health Organization, smoking is the largest preventable cause of premature death worldwide. It is a common habit among teenagers, adults, and even health professionals[1]. In addition to the well known adverse effects of smoking on cardiovascular and respiratory systems, some studies have shown that smoking is a major risk factor for some gastrointestinal diseases[2-4]. Several clinical and epidemiological studies have provided evidence suggesting that smokers are more susceptible to peptic ulcer disease and respond to anti-ulcer drugs less efficiently than non-smokers[4-9]. In experimental animals, some studies have shown that cigarette smoking potentiates the damaging effects of ethanol or corticosteroids on the gastric mucosa[10,11].

The mechanisms by which cigarette smoking adversely affects the gastric mucosa have not been fully elucidated. It has been shown that free radical production, infiltration of neutrophils, stimulation of angiotensin II production, down-regulation of epidermal growth factor and reduction of gastric blood flow play an important role in the harmful effects of smoking[7,12-14]. However, it is not

known whether cigarette smoking or nicotine adversely affects some other factors that may influence the integrity of the gastric mucosa. The hydrochloric acid secreted by parietal cells is one of the main aggressive factors that play an important role in gastric mucosal damage and the pathogenesis of peptic ulcer disease[15,16]. The major protein involved in this process of acid secretion is the proton pump or H,K-ATPase. It is not known whether the H,K-ATPase of gastric parietal cells is altered by cigarette smoking.

In the stomachs of rodents and humans, parietal cells are scattered throughout the gastric glands, made of pit, isthmus, neck and base regions (Figure 1). They develop from epithelial progenitors which are anchored in the isthmus region[17-19]. During their development, parietal cells concomitantly synthesize the catalytic α- and regulatory β-subunits of H,K-ATPase[20]. Following their maturation in the isthmus, parietal cells bidirectionally migrate to become scattered throughout the glandular regions of the gastric epithelium. In mice, the turnover time of parietal cells averages 54 d. Old parietal cells undergo progressive physiological deterioration and eventually die and are eliminated at the luminal surface by extrusion into the gastric lumen or deep at the gland bottom by phagocytosis *via* a neighboring healthier glandular cell or an invasive connective tissue macrophage[21].

In the cytoplasm of parietal cells, both α- and β-subunits of H,K-ATPase are targeted to the membranes of tubulovesicles. Upon stimulation by histamine, acetylcholine, or gastrin, the tubulovesicles translocate from the cytoplasm to the apical and canalicular membranes of the parietal cell. Therefore, expansion of the canalicular system and elongation of the microvilli are features of a stimulated parietal cell. H,K-ATPase of the apical and canalicular membranes of stimulated parietal cells utilizes ATP generated by the numerous large mitochondria to pump protons into the lumina of canaliculi and gastric glands in exchange for potassium[22]. While much research is directed to discover new inhibitors of gastric H,K-ATPase, little is known about the extrinsic factors involved in its dysregulation.

Since smokers are more susceptible than nonsmokers to developing peptic ulcer disease and more likely to experience delays in ulcer healing, and since parietal cells are considered a key player during the pathogenesis and healing of this disease, we hypothesized that smoking alters H,K-ATPase, the major protein of the parietal cell which is responsible for acid secretion. Therefore, the aim of this study was to test whether the expression and activity of H,K-ATPase of parietal cells would be affected in animals administered with cigarette smoke extract.

MATERIALS AND METHODS

Preparation of cigarette smoke extracts

The method of Shin *et al*[23] was slightly modified to prepare ethanol and aqueous extracts of cigarette smoke. The smoke of burning red Marlboro cigarettes (Philip Morris, Inc., Richmond, VA, USA) was bubbled into ethanol or water by using a vacuum system. The ethanol extract was allowed to evaporate and the precipitate was dissolved in 0.1% dimethyl sulfoxide (DMSO).

Animals and experimental design

The procedures follow experiment in this study were in accordance with the guidelines for the care and use of laboratory animals and were approved by the Animal Research Ethics Committee of the Faculty of Medicine and Health Sciences, UAE University. In this study, C57BL mice of both sexes were used at two different age groups. (1) Young adult 8-wk-old mice (n = 12) received the ethanol smoke extract, 5 mg/kg body weight/day, *via* orogastric gavage needle on 3 consecutive days. (2) Weaning-age mice (3-wk-old, n = 32) received the aqueous smoke extract in their drinking bottles which were made freely accessible for 7 or 14 continuous days. Fresh extract-containing water was used daily. It was estimated that every day, on average, each weaned mouse consumed 5 mL of water containing smoke extract of two cigarettes. For both age groups of smoke-treated mice, weight- and sex-matched littermate (8- or 3-wk-old) mice were used as controls and received only vehicle (0.1% DMSO or water, respectively). One day after treatment, each pair of smoke-treated and control littermate mice was killed by an overdose of ether. The stomachs were immediately removed and processed for biochemical and immunohistochemical analyses.

Mucosal homogenate preparation

Gastric mucosal homogenates were prepared as previously described[20,24]. Part of the oxyntic mucosa of the stomach was scraped, minced and then homogenized on ice-cold hypotonic buffer (pH 6.7) containing 113 mmol/L mannitol, 37 mmol/L sucrose, 0.4 mmol/L EDTA, 5 mmol/L piperazine-N,N'-bis(2-ethanesulfonic acid)-tris (hydroxymethyl) aminomethane. The crude homogenate was centrifuged at a low speed (35 *g*) for 5 min to remove unbroken cells and tissues. To obtain pellets enriched in H,K-ATPase, some solubilized crude homogenates of control and smoke-treated mice were centrifuged at a higher speed (25 000 *g*) for 2 h. Portions of the low-speed supernatants and the re-suspended high-speed pellets of control and smoke-treated mice were processed for measurement of protein concentration using Bradford's method and then quantification of H,K-ATPase using Western blotting.

Western blotting analysis

Portions of the homogenates or re-suspended pellets of control and smoke-treated mice were solubilized in buffer containing 1% SDS, 0.5 mol/L urea, 5% 2-mercaptoethanol, 0.25 mmol/L EDTA, 10% glycerol, 0.0025% bromophenol blue, and 30 mmol/L Tris-HCl, pH 6.8 and run through 8% or 10% acrylamide[20]. Proteins were subsequently electro-transferred onto nitrocellulose membranes (Schleicher & Schuell Bioscience, Dassel, Germany) and probed with mouse monoclonal antibodies specific for the α- (97 kDa) or β- (60-80 kDa) subunits of H,K-ATPase (Medical & Biological Laboratories Co., Woburn, MA, USA) at a

dilution of 1:2000. After washing, blots were incubated with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Ig) G (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) at 1:10 000 dilution. To control equal loading of proteins in both smoke-treated and control mice, anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used at 1:2000 dilution. Bands of protein-antibody complexes were visualized using SuperSignal West Pico chemiluminescence detection kit (Thermo Fisher Scientific, Rockford, IL, USA) and their relative intensities were quantified by densitometry using the Scion Imaging program for Windows (version β 4.0.3.2; developed by Scion, Frederick, MD, USA). Data were expressed as means in arbitrary units and the level of significance of differences between control and smoke-treated groups were determined by using the Student's *t* test. $P < 0.05$ was taken as significant.

Light microscopic and immunohistochemical analyses

Pieces of the oxyntic regions of the stomachs of control and smoke-treated mice were fixed in Bouin's solution and embedded in paraffin. For general histology, some sections (5 μm) were stained with hematoxylin-eosin or periodic acid Schiff technique. Adjacent sections were used for immunohistochemical analysis using antibodies specific for the α- or β-subunits of the gastric H,K-ATPase. Antigen-antibody binding sites were visualized by using fluorescine isothiocyanate (FITC)-labeled donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA).

To measure the intensity of H,K-ATPase immunolabeling of the cells, image analysis was performed on probed gastric mucosal tissue sections of some control ($n = 4$) and smoke-treated littermate mice ($n = 4$) using the Scion Imaging program as previously described[20]. Probed sections were examined at 40 × magnification with an Olympus microscope and photographed with a DP-70 digital camera with the option of fixed manual exposure to ensure equal exposure of control and smoke-treated tissue sections. Digitalized TIFF images of immunolabeled parietal cells were stored at a resolution of 300 dpi. Labeled parietal cells cut through their nuclei were only considered for measurement by using the freehand tool and drawing a line around the periphery of the cell. The number of cells examined per animal ranged from 20 to 35. Following measurement of the immunostaining intensity of various cells in one section, the background intensity was subtracted. Quantitative results of the optical density were reported in arbitrary units corresponding to immunostaining intensity which is indicative of the amount of H,K-ATPase in the sectioned cells. For each stained cell examined, the area was also measured and expressed in arbitrary units. Data are presented as mean ± SE.

Enzymatic activity assay

In some experiments, the gastric mucosa of control and smoke-treated mice were homogenized and briefly centrifuged at low speed as mentioned above. Some of the supernatant was processed by the Bradford method for measurement of protein concentration and the remainder for K^+-dependent *p*-nitrophenylphosphatase (*p*NPPase) activity assay which was used as an index of H,K-ATPase activity[20]. The *p*NPPase activity was measured at 37℃ in buffer containing 7.5 mmol/L Tris-HCl (pH 7.5), 3.5 mmol/L $MgSO_4$, 30 mmol/L sucrose, and 0.02 mmol/L EDTA. To eliminate the contribution of Na,K-ATPase, 0.1 mmol/L ouabain was included in the incubation buffer. The K-dependent and Na-dependent *p*NPPase activity was assayed with 20 mmol/L KCl and NaCl in the buffer, respectively. The reaction was initiated by the addition of 5 mmol/L sodium *p*-nitrophenyl phosphate and terminated by 1.5 mL of 0.5 mol/L NaOH. Liberated *p*-nitrophenol was read at 410 nm by a Beckman Du 70 spectrophotometer. The K-dependent *p*NPPase activity was obtained from the difference of the values obtained with and without 20 mmol/L KCl. The enzymatic activity was expressed in micromoles per milligram protein per minute. Student's *t* test was used to compare values in control *vs* smoke-treated mice.

RESULTS

In the present study, extracts of cigarette smoke were administered to adult and weaned mice in two different modes: (1) aqueous extract in drinking water, and (2) ethanol extract *via* orogastric gavage needle. These two different modes of administration showed more or less similar effects on gastric H,K-ATPase. However, when the smoke extract was introduced with the drinking water, a duration-dependent effect was noted. The 7-d exposure of the gastric mucosa to smoke extract in the drinking water produced no significant difference between control and smoke-treated mice. When the duration of administration of smoke-containing drinking water was doubled, a significant difference was noted in the H,K-ATPase of smoke-treated *vs* control mice.

Effect of cigarette smoke extract on the amount of H,K-ATPase

Proteins of the mucosal homogenates of mice treated with ethanol or aqueous extracts of cigarette smoke and their control littermates were separated on polyacrylamide gels and transblotted on nitrocellulose membranes. Probing of the membranes with antibodies specific for the α- or the β-subunits of H,K-ATPase revealed an increase in the amount of this protein in smoke-treated mice (Figure 2). Densitometric analysis of the protein bands of H,K-ATPase showed that the amounts of both the α- and β-subunits were significantly increased. It was estimated that the percent increase of the α subunit averaged 220% and the percent increase of the β subunit averaged 350%. However, it should be noted that in the case of aqueous extract-treated mice, there was a dose/duration-dependent effect on H,K-ATPase expression. While the 7-d-treatment showed no significant difference as compared with control littermate mice (data not shown), 14 d of treatment showed a significant increase in the amount of H,K-ATPase ($P < 0.05$).

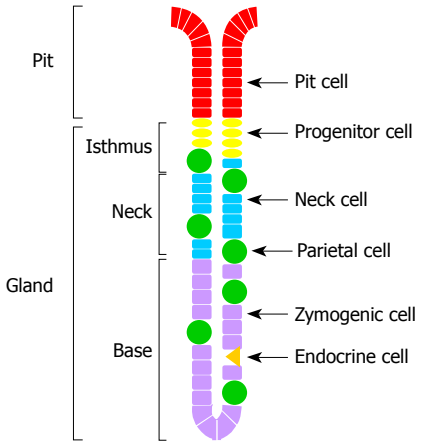

Figure 1 Schematic drawing of the structural unit of the gastric epithelium showing the pit and three glandular regions and the scattered H,K-ATPase-containing parietal cells.

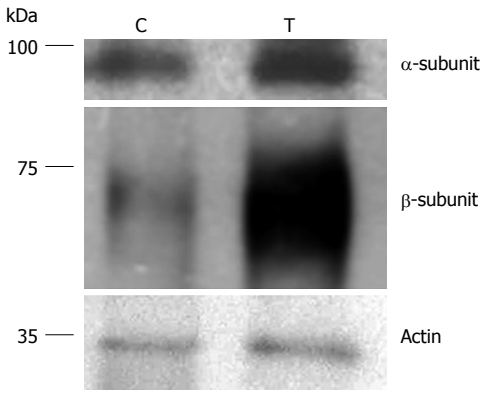

Figure 2 Representative transblots showing protein expression of the α- and β-subunits of H,K-ATPase in control (C) and smoke-treated (T) mice. Note the differences in the intensity of the protein bands in control *vs* smoke-treated samples. Ten micrograms of proteins were loaded per lane. Beta actin was detected by a mouse monoclonal antibody and used as a loading control.

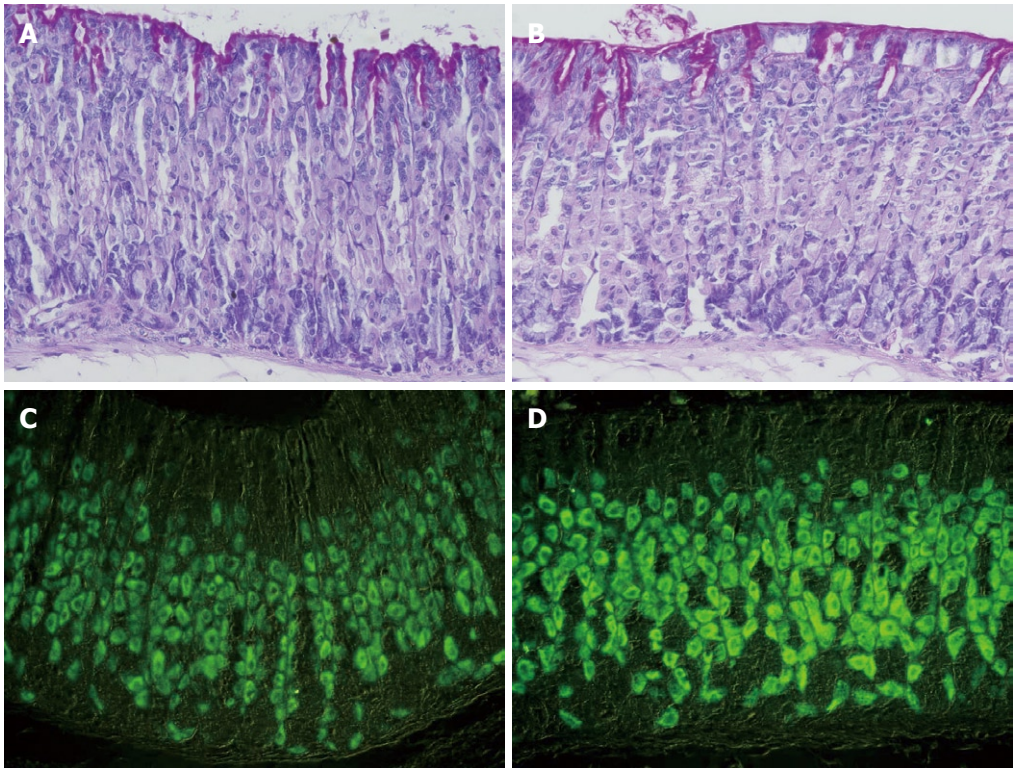

Figure 3 Gastric mucosal tissue sections of control (A, C) and smoke-treated (B, D) mice. A and B demonstrate the gastric mucosae of control and treated mice stained with periodic acid-Schiff and hematoxylin. No difference is noted in parietal cells of control and treated tissues. Immunohistochemical labeling of parietal cells in the gastric mucosa of control (C) and smoke-treated (D) mice with antibodies specific for H,K-ATPase β-subunit. Labeled parietal cells are distributed throughout the gastric glands. Note the difference in the labeling intensity of parietal cells in control *vs* smoke-treated mice, × 400.

Effect of cigarette smoke extract on the immuno-labeling of parietal cells

To test whether the increase in the amount of H,K-ATPase is due to an increase in parietal cell number or due to an increase in the amount of protein per cell, oxyntic mucosal tissue sections of control and smoke-treated mice were probed with antibodies specific for the α- or β-subunit of H,K-ATPase. To eliminate the possible variations in immunostaining conditions, stomach tissues obtained from smoke-treated and their littermate sex and weight-matched control mice were processed simultaneously and embedded in the same paraffin blocks. Tissue sections of control and treated mice were de-waxed and immuno-probed together on the same slides.

Microscopic examination demonstrated the usual pattern of distribution of labeled parietal cells in both control and smoke-treated tissues (Figure 3). Parietal cells were scattered throughout the mucosa of all tissues examined. Counts of parietal cells in control and smoke-treated tissues showed no significant difference. The number of H,K-ATPase-labeled parietal cells averaged

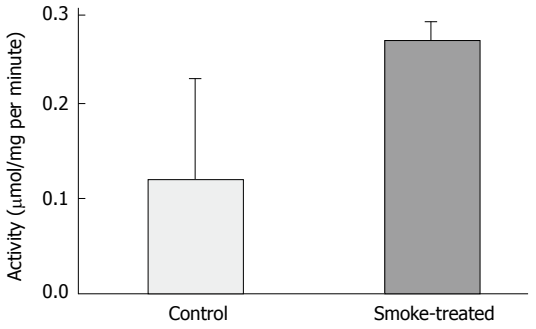

Figure 4 Analysis of the enzymatic activity of the H,K-ATPase of gastric mucosae in control and smoke-treated mice. Note the increased activity in the cigarette smoke-treated homogenate.

12.3 cells per gland in control mice and 11.7 cells in the gland of smoke-treated mice. However, when the intensity of the immuno-labeling was compared in control and smoke-treated tissues, an apparent difference was noted. In general, immuno-stained cells of smoke-treated tissues appeared darker than in control tissues (Figure 3). This difference reflected an increase in the amount of H,K-ATPase per cell after treatment with cigarette smoke extract. Quantification of the intensity of H,K-ATPase immunostaining confirmed this difference. Measurements of parietal cell density in each pair of control and smoke-treated mice showed that the percentages of increase in staining intensity after smoke treatment are highly significant ($P < 0.001$) and varied from 150% to 200%. In all mice examined, a similar staining pattern was obtained with antibodies specific for the α- and β-subunits.

Effect of cigarette smoke extract on the activity of the proton pump

To test whether the increase in the expression of H,K-ATPase protein is associated with a change in its enzymatic activity, some of the mucosal homogenates were processed for *p*NPPase activity assay. The results indicated that while the enzymatic activity in control tissues averaged 0.12 μmol/mg per minute, smoke-treated tissues showed more than 2-fold increased activity, 0.27 μmol/mg per minute ($P < 0.05$, Figure 4).

DISCUSSION

The present study demonstrates that cigarette smoke extract enhances the expression and activity of H,K-ATPase in the oxyntic mucosa of the mouse stomach which may explain the susceptibility of smokers to development of peptic ulcer disease.

The pathogenesis of peptic ulcer disease involves several factors including enhanced acid secretion, which is regarded as one of the major ulcerogenic factors. Acid secretion is also considered the primary target of contemporary drug therapy for peptic ulcer disease.

The available data concerning the effects of cigarette smoking on gastric acid secretion are controversial. Clinical studies showed that smoking may stimulate[25-28], inhibit[29,30] or have no effect[31,32] on gastric acid secretion. Such disparity between studies may be explained, in part, by the differences in nicotine content and number of cigarettes used, and by the lack of adequate controls due to marked individual variability of basal gastric secretion. In the current study, we did not intend to measure acid secretion, but the plan was to dissect the event down stream and examine the major enzyme of the parietal cell, H,K-ATPase, which is responsible for acid secretion.

Nicotine is one of the main constituents of cigarette smoke responsible for the adverse effects of smoking. The effect of nicotine on gastric acid secretion is also controversial. Several lines of evidence have suggested that it has a stimulating effect. Nicotine administered to rats for 10 d caused an increase in gastric secretory volume and acid output. This finding was attributed to increased muscarinic receptor sensitivity, and consequently, basal acid secretion[33]. Lindell *et al*[34] also noted that nicotine administration enhances gastric acidity and impairs postprandial gastric neutralization in humans. Likewise, nicotine treatment abolishes in a dose-dependent manner the depressing effect of ethanol on acid secretion in rats[35] and significantly stimulates basal gastric acid output in cats[36]. *In vitro* study demonstrated that nicotine could exert direct stimulatory effects on parietal cells and potentiate the histamine-mediated response in the isolated cell model[36].

Some other studies showed contrasting findings. In one study, the gastric acid secretion stimulated by intravenous pentagastrin was completely inhibited by nicotine[37]. Another study also showed that acid output together with gastric secretory volume one hour after vagal stimulation induced by modified sham feeding was lower in human subjects on smoking than non-smoking days[38]. Based on the contrasting nature of evidence, the effect of nicotine or smoking on gastric acid secretion remained elusive.

The present study supports the view that smoking enhances gastric acid secretion by up-regulating the expression and activity of H,K-ATPase. Based on previous studies, the mechanism by which the smoke extract affected the acid secreting parietal cells could be a combination of receptor mediated and direct effects on the parietal cell.

Therefore, the present study provides an answer to some questions which were raised by some epidemiological studies. Why are cigarette smokers more susceptible to development of peptic ulcer disease? Why is recurrence of peptic ulcer more common in smokers than non-smokers? Why is the healing of peptic ulcer disease delayed in smokers? As an answer to these questions, we hypothesized that H,K-ATPase in the parietal cells is more aggressive in smokers than non-smokers. Then we tested this hypothesis by using an ethanol or aqueous extract of cigarette smoke, which were previously found to be rich in nicotine by various chromatography techniques[39]. The extract was administered to mice orally and three methods were carried out to characterize the anticipated alteration of the gastric H,K-ATPase. First, Western blotting analysis of homogenates obtained from the gastric mucosae of control and smoke-treated mice showed an increase in the amount of H,K-ATPase in the 3-d-treated adult mice and 14-d-treated weaning-age mice. However, when the

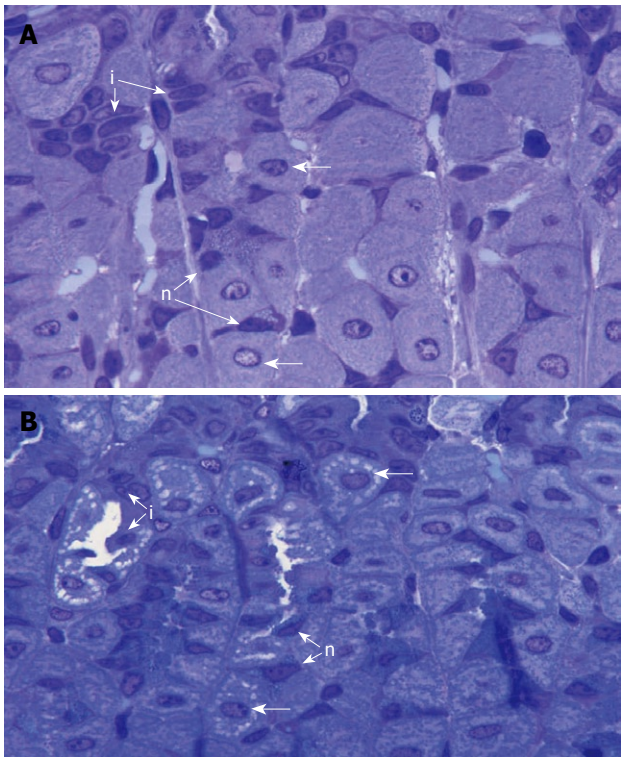

Figure 5 Semithin (0.5-micron-thick) sections of the gastric mucosae of control (A) and smoke-treated (B) mice stained with toluidine blue to demonstrate the isthmus and neck regions of the gastric glands. The large numerous parietal cells (horizontal arrows) are separated by progenitor or isthmal cells (i) and neck cells (n). Note that, in the smoke-treated tissue but not control tissue, there are pale areas in the cytoplasm of parietal cells which represent expanded lumen of the intracellular canaliculi, × 1000.

weaned mice were treated for only 7 d with half the dose, there was no significant change in the amount of H,K-ATPase as compared to their control littermates. Therefore, there is a duration- and dose-dependent effect of cigarette smoke extract in weaning-age mice. During this age, the developing gastric glands undergo compartmentalization into isthmus, neck and base regions[40]. In these developing glands, parietal cells might not have acquired all the machinery to respond to an extrinsic stimulus and therefore a longer duration of treatment with cigarette smoke extract is needed. Second, immunohistochemical analysis of gastric parietal cells using antibodies specific for their H,K-ATPase revealed an enhancement in the immunostaining of parietal cells of the smoke-treated mice. Since tissues of these treated mice and their weight- and sex-matched littermate control mice were processed together, embedded in the same tissue blocks and immunoprobed simultaneously on the same slides, the differences in immunostaining intensity were taken to represent an increase in the amount of H,K-ATPase per parietal cell. Third, the proton pump activity, measured by *p*NPPase assay was enhanced in the gastric mucosal homogenates of smoke-treated mice compared to control mice.

The question was raised whether the increased amount and activity of H,K-ATPase was associated with the translocation of tubulovesicles into the canalicular membranes. Some gastric mucosal tissues obtained from three pairs of smoke-treated and control mice were fixed in a Karnovesky's solution and processed for Araldite embedding and semithin sectioning. Microscopic examination of parietal cells located in the isthmus and neck regions of the gastric glands (known to be involved in acid secretion[41]) revealed that those of the smoke treated mice tend to acquire stimulated morphology with expanded canalicular system as compared to those of control mice (Figure 5).

In conclusion, the present study demonstrates a possible explanation for the susceptibility of smokers to develop peptic ulcer disease. Therefore, we propose the following scenario. It seems that smokers develop parietal cells with an increased amount and activity of H,K-ATPase and tendency to acquire an extended canalicular system. Accordingly, parietal cells of smokers are enhanced to produce much acid upon stimulation and their gastric and duodenal mucosae become more vulnerable to development of peptic ulcer disease.

COMMENTS

Background

Clinical, epidemiological and experimental studies have shown that smokers are more susceptible to peptic ulcer disease and respond to anti-ulcer drugs less efficiently than non-smokers. Parietal cells are targets for anti-ulcer drugs and their inhibition is an important modality for peptic ulcer treatment.

Research frontiers

Parietal cell activation may be associated with gastric mucosal injury and peptic ulcer disease. Since smoking is a predisposing factor to peptic ulcer, it is hypothesized that cigarette smoke extract may cause activation of gastric parietal cells. In this study, the authors demonstrated that over-expression of the gastric proton pump could be a possible mechanism for the susceptibility of smokers to peptic ulcer disease.

Innovations and breakthroughs

Previous reports have demonstrated the role of epidermal growth factors, blood flow, neutrophils, angiotensin Ⅱ, and free radicals in the adverse effects of smoking on the gastrointestinal mucosa. In this study, the authors have demonstrated an additional possible effect of smoking on the expression and activity of the gastric proton pump.

Applications

By understanding how smoking may alter the biological features of the gastric mucosa, this study may help in improving the current preventive and therapeutic modalities of peptic ulcer disease.

Terminology

The proton pump or H,K-ATPase is the major protein of parietal cells responsible for acid secretion. It is the main target for anti-ulcer drugs. Better understanding of the biological features of these cells and defining factors responsible for the regulation or dysregulation of their proton pump is important for designing new modalities for prevention and treatment of peptic ulcer disease.

Peer review

The authors used a smoking extract to study parietal cell H,K-ATPase expression and activity. It is a very interesting study.

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Risk factors for rebleeding after angiographically negative acute gastrointestinal bleeding

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Abstract

AIM: To identify possible predictive factors for rebleeding after angiographically negative findings in patients with acute non-variceal gastrointestinal bleeding.

METHODS: From January 2000 to July 2007, 128 patients with acute non-variceal gastrointestinal bleeding had negative findings after initial angiography. Clinical and laboratory parameters were analyzed retrospectively.

RESULTS: Among 128 patients, 62 had no recurrent gastrointestinal bleeding and 66 had recurrent gastrointestinal bleeding within 30 d. As determined by the use of multivariate analysis, an underlying malignancy, liver cirrhosis and hematemesis were significant factors related to recurrent gastrointestinal bleeding.

CONCLUSION: Clinical factors including underlying malignancy, liver cirrhosis, and hematemesis are important predictors for rebleeding after angiographically negative findings in patients with acute non-variceal gastrointestinal bleeding.

Key words: Angiography; Gastrointestinal hemorrhage; Predictive factor

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INTRODUCTION

Acute non-variceal gastrointestinal bleeding accounts for approximately 20% of emergency room visits and 5% of admissions[1]. Although endoscopy (including the use of upper endoscopy and colonoscopy) has been used as a first-line treatment option in patients with gastrointestinal bleeding[2,3], angiographic intervention can be used as a safe diagnostic and treatment method in patients with gastrointestinal bleeding that is refractory to endoscopic treatment[4-6].

Angiography requires a bleeding rate of 0.5-1 mL/min for detection. When neither extravasation nor vascular abnormality such as pseudoaneurysm is found, the bleeding site cannot be embolized selectively. Thus, intermittent bleeding is likely to result in a negative angiographic study[7,8]. The incidence of rebleeding in patients with negative initial angiography has been reported in up to 60% of cases[9]. However, little is known about the predictive factors for rebleeding, to determine if further investigations should be performed. The aim of this retrospective study was to identify the factors related to rebleeding in patients with gastrointestinal bleeding and normal angiographic findings.

MATERIALS AND METHODS

From January 2000 to July 2007, 341 patients with acute non-variceal gastrointestinal bleeding were referred to the angiography unit of our institution for possible transcatheter arterial embolization. We excluded 193 patients as they had active bleeding detected by angiography, and these patients received selective or empirical embolization. Among 148 patients with negative findings upon initial angiography, 20 were

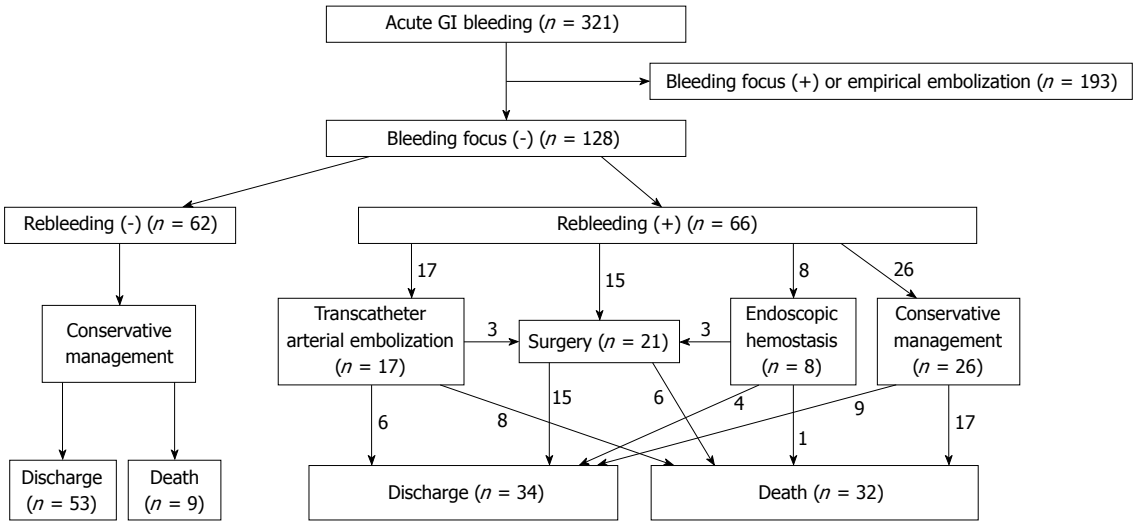

Figure 1 Flow diagram of patient outcome.

excluded because of limited medical records or suspected variceal gastrointestinal bleeding. A total of 128 patients (94 male, 34 female; age range, 18-85 years, mean age, 57.8 years) who had no active bleeding detected by initial angiography were included in this study. Approval was obtained from the ethical board committee of Seoul National University Hospital and patient informed consent was waived because of the retrospective nature of the study.

Clinical and laboratory parameters were reviewed retrospectively. The variables assessed included the following: patient age, sex, history of hematemesis, hematochezia or melena, shock, hemoglobin level, platelet count, prothrombin time, partial thromboplastin time, recent surgery, potential bleeding diatheses (liver cirrhosis or chronic renal failure), underlying malignancy, use of nonsteroidal anti-inflammatory drugs, and use of other antiplatelet agents or anticoagulants at the time of evaluation. Rebleeding was confirmed by endoscopy or surgery, and was clinically defined as: (1) fresh hematemesis; (2) fresh melena with systolic blood pressure < 100 mmHg; (3) a decrease in hemoglobin level of > 4 g/dL within 24 h; and (4) a requirement of two more red blood cell transfusions within 24 h.

The univariate association between clinical and laboratory variables and rebleeding was examined using Fisher's exact test for categorical variables and the *t* test for continuous variables. Variables with $P < 0.25$ as determined by univariate analysis were subjected to multiple logistic regression analysis with the use of a backward stepwise method. For univariate and multiple logistic regression analysis, $P < 0.05$ was regarded as statistically significant. Statistical analyses were performed using commercially available software (SPSS for Windows version 10.0 (Chicago, IL USA). All reported *P* values were two tailed.

RESULTS

Before the initial angiography examination, endoscopy was performed in 97 patients, endoscopic hemostasis was attempted in 18 patients, tagged red blood cell scintigraphy was performed in 21 patients, and a computed tomography (CT) scan was obtained for 40 patients. After initial angiography with negative findings, endoscopy was performed in 81 patients, endoscopic hemostasis was attempted in eight patients, tagged red blood cell scintigraphy was performed in 18 patients, a CT scan was obtained for 18 patients, and surgical treatment was performed in 21 patients.

The location of bleeding was the esophagus ($n = 3$), stomach ($n = 31$), duodenum ($n = 25$), small intestine ($n = 12$), colon ($n = 13$) and unknown ($n = 44$). The cause of bleeding was a benign ulcer ($n = 50$), gastrointestinal tumor ($n = 7$), ischemic enteritis ($n = 3$), radiation colitis ($n = 2$), iatrogenic injury ($n = 2$), angiodysplasia ($n = 2$) and undetermined ($n = 62$).

Among 128 patients who had no active bleeding detected by angiography, 62 had no recurrent gastrointestinal bleeding and 66 had recurrent gastrointestinal bleeding. Figure 1 documents the clinical course of the 128 patients. For the 66 patients with rebleeding, 40 received interventions including surgery, transcatheter arterial embolization and endoscopic hemostasis. The 4-wk mortality rate was 48% (32/66) for patients with rebleeding and 15% (9/62) for those without rebleeding. Among 41 expired patients, the cause of death was uncontrolled bleeding in 11, pneumonia or sepsis in 13, aggravation of the underlying malignancy in 10, cardiac failure in one, hepatic failure in one, and unknown in five.

Based on univariate analysis, the hemoglobin level, partial thromboplastin time, use of antiplatelet medication, underlying malignancy, presence of liver cirrhosis and shock were significant factors related to recurrent gastrointestinal bleeding. Based on multivariate analysis, underlying malignancy ($P = 0.002$, OR = 3.81), liver cirrhosis ($P = 0.017$, OR = 4.81) and hematemesis ($P = 0.042$, OR = 2.59) were significant factors related to recurrent gastrointestinal bleeding (Table 1).

Table 1 Prediction of rebleeding in patients with angiographically negative gastrointestinal bleeding

Variable		Rebleeding		*P* value at univariate analysis	Multiple logistic regression		
		Absent	Present		*P* value	OR	CI
Age (yr)		59.2 ± 15.1	56.9 ± 15.9	0.414			
Hemoglobin (g/dL)		8.2 ± 1.9	7.3 ± 1.9	0.009			
Platelet (× 1000/mm)		173.5 ± 98	151 ± 140	0.301			
PT (INR)		1.25 ± 0.31	1.38 ± 0.66	0.063			
aPTT (s)		38.7 ± 9.9	48.6 ± 31.8	0.027			
Sex	Female	17	18	1.000			
	Male	45	48				
NSAID	No use	59	64	0.673			
	Use	3	2				
Anticoagulation	No	56	64	0.155			
	Yes	6	2				
Antiplatelet therapy	No	47	61	0.014			
	Yes	15	5				
Malignancy	Absent	42	26	0.002	0.002	3.81	1.63-8.91
	Present	20	40				
GI tumor bleeding	Absent	57	56	0.275			
	Present	5	10				
Recent surgery	Absent	44	41	0.350			
	Present	18	25				
Recent GI surgery	Absent	54	52	0.247			
	Present	8	14				
Past GI bleeding history	Absent	47	51	1.000			
	Present	15	15				
Chronic renal failure	Absent	54	58	1.000			
	Present	8	8				
Liver cirrhosis	Absent	56	47	0.007	0.017	4.81	1.32-17.54
	Present	6	19				
Hematemesis	Absent	46	38	0.063	0.042	2.59	1.04-6.07
	Present	16	28				
Hematochezia	Absent	32	34	1.000			
	Present	30	32				
Melena	Absent	40	43	1.000			
	Present	22	23				
Shock	Absent	37	24	0.013			
	Present	25	42				

PT: Prothrombin time; aPTT: Activated partial thromboplastin time; NSAID: Non-steroidal anti-inflammatory drug.

DISCUSSION

Acute non-variceal gastrointestinal bleeding is one of the common emergency conditions for inpatients as well as outpatients[1]. Superselective angiography and transcatheter embolization have been used widely for upper and lower gastrointestinal bleeding refractory to endoscopic therapy[4,5]. In the case of failure of endoscopic management caused by a large number of blood clots or poor bowel preparation, angiography may be the choice of diagnostic or therapeutic method[4]. The angiographic procedure can provide accurate localization of the bleeding focus and immediate hemostasis, and localization of the bleeding site prior to surgery can prevent "blind" bowel resection. In addition, the use of angiography is less invasive than surgery, and is a good option for poor surgical candidates[10]. Recently, the use of improved techniques and instruments has decreased the number of complications such as bowel ischemia within an acceptable range[5,6].

Unfortunately, blood extravasation is not always visualized. In recent reviews of angiographic findings, blood extravasation or intraluminal blush was seen in 40%-60% of angiographic cases of non-variceal upper gastrointestinal bleeding[1,7,8]. There have been many studies of patients with normal angiograms, but a gold standard for management has not been determined. Some suggest that, in the case of negative angiographic findings in patients with intermittent or slow flow bleeding, use of nuclear scintigraphy seems reasonable to help confirm and localize the lesion[11,12]. The use of CT angiography may add to the detection of intermittent bleeding with possible better localization of the source and etiology of the bleeding. Ettorre *et al*[13] have shown a detection rate of 72% in patients with obscure gastrointestinal bleeding, in whom endoscopic and nuclear imaging failed to localize the bleeding site. The use of angiography has been reported with intra-arterial or intravenous injection of vasodilators, heparin, and even thrombolytic drugs to improve the rate of positive angiographic findings in occult lower gastrointestinal bleeding, although these modifications have been considered provocative[14-16]. Some investigators have suggested that blind embolization of the left gastric artery after endoscopic localization can show a decrease in the rebleeding rate[17]. As a result of the safety of the procedure, empiric embolization of the upper gastrointestinal tract for acute bleeding has been recommended when guided by endoscopic findings.

We expect that determination of the predictive factors for rebleeding may help in the selection of patients for further work-up or treatment, and consequently, may increase the success rate and decrease the rate of complications. Several studies have demonstrated clinical and endoscopic factors including liver cirrhosis, recent surgery, hypovolemic shock, hematemesis, large ulcer size, non-bleeding visible vessel, and the presence of an adherent clot on an ulcer base as significant predictive factors for the recurrence of hemorrhage in patients with peptic ulcer[18,19]. In our study, underlying malignancy, liver cirrhosis and hematemesis were significant factors related to recurrent gastrointestinal bleeding. As we did not perform endoscopy in all patients, and the study population was heterogeneous, including upper and lower gastrointestinal bleeding, endoscopic factors were not included in the analysis.

Rebleeding rates reported in the literature vary from 7% to 25% in patients with peptic ulcer or lower gastrointestinal bleeding[18-20]. In our study, the incidence of rebleeding within 1 mo was 52% (66/128). We think that the rebleeding rate was high because many severely ill patients were included in our study population.

The mortality rate in our study was 48% for patients with rebleeding and 15% for those without. Since there are many variables, we cannot state that the rebleeding itself affected mortality. However, prediction of rebleeding seems to have a relation to the prediction of prognosis.

This study had some limitations. First, the variable diagnostic and therapeutic modalities were performed without a settled sequence or principle. Most of the patients (120/128) received variable transfusions of red blood cells, fresh frozen plasma or platelet concentrate before angiography. Tagged red blood cell scintigraphy was performed in 21 patients and CT angiography in 40. Endoscopy was performed in 97 patients and 18 underwent endoscopic treatment. The selection of endoscopy or angiography in acute gastrointestinal bleeding is not well established. In our retrospective review, in cases in which postoperative CT showed an active bleeding focus, or the condition of the patient was inappropriate for endoscopy, angiography was performed as the first-choice method for diagnosis and treatment of acute gastrointestinal bleeding. Second, patients were enrolled in the study from only a single referral hospital. Many of the patients were elderly and had numerous medical problems. These conditions may have influenced the relatively high rebleeding rate and high mortality rate. Third, although the clinical features of upper gastrointestinal bleeding are quite different from lower gastrointestinal bleeding, both upper and lower gastrointestinal bleeding were included in this study population, as we could not determine the location of the bleeding site in 44 of 128 patients.

In conclusion, clinical factors including underlying malignancy, liver cirrhosis, and hematemesis are important predictors of recurrent bleeding after negative angiographic findings in patients with acute non-variceal gastrointestinal bleeding.

COMMENTS

Background

Acute non-variceal gastrointestinal bleeding is a common emergency condition. Superselective angiography and transcatheter embolization have been used widely for gastrointestinal bleeding. Unfortunately, blood extravasation is not always visualized.

Research frontiers

To predict the risk of gastrointestinal rebleeding after negative angiography may be important clinically. However, little is known about the predictive factors for rebleeding, to determine if further investigations should be performed.

Innovations and breakthroughs

This study is believed to be the first to establish predictive factors for rebleeding after angiographically negative gastrointestinal bleeding.

Applications

Determination of the predictive factors for rebleeding may help in the selection of patients for further work-up or treatment, and consequently, might increase the success rate and decrease the rate of complications.

Peer review

This study evaluated retrospectively the risk factors for gastrointestinal rebleeding after negative angiography.

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BRIEF ARTICLES

Hepatitis B virus subgenotypes and basal core promoter mutations in Indonesia

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Abstract

AIM: To identify the distribution of hepatitis B virus (HBV) subgenotype and basal core promoter (BCP) mutations among patients with HBV-associated liver disease in Indonesia.

METHODS: Patients with chronic hepatitis (CH, *n* = 61), liver cirrhosis (LC, *n* = 62), and hepatocellular carcinoma (HCC, *n* = 48) were included in this study. HBV subgenotype was identified based on S or preS gene sequence, and mutations in the HBx gene including the overlapping BCP region were examined by direct sequencing.

RESULTS: HBV genotype B (subgenotypes B2, B3, B4, B5 and B7) the major genotype in the samples, accounted for 75.4%, 71.0% and 75.0% of CH, LC and HCC patients, respectively, while the genotype C (subgenotypes C1, C2 and C3) was detected in 24.6%, 29.0%, and 25.0% of CH, LC, and HCC patients, respectively. Subgenotypes B3 (84.9%) and C1 (82.2%) were the main subgenotype in HBV genotype B and C, respectively. Serotype adw2 (84.9%) and adrq+ (89.4%) were the most prevalent in HBV genotype B and C, respectively. Double mutation (A1762T/G1764A) in the BCP was significantly higher in LC (59.7%) and HCC (54.2%) than in CH (19.7%), suggesting that this mutation was associated with severity of liver disease. The T1753V was also higher in LC (46.8%), but lower in HCC (22.9%) and CH (18.0%), suggesting that this mutation may be an indicator of cirrhosis.

CONCLUSION: HBV genotype B/B3 and C/C1 are the major genotypes in Indonesia. Mutations in BCP, such as A1762T/G1764A and T1753V, might have an association with manifestations of liver disease.

Key words: Basal core promoter mutation; Hepatitis B virus; Indonesia; Liver disease; Subgenotype

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INTRODUCTION

Hepatitis B virus (HBV) infection is associated with a diverse clinical spectrum of liver damage ranging from asymptomatic carriers, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC)[1]. HBV, a member of the *hepadnaviridae*, is a relaxed circular double-stranded DNA virus, and is currently classified into 8 genotypes (A to H), which reflect its geographical distribution[2,3]. For instance, HBV genotype A is prevalent in Europe, Africa, and India[4]. HBV genotypes B and C are predominant in most parts of Asia, including China, Japan, and Indonesia[4-10]. Genotype D is common in the Mediterranean area, the Middle East and India, whereas genotype E is localized in sub-Saharan Africa[4,11-13]. Genotype F and H are only identified in Central and South America[4,14,15]. Genotype G has been found in France, Germany, and the United States[4,16-18].

Besides the differences in geographical distribution, there is growing evidence that the HBV genotype may also influence the clinical outcomes of liver disease. Among Asian patients who constitute approximately 75% of HBV carriers worldwide, it has been shown that HBV genotype C is more commonly associated with severe liver disease and the development of cirrhosis and HCC than HBV genotype B[19-23]. However, most of these studies were carried out in Taiwan and Japan, thus can not be generalized even for Asian countries.

In addition to HBV genotype, mutations in the core promoter, precore or HBx gene have been shown to have an association with severe liver disease. For instance, many studies have revealed that the double mutation in BCP (A1762T/G1764A) is associated with an increased risk of severe liver disease including HCC, and can be used as a prediagnostic biomarker of HCC[24-28]. The predominant mutation in the precore region of HBV which involved a G-to-A change at nucleotide 1896, and resulted in a premature stop codon at codon 28, was proved to be associated with increased HCC risk[23,28-30]. In addition, among HBV carriers, the A1762T/G1764A mutation is more frequently found in genotype C than genotype B[19,31]. However, an independent study on a comparison of HBV genotype C from Vietnam and Japan showed mutations at different positions in the core promoter/precore region of HBV[32], indicating that the effect of mutation on liver carcinogenesis may not be universal. In addition, some mutations in HBx protein, in particular for HBV genotype C, have been shown to be significantly associated with HCC. A Serine-to-Alanine mutation at codon 31 (S31A) in HBx protein[33], a Proline-to-Serine mutation at codon 38 (P38S) in HBx protein of HBV genotype C[34], and some other particular mutations in HBx protein were found to be associated with increased risk of HCC[35]. Those studies, however, were independently carried out in different countries (China Taiwan, Japan, and Korea), and resulted in three different results.

Despite various reports about the effect of HBV genotype and/or mutations on liver disease progression, the virological significance on liver carcinogenesis is not yet fully elucidated. In particular for Indonesia, some reports had been published regarding the distribution of HBV genotype[7-10,36], and only one study reported samples from CH, LC, and HCC[8]. Moreover, to the best of our knowledge there is no report on the distribution of BCP mutations and their possible association with clinical manifestations of liver disease. Thus, the aims of the present study were to identify the distribution of HBV genotype/subgenotype and BCP mutations in patients with different clinical status, and to investigate the association of HBV genotype/subgenotype or BCP mutations and liver disease progression in Indonesia.

MATERIALS AND METHODS

Samples

Serum samples were obtained from 171 patients with HBV-associated liver disease, comprising 61 CH patients (mean age 37.8 ± 13.0 years; male/female: 40/21), 62 LC patients (mean age 50.2 ± 11.6 years; male/female: 44/18), and 48 HCC patients (mean age 49.6 ± 10.4 years; male/female: 43/5). CH was defined as persistent seropositivity for HBsAg for at least 6 months. LC was diagnosed by liver function tests and ultrasonography. The diagnosis of HCC was on the basis of ultrasonography as well as an elevated serum α-fetoprotein (AFP) level (≥ 200 ng/mL), or liver biopsy samples by needle aspiration for samples in which the AFP level was low. Sera of CH, LC, and HCC patients were collected from Cipto Mangunkusumo Hospital, Gatot Soebroto Hospital, Klinik Hati, Jakarta, Siloam Hospital Lippo Karawaci, Tangerang, Mataram General Hospital, Mataram, and Wahidin Sudirohusodo Hospital, Makassar, from May 2006 until November 2008. All sera were hepatitis B surface antigen (HBsAg)-positive as determined by a commercially available enzyme-linked immunosorbent assay kit (Abbott Laboratories, Chicago, IL, USA). Blood samples were collected from each patient at the time of their clinical evaluation, separated into sera and stored at -70℃ until viral DNA extraction. The study was approved by the Institutional Ethic Committee and informed consent was obtained from each patient.

Viral DNA extraction and PCR amplification

HBV DNA was extracted from 200 μL serum using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, and 80 μL of eluted DNA was stored at -70℃ until use. Full S gene was amplified by PCR with primers Fgp2 and Rgp2 (Table 1). The following cycling parameters were used for 40 cycles of PCR: denaturation at 95℃ (30 s), annealing at 55℃ (45 s) and elongation at 72℃ (2 min). When the PCR amplification was negative, a nested PCR was carried out to amplify the preS region.

Table 1 Primers used in this study

Primer	Nucleotide sequence (5′→3′)	Position	Polarity	Reference
Full S				
Fgp2	CGCCATGGGAGGTTGGTCTTCCAAACCTCG	2848-2873	Forward	This study
Rgp2	GACAAGCTTAATGTATACCCAAAGACAAAAGAAAATTGG	803-835	Reverse	
PreS (nested PCR)				
HBPr94	GGTAAAAAGGGACTCACGATG	775-795	Reverse	[2]
HBPr134	TGCTGCTATGCCTCATCTTC	414-433	Forward	
HBPr135	CAAAGACAAAAGAAAAATTGC	803-822	Reverse	
HBx				
Fgp3	CGCCATGGCTGCTAGGCTGTGCTGCCAAC	1374-1398	Forward	This study
Rgp3	CGCTCGAGGGCAGAGGGGAAAAAGTTGCATGGT	1811-1838	Reverse	
HBx (nested PCR)				
HB1	GCCAAGTGTTTGCTGACGC	1175-1193	Forward	[37]
HB2	CCATACTGCGGAACTCCTAG	1266-1285	Forward	
HB3	AAAGTTGCATGGTGCTGGTG	1804-1823	Reverse	

Primers HBPr134 and HBPr135 (Table 1) were used as previously described for the first-round 35 cycles of PCR by the following cycling parameters[2]: denaturation at 95℃ (1 min), annealing at 48℃ (30 s) and elongation at 72℃ (1 min). The second-round PCR was then performed using primers HBPr94 and HBPr134 (Table 1) with the same conditions as the first-round PCR except for annealing at 56℃ (30 s). Similarly, HBx gene was amplified using primers Fgp3 and Rgp3 (Table 1). The cycling parameters were the same as that for S gene amplification, except with an elongation time of 1 min. A nested PCR was performed for PCR negative samples using primers HB1 and HB3 for the first round PCR [35 cycles: denaturation at 95℃ (1 min), annealing at 48℃ (30 s) and elongation at 72℃ (1 min)] and using primers HB2 and HB3 for the second round PCR with the same parameters as the first-round PCR, but the annealing temperature was 46℃, as described previously[37]. Both sets of primers could amplify the full HBx gene. All PCR reactions were carried out by the PCR Core System (Promega, Madison, WI, USA). The PCR products were visualized on 1% agarose gel stained with ethidium bromide and purified using Wizard® SV Gel and the PCR Clean-Up System (Promega, Madison, WI, USA).

Analysis of HBV genotype/subgenotype, serotype and HBx mutations

Nucleotide sequences of the PCR fragments were determined with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and the appropriate primers, and sequenced with 3130xl DNA sequencer (Applied Biosystems). All HBs and HBx gene sequences were edited manually and were aligned with reference sequences retrieved from GenBank, using the ClustalW program incorporated in Bioedit v7.0. HBV genotypes/subgenotypes were determined based on the homology in the S or preS gene. Phylogenetic trees were constructed by the neighbor-joining method. HBV serotypes were deduced on the basis of predicted amino acid sequences of HBsAg[3,38,39].

Statistical analysis

All statistical analyses were performed using SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc® version 10.1.0.0 for Windows (MedCalc Software, Broekstraat, Mariakerke, Belgium). Significance differentiations for continuous variables were analyzed using *t*-test analysis. While the categorical variables were analyzed using the Fisher's exact test and chi-square test. $P < 0.05$ were considered significant.

RESULTS

HBV genotypes/subgenotypes distribution and clinical diagnosis

Only HBV genotype B and C were detected in the samples, which were respectively distributed in 73.7% and 26.3% of the samples (Table 2). Among HBV genotype B, subgenotypes B2, B3, B4, B5 and B7 were identified, although subgenotype B3 was the major subgenotype identified (84.9% of all genotype B or 62.6% of total samples). HBV subgenotypes B2 and B4 were only found in CH and LC, respectively. HBV subgenotype B5 was found in LC and HCC, while subgenotype B7 was detected in all different clinical diagnoses of the samples. On the other hand, among HBV genotype C, subgenotypes C1, C2, and C3 were found, but subgenotype C1 was dominant (82.62% of all genotype C or 21.6% of total samples). HBV subgenotype C1 was distributed in all samples, but subgenotype C2 and C3 were not detected in HCC samples. Based on statistical analysis, there was no significant association between HBV genotype/subgenotype and a clinical diagnosis of liver disease (Table 2). Serotype distribution demonstrated that adw2 was the major serotype (62.6%) in the samples, followed by adrq+ (24.6%) (Table 2). Other serotypes such as adw, adw3, ayw, ayw1, and ayr were also found in a small number of the samples. Similar to genotype results, no association between serotype and clinical status of the liver disease was observed (Table 2).

HBx and basal core promoter mutations

Initially, amino acid sequences of HBx from the samples were aligned and compared with reference sequences of amino acids retrieved from GenBank (accession no. BAA23459 and BAD86602 for HBV genotype B and C,

Table 2 HBV genotype and serotype distribution in samples with different clinical diagnosis

Characteristics		*n* (%) in each clinical diagnosis						*P*			
				HCC							
		CH (*n* = 61)	LC (*n* = 62)	With LC (*n* = 12)	Without LC (*n* = 36)	All HCC (*n* = 48)	Total (*n* = 171)	CH *vs* LC	CH *vs* All HCC	LC *vs* All HCC	CH *vs* LC *vs* All HCC
Genotype and subgenotype											
B	B2	5 (8.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (2.9)	NS	NS	NA	0.007
	B3	38 (62.3)	41 (66.1)	8 (66.7)	20 (55.6)	28 (58.3)	107 (62.6)	NS	NS	NS	NS
	B4	0 (0.0)	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	NS	NA	NS	NS
	B5	0 (0.0)	0 (0.0)	2 (16.7)	3 (8.3)	5 (10.4)	5 (2.9)	NA	0.034	0.032	0.002
	B7	3 (4.9)	2 (3.2)	0 (0.0)	3 (8.3)	3 (6.3)	8 (4.7)	NS	NS	NS	NS
Total genotype B		46 (75.4)	44 (71.0)	10 (83.3)	26 (72.2)	36 (75.0)	126 (73.7)	NS	NS	NS	NS
C	C1	12 (19.7)	13 (21.0)	2 (16.7)	10 (27.8)	12 (25.0)	37 (21.6)	NS	NS	NS	NS
	C2	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	NS	NS	NA	NS
	C3	2 (3.3)	5 (8.1)	0 (0.0)	0 (0.0)	0 (0.0)	7 (4.1)	NS	NS	NS	NS
Total genotype C		15 (24.6)	18 (29.0)	2 (16.7)	10 (27.8)	12 (25.0)	45 (26.3)	NS	NS	NS	NS
No. HBV genotype B/C (%-B)		46/15 (75.4)	44/18 (71.0)	10/2 (83.3)	26/10 (72.2)	36/12 (75.0)	126/45 (73.7)	NS	NS	NS	NS
Genotype and serotype											
B	adw	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.8)	1 (2.1)	1 (0.6)	NA	NS	NS	NS
	adw2	38 (62.3)	39 (62.9)	9 (75.0)	21 (58.3)	30 (62.5)	107 (62.6)	NS	NS	NS	NS
	adw3	3 (4.9)	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.3)	NS	NS	NS	NS
	ayw	0 (0.0)	1 (1.6)	0 (0.0)	1 (2.8)	1 (2.1)	2 (1.2)	NS	NS	NS	NS
	ayw1	5 (8.2)	3 (4.8)	0 (0.0)	4 (11.1)	4 (8.3)	12 (7.0)	NS	NS	NS	NS
C	adrq+	14 (23.0)	17 (27.4)	2 (16.7)	9 (25.0)	11 (22.9)	42 (24.6)	NS	NS	NS	NS
	adw2	0 (0.0)	1 (1.6)	0 (0.0)	1 (2.8)	1 (2.1)	2 (1.2)	NS	NS	NS	NS
	ayr	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.6)	NS	NS	NA	NS

CH: Chronic hepatitis; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma; NS: Not significant; NA: Not applicable; HBV: Hepatitis B virus.

Table 3 Frequencies of some HBx mutations in HBV genotype B according to different clinical diagnosis

Amino acid substitutions	*n* (%) in each clinical diagnosis						*P*			
			HCC							
	CH (*n* = 46)	LC (*n* = 44)	With LC (*n* = 10)	Without LC (*n* = 26)	All HCC (*n* = 36)	Total (*n* = 126)	CH *vs* LC	CH *vs* All HCC	LC *vs* All HCC	CH *vs* LC *vs* All HCC
T118N	23 (50.0)	6 (13.6)	4 (40.0)	7 (26.9)	11 (30.6)	40 (31.7)	< 0.001	NS	NS	0.028
I127N/T/S	5 (10.9)	16 (36.4)	2 (20.0)	2 (7.7)	4 (11.1)	25 (19.8)	0.009	NS	0.019	NS
K130M	8 (17.4)	23 (52.3)	5 (50.0)	11 (42.3)	16 (44.4)	47 (37.3)	0.001	0.015	NS	0.006
V131I	8 (17.4)	22 (50.0)	4 (40.0)	11 (42.3)	15 (41.6)	45 (35.7)	0.002	0.030	NS	0.012

respectively). Several amino acid changes were observed in both HBV genotype B and C. The prevalence of four amino acid substitutions (T118N, I127N/T/S, K130M and V131I) in HBV genotype B were significantly different between CH and LC, and three of them (T118N, K130M and V131I) showed a significant difference in prevalence between CH, LC and HCC, but none of them was significantly different between LC and HCC (Figure 1, Table 3). In contrast, none of the amino acid substitutions showed any significant difference in prevalence in the different clinical status in HBV genotype C (data not shown).

The four substituted amino acids located in BCP region, the corresponding nucleotides (C1726A/T1727(C/T) corresponding to T118N, T1753V corresponding to I127N/T/S, and A1762T/G1764A corresponding to K130M and V131I) were analyzed (Table 4). Mutations at positions 1762 and 1764 (corresponding to K130M and V131I amino acid substitutions), either as a double mutation or an independent mutation, were significantly higher in LC and HCC than CH. Particularly, the double mutation (A1762T/G1764A) which was found in 19.7%, 59.7% and 54.2% of CH, LC and HCC, respectively ($P < 0.001$). There was no significant difference in the prevalence of the double mutation between HCC with and without cirrhosis (41.7% and 58.3%). Analysis of the nucleotide at position 1753 showed that a T-to-V (A/G/C) mutation (corresponding to I127N/T/S amino acid substitutions) was significantly higher in LC (46.8%) compared with CH (18.0%) and HCC (22.9%) ($P = 0.004$), suggesting that this mutation could be an indicator of liver cirrhosis. Moreover, the prevalence of T1753V mutation was also not significantly different between HCC with cirrhosis (16.7%) and that without cirrhosis (25.0%) (data not shown).

In addition, C1726A/T1727 (C/T) mutations

Table 4 Prevalence of HBx and core promoter mutations in samples with different clinical diagnosis

Characteristics	*n* (%) in each clinical diagnosis									
			HCC				*P*			
	CH (*n* = 61)	LC (*n* = 62)	With LC (*n* = 12)	Without LC (*n* = 36)	All HCC (*n* = 48)	Total (*n* = 171)	CH *vs* LC	CH *vs* All HCC	LC *vs* All HCC	CH *vs* LC *vs* All HCC
Genotype B/C (%B)	46/15 (75.4)	44/18 (71.0)	10/2 (83.3)	26/10 (72.2)	36/12 (75.0)	126/45 (73.7)	NS	NS	NS	NS
BCP mutations										
C1726A/T1727(C/T)	24 (39.3)	8 (12.9)	4 (33.3)	7 (19.4)	11 (22.9)	43 (34.1)	0.002	NS	0.015	0.003
T1753V	11 (18.0)	29 (46.8)	2 (16.7)	9 (25.0)	11 (22.9)	51 (40.5)	0.015	NS	0.018	0.004
A1762T	12 (19.7)	38 (61.3)	6 (50.0)	21 (58.3)	27 (56.3)	77 (61.1)	< 0.001	< 0.001	NS	< 0.001
G1764A	13 (21.3)	38 (61.3)	5 (41.7)	21 (58.3)	26 (54.2)	77 (61.1)	< 0.001	0.0002	NS	< 0.001
C1766T	1 (1.6)	3 (4.8)	1 (8.3)	1 (2.8)	2 (4.2)	6 (4.8)	NS	NS	NS	NS
T1768A	1 (1.6)	2 (3.2)	1 (8.3)	2 (5.6)	3 (6.3)	6 (4.8)	NS	NS	NS	NS
A1762T/G1764A	12 (19.7)	37 (59.7)	5 (41.7)	21 (58.3)	26 (54.2)	75 (59.5)	< 0.001	0.0004	NS	< 0.001

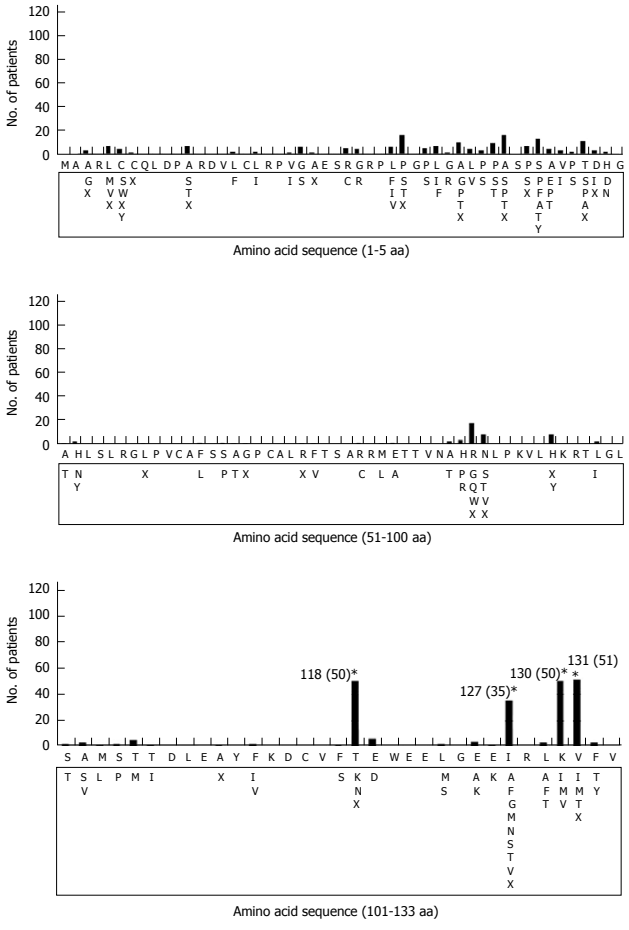

Figure 1 Distribution and frequencies of the amino acid mutations in the 133 amino acids of HBx protein of HBV genotype B observed in the present study. Reference sequence of HBV genotype B (accession no. BAA23459) is shown at the top and mutations are shown below the reference sequence. Stars indicate the major substitutions observed and values in parentheses are number of patients with respective mutation.

(corresponding to T118N substitution) were significantly higher in CH (39.3%) than in LC (12.9%) and HCC (22.9%). These results suggested that these mutations were reversely associated with severity of liver disease. In another words, single nucleotide polymorphisms (SNPs) in C1726/T1727 have an association with liver disease manifestations. The distribution of SNPs in 1726/1727 is shown in Table 5. In HBV genotype B, most of nucleotides in 1726 were A or C. The percentage of 1726A was significantly higher in CH (52.2%) than in LC (20.5%) and HCC (33.3%) ($P = 0.009$), while 1726C was more prevalent in LC (79.5%) and HCC (66.7%) than in CH (41.3%) ($P = 0.001$). On the other hand, most of the nucleotides in 1727 were T, however there was no significant difference in the percentage of 1727T in CH (95.7%), LC (88.6%), and HCC (97.2%) ($P = 0.279$). These results suggested that SNP in 1726, but not in 1727, of HBV genotype B was associated with the development of liver disease. In contrast, no association between SNP in the same positions in HBV genotype C and progression of liver disease was observed (Table 5).

Comparison of BCP and HBx mutations between genotype B and C

The percentage of cases with A1762T/G1764A mutation was significantly higher in genotype C than genotype B, regardless of clinical status: 53.3% *vs* 8.7% in CH, 83.3% *vs* 50.0% in LC, and 83.3% *vs* 45.5% in HCC (Figure 2A). From an analysis of total samples of these two genotypes, it was shown that the percentage of A1762T/G1764A mutation in genotype C was higher than that of genotype B (73.3% *vs* 33.3%, $P < 0.001$). Similar to the results of A1762T/G1764A mutation, T1753V mutation also showed a significantly different distribution between genotypes C (55.6%) and B (20.6%) with $P < 0.001$ (Table 6 and Figure 2B). When T1753V mutation was observed in each clinical status, its prevalence in HBV genotype C and B were 40.0% *vs* 10.9% in CH, 66.7% *vs* 38.6% in LC, and 58.3% *vs* 11.1% in HCC. In contrast, C1726A/T1727(C/T) mutation was more frequent in HBV genotype B (31.7%) than genotype C (6.7%) ($P = 0.002$).

DISCUSSION

Identification of viral as well as host factors associated with the development of severe liver disease including HCC may have important clinical implications in the management of patients with HBV infection. Many studies have suggested that HBV genotype might play

Table 5 Single nucleotide polymorphisms in 1726/1727 of HBV Genotype B and C

SNP	*n* (%) in each HBV genotype and clinical diagnosis											
	Genotype B						Genotype C					
			HCC						HCC			
	CH (*n* = 46)	LC (*n* = 44)	With LC (*n* = 10)	Without LC (*n* = 26)	All HCC (*n* = 36)	*P*	CH (*n* = 15)	LC (*n* = 18)	With LC (*n* = 2)	Without LC (*n* = 10)	All HCC (*n* = 12)	*P*
1726A	24 (52.2)	9 (20.5)	4 (40.0)	8 (30.8)	11 (33.3)	0.009	13 (86.7)	13 (72.2)	2 (100.0)	7 (70.0)	9 (75.0)	NS
1726C	19 (41.3)	35 (79.5)	6 (60.0)	18 (69.2)	24 (66.7)	0.001	2 (13.3)	5 (27.8)	0 (0.0)	3 (30.0)	4 (33.3)	NS
1726T	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NS	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
1727A	1 (2.2)	2 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	NS	8 (53.3)	11 (61.1)	2 (100.0)	2 (20.0)	4 (33.3)	NS
1727C	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	NS	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
1727G	1 (2.2)	2 (4.5)	0 (0.0)	1 (3.8)	1 (2.8)	NS	4 (26.7)	2 (11.1)	0 (0.0)	5 (50.0)	5 (41.7)	NS
1727T	44 (95.7)	39 (88.6)	10 (100.0)	25 (96.2)	35 (97.2)	NS	3 (20.0)	5 (27.8)	0 (0.0)	3 (30.0)	3 (25.0)	NS
1726A/1727A	1 (2.2)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	NS	7 (46.7)	10 (55.6)	2 (100.0)	2 (20.0)	4 (33.3)	NS
1726A/1727G	1 (2.2)	1 (2.3)	0 (0.0)	1 (3.8)	1 (2.8)	NS	4 (26.7)	2 (11.1)	0 (0.0)	5 (50.0)	5 (41.7)	NS
1726A/1727T	22 (47.8)	7 (15.9)	4 (40.0)	7 (26.9)	11 (30.6)	0.007	2 (13.3)	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	NS
1726C/1727A	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	NS	1 (6.7)	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	NS
1726C/1727G	0 (0.0)	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	NS	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
1726C/1727T	19 (41.3)	32 (72.7)	6 (60.0)	18 (69.2)	24 (66.7)	0.006	1 (6.7)	4 (22.2)	0 (0.0)	3 (30.0)	3 (25.0)	NS

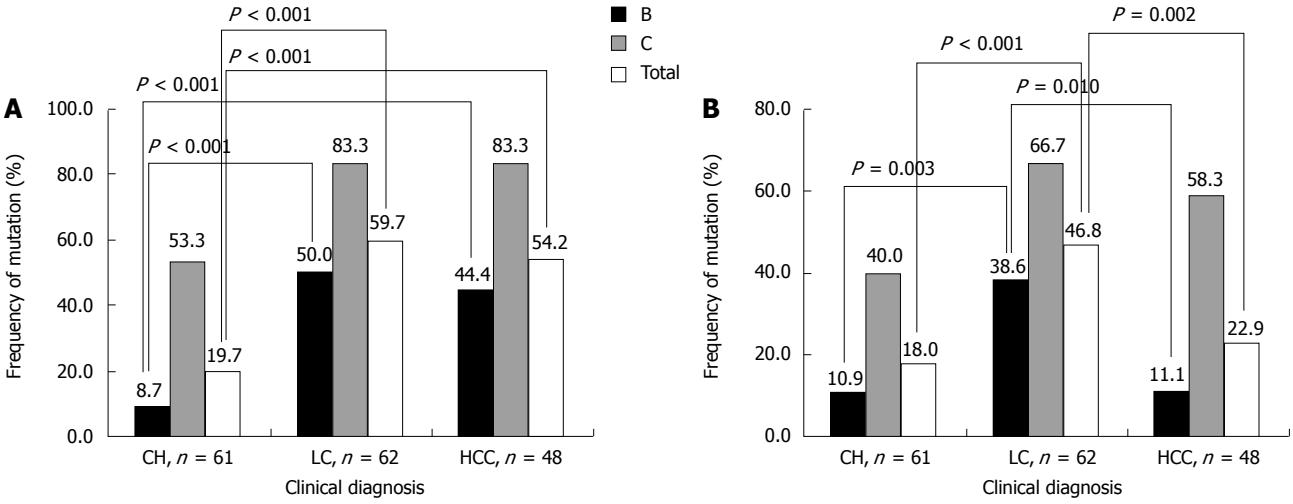

Figure 2 The prevalence of A1762T/G1764A (A) and T1753V (B) mutations in the samples with different clinical diagnoses. The number on each histogram represents the percentage of each mutation in each group. *P* values between the groups are shown respectively.

an important role in the development of severe liver diseases. However, it is also widely accepted that HBV genotypes appear to show varying geographic patterns in their distribution which means the association between HBV genotype and the severity of liver disease may differ from one region to another. For instance, studies from Taiwan and Japan demonstrated that HBV genotype C is associated more with severe liver disease that HBV genotype B[19-23]. Since HBV genotype B and C are the main genotypes transmitted in these areas, the investigators compared between genotype B and C. However, another study from Alaska showed that there was a significant association between HBV genotype F and the development of HCC among native Alaskan people[40]. This means that the association between HBV genotype and severity of liver disease could be different, depending on the area and the genotype of HBV transmitted in that particular area. From this study, a cross-sectional analysis of subjects from several different centers in Indonesia, it was found that HBV genotype B was the major genotype and no association between HBV genotype as well as serotype and clinical status of liver disease was observed. Analysis of data from a prospective cohort study, however, is needed to further elucidate the association between HBV genotype and manifestations of liver disease in Indonesian HBV carriers.

Many studies have demonstrated that virus mutations, including mutations of HBx protein, BCP, and precore are linked to the severity and outcome of HBV infection. A study from Taiwan reported that the amino acid substitution at codon 31 of HBx protein (S31A) was frequently found in HCC patients and was predicted to have an association with HCC development[33]. A Japanese group also reported that a mutation at codon 38 (P38S) of HBV genotype C was associated with HCC development[34]. Recently, it was reported that mutations in HBx protein (V5M/L, P38S, H94Y, I127T/N, K130M

Table 6 Comparison of core promoter mutations in HBV genotype B and C

Characteristics	HBV genotype			*P*
	B	C	All	
No. (%) of patients	126 (73.7)	45 (26.3)	171 (100.0)	< 0.001
Age (mean ± SD)	44.3 ± 12.8	49.4 ± 13.3	45.6 ± 13.1	0.024
Male/Female (%Male)	95/31 (75.4)	32/13 (71.1)	127/44 (74.3)	NS
No. (%) of A1762T/G1764A	42 (33.3)	33 (73.3)	75 (43.9)	< 0.001
No. (%) of T1753V	26 (20.6)	25 (55.6)	51 (29.8)	< 0.001
No. (%) of C1726A/T1727 (C/T)	40 (31.7)	3 (6.7)	43 (25.1)	0.002

and V131I) from Korean patients are linked with severity of liver disease[35]. HBx protein analysis of samples in the present study showed that I127N/T/S, K130M and V131I amino acid substitutions are associated with severe liver disease, especially with liver cirrhosis (Table 3). However, no association between S31A as well as P38S mutations and liver disease progression was found, which is different from previous studies in Taiwan, Japan, and Korea[33-35].

It is well known that the double mutation (A1762T/G1764A) in BCP is associated with an increased risk of liver disease. For instance, the frequency of double mutation (A1762T/G1764A) increased with advancing clinical status in Taiwanese patients [3%, 11%, 32% and 64% in asymptomatic carriers (AC), LC, CH, and HCC groups, respectively][24]. A recent report from China has also demonstrated that the incidence of double mutation increased along with the progression of liver disease; the percentage of the double mutation was 33%, 56% and 85% in CH, LC, and HCC groups, respectively[31]. In Indonesian patients, however, the A1762T/G1764A double mutation was increased in CH from 19.7% to 59.7% in LC and was slightly decreased in HCC (54.2%) (Table 4). These results suggest that the double mutation is associated with severe liver disease. In addition, analysis of the nucleotide at position 1753 showed that a T-to-V (A/G/C) mutation increased to 46.8% in LC from 18.0% in CH, but dramatically decreased in HCC (22.9%) (Table 4), suggesting that this mutation is associated with liver cirrhosis rather than HCC. In contrast, analysis of sera or plasma from Japanese subjects with AC, CH, LC and HCC infected with HBV genotype C showed that the percentage of T1753V mutation increased with progression of liver disease[41]. It is also reported that T1753V mutation was higher in HCC (53.2%) compared with LC (18.8%) and CH (9.8%)[31]. These results were inconsistent with the present study, particularly in LC and HCC. These discrepancies might be associated with HCC status; most of HCC cases in the present study were without cirrhosis. Another possibility is that most of the samples analyzed in the previous reports were HBV genotype C, whereas most of samples in the present study were HBV genotype B.

The most interesting finding of the present study is the association of SNP at position 1726 of HBV genotype B, but not genotype C, and severity of liver disease. Since HBV genotype B is the major genotype in Indonesia, this finding is important for the management and prevention of HBV carriers from developing more advanced disease such as liver cirrhosis and HCC in Indonesia. This association, however, has to be confirmed by analyzing more samples. A comparison of mutation prevalence between HBV genotype B and C showed that the percentage of T1753V and A1762T/G1764A mutations were higher in genotype C than in genotype B (Table 6). These results are in accordance with previous findings from Taiwan and China[20,31].

In summary, the present study demonstrated that HBV genotype B and C were detected among HBV-associated liver disease patients in Indonesia, and genotype B was predominant. It was found that HBV genotype, as well as the serotype, might not be associated with an increased risk of HCC. The A1762T/G1764A and T1753V mutations in BCP can be used as an indicator for progression of liver disease in Indonesian patients.

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COMMENTS

Background

Hepatitis B virus (HBV) genotype, mutations in the core promoter, precore or HBx gene have been shown to have an association with severe liver disease. The aims of the study were to identify the distribution of HBV subgenotype and basal core promoter (BCP) mutations among patients with HBV-associated liver disease in Indonesia, and analyze the possible association between HBV genotype and/or BCP mutations and severity of liver disease among Indonesian patients.

Research frontiers

Although there were some reports on the distribution of HBV genotype in Indonesia, the association between HBV genotype and/or BCP mutations and liver disease progression has not been investigated. Therefore it is important to have information not only related to the distribution of HBV genotype/subgenotype and BCP mutations in patients with different clinical status, but also the association of HBV genotype/subgenotype and/or BCP mutations and liver disease progression in Indonesia.

Innovations and breakthroughs

The present study demonstrated that only HBV genotype B and C were detected among HBV-associated liver disease patients in Indonesia, and genotype B was predominant. It was found that HBV genotype, as well as the serotype, might not be associated with an increased risk of hepatocellular carcinoma (HCC). The double mutation (A1762T/G1764A) was associated with progression of liver disease, while T1753V mutation could be used as an indicator of liver cirrhosis rather than HCC. In addition, SNP in 1726 has an association with manifestations of liver disease.

Applications

The double mutation (A1762T/G1764A) can be used for the prediction of severe liver disease including cirrhosis and HCC, whereas the T1753V mutation is a predictor of liver cirrhosis in Indonesian patients. In addition, SNP in 1726 can also be used for the prediction of liver disease severity.

Terminology

HBs; HBs gene encode the surface protein of HBV that consist of preS1, preS2, and S. HBx; HBx gene encode functional X protein. BCP; BCP can be

considered a part of HBx gene that regulate the core gene expression.

Peer review

The study provides a identify HBV subgenotype and basal core promoter (BCP) mutations distribution among HBV-associated liver disease patients in Indonesia. The work is of theoretical and practical importance.

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Imaging features of intraductal papillary mucinous neoplasms of the pancreas in multi-detector row computed tomography

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Abstract

AIM: To retrospectively evaluate the imaging features of pancreatic intraductal papillary mucinous neoplasms (IPMNs) in multi-detector row computed tomography (MDCT).

METHODS: A total of 20 patients with pathologically-confirmed intraductal papillary mucinous neoplasms (IPMNs) were included in this study. Axial MDCT images combined with CT angiography (CTA) and multiplanar volume reformations (MPVR) or curved reformations (CR) were preoperatively acquired. Two radiologists (Tan L and Wang DB) reviewed all the images in consensus using an interactive picture archiving and communication system. The disputes in readings were resolved through consultation with a third experienced radiologist (Chen KM). Finally, the findings and diagnoses were compared with the pathologic results.

RESULTS: The pathological study revealed 12 malignant IPMNs and eight benign IPMNs. The diameters of the cystic lesions and main pancreatic ducts (MPDs) were significantly larger in malignant IPMNs compared with those of the benign IPMNs ($P < 0.05$). The combined-type IPMNs had a higher rate of malignancy than the other two types of IPMNs ($P < 0.05$). Tumors with mural nodules and thick septa had a significantly higher incidence of malignancy than tumors without these features ($P < 0.05$). Communication of side-branch IPMNs with the MPD was present in nine cases at pathologic examination. Seven of them were identified from CTA and MPVR or CR images. From comparison with the pathological diagnosis, the sensitivity, specificity, and accuracy of MDCT in characterizing the malignancy of IPMN of the pancreas were determined to be 100%, 87.5% and 95%, respectively.

CONCLUSION: MDCT with CTA and MPVR or CR techniques can elucidate the imaging features of IPMNs and help predict the malignancy of these tumors.

Key words: Computed tomography; Diagnostic imaging; Intraductal papillary mucinous neoplasm; Pancreatic neoplasms

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INTRODUCTION

Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas, defined by the World Health Organization (WHO), are a broad spectrum of neoplasms arising from the pancreatic duct epithelia and characterized by cystic dilatation of the main and/or branch pancreatic ducts, and occasionally by the presence of mural nodules (papillary excrescence or protuberances)[1-6]. According to

the site and extent of involvement, IPMNs are classified into three subtypes: main duct type, branch duct type, and combined-type (mixed type) with both main and branch duct lesions[2,7,8]. Although an IPMN is a rare tumor, the increasingly common use of imaging techniques has contributed to the identification of an increasing number of the lesions, even in entirely asymptomatic cases[9-13].

According to the International Guidelines for management of IPMN[2], magnetic resonance imaging (MRI) along with magnetic resonance cholangiopancreatography (MRCP) is referred to as the best imaging modality to outline the gross appearance of IPMN. However, computed tomography (CT) is still the mainstay in the evaluation of patients with IPMNs of the pancreas. Improvements in CT such as the evolution of post-processing techniques in multi-detector row CT (MDCT), have enhanced the capability of CT in the evaluation of abnormalities of the pancreatic parenchyma and the pancreatic ducts in patients with IPMNs[10]. Axial CT images combined with multiplanar volume reformations (MPVR) or curved reformations (CR) and CT angiography (CTA) with maximum intensity projection should display the imaging features of IPMNs in more detail. Recent studies assessed the capability of MDCT in defining the invasiveness of the IPMN and in evaluating the resectability of IPMNs[14,15]. The IPMN can evolve through all biological stages, from slight dysplasia to carcinoma. As one of the few surgically curable pancreatic tumors, accurate preoperative prediction of malignancy remains one of the major issues in the optimal treatment of IPMNs, and it also influences the outcome of the resection[16,17].

The present study retrospectively assessed the imaging findings of IPMNs with MDCT and evaluated the capabilities of MDCT with emphasis on the postprocessing techniques to predict the malignancy of IPMNs.

MATERIALS AND METHODS

Patient population

Between December 2005 and March 2008, IPMN of the pancreas was diagnosed in 31 cases at our institution based on surgico-pathological examination. Of these 31 cases, 20 patients [11 male, 9 female; mean age, 62 years (range, 41-81 years)] who underwent MDCT within a month prior to surgery were recruited into this study. At our hospital, surgical planning requires that most patients in whom IPMN of the pancreas is suspected undergo MDCT including CTA and MPVR or CR processing rather than dynamic MRI with MRCP for evaluation of the extent of the disease and the relationship between affected parts of the pancreas and surrounding vessels and organs. The lesions revealed by the subsequent pathological examinations were divided into three subtypes: benign adenoma, noninvasive carcinoma including borderline malignancy and carcinoma *in situ*, and invasive carcinoma in accordance with the WHO classification. A borderline lesion was defined as a tumor which was not overtly malignant but had some foci of severe cellular atypia, indicating that it should be treated as malignant. Thus, an IPMN considered at imaging to be malignant could include a borderline malignancy, carcinoma *in situ*, and invasive carcinoma on pathology in this study. The mean interval between the imaging and surgery was 22 d (range, 10-31 d). Institutional Review Board approval and waiver of informed consent for this retrospective study were obtained.

CT techniques

All CT examinations were performed with a 4- or 16-slice MDCT scanner (Lightspeed QX/I or Lightspeed 16; GE Medical Systems, Milwaukee, Wis). The parameters applied in the CT scan were as follows: a tube voltage of 120 kVp, a tube current of 280 mA, and tube rotation time of 0.5-0.8 s. Prior to administration of contrast agents, non-enhanced CT of the upper abdomen was performed with 10 mm slice thickness and 10 mm spacing. Non-ionic contrast materials with an iodine concentration of 300 mgI/mL were injected into the antecubital vein at a rate of 3-4 mL/s with an 18-20-gauge cannula for all the patients. In addition, 40 mL dextrose was administered at the same rate immediately after the administration of contrast agents. The pancreatic phase of dual phased CT was started at 35 s after the initiation of contrast material injection, whereas the venous phase was performed afterwards with a delayed time of 65-70 s. For the pancreatic phase, a slice thickness of 1.25 mm and a table speed of 11.25 mm/s were utilized while a slice thickness of 5 mm and a table speed of 18.75 mm/s were employed for the venous phase. Axial images were retrospectively reconstructed at a 50% overlap, using a 1.25 mm slice thickness, and 0.625 mm spacing.

In all patients, CTA and 2-dimensional (2D) MPVR or CR images were generated from the source axial images at a commercially available dedicated workstation (ADW 4; GE Medical Systems) by a radiologist (Zhao YE).

Image analysis

Two radiologists (Tan L and Wang DB) reviewed all the images in consensus at an interactive picture archiving and communication system workstation. The readers were aware of the diagnosis of an IPMN but were blinded to the findings of surgery and pathological examination. In cases of interobserver disagreement, final decisions were made through consultation with a third experienced radiologist (Chen KM).

Each reader recorded the following items: (1) the largest diameter of the cystic lesion; (2) the largest diameter of the main pancreatic duct (MPD); (3) classification of the tumors as main duct type, branch duct type, and combined type (mixed type). The main duct type was diagnosed when dilatation of the MPD had increased its diameter to more than 5 mm. The presence of one or multiple cystic lesions in the pancreatic parenchyma, without dilatation of the MPD, indicated that a branch duct-type tumor was present. The combined type was diagnosed when the pancreas contained one or more cystic lesions and the diameter of the dilated MPD was more than 5 mm; (4) locations of the lesions: in the head or uncinate process, the body, or the tail of the pancreas. Diffuse involvement was denoted when the lesion involved the entire gland; (5) internal solid structures: presence or absence of mural nodules and thick septa; (6) other findings including vascular involvement, lymph node enlargement,

Table 1 Relationship between imaging findings on MDCT and surgico-pathological diagnosis of pancreatic IPMNs

MDCT findings	Surgical and pathological results		*P*
	Benign (*n* = 8)	Malignant (*n* = 12)	
Largest diameter of cystic lesion	21.6 ± 10.3 mm	43.5 ± 16.5 mm	< 0.05
Caliber of main pancreatic duct	3.3 ± 1.6 mm	7.5 ± 5.5 mm	< 0.05
Morphologic type			< 0.05
Branch duct	4	1	
Combined	2	10	
Main duct	2	1	
Location of cystic lesion			> 0.05
Head and uncinate	4	10	
Body	0	1	
Tail	2	0	
Diffuse	2	1	
Solid structures inside lesion			< 0.05
Mural nodules and thick septa	2	10	
No mural nodules or thick septa	4	1	

MDCT: Multi-detector row computed tomography; IPMNs: Intraductal papillary mucinous neoplasms.

and duodenal involvement, as well as dilatation of the bile duct and distant metastasis. All the imaging diagnoses were compared with the surgico-pathological outcomes.

Statistical analysis

The unpaired Student's *t*-test was used to assess the differences in the largest diameters of the cystic lesion and MPDs between the benign and malignant groups. Fisher's exact test was used to evaluate the differences in classification, location, and internal solid structures between the benign and malignant tumors confirmed by pathology. The sensitivity, specificity, and accuracy of the MDCT diagnosis for IPMNs of the pancreas were calculated. $P < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 13.0 computer software (Chicago, IL, USA).

RESULTS

Pathologic results

Five patients (5 of 20, 25%) had invasive carcinoma, whereas seven patients (7 of 20, 35%) had a noninvasive carcinoma (including three borderline lesions and four carcinoma *in situ*). Both of these lesions were regarded as malignant in this study. The lesions in the other eight patients (8 of 20, 40%) were classified as benign IPMNs by pathology. Among the 12 malignant IPMNs, two patients had duodenal involvement with one also having vascular involvement, another two patients had regional lymph node metastasis on surgico-pathological examination, and one patient only had vascular involvement.

Imaging features of IPMNs compared with surgico-pathological results

The imaging features of IPMNs at MDCT are summarized in Table 1. The largest diameters of the cystic lesions were 21.6 ± 10.3 mm and 43.5 ± 16.5 mm for benign and malignant IPMNs, respectively. A statistically significant difference has been demonstrated between the sizes of the lesions of benign and malignant IPMNs ($P < 0.05$). The mean diameter of most dilated segments of MPD was 3.3 ± 1.6 mm in patients with benign IPMNs, and 7.5 ± 5.5 mm in patients with malignant IPMNs. The diameter of the MPD was significantly larger in patients with malignant IPMNs compared with that of benign cases ($P < 0.05$) (Figure 1). Based on MDCT findings, five patients (5 of 20, 25%) were classified as branch duct type, three patients (3 of 20, 15%) as main duct type, and 12 patients (12 of 20, 60%) as combined type. With pathological correlation, malignant IPMNs presented in one of the five patients with branch duct type, one of three patients with main duct type, and 10 of the 12 patients with combined type. Significant correlation was shown between the type of ductal involvement and the pathological results. The combined type also had a higher rate of malignancy ($P < 0.05$) (Figure 2). Three combined-type cases had accompanying marked dilatation of the biliary tracts including extrahepatic and intrahepatic bile ducts (Figure 3). The pancreatic head and uncinate process were the most common locations of IPMNs, accounting for 70% (14 of 20). Malignant IPMNs presented in 10 of these 14 patients with an incidence of 83.3% (10 of 12) among all the malignant cases. Only one malignant IPMN identified

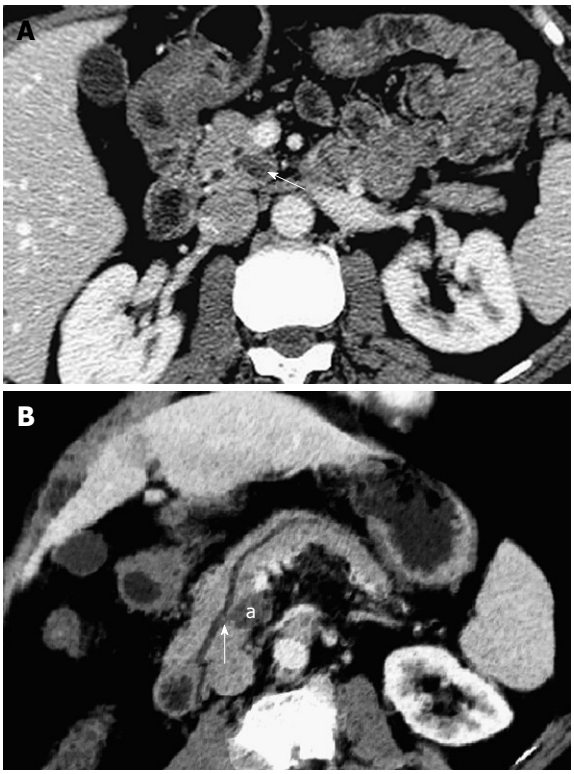

Figure 1 Pathologically confirmed benign branch-duct-type IPMN in a 47-year-old woman with abdominal discomfort for about 6 mo. A: There was a 2-cm cystic mass (white arrow) in the uncinate process of the pancreas at the axial abdominal MDCT image; B: The cystic mass (a) in the posterior pancreatic parenchyma was demonstrated with a communication (white arrow) between the mass and the main pancreatic duct which was slightly dilated in the curved reformed (CR) image.

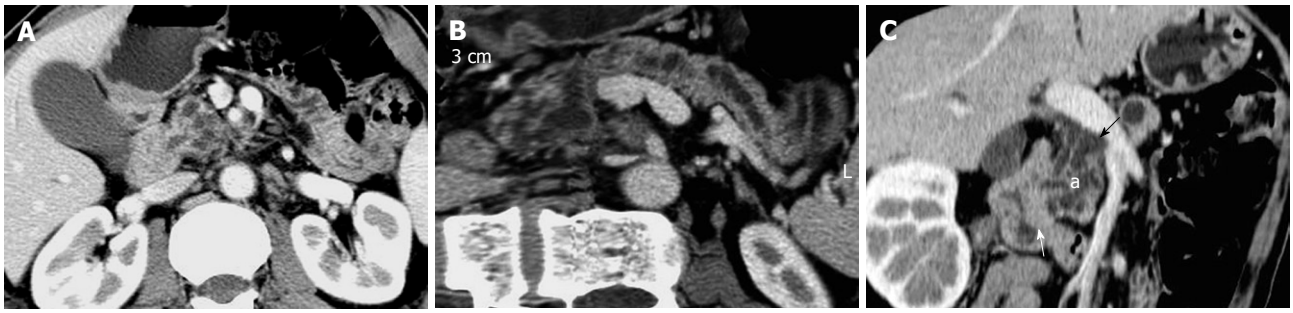

Figure 2 Pathologically confirmed malignant combined-type IPMN in a 41-year-old man with abdominal and back pain for about 2 years. A: A 4 cm cystic mass with multiple septa arising from the pancreatic head was revealed in the axial MDCT image; B: Besides the cystic mass in the pancreatic head, the profile of the main pancreatic duct, which was severely dilated, was depicted on the CR image; C: The cystic lesion (a) in the pancreatic head invaded the duodenum and main portal vein resulting in the duodenal wall thickening (white arrow) with marked enhancement and irregular narrowing (dark arrow) of the vessel in the multiplanar volume reformation (MPVR) image.

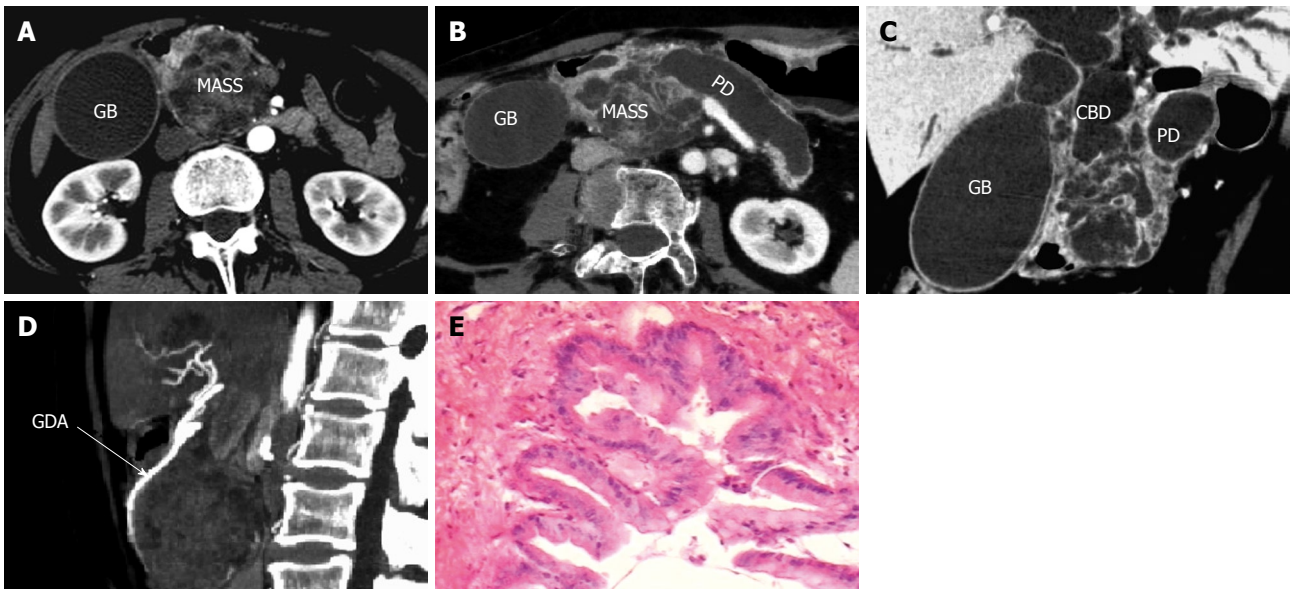

Figure 3 Pathologically confirmed malignant combined-type IPMN in a 65-year-old man with jaundice and abdominal pain for about 1 year. A: An 8 cm cystic and solid mass (MASS) was seen in the axial arterio-phased MDCT image with contrast agents. The gallbladder (GB) was distended; B: The heterogeneous mass was shown with severe dilatation of the main pancreatic duct (PD) and the gallbladder (GB) in the CR image; C: The profile of the dilatation of pancreatobiliary system (CBD, common bile duct) was entirely depicted in the MPVR image; D: The gastroduodenal artery (GDA) showed irregularity as a result of infiltration of the tumor; E: The tumor consisted of papillary proliferations of tall columnar mucin-producing epithelium. Atypical epithelium characterized by enlarged nuclei (HE, × 150).

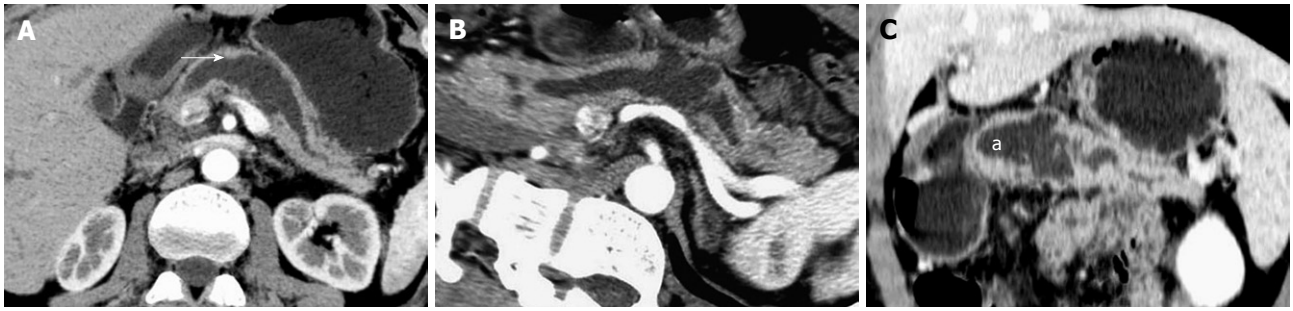

Figure 4 Pathologically confirmed malignant combined-type IPMN in a 55-year-old man with abdominal pain for 1.5 years. A: A 3 cm × 10 cm longitudinally cystic mass in the pancreatic body was shown with a mural nodule (white arrow) in the axial MDCT images; B: The classification of combined type for this case was accurately defined by the CR image; C: The profile of the cystic mass (a) and dilatation of the branch duct and the upstream pancreatic duct were identified in the MPVR images.

by MDCT was located at the body of the pancreas (Figure 4), whereas the IPMNs arising from the pancreatic tail in two cases were both benign and verified by pathology. The other three (3 of 20, 15%) cases had diffuse lesions, and one of these (33.3%) was malignant. Among 17 branch duct-type and combined-type lesions, mural nodules and thick septa were seen in 12 cases (Figure 4). Ten of these 12 were referred to as malignant IPMNs on pa-

thology. Tumors with mural nodules and thick septa had a significantly higher incidence of malignancy than tumors without them ($P < 0.05$).

No distant metastasis was revealed on MDCT in any of the patients. Vascular invasion was revealed in two patients from CTA images (Figure 2), and was proven by surgico-pathological studies. Lymph node enlargement was seen in three patients on axial CT images, but one was a false-positive when compared with the results of pathological examination. Two patients had duodenal involvement depicted by MPVR images and confirmed by pathological examination (Figure 2), and one of these had vascular involvement. Vascular involvement was also detected in another case. Communication of side-branch IPMNs with the MPD was present in nine cases at pathological examination. Seven of these (77.7%, 7 of 9) were identified from CTA and MPVR images (Figure 1).

From a comparison with the pathological outcomes, the sensitivity, specificity, and accuracy of MDCT in characterizing the malignancy of IPMN of the pancreas were determined to be 100%, 87.5% and 95%, respectively.

DISCUSSION

After the first report of IPMN in 1982, this tumor entity has been increasingly recognized over the past decades as a result of the markedly improved imaging modalities[18,19]. IPMNs mainly occur in the 6th to 7th decades of life, affecting males slightly more frequently than females. IPMNs account for 0.5% of all pancreatic neoplasms found at autopsy, 7.5% of clinically diagnosed pancreatic neoplasms, and 16.3% of surgically resected pancreatic neoplasms[20]. The IPMN was histologically defined by the WHO as "intraductal mucin-producing neoplasm with tall columnar mucin-containing epithelium with or without papillary projections, involving the MPD and/or major side branches"[21]. The IPMN is also believed by some to follow the so-called "adenoma-carcinoma" sequence[19]. Sohn *et al*[22] reported that there appears to be a lag time of approximately 5 years in the progression from adenoma to invasive carcinoma and that progression to invasive carcinoma occurs relatively quickly once moderate dysplasia is found. Kawai *et al*[17] reported that the malignancy rate of IPMNs was 48% while it was 67% in the study conducted by Lopez Hänninen *et al*[23]. In our study, the malignancy rate was 60% (12 of 20). The treatment decision with regard to IPMN is often based on the patient's age at presentation, the lesion location in the pancreas, the extent of ductal involvement, and also the presence or absence of malignant features[24]. Since most of the main duct IPMNs will progress into invasive carcinomas, the resection of main duct IPMNs and mixed variant IPMNs is recommended if the patient is a good surgical candidate with a reasonable life expectancy[2,25,26]. Currently, the cross-sectional imaging studies including MDCT and MRI with MRCP play a crucial role in structuring the treatment protocol for the patients with IPMNs.

Since the MDCT is widely used in clinical practice, more and more IPMNs are detected. Because of the overproduction of mucus, MPD dilatation can occur both proximal and distal to the tumor. In our study, most (14 of 20) of the IPMNs were located at the head and uncinate process of the pancreas, but there was no statistically significant differences in the distribution of the lesion locations because of the bias resulting from the small size of the patient population in this group. However, the cystic lesions in the pancreatic parenchyma and the diameter of the MPD in malignant IPMNs presented with significantly larger sizes than the benign IPMNs ($P < 0.05$). More combined-type IPMNs were malignant than the other two subtypes ($P < 0.05$). The MDCT features most specific for a malignant IPMN were mural nodules and thick septa inside the lesion (10 of 20) in this group as indicated in the literature[27,28]. Thinner-slice reformed CTA and MPVR or CR images showed other malignant signs clearly, including vascular invasion, lymph node enlargement, and duodenal involvement, as well as dilatation of the common bile duct and common hepatic duct (Figure 3). Ogawa *et al*[15] reported that the sensitivity, specificity, and accuracy for identifying malignancy were 83%, 81% and 82% and for identifying pancreatic parenchymal invasion were 90%, 88% and 89%, respectively. According to the International Consensus Guidelines for management of IPMN, the branch duct IPMNs are benign more frequently than the main duct IPMNs[2]. The data in this study also complied with this rule. However, compared with the published series from Europe, Japan, and the USA[9,29-32], the main duct IPMN had a lower rate of malignancy (1 of 3) in the present study, whereas the combined-type IPMNs demonstrated malignancy in more cases (10 of 12) on pathology. The sensitivity, specificity, and accuracy of MDCT in characterizing the malignancy of IPMNs were 100%, 87.5% and 95%, respectively. They were a little bit higher than the data in the published literature[2,15]. This could be explained possibly by the bias generated by the small number of purely main duct IPMNs and the limited size of the entire patient population in this group. Moreover, because of the limited cases of IPMN recruited in the present study, we considered the noninvasive carcinoma including borderline malignancy and carcinoma *in situ*, and invasive carcinoma together as a malignancy for analysis. On the other hand, this strategy without separation of the noninvasive and invasive carcinoma should be quite reasonable in the clinical context since progression from noninvasive malignancy to invasive carcinoma occurs relatively quickly once moderate dysplasia is found[22]. To some extent, the noninvasive carcinoma including borderline malignancy and carcinoma *in situ* should attract as much attention as invasive carcinoma in this setting where even the benign adenoma can progress to carcinoma in only 5 years[22]. Therefore, more prospective studies with a big IPMN population are required in order to reveal the more accurate profile of Chinese patients in the future.

Compared with the combined type IPMNs, patients with the branch-duct type IPMN without malignant features can be managed by follow-up examinations instead of surgery, particularly when the patient refuses surgery, or when the patient is in poor condition with other severe concomitant disease[33]. Thus, the imaging

modalities such as MDCT could be valuable in deciding whether the patient should undergo surgery or follow-up in most cases. The presence of communication of the pancreatic cystic lesion with the MPD is one of the most reliable findings for the diagnosis of branch duct IPMN[34]. With routine transverse CT scanning of the pancreas, the communicating duct is not easily seen. MPVR or CR images can markedly increase the chance of identification of the interaction.

Normally, IPMNs have a better outcome and prognosis compared to pancreatic ductal adenocarcinomas. The overall 5-year survival rate has been reported to exceed 80% for noninvasive IPMNs and 50% for the invasive malignant IPMNs[19]. Therefore, correct evaluation of IPMN is extremely important as a recent analysis has suggested that this entity is one of the few surgically curable pancreatic neoplasms[18]. Prior investigations showed MRI with MRCP was useful for assessment of IPMN as a noninvasive approach[23,35,36]. However, its spatial resolution is not as high as that of CT. Moreover, MDCT scanning is fast enough to acquire all necessary imaging data during a single breath-hold in which much thinner sliced images can be generated. It is less subject to respiratory motion and partial-volume effects than MRI[37,38]. Since most of the patients are elderly, the abovementioned characteristics of MDCT seem to be important for patients with IPMNs. With CTA and MPVR or CR images using 3D or 2D modes in different planar directions, MDCT can show the lesion itself, the surrounding structures, and the nearby vessels, as well as the bile duct during one set of CT scans. The reformed images of bile ducts were similar to the MRCP image (Figure 3). Dilatation of the bile ducts and the enlarged gallbladder could be clearly depicted. With the postprocessing techniques, CTA and MPVR or CR images can be generated with more details about the IPMN and the surrounding parenchyma or adjacent vessels.

In conclusion, MDCT scanning with CTA and MPVR or CR techniques can help predict malignant IPMN by differentiating the various types of ductal involvement and demonstrating the mural nodules and thick septa of the lesion, the MPD dilatation of the combined subtypes, the size of the cystic lesion as well as the involvement of surrounding structures. MDCT can be referred to as the diagnostic tool of choice for accurate evaluation of IPMN before treatment.

COMMENTS

Background

Intraductal papillary mucinous neoplasm (IPMN) mainly occurs in the 6th to 7th decades of life, affecting males slightly more frequently than females. IPMN accounts for 0.5% of all pancreatic neoplasms found at autopsy, 7.5% of clinically diagnosed pancreatic neoplasms, and 16.3% of surgically resected pancreatic neoplasms. The increasingly common use of imaging techniques has contributed to the identification of an increasing number of the lesions, even in entirely asymptomatic cases.

Research frontiers

Accurate preoperative evaluation of an IPMN is extremely important for the clinician involved in the diagnosis and further evaluation and intervention, as it is one of the few surgically curable pancreatic neoplasms. However, main duct and combined type IPMNs are more likely to be malignant with biological aggressiveness. A noninvasive imaging approach is the tool of choice for assessing an IPMN of the pancreas preoperatively and can provide accurate information for planning treatment protocols. Although magnetic resonance imaging with magnetic resonance cholangiopancreatography is referred to as the best imaging modality to outline the gross appearance of IPMNs, computed tomography (CT) is still the mainstay in evaluation of patients with IPMNs of the pancreas. Moreover, multi-detector row CT (MDCT) scanning is fast enough to acquire all the necessary imaging data during one single breath-hold in which much thinner sliced images can be generated for reformations.

Innovations and breakthroughs

There is limited published literature concerning the prediction of malignancy of IPMNs of the pancreas. In this series, the combined-type IPMNs had a higher rate of malignancy than the other two types of IPMNs ($P < 0.05$). The diameters of the cystic lesion and main pancreatic duct (MPD) were significantly larger in malignant tumors compared with those of benign IPMNs ($P < 0.05$). Tumors with mural nodules and thick septa had a significantly higher incidence of malignancy than tumors without these features ($P < 0.05$). In comparing with the pathological results, the sensitivity, specificity, and accuracy of MDCT in characterizing the malignancy of IPMN of the pancreas were determined as 100%, 87.5% and 95%, respectively. The imaging findings were predictive of the malignancy of the IPMNs.

Applications

Based on their research, MDCT scanning with CT angiography (CTA) and MPVR or CR techniques can help predict malignant IPMN by differentiating the various types of ductal involvement and demonstrating the mural nodules and thick septa of the lesion, the MPD dilatation of the combined subtypes, the size of the cystic lesion as well as the involvement of surrounding structures. MDCT can be referred to as the diagnostic tool of choice in accurate evaluation of IPMN before treatment.

Terminology

IPMN: histologically defined by the World Health Organization as an "intraductal mucin-producing neoplasm with tall columnar mucin-containing epithelium with or without papillary projections, involving the MPD and/or major side branches"; MDCT: multiple detectors applied to CT. This modality can improve the scanning speed and spatial resolution dramatically. Furthermore, MDCT is intrinsically suitable for CTA scanning.

Peer review

The authors evaluated the predictive factors for the presence of malignancy associated with IPMN based on their own data. It is well organized and an overall theoretical analysis is given. The conclusions are scientifically reliable and valuable.

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BRIEF ARTICLES

Conservative resection for benign tumors of the proximal pancreas

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Abstract

AIM: To evaluate the safety and long-term prognosis of conservative resection (CR) for benign or borderline tumor of the proximal pancreas.

METHODS: We retrospectively analyzed 20 patients who underwent CR at the Second Affiliated Hospital of Zhejiang University School of Medicine between April 2000 and October 2008. For pancreaticojejunostomy, a modified invagination method, continuous circular invaginated pancreaticojejunostomy (CCI-PJ) was used. Modified continuous closed lavage (MCCL) was performed for patients with pancreatic fistula.

RESULTS: The indications were: serous cystadenomas in eight patients, insulinomas in six, non-functional islet cell tumors in three and solid pseudopapillary tumors in three. Perioperative mortality was zero and morbidity was 25%. Overall, pancreatic fistula was present in 25% of patients. At a mean follow up of 42.7 mo, all patients were alive with no recurrence and no new-onset diabetes mellitus or exocrine dysfunction.

CONCLUSION: CR is a safe and effective procedure for patients with benign tumors in the proximal pancreas, with careful CCI-PJ and postoperative MCCL.

Key words: Adenoma; Islet cell; Conservative resection; Cystadenoma; Enucleation; Insulinoma; Pancreatectomy; Pancreatic fistula; Pancreatic neoplasms

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INTRODUCTION

Pancreaticoduodenectomy (PD) and distal pancreatectomy (DP) are standard operations for tumors located in the proximal portion of the pancreas. However, these standard techniques are associated with a significant risk of long-term endocrine and exocrine impairment. Recently, there has been increased interest in conservative surgery in an attempt to preserve as much as possible the pancreatic parenchyma and integrated upper digestive system anatomy. For patients with benign or low-grade malignant tumors, conservative resection (CR) such as central pancreatectomy or enucleation has been investigated to maintain the normal upper digestive system anatomy, and to reduce the risk of development of exocrine and endocrine functional insufficiency. Although CR of the proximal pancreas may be more appropriate and less time-consuming, it has not been used widely previously because of its high morbidity, especially pancreatic fistula. The focus of this study was to evaluate the safety and outcomes of CR for benign or borderline tumors of the head, neck and proximal part of the pancreas.

MATERIALS AND METHODS

Twenty patients with benign or borderline tumors

localized in the head, neck and proximal part of the pancreas, who were treated with central pancreatectomy (n = 11) and enucleation (n = 9), between April 2000 and October 2008, were analyzed retrospectively. Data on preoperative, intraoperative and postoperative care were collected and maintained on a secure database. Preoperative parameters included demographics, clinical presentation, and exocrine and endocrine evaluation. Preoperative imaging modalities such as abdominal spiral contrast CT and ultrasound imaging were used to evaluate the suitability for surgical resection in all cases. Intraoperative details including operative time, total blood loss, transfusion and the method of surgery were recorded. Postoperative events and clinical outcomes such as surgical complications, mortality, pathological data and long-term follow-up were recorded carefully.

Postoperative pancreatic fistula was defined as drainage of > 50 mL per 24 h of fluid, with amylase content > 3 times serum amylase activity for > 10 d after operation[1,2]. Perioperative mortality was defined as death in the hospital or within 30 d. Delayed gastric emptying (DGE) was defined to be present when nasogastric intubation was maintained for ⩾ 10 d, combined with at least one of the following: vomiting after removal of the nasogastric tube, reinsertion of nasogastric tube, or failure to restore oral feeding[3]. Fasting glucose blood level was used for the diagnosis of new-onset diabetes[4]. Exocrine insufficiency was defined as steatorrhea and weight loss requiring pancreatic enzymes supplementation.

Indications for CR of the pancreas

CR was indicated for benign or low-grade malignant lesions localized in the proximal pancreas, especially in young patients. Simple enucleation was used when the tumor was near or at the surface of the pancreas. Great care had to be taken to avoid the main pancreatic duct injury and obtain complete excision during surgery, otherwise central pancreatectomy was chosen. Central pancreatectomy was indicated when the tumor was deeply embedded in the pancreatic parenchyma without clear margin, and was not suitable for enucleation. The distal remnant of the pancreas was kept at least 5 cm in length.

Surgical technique for central pancreatectomy

Central pancreatectomy was performed as reported previously[5-7]. After transecting the pancreas by electrocautery to the left and right of the tumor, the main pancreatic duct was ligated using a 3-0 silk suture, and the proximal pancreatic stump was oversewn with 3-0 polypropylene in a continuous running fashion. Reconstruction of the distal pancreatic remnant was accomplished with a retrocolic Roux-en-Y pancreaticojejunostomy. For pancreaticojejunostomy, a modified invagination method, continuous circular invaginated pancreaticojejunostomy (CCI-PJ), was used. The remnant of the pancreas was dissected for about 1-3 cm from the cut edge, and several small veins running between the pancreas and the splenic vein had to be divided and ligated. The pancreatic stump was invaginated into the jejunum, and a single-layer continuous circular anastomosis with 3-0 polypropylene was performed between the full thickness of the resected jejunum and the body of the pancreas. The largest silicon stent that could be passed into the main pancreatic duct was used in all these patients. Finally, four 24-F drainage catheters were placed near the pancreatic anastomosis.

Postoperative management

Antibiotics were used prophylactically for 5 d after operation, but octreotide was not used routinely. Parenteral nutrition and early enteral feeding were administered. Enteral feeding usually began gradually on postoperative day 3. The fluid from the drain tube placed near the pancreatic anastomosis was monitored routinely for volume and amylase level. For those patients with pancreatic fistulas, modified continuous closed lavage (MCCL) was performed as reported in our previous study[8]. A long 6/8-F silicon tube was inserted into each of the 24-F drainage catheters and pulled out from the lateral part of the catheter about 3-5 cm to the external end. The drainage catheter was connected with a drainage pack. A high volume (20-50 L/d) of normal saline was infused through the silicon tube and eventually ran out into the drainage pack through the same rubber catheter. The lavage volume and duration was adjusted according to the appearance and quality of the outflowing liquid.

Statistical analysis

Results are presented as mean ± SD. Statistical analysis was performed using SPSS version 15.0 statistical software. Student's t test was used for comparison of two independent samples. Categorical variables were compared using the χ^2 or Fisher exact test. $P < 0.05$ was considered statistically significant.

RESULTS

The characteristics of CR patients are summarized in Table 1. Eight patients had serous cystadenoma, six had insulinoma, three had solid pseudopapillary tumors, and three had non-functional islet cell tumors. Each tumor was resected with clear margins, as determined by intraoperative frozen section and confirmed by final pathological examination. Mean tumor diameter was 2.9 cm (range 0.8-10.0 cm). Mean operating time was 236.5 min (range 75-405 min). The mean intraoperative blood loss was 350 mL (range 100-1200 mL). Three patients (15%) required intraoperative blood transfusion. The mortality was zero and morbidity was 25%. Overall occurrence of pancreatic fistula was 25% and DGE was 20%. Compared with 12 cases of PD for benign tumors in the proximal pancreas during the same period in our hospital, the characteristics did not differ significantly between the two groups. The mortality was zero and the morbidity was 25% in both groups.

It should be pointed out that, among five cases of

Table 1 Characteristics of the 20 patients who underwent CR

Patient No.	Age (yr)	Sex	Pathological description	Size (cm)	Lesion location	Operation	Complication	Follow up (mo)
1	75	F	Serous cystadenoma	2.0	Neck	Central pancreatectomy	Pancreatic fistula (significant case) Hemorrhage DGE	100
2	66	M	Non-functional islet cell tumor	2.6	Neck	Enucleation	No	89
3	38	F	Insulinoma	1.0	Proximal body	Enucleation	No	76
4	39	M	Insulinoma	0.8	Uncinate process	Enucleation	No	75
5	62	M	Non-functional islet cell tumor	2.5	Proximal body	Central pancreatectomy	Pancreatic fistula (significant case), perianastomotic fluid collection with infection, DGE	62
6	45	F	Serous cystadenoma	2.8	Proximal body	Central pancreatectomy	No	54
7	12	F	Solid pseudopapillary tumor	3.0	Neck	Enucleation	No	46
8	28	F	Solid pseudopapillary tumor	10.0	Head	Enucleation	Pancreatic fistula (the largest daily volume > 1000 mL in the early postoperative period)	46
9	40	F	Insulinoma	2.0	Proximal body	Central pancreatectomy	No	45
10	61	F	Solid pseudopapillary tumor	1.8	Neck	Central pancreatectomy	No	45
11	39	M	Insulinoma	1.7	Proximal body	Enucleation	No	43
12	62	F	Non-functional islet cell tumor	2.0	Neck	Central pancreatectomy	No	34
13	47	F	Serous cystadenoma	3.0	Neck	Central pancreatectomy	No	34
14	29	F	Insulinoma	2.0	Neck	Central pancreatectomy	No	24
15	29	M	Insulinoma	1.5	Head	Enucleation	No	23
16	55	F	Serous cystadenoma	2.0	Neck	Central pancreatectomy	Pancreatic fistula, DGE	21
17	32	M	Serous cystadenoma	4.0	Proximal body	Central pancreatectomy	No	13
18	39	F	Serous cystadenoma	5.0	Neck	Enucleation	Pancreatic fistula, DGE	10
19	66	M	Serous cystadenoma	4.0	Proximal body	Central pancreatectomy	No	7
20	42	M	Serous cystadenoma	5.0	Head	Enucleation	No	6

CR: Conservative resection; DGE: Delayed gastric emptying.

fistula from 20 CR operations, there were two from nine enucleations and three from 11 central pancreatectomies. Fistula after enucleation usually healed within 7-15 d, by multiple drainage without special intervention. However, in one case of enucleation for a 10-cm solid pseudopapillary tumor located at the head of the pancreas, a small rupture in the main pancreatic duct was found during surgery, and it was repaired by primary suturing with 5-0 polypropylene. The volume of drainage came up to > 1 L/d at the early stage after operation. With MCCL, the fistula healed 15 d later. In the three fistulas from 11 central pancreatectomies, two cases presented with clinically significant pancreatic fistulas that required further medical intervention (Table 1).

After a postoperative follow up of 42.7 mo (range 6-100 mo), all patients were alive without tumor recurrence. No patients developed new-onset diabetes mellitus. However, two cases of new-onset diabetes developed in the PD group. None experienced clinical exocrine insufficiency or required pancreatic enzyme supplements, and three patients needed exocrine substitution in the PD group. Exocrine function of the pancreas was better preserved in the CR than PD group ($P < 0.05$).

DISCUSSION

For tumors located in the head, neck and proximal pancreas, standard or extended pancreatectomy, such as PD, DP and extended DP, which involves resection of a notable amount of normal parenchyma, has been indicated for benign lesions. It has been reported that the rates of new-onset diabetes mellitus and exocrine insufficiency after PD were 10%-40% and 22%-60% respectively[7,9-12]. Previous reports have shown that 72% of patients became insulin dependent after subtotal left pancreatectomy, whereas 85%-95% resection caused diabetes in all patients[13,14]. Besides pancreatic parenchyma, the integrated upper digestive and biliary anatomy also plays a key role in maintaining consequent digestive, immunological and coagulative function and neurohormonal regulation of insulin activity[15]. In addition, Reid-Lombardo *et al*[16] have reported that the 5- and 10-year cumulative probability of biliary stricture after PD for benign lesions was 8% and 13%; that is why, in recent years, CR, such as central pancreatectomy and enucleation, has been investigated with great interest. In the present study, with a mean of 42.7 mo follow-up, there was no new-onset diabetes mellitus

Table 2 Postoperative morbidity and mortality rates of CR for benign tumors in the proximal pancreas: summary of cases in the literature

Author	Year	No. of cases	Morbidity rate (%)	Pancreatic fistula (%)	Mortality rate (%)
Sperti *et al*[21]	2000	10[1]	40	30	0
Balzano *et al*[6]	2003	46[2]	51	39	0
Efron *et al*[24]	2004	14[1]	50	36	0
Roggin *et al*[23]	2006	10[1]	60	30	0
Brown *et al*[25]	2006	10[1]	60	40	0
Christein *et al*[22]	2006	8[1]	63	63	0
Falconi *et al*[7]	2007	36[3]	44	31	0
Present series	2007	20	25	25	0

[1]Central pancreatectomy; [2]Thirty two central pancreatectomies and 14 enucleations; [3]Twenty one central pancreatectomies and 15 enucleations.

and pancreatic exocrine insufficiency, which is the same as previously reported[17-23]. Moreover, in our study, the mean age of patients was only 45.3 years old. For these young patients with benign pancreatic disease, CR might be much more significant in achieving good quality of life, because CR can avoid long-term anastomotic complications and pancreatic insufficiency.

However, conservative pancreatectomy has not been used widely to date because of its high morbidity rate, especially pancreatic fistula. It has been reported that the morbidity rate of CR ranged from 35% to 63% (Table 2), which is much higher than that for PD and DP[6,7,22-25]. In our study, the morbidity rate of CR was 25%. Overall pancreatic fistula rate was 25%, and DGE rate was 20%.

The pancreatic fistula rate in our study was a little better than that reported since 2000 (Table 2), which has ranged from 30% to 63%[6,7,22-25]. In our study, although fistula developed in five of 20 patients, only two with clinically significant fistula required further intervention. According to our experience here, CCI-PJ and efficient MCCL might be responsible for lower morbidity in CR. With CCI-PJ, the stump of the pancreas and the cut edge of the jejunum could be connected closely with polypropylene sutures, which were used for anastomosis of the blood vessels. After postoperative pancreatic fistula has been diagnosed clinically, postoperative MCCL with a high volume of normal saline (20-50 L/d) was necessary to control the pancreatic fistula effectively. The advantage of MCCL compared with general lavage is that there was no concern about the imbalance between ingoing and outgoing fluids because the small silicon tube for irrigation was placed at the tip of the large catheters for outgoing fluid. With MCCL, most patients with pancreatic fistula recover without further surgical intervention.

In conclusion, CR for benign or borderline tumors of the proximal pancreas could be performed safely with careful CCI-PJ and postoperative MCCL. It is useful for preserving long-term pancreatic function.

COMMENTS

Background

In recent years, there has been a marked increase of incidentally discovered benign or borderline tumor of the pancreas using advanced diagnostic imaging techniques. The resection of benign lesions located in the proximal portion of the pancreas traditionally has been accomplished by pancreaticoduodenectomy and distal pancreatectomy. These extended resections result in removal of normal pancreatic tissue, which increased the risk of loss of exocrine and endocrine function.

Research frontiers

Conservative pancreatic resection including pancreatic enucleation and central pancreatectomy has evolved as a means of preserving as much as possible the pancreatic parenchyma and integrated upper digestive system anatomy. In the present study, the authors evaluated the safety and long-term prognosis of patients who underwent conservative resection (CR) for benign or borderline tumors of the proximal pancreas.

Innovations and breakthroughs

CR for benign or borderline tumors of the proximal pancreas could be performed safely with careful continuous circular invaginated pancreaticojejunostomy and postoperative modified continuous closed lavage (MCCL). It is effective for preserving long-term pancreatic function.

Applications

CR is a safe and reasonable technique for benign tumors or lesions of low malignant potential in the proximal pancreas. To obtain good results, careful patient selection and experience in pancreatic surgery are of paramount importance in this setting.

Terminology

Central pancreatectomy, also known as middle pancreatectomy, is a segmental resection of the pancreas. Dagradi and Serio performed the first central pancreatectomy with an oncological indication in 1984. The main advantage of this operation is that it permits preservation of most of the pancreatic parenchyma, extrahepatic bile duct, duodenum, and spleen.

Peer review

The article is well written and demonstrates the results of CR of benign tumor of the proximal pancreas. CR is a procedure that offers benefits for benign and low-grade malignant pancreatic tumors, because it allows preservation of endocrine and exocrine functions. A lower risk of typical postoperative complications following pancreatectomy is especially important in young patients, which is stressed by authors of this study. A presented method (MCCL) of management in patients with pancreatic fistula may be useful for other researchers.

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Stability of a rat model of prehepatic portal hypertension caused by partial ligation of the portal vein

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Abstract

AIM: To study the stability of portal hypertension (PHT) caused by partial ligation of the portal vein ligation (PVL) in a rat model.

METHODS: Thirty male adult Wistar rats were divided into two groups: 10 in Group Ⅰ received a sham operation; and 20 in Group Ⅱ received partial PVL. Portal vein pressure (PVP) was measured at four time periods: before ligation, 2 wk, 6 wk and 10 wk post-surgery. Portal venography, blood sampling and liver and spleen pathological examinations were conducted at 10 wk after surgery.

RESULTS: The PVP was 9.15 ± 0.58 cmH_2O before ligation, and increased to 17.32 ± 0.63 cmH_2O 2 wk after PVL. By repeat measurement of the PVP in each rat, it was shown to remain elevated for 10 wk. There were no significant differences in the pressure measurements at 2 wk, 6 wk and 10 wk. Varices were found mainly in the mesenteric vein 2 wk after PVL, which were more obvious later, while these manifestations were similar at week 6 and week 10. Portal venography demonstrated the varices and collaterals. There was no significant change in liver pathology. The volume of the spleen was enlarged 2-fold after ligation, and the sinus of the spleen was enlarged due to congestion. Significant sinus endothelial cell proliferation was observed, but no evidence of hypersplenia was found on hemogram and biochemical examination.

CONCLUSION: These findings suggest that a satisfactory prehepatic PHT rat model can be obtained by partial ligation of the portal vein, and this PHT rat model was stable for at least 10 wk.

Key words: Ligation; Portal hypertension; Portal vein; Rat

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INTRODUCTION

A rat model of prehepatic portal hypertension (PHT) produced by partial portal vein ligation (PVL) is an important tool in studying PHT. The PVL model has been used to study alterations in the splanchnic circulation and in the pathophysiology of the hyperdynamic circulation after ligation[1,2]. However, from the literature almost all of these studies were carried out around 2 wk after PVL; and are rarely reported beyond this time period. It is suspected that elevated portal vein pressure (PVP) can last for a longer period of time. The aim of this study was to determine the stability of artificially elevated PVP using rigidly controlled experiments. Furthermore, we used this

model to evaluate the effect of a newly designed splenohepatopexy in PHT treatment.

MATERIALS AND METHODS

Thirty male Wistar rats weighing 280-320 g (average 302 g) were kept under temperature controlled conditions and an artificial 12-h light-dark cycle. They were allowed standard chow and water *ad libitum*. All animals and procedures were approved by the Ethics Committee of the Animal Experiment Center of Capital Medical University.

Animals were randomized into two groups: Ten rats in Group Ⅰ had a sham operation; and twenty rats in Group Ⅱ had partial portal-vein ligation. PVP was measured at four time periods: before ligation, 2 wk, 6 wk, and 10 wk after surgery. Portal venography was performed in rats at 10 wk. Other parameters measured included: weight and volume of spleen, hemogram, biochemical parameters and pathologic examination of the liver and spleen.

Model establishment

Animals were anesthetized with intramuscular administration of 2% pentobarbital solution. The model was established according to Harvolsen and Myking[3,4]. A 1.5 cm midline incision was made and the portal vein was exposed. The diameter of the portal vein was measured by Vernier calipers at the point where the ligation would be carried out. A No.4 silk thread was placed around the portal vein together with a pre-placed 21G blunt-tipped needle lying along the portal vein. By tying the ligature snugly beyond the left gastric vein, and then pulling out the needle, a stenosis of the portal vein with a constriction corresponding to the thickness of the pre-placed needle was left behind.

PVP measurement

After anesthesia, a midline incision was made. A segment of the mesenteric branch vein was cannulated with a 24-g cannula needle, and the tip of the cannula was advanced just into the trunk of the superior mesenteric vein. The PVP was recorded, *via* a pressure transducer, by the BL-420E+ biophysical function experiment system (manufactured by Taimeng Technology Limited Company, Chengdu, China). Pressure measurement lasted for 3 min, and the average value was regarded as the PVP. To maintain stable anesthesia, the measurement was started 20 min after the injection of pentobarbital.

Portal venography

A midline incision was made after anesthesia; the ileocolic vein was cannulated with a 20-g cannula, and the tip of the cannula was advanced into the lower part of the portal vein just above the entrance to the splenic vein. Seventy-six percent meglumine diatrizoate was injected at a speed of 2 mL/5 s, and the intrahepatic and extrahepatic portal vein system could be seen.

Blood samples and liver, spleen pathological examination

At week ten, blood samples from the rats were obtained for hemogram and biochemical examination. Both liver and spleen biopsy were taken for pathological examination, (using Hematoxylin-eosin staining). The rats were sacrificed at the end of the experiment.

Table 1 PVP changes (cmH_2O), (mean ± SD)

Groups	wk-0	wk-2	wk-6	wk-10
G-Ⅰ (SO, n = 10)	9.15 ± 0.58	9.22 ± 0.49	9.27 ± 0.43	9.21 ± 0.49
G-Ⅱ (PVL, n = 16)	9.21 ± 0.63	17.32 ± 1.77[a]	17.36 ± 1.93[a,b]	16.82 ± 2.20[a,b]

Portal vein pressure (PVP) changes at four time periods were compared. [a]P < 0.001 *vs* value of G-Ⅰ; [b]P < 0.001 *vs* value of wk-2 in G-Ⅱ. wk-0: before ligation; wk-2, wk-6, wk-10: 2 weeks, 6 weeks and 10 weeks post-surgery, respectively.

Statistical analysis

The data were expressed as mean ± SD. Repeated analysis of variance was used to compare the differences between groups by SPSS 11.5 software. Values of P < 0.05 were considered significant.

RESULTS

Constriction rate of the portal vein

The average diameter of the portal vein before ligation was 2.40 ± 0.18 mm, and the outer diameter of the 21-g needle was 0.80 mm. According to the formula, the constriction rate = $(1-\pi r^2/\pi R^2) \times 100\%$[5] = 88.9%.

Survival rate of animals

None of the rats in Group Ⅰ died, while 4 rats in Group Ⅱ died after PVL. The survival rate was 80% (16/20). Three rats died within 24 h of ligation, the other rat died between 24 and 48 h after surgery. Autopsy found severe congestion of the mesenteric and splenic veins, as well as marked cyanosis of the gut and spleen. No mortality occurred after this period.

PVP

Partial ligation of the portal vein resulted in an immediate increase in PVP up to 25-30 cmH_2O. Two weeks after surgery, PVP dropped to about 17.32 cmH_2O, which was about twice the value of the control (P < 0.01). The pressure at week 6 and week 10 was maintained at a similar level, with no significant difference compared with the value for week 2. PVP in Group Ⅱ was significantly higher than that in Group Ⅰ. (Table 1 and Figure 1).

Varices and collateral circulation after PVL

Gross observations: In Group Ⅰ, in the mesenteric vein, there was no distortion, no varices and no visible collaterals. In Group Ⅱ, in the mesenteric and gastric veins, varices were found mainly in the mesenteric vein 2 wk after PVL, and collaterals could be seen mainly between the spleen and left kidney. The left adrenal vein was markedly engorged. Collaterals could also be seen between the inferior mesenteric vein and the posterior peritoneum (Figures 2 and 3). Collateral

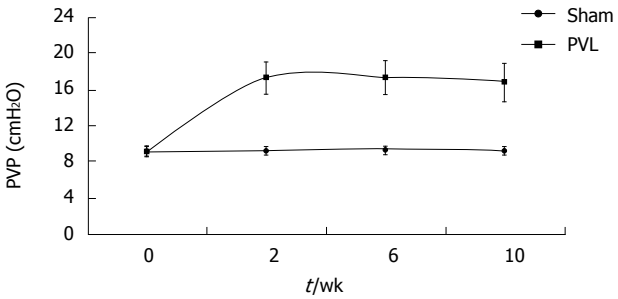

Figure 1 Pressure tendency of PVP after PVL.

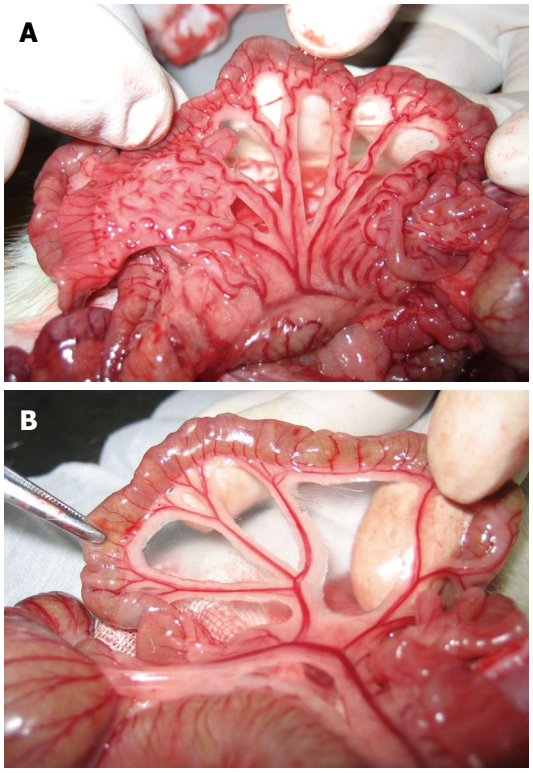

Figure 2 Changes in the mesenteric vein. A: There was no distortion, no varices, no visible collaterals in the mesenteric vein before portal vein ligation; B: Varices were found mainly in the mesenteric vein 2 wk after portal vein ligation.

vessels were found in the porta hepatis in some rats. These manifestations were more obvious later, and were similar at week 6 and week 10. The spleen was markedly enlarged 2 wk after surgery with some white fibrin deposits on the splenic capsule.

Portal venography

Group Ⅰ: An angiographic study revealed normal mesenteric and portal vein image patterns in control rats. The intrahepatic portal vein was shown as tree twig branches. There was only a little contrast medium in the mesenteric vein and the splenic vein. After ligation of the portal vein, images of the superior and inferior mesenteric veins and the splenic vein appeared simultaneously, but no collateral circulation could be seen (Figure 4).

Group Ⅱ: At week 10, portal venography showed the mesenteric vein with varices and collaterals, and a lot of

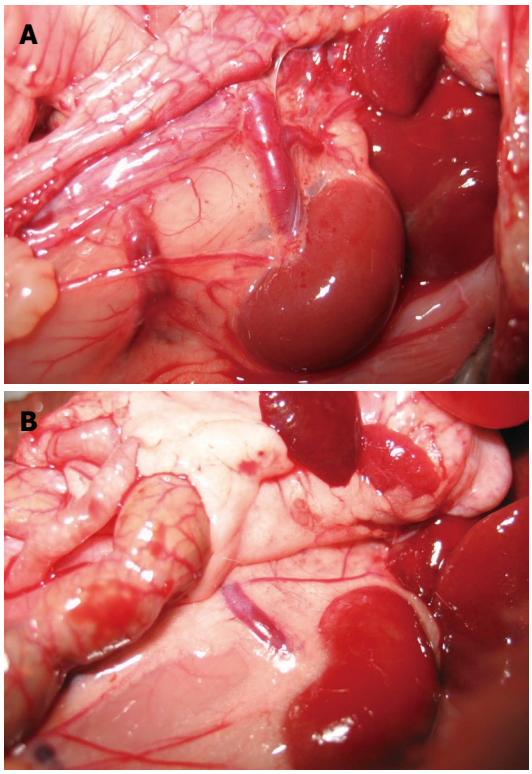

Figure 3 Collaterals after PVL. A: In the control rat, the left renal vein was thin and of normal size, no collateral vessels could be seen between the spleen and the left kidney; B: Collaterals could be seen mainly between the spleen and the left kidney 10 wk after PVL. The left adrenal vein was markedly engorged. The collaterals could also be seen between the inferior mesenteric vein and the posterior peritoneum.

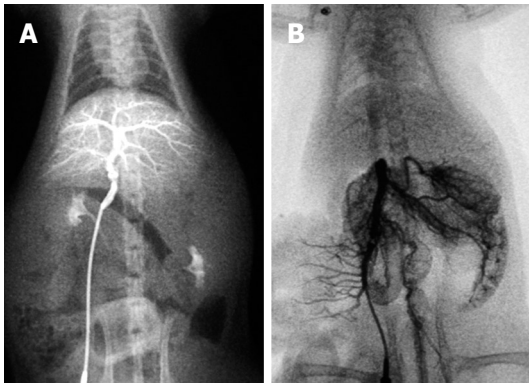

Figure 4 Portal venography in control rat. A: The intrahepatic portal vein was shown as tree twig branches. There was little contrast medium in the mesenteric vein and the splenic vein; B: After ligation of the portal vein, images of the superior and inferior mesenteric vein and the splenic vein appeared simultaneously, but no collateral circulation could be seen.

the contrast medium was seen in the vena cava, which indicated the establishment of the collateral circulation. These observations were not seen in the control rats. Following PVL, the left adrenal vein was clearly seen and had an enlarged diameter, while this vein was not seen in the control group. On continuous observation, some contrast medium was seen in the portal vein system which diffused *via* the left adrenal vein into the left renal vein and the vena cava. Additionally, collateral vessels between the inferior mesenteric vein and the

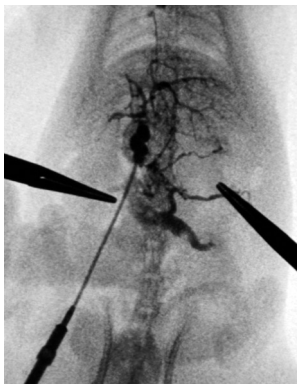

Figure 5 At week 10, portal venography showed the mesenteric vein with varices and collaterals. A lot of contrast medium was seen in the vena cava, which indicated the establishment of the collateral circulation. These observations were not seen in the control rats. The left adrenal vein after PVL was clearly shown with an enlarged diameter, while this vein was not seen in the control group.

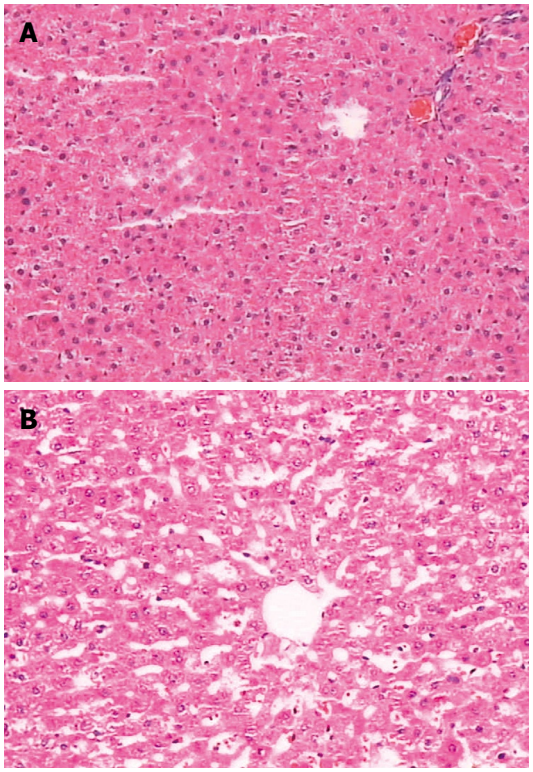

Figure 6 There were no significant changes in liver pathology. A: Pathologic image of rat in Group-Ⅰ; B: Image of Group-Ⅱ.

posterior peritoneum were also seen in the angiograph. No obvious esophageal varices were found. Contrast medium in the liver decreased significantly (Figure 5).

Splenic volume

The splenic volume was slightly increased when the weight of the animal increased in Group Ⅰ, and was almost twice the original size in Group Ⅱ after PVL. The difference in volume in Group Ⅱ was significantly different from that in Group Ⅰ ($P < 0.01$) (Table 2).

Hemogram and biochemical parameters

There were no significant differences between Group Ⅰ and Ⅱ in hemogram and biochemical parameters. No evidence of hypersplenia was found.

Pathology study

There was no significant change in liver pathology. The spleen capsule was thickened, the sinus of the spleen was enlarged due to congestion, there was significant sinus endothelial cell proliferation, and the spleen trabeculae were widened. The white pulp and germinal center had significant atrophy (Figures 6 and 7).

Table 2 Splenic volume changes (cm^3), (mean ± SD)

Groups	wk-0	wk-2	wk-6	wk-10
G-Ⅰ (SO, n = 10)	1.90 ± 0.28	1.93 ± 0.24	2.03 ± 0.23	2.04 ± 0.25
G-Ⅱ (PVL, n = 16)	2.07 ± 0.40	3.94 ± 1.33[a,b]	4.07 ± 1.37[a,b]	4.17 ± 1.44[a,b]

[a]$P < 0.01$ *vs* value of G-Ⅰ; [b]$P < 0.01$ *vs* value of wk-0 in G-Ⅱ. wk-0: before ligation; wk-2, wk-6, wk-10: 2 weeks, 6 weeks and 10 weeks post-surgery, respectively.

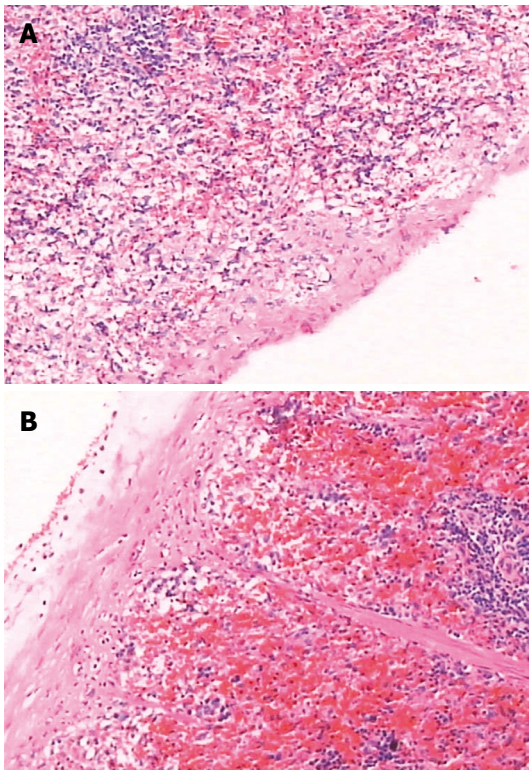

Figure 7 Pathologic image of the spleen. A: Group-Ⅰ; B: Group-Ⅱ, the capsule was thickened, the spleen sinus was enlarged due to congestion, there was significant sinus endothelial cell proliferation, and the spleen trabeculae were widened. The white pulp and germinal center had significant atrophy.

DISCUSSION

The rat model of PHT produced by partial PVL is an important tool in studying PHT. It is fast, economic, simple and repeatable. Some authors have applied the model to the study of alterations in splanchnic circulation and the pathophysiology of the hyperdynamic circulation[1]. However, most of these studies were short-term experiments, which were carried out 2-3 wk after ligation. There are few reports of long-term experiments, two months or more after ligation. Some authors even doubt the long-term stability of the model, and are concerned that PVP will drop to the normal level due to establishment of the collateral circulation. For experiments on the surgical treatment of PHT,

such as spleno-hepatopexy, a long-term stable model is needed. The aims of the present study were to prove the stability of the PHT rat model under rigidly controlled experimental conditions, and to prove that this model is suitable for evaluating the effect of our newly designed spleno-hepatopexy for PHT treatment. For spleno-hepatopexy, a peripheral cut on the surface of the spleen and the same on the liver are made which adhere together, it is hoped that the high pressure blood in the spleen will diffuse into the liver through the collateral circulation instead of porto-systemic venous shunting to allow detoxification of the liver.

In our experiment, the PVP value before ligation and in control animals was within the same range as that found by other investigators[4,6,7]. The establishment of a partial PVL resulted in instant congestion in the portal vein system, and the PVP immediately increased to 25-30 cmH_2O. At week 2 after surgery, following compensation by the animal itself, the pressure dropped to about 17 cmH_2O. In subsequent observations from week 2 to week 10, the elevated PVP value was maintained at the increased level of about 17 cmH_2O, which was about twice the level before ligation.

Several factors affected the results of these experiments. The portal vein constriction rate is a critical factor in rat survival in the PHT model. After complete portal vein ligation, the portal vein system was suddenly blocked, and this resulted in a serious blood deficiency in the circulation, and the rat died within one hour. For partial ligation, the survival rate following PVL was positively related to the constriction rate[4]. We obtained different constriction rates by using different-sized needles. With a 26-g (diameter = 0.45 mm) or a 23-g needle (diameter = 0.6 mm), all the rats died within 1-2 h after ligation. Using a 22-g needle (diameter = 0.7 mm), mortality was about 80%, which was obviously not acceptable for these experiments. Using a 21-g needle (diameter = 0.8 mm), the constriction rate was 88.9%, and the mortality dropped to 20%-30%, and was acceptable for this model of PTH. By decreasing the constriction rate using a 19-g needle (diameter = 1.0 mm), the survival rate was increased, but the PVP did not reach an ideal level to meet the experimental demand.

Constriction of the portal vein resulted in increased resistance in the portal vein, and therefore increased PVP. The resistance of the portal vein was mainly determined by the constriction rate of the portal vein in the experiment. Other factors such as the thickness of the thread were also important. We tried different sizes of thread in our preliminary experiments, including 3-0, 1-0 and No.1, No.4 and No.7 silk. For thin thread less than No.1, the PVP was unstable, and the pressure was usually low, because the resistance of a tube is positively related to the obstructive length. However, for thread thicker than No.7 silk, it was difficult to make a tight knot on such a small portal vein of nearly 1.0 mm. Eventually, we selected the No.4 thread and obtained satisfactory results. Some authors use triple-ligation on the portal vein to obtain the PVL model. Additionally, right ligation against the portal vein axis is also quite important. The strength used for ligation is important and should be the same in every experiment. Too much strength could damage the portal vein, which may result in thrombosis, while too little strength would be too loose and inaccurate for standard constriction. Cleaning the surrounding tissue next to the portal vein is also useful to reduce errors in constriction diameter.

There have been different reports on the PVP level after PVL. Orda *et al*[8] induced PVL in a rat model with an elevated pressure for 2-3 wk, which then dropped to normal. This was thought to be due to a large amount of collateral circulation formation. Canty[9] summarized the previous experience and thought that simple ligation or application of a meroid constrictor to the portal vein could only maintain a high pressure for 4-6 wk. He designed a method of using the ameroid constrictor accompanied by portal lymph node ligation, and hence a high portal pressure could be maintained for about 12 months. Myking *et al*[3] and Halvorsen *et al*[7] performed serial experiments on the PVL rat model, and successfully elevated PVP for 12 mo after simple ligation, and was considered a stable and repeatable rat model.

The difference in results in the literature might be due to multiple factors, e.g. the animal strain and body weight. In a very low weight rat, a stable elevated pressure is difficult to maintain[3]. With regard to the constriction rate, this might be influenced by the size of the needle, the thickness of the thread, and the design of the experiments.

In the PVL model of Myking and Halvorsen[3,7], only one pressure measurement was carried out for each rat. In our experiment, four measurements were carried out for each rat. By this method, we could observe the PVP changes in every rat, which was much more accurate and reliable than a single measurement. Following surgery, there was little adhesion in the rat abdominal cavity except at the local area of ligation or puncture. Because the mesenteric vein branch can be used repeatedly, multiple measurements were possible for each rat.

The peak PVP level after PVL was usually 24 h post-surgery[10]. Under natural compensation, the collateral circulation is established to release the high pressure. It was proved that the collateral circulation could be observed two days after PVL, and was fully established 3-4 wk post-surgery[10]. The diverted volume of portal vein blood flow was about 95%[3]. In our experiments, we also observed that varices appeared in the mesenteric vein 2 wk after surgery. The collateral vessels between the spleen and left kidney, between the inferior mesenteric vein and the posterior peritoneum, and at the site of porta hepatis were also observed at this time, but were more apparent at 6 wk after ligation. The manifestations at week 10 were similar to those at week 6. Portal venography further proved the establishment of the collateral circulation. The left adrenal vein was significantly enlarged and became an important shunting vessel between the splanchnic circulation and the systemic circulation.

Some researchers[1] have studied the splanchnic and

systemic hemodynamics in the PVL rat model. They found that, after 2 wk of PVL, the rats with portal hypertension and greater than 93% portal-systemic shunting had an increase in portal venous inflow of 50%, and a concomitant 40% decrease in splanchnic arteriolar resistance. Cardiac index was elevated by 50%, and total peripheral resistance was decreased by 40%. The resistance to portal blood flow in portal vein-constricted rats was similar to that in control rats, indicating that the hyperdynamic portal venous inflow, and not resistance, was the mainstay of the elevated portal venous pressure. Some researchers[10] have suggested that many vessel-activated substances which accumulate in the systemic system, without inactivation by the liver, were the direct cause of the hyperdynamic circulation. Therefore, it is considered that, in the early period of PVL, portal vein obstruction is the direct cause of elevated PVP, but with the subsequent establishment of the collateral circulation, the hyperdynamic circulation becomes the important factor in maintaining the elevated pressure. Although portal-systemic shunting can reach to more than 90%, the PVP can still maintain an elevated level.

Other features of the PVL portal hypertensive rat model were noted: Hepatic function remained normal, which is similar to the clinical manifestation of PHT. Although the spleen volume increased almost 2-fold due to enlargement and congestion of splenic sinusoids, proliferation of sinus endothelial cells, hypersplenism was not shown by hemogram, which was similar to findings in the dog PHT model[5]. The reason for this may be due to species and anatomy differences to that in humans, or may be due to the short observation period.

In conclusion, portal vein partial ligation increases PVP to around twice the normal value and can be maintained for more than 10 wk. This method can provide a satisfactory model for investigating the surgical treatment of PHT.

COMMENTS

Background

Portal hypertension (PHT) is a serious disease in children. In about half of children with PHT it is prehepatic. However, the results of surgery for PHT are unsatisfactory. To investigate and to improve the results of surgery, a long-term stable animal model of PHT is needed. Partial portal vein ligation is a good method of producing a prehepatic PHT rat model. The technique is fast and economic. However, researchers have had different experiences in the long-term stability of the model. Some authors have suggested that the portal vein pressure (PVP) would drop to normal 2 wk after ligation, but others think that the models would be stable for more than a year.

Research frontiers

Several types of PHT animal models have already been established. But focusing on the long-term stability of these models it is still a frontier of research.

Innovations and breakthroughs

In their experiments, the prehepatic PHT rat model was proved to be stable for more than 10 wk. The anatomic features of this model were described in detail. Moreover, it is interesting that almost no adhesion in the abdominal cavity and no blood thrombosis in the mesenteric vein were found after repeated operations. Thus, it is possible to measure the PVP repeatedly in one rat, providing a better comparison in the same animal. This is superior to the method commonly used, i.e. a single measurement in each experimental rat.

Applications

The prehepatic PHT rat model is quite useful in the study of PHT, as the alterations in the splanchnic circulation and the pathophysiology of the hyperdynamic circulation can be studied. It is a fast and economic model and can be widely used in this research field. In addition, it can also be used in clinical research, e.g. a new design for spleno-hepatopexy, and to explore new surgical techniques for PHT in children.

Terminology

Portal hypertension: A series of syndromes with abnormal circulation and increased blood pressure in the portal system. According to its pathology, PHT can be divided into three types, prehepatic, intrahepatic and posthepatic. Varices: Abnormally dilated/stretched veins, frequently caused by the development of portal collateral vessels as a result of portal hypertension. It often occurs in the portal system, such as the mesenteric vein and the splenic vein. The most clinically important varices in humans are found in the esophagus and stomach-submucosal varices of the lower esophagus or gastric fundus sub-mucosa. Spleno-hepatopexy: Is a newly designed surgical procedure which allows communication of the spleen with the liver to establish a compensatory collateral circulation by-pass which crosses over the blockage of the portal vein. Thus, the blood in the distal portal system may be drawn into the liver, instead of into the systemic circulation allowing liver detoxification. This surgical technique would be beneficial for patients with prehepatic portal hypertension, especially in children.

Peer review

The study by Dr. Wen *et al*, examines the natural history of portal hypertension in a rat model subjected to partial portal vein occlusion. It may be worth the authors effort as the paper provides valuable information on a common animal model of portal hypertension.

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BRIEF ARTICLES

Significance and relationship between Yes-associated protein and survivin expression in gastric carcinoma and precancerous lesions

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Abstract

AIM: To analyze the differences and relevance of Yes-associated protein (YAP) and survivin, and to explore the correlation and significance of their expression in gastric carcinoma and precancerous lesions.

METHODS: The PV9000 immunohistochemical method was used to detect the expression of YAP and survivin in 98 cases of normal gastric mucosa, 58 intestinal metaplasia (IM), 32 dysplasia and 98 gastric carcinoma.

RESULTS: The positive rates of YAP in dysplasia (37.5%) and gastric carcinoma (48.0%) were significantly higher than that in normal gastric mucosa (13.3%), $P < 0.01$. The positive rates of survivin in IM (53.4%), dysplasia (59.4%) and gastric carcinoma (65.3%) were significantly higher than in normal gastric mucosa (11.2%), $P < 0.01$. Survivin expression gradually increased from 41.7% in well differentiated adenocarcinoma through 58.3% in moderately differentiated adenocarcinoma to 75.6% in poorly differentiated adenocarcinoma, with significant Rank correlation, $r_k = 0.279$, $P < 0.01$. The positive rate of survivin in gastric carcinoma of diffused type (74.6%) was significantly higher than that in intestinal type (51.3%), $P < 0.05$. In gastric carcinoma with lymph node metastasis (76.9%), the positive rate of survivin was significantly higher than that in the group without lymph node metastasis (41.2%), $P < 0.01$. In 98 cases of gastric carcinoma, the expression of YAP and of survivin were positively correlated, $r_k = 0.246$, $P < 0.01$.

CONCLUSION: YAP may play an important role as a carcinogenic factor and may induce survivin expression. Detecting both markers together may help in early diagnosis of gastric carcinoma.

Key words: Apoptosis; Cell proliferation; Gastric cancer; Immunohistochemistry; Neoplastic processes; Survivin protein; Yes-associated protein

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Da CL, Xin Y, Zhao J, Luo XD. Significance and relationship between Yes-associated protein and survivin expression in gastric carcinoma and precancerous lesions. *World J Gastroenterol* 2009; 15(32): 4055-4061 Available from: URL: http://www.wjgnet.com/1007-9327/15/4055.asp DOI: http://dx.doi.org/10.3748/wjg.15.4055

INTRODUCTION

Yes-associated protein (YAP) is a type of cellular adaptor protein and transcriptional co-activator, which was initially isolated by Sudol *et al*[1] in 1994, as a result of its binding to the Src family member non-receptor tyrosine kinase YES (Yes kinase-associated protein). In biological conditions, YAP was described as a target of the Hippo (Hpo)-Salvador-Warts pathway and was phosphorylated by the pathway to negatively regulate growth by simultaneously inhibiting proliferation and promoting apoptosis. In recent years, some investigators have found YAP to be overexpressed and highly activated in hepatic cancers and mammary cancers[2-4], suggesting its carcinogenicity. Survivin is a new member of the inhibitor of apoptotic protein (IAP) family, and was initially cloned by the cDNA of effector cell protease receptor-1 in the human genomic library in 1997[5]. Many

investigations have found survivin to be overexpressed in most common tumors, but almost never in normal tissues[6]. The overexpression of survivin was closely related to tumorigenesis and progression, and was one of the strongest apoptotic inhibitors identified. In tumors, lack of, or mutation of, any factor(s) in the Hpo signaling pathway can lead to dephosphorylation and activation of YAP, which then induces a high expression of Ki67, c-myc, SOX4, H19, AFP, BIRC5/survivin, BIRC2/cIAP1 and other cellular proliferation-related genes and inhibitors of apoptosis. Of note is the massive induction (30-fold) of BIRC5/survivin, leading to breakdown in the balance of cellular proliferation and apoptosis, and an increase in the occurrence and development of tumors[7].

Gastric cancer is one of the malignant diseases with the highest incidence and mortality rates, but its pathophysiology remains to be clarified. We measured the expression level of YAP and survivin in normal gastric mucosa, precancerous lesions and gastric carcinoma using an immunohistochemical (IHC) method in order to analyze the significance and correlations of these two factors with gastric tumorigenesis.

MATERIALS AND METHODS

Clinicopathological data

We collected gastric carcinoma specimens from the First Affiliated Hospital of China Medical University: 98 cases of gastric carcinoma, including 29 cases of early gastric carcinoma (EGC) and 69 cases of advanced gastric carcinoma (AGC), with matched normal gastric mucosa, 58 cases of intestinal metaplasia (IM), and 32 cases of dysplasia (DYS). There were 66 males and 32 females, mean age 60 years. Gross types were as follows: EGC cases: 18 cases of type Ⅰ + Ⅱc, 10 cases of type Ⅲ, one case of extensive superficial type; AGC cases: seven cases of Borrmann Ⅰ + Ⅱ, 62 cases of Borrmann Ⅲ + Ⅳ. According to the World Health Organization histological classification of GC, the 98 cases were classified as follows: two papillary adenocarcinoma, 12 well differentiated adenocarcinoma, 25 moderately differentiated adenocarcinoma, 41 poorly differentiated adenocarcinoma, two undifferentiated adenocarcinoma, seven signet ring cell carcinomas and nine mucinous adenocarcinoma.

Tissue microarray construction and IHC staining

Samples were fixed in 10% formalin, embedded in paraffin, cut into 4 μm thick sections and constructed in blocks for tissue microarray. All the samples were evaluated by two experienced pathologists for diagnosis.

Expression of YAP and survivin in gastric carcinomas, precancerous lesions and normal gastric mucosa were detected using an IHC method. A PV-9000 kit was purchased from Beijing Zhongshan Golden Bridge Biotechnology Company. Anti-human rabbit YAP polyclonal antibody was purchased from the Cell Signaling Technology Company (working dilution 1:25). Anti-human rabbit polyclonal antibody survivin (ready to use) was purchased from Fuzhou Maixin Company (China). All procedures were implemented according to the manufacturer's instructions. For negative controls, sections were treated with 0.01 mol/L phosphate-buffered saline instead of primary antibodies.

IHC staining evaluation

YAP was specifically located in the cytoplasm and nucleus of carcinoma cells; survivin was specifically located in the cytoplasm of carcinoma cells. Staining intensity (A) was classified as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The percentage of positive cells (B) examined in 200 cells were divided into 0 (< 5%), 1 (5%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (> 75%). According to the product of A and B, the IHC result was classified as 0, negative (-); 1-4, weakly positive (+); 5-8, moderately positive (++) and 9-12, strongly positive (+++).

Statistical analysis

Statistical analysis was performed using SPSS 11.5 Package, χ^2 test, Fisher's exact test and Kendall's *tau-b* test were used to differentiate the rates of different groups and test the correlation between the two factors. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of YAP in normal gastric mucosa, IM, DYS and gastric carcinoma

The positive rates of YAP presence in dysplasia (37.5%) and gastric carcinoma (48.0%) were significantly higher than that in normal gastric mucosa (13.3%), $P < 0.01$; there was no statistically significant difference between YAP expression in the normal gastric mucosa and IM (16/58, 27.6%), dysplasia and gastric carcinoma, $P > 0.05$. YAP expression showed an increasing trend from well differentiated adenocarcinoma (4/12, 33.3%), through moderately differentiated adenocarcinoma (11/25, 44.0%) to poorly differentiated adenocarcinoma (24/41, 58.5%), although without significant Rank correlation. The positive rate of YAP expression showed an increasing trend from gastric carcinoma without lymph node metastasis (5/17, 29.4%), to gastric carcinoma with lymph node metastasis (24/52, 46.2%), though without statistical significance, $P > 0.05$. There was no significant correlation of the expression of YAP with patients' gender, age, Borrmann's classification of gastric carcinoma or Lauren classification, $P > 0.05$ (Tables 1 and 2, Figure 1).

Expression of survivin in normal gastric mucosa, IM, DYS and gastric carcinoma

The positive rates of survivin in IM (53.4%), dysplasia (59.4%) and gastric carcinoma (65.3%) were significantly higher than that in normal gastric mucosa (11.2%), $P < 0.01$. The expression level gradually increased from well differentiated adenocarcinoma (41.7%), through moderately differentiated adenocarcinoma (58.3%) to poorly differentiated adenocarcinoma (75.6%), with significant Rank correlation, $r_k = 0.279$, $P < 0.01$.

Table 1 Correlation of Yes-associated protein (YAP) expression with normal gastric mucosa, intestinal metaplasia (IM), dysplasia (DYS) and gastric carcinoma (GC)

Groups	*n*	YAP expression				Positive (%)	*P*
		-	+	+ +	+ + +		
Normal mucosa	98	85	10	2	1	13.3	0.110[a]/0.009[b]
IM	58	42	12	3	1	27.6	0.625[c]/0.083[d]
DYS	32	20	9	3	0	37.5	0.653[e]
GC	98	51	30	12	5	48.0	0.0001[f]

Fisher's exact test. [a]Normal mucosa *vs* IM; [b]Normal mucosa *vs* DYS; [c]IM *vs* DYS; [d]IM *vs* GC; [e]DYS *vs* GC; [f]Normal mucosa *vs* GC.

Table 2 Correlation of YAP expression with clinicopathologic features of gastric carcinoma

Groups	*n*	YAP expression				Positive (%)		*P*
		-	+	+ +	+ + +			
Sex								0.309
Male	66	31	22	8	5	53.0		
Female	32	20	8	4	0	37.5		
Age								0.304
< 60	54	30	15	8	1	45.5		
⩾ 60	44	21	15	4	4	51.2		
Gross type								
EGC								0.937
Type Ⅰ + Ⅱc	18	6	8	3	1	66.7		
Type Ⅲ	10	4	3	2	1	60.0		
AGC								0.074
Type Bor Ⅰ + Ⅱ	7	2	2	2	1	71.4		
Type BorⅢ + Ⅳ	62	38	17	5	2	38.7		
WHO's histological types							r_k = 0.181	0.079
Well-diff. ade.	12	8	4	0	0	33.3		0.635[a]
Moderately-diff. ade.	25	14	6	3	2	44.0		0.673[b]
Poorly-diff. ade.	41	17	15	6	3	58.5		0.406[c]
Undiff. ade.	2	2	0	0	0	0.0		
Papillary ade.	2	0	1	1	0	100.0		
Signet ring cell carcinoma	7	4	2	1	0	42.9		
Mucinous ade.	9	6	2	1	0	33.3		
Lauren types								0.669
Intestinal type carcinoma	39	23	11	4	1	41.0		
Diffused type carcinoma	59	28	19	8	4	52.5		
Lymph node metastasis								0.602
Yes	52	28	16	5	3	46.2		
No	17	12	3	2	0	29.4		

Fisher's exact test. ade.: Adenocarcinomas; diff.: Differentiated; EGC: Early gastric carcinoma; AGC: Advanced gastric carcinoma. [a]Well-diff. *vs* Moderately-diff. ade.; [b]Moderately-diff. *vs* Poorly-diff. ade.; [c]Well-diff. *vs* Poorly-diff. ade..

The positive rate of survivin in gastric carcinoma of diffused type (74.6%) was significantly higher than that in intestinal type (51.3%), $P < 0.05$. In gastric carcinoma with lymph node metastasis (76.9%), the positive rate of survivin was significantly higher than that in the group without lymph node metastasis (41.2%), $P < 0.01$, There was no relationship between gastric carcinoma and sex, age and gross type of carcinoma (Tables 3 and 4, Figures 2-4).

DISCUSSION

The Hpo pathway was originally identified in Drosophila as a potent regulator of inhibition of cell growth and promotion of apoptosis. The pathway consists of a tumor suppressor kinase cascade which negatively regulates growth and results in inactivation of a transcriptional co-activator, Yorkie (Yki)[8]. The human ortholog of Yki, YAP, has a 31% sequence homology and similar biologic activity. YAP is a 65 kDa phosphoprotein which is rich in proline. In biological conditions, YAP is phosphorylated by the Hpo signaling pathway, and is highly conserved with other components of this pathway, regulating the balance between cell proliferation and apoptosis to maintain the steady-state of the cellular environment[2,9,10]. Dysregulation of any factor(s) in this pathway can lead to tumorigenesis. Overholtzer *et al*[3] introduced the YAP gene by retroviral infection into the immortalized, but non-tumorigenic, human mammary epithelial cell line MCF10A and found that overexpression of YAP induced epithelial-to-mesenchymal transition, suppression of apoptosis, growth factor-independent proliferation, and anchorage-independent growth in soft agar, which suggests that YAP contributes to malignant transformation in cancers, and supports the potential significance of this pathway in human cancer.

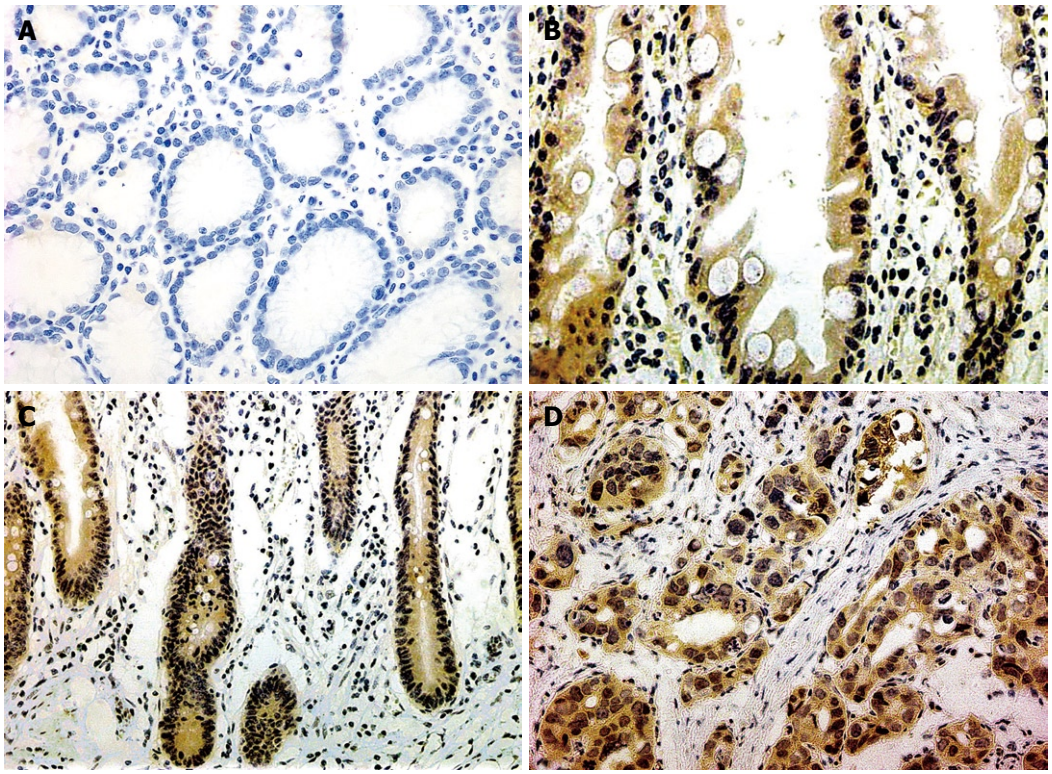

Figure 1 Expression of Yes-associated protein (YAP) in normal gastric mucosa (A), intestinal metaplasia (B), dysplasia (C) and gastric carcinoma (D). IHC PV9000, × 200.

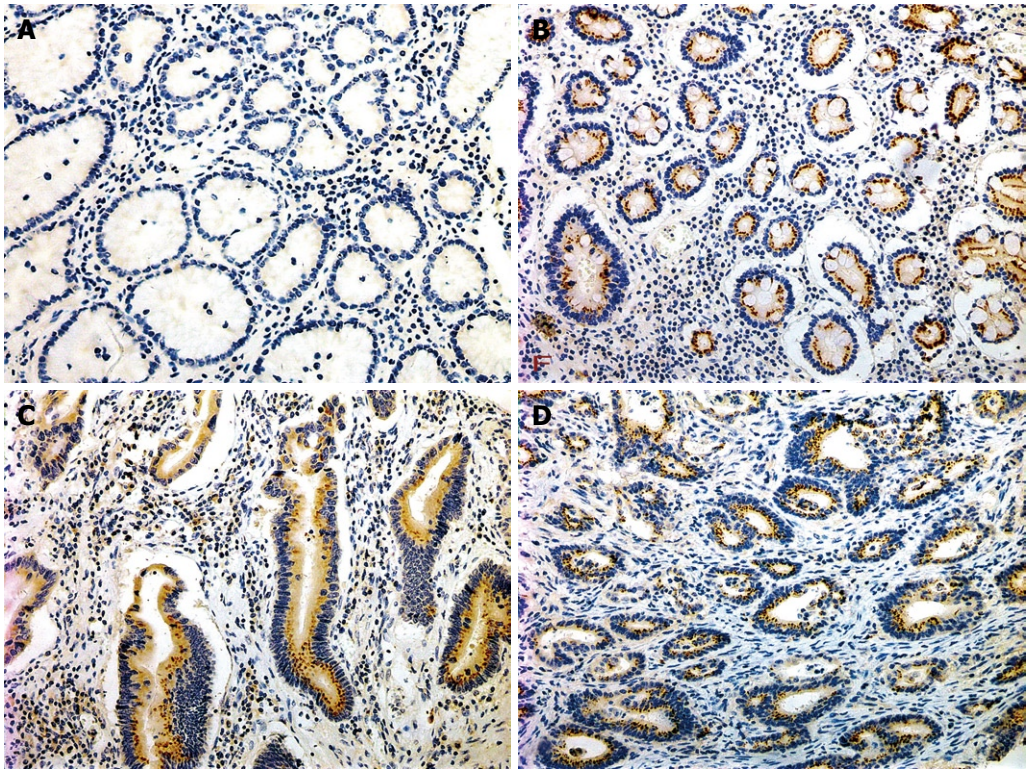

Figure 2 Expression of survivin in normal gastric mucosa (A), intestinal metaplasia (B), dysplasia (C) and gastric carcinoma (D). IHC PV9000, × 200.

Zhao *et al*[12] evaluated YAP expression in 115 cases of human hepatocellular carcinoma (HCC) samples by IHC staining of tissue microarrays. Among the 115 cases of human HCC samples examined, 54% showed strong YAP staining, while 95% of normal liver tissue samples (40 out of 42 cases) showed very weak staining, indicating a significant difference in YAP levels between normal and cancerous tissues. Similar observations were made in prostate cancer tissues. Up to now, we have found only one report on the expression and role of YAP expression in gastric carcinoma, where YAP expression in 78 normal gastric mucosa was 14%, while in 55 gastric carcinomas and 92 gastric metastatic disease the expression was 30% and 35% respectively, significantly higher than that in normal gastric mucosa[11]. Our IHC investigation found that the expression of YAP in DYS and gastric carcinoma was significantly higher than in normal gastric mucosa, suggesting that an

Table 3 Correlation of survivin expression with normal gastric mucosa, intestinal metaplasia, dysplasia and gastric carcinoma

Groups	*n*	Survivin expression				Positive (%)	χ^2	*P*
		-	+	++	+++			
Normal mucosa	98	87	10	1	0	11.2		0.0001[1,a]/0.0001[1,b]
IM	58	27	19	8	4	53.4	2.683	0.486[1,c]/0.443[d]
DYS	32	13	8	6	5	59.4	1.584	0.663[e]
GC	98	34	34	20	10	65.3	67.944	0.0001[f]

[1]Fisher's exact test. [a]Normal mucosa *vs* IM; [b]Normal mucosa *vs* DYS; [c]IM *vs* DYS; [d]IM *vs* GC; [e]DYS *vs* GC; [f]Normal mucosa *vs* GC.

Table 4 Correlation of survivin expression with clinicopathologic features of gastric carcinoma

Groups	*n*	Survivin expression				Positive (%)	χ^2	*P*
		-	+	++	+++			
Sex							4.87	0.172
Male	66	20	27	14	5	69.7		
Female	32	14	7	6	5	56.2		
Age							2.74	0.434
< 60	54	20	15	13	6	63.0		
⩾ 60	44	14	19	7	4	68.2		
Gross type								
EGC								0.310[1]
Type Ⅰ + Ⅱc	18	5	7	3	3	72.2		
Type Ⅲ	10	6	1	2	1	40.0		
AGC								0.696[1]
Type Bor Ⅰ + Ⅱ	7	3	2	1	1	57.1		
Type BorⅢ+ Ⅳ	62	19	24	14	5	69.4		
WHO's histological types							r_K = 0.279	0.006
Well-diff. ade.	12	7	4	1	0	41.7		0.824[1,a]
Moderately-diff. ade.	25	11	10	2	2	56.0		0.223[1,b]
Poorly-diff. ade.	41	10	16	10	5	75.6		0.149[1,c]
Undiff. ade.	2	1	1	0	0	50.0		
Papillary ade.	2	1	0	1	0	50.0		
Signet ring cell carcinoma	7	1	2	3	1	85.7		
Mucinous ade.	9	3	1	3	2	66.7		
Lauren types							8.61	0.035
Intestinal type carcinoma	39	19	14	4	2	51.3		
Diffused type carcinoma	59	15	20	16	8	74.6		
Lymph node metastasis								0.005[1]
Yes	52	12	19	15	6	76.9		
No	17	10	7	0	0	41.2		

[1]Fisher's exact test. [a]Well-diff. *vs* Moderately-diff. ade.; [b]Moderately-diff. *vs* Poorly-diff. ade.; [c]Well-diff. *vs* Poorly-diff. ade..

abnormality of the Hpo pathway leads to overexpression of YAP, resulting in malignant transformation of the gastric mucosa. We speculate that YAP may play an important role as a tumorigenic factor and early gastric tumorigenic molecule during gastric carcinogenesis.

Survivin is a new member of the IAP family, and has been implicated to have a role in protection from apoptosis and regulation of mitosis[12]. The survivin gene has been located on the 17q25 chromosome, encoding a 16.5 kDa protein. Survivin is characterized by a unique structure with a single BIR on the N terminal and an α-helix structure on the C terminal; the BIR structure is thought to play a role during anti-apoptosis, while the helix structure may participate in the microtubule binding structure[13,14]. Data from a large analysis of human transcripts revealed survivin as the fourth most highly expressed protein in human cancer tissue compared with normal tissue[15-18]. Xiao *et al*[19] found that the positive rates of survivin expression in tumors with metastases (in lymph node metastasis 86.2%, liver metastasis 100% and ovarian metastasis 100%) were significantly higher than that in tumors without metastasis (64.3%). Our data indicated that the positive rates of survivin in IM, atypical hyperplasia and gastric carcinoma were significantly higher than that in normal gastric mucosa. The expression level gradually increased from well differentiated adenocarcinoma, through moderately differentiated adenocarcinoma to poorly differentiated adenocarcinoma, with significant Rank correlation. The positive rate of survivin in gastric carcinoma of the diffused type was significantly higher than that in the intestinal type. In gastric carcinoma with lymph node metastasis, the positive rate of survivin was significantly higher than that in the group without lymph node metastasis, indicating that survivin may be involved in the occurrence, development and lymph node metastasis of gastric carcinoma. Survivin can act as a prognostic and predictive indicator for gastric carcinoma patients.

Dong *et al*[7] used microarray analysis to identify YAP-induced genes in murine livers. Selected genes

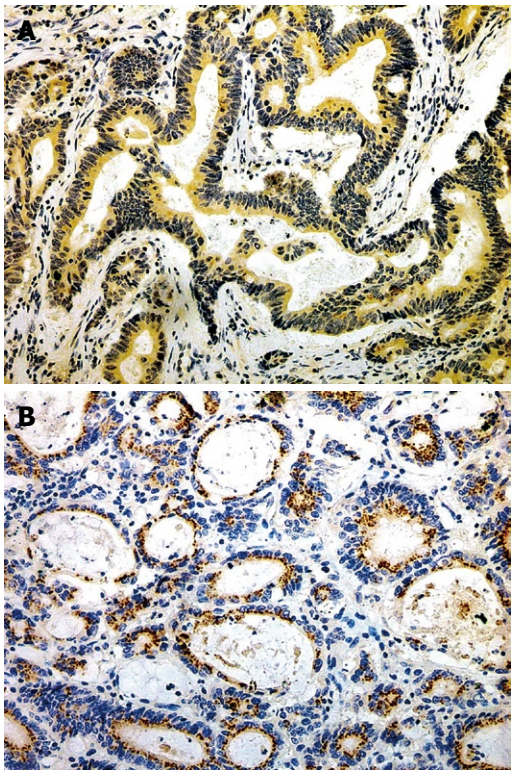

Figure 3 The expression of survivin in gastric carcinoma without lymph node metastasis (A, × 200) and with lymph node metastasis (B, × 100). IHC PV9000.

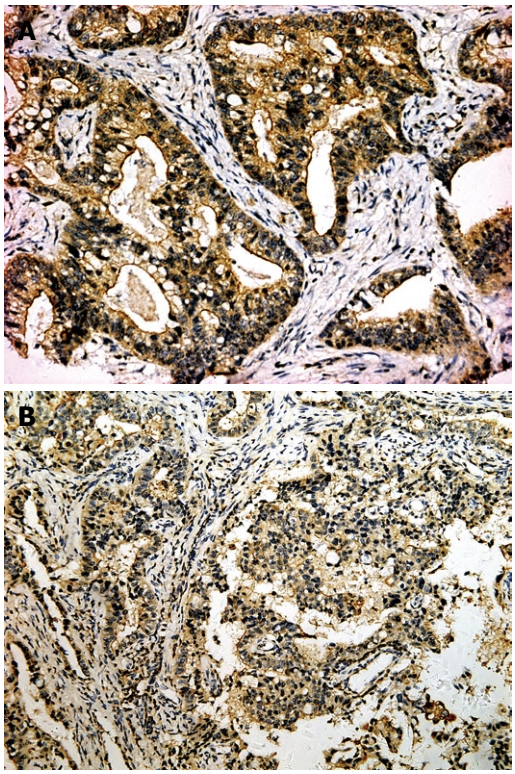

Figure 4 The expression of survivin in primary gastric carcinoma (A) and relevant lymph node metastasis (B). IHC PV9000, × 200.

from the microarray analysis were validated by real-time quantitative polymerase chain reaction analysis. YAP induced the transcription of many genes which are normally associated with hepatocyte proliferation, such as Ki67, c-myc, SOX4, H19, and AFP. It also induced the expression of several negative regulators of apoptosis, such as the IAP family members BIRC5/survivin and BIRC2/cIAP1, and the BCL2 family gene MCL1. Of note is the massive induction (30-fold) of BIRC5/survivin. To determine whether cIAP1 and YAP might cooperate during tumorigenesis, $p53^{-/-}$, myc liver progenitor cells were infected with either YAP and control vector or YAP plus cIAP1 and assayed for their ability to form tumors *in vivo*. Tumors arising from $p53^{-/-}$, myc hepatoblasts coexpressing cIAP1 and YAP grew faster than those expressing either oncogene alone, suggesting that they may collaborate to contribute to tumorigenesis and progression[20].

Our investigation found that the expression of YAP and survivin in gastric carcinoma were positively correlated, and we speculate that YAP may induce a high expression of cell proliferation-related factors and apoptotic inhibitors, such as Ki67, cIAP1 and survivin. Survivin may participate in gastric carcinogenesis, progression and metastasis by inhibiting apoptosis of gastric carcinoma and regulating cellular mitosis. Whether YAP and survivin collaborate to contribute to gastric carcinogenesis and progression require further study.

Previous reports have found that YAP was an activator of cell death in mammalian cells. YAP was shown to activate apoptosis in response to DNA damage by interacting with p73 in several cancer cell lines[21,22]. This is in direct contrast to the results of our investigation and other previous reports. The roles of YAP in biological and pathological conditions remain to be clearly defined.

COMMENTS

Background

Yes-associated protein (YAP) is a type of cellular adaptor protein and transcriptional co-activator. In recent years, some investigators have found YAP to be overexpressed and highly activated in hepatic cancers and mammary cancers, suggesting its tumorigenicity. Survivin is a new member of the inhibitor of apoptotic protein (IAP) family, which was initially cloned by the cDNA of the effector cell protease receptor-1 in the human genomic library in 1997. The authors measured the expression of YAP and survivin in normal gastric mucosa, precancerous lesions and gastric carcinoma using an immunohistochemical method, to analyze the significance and correlations of the two factors with gastric carcinogenesis.

Research frontiers

The Hippo (Hpo) pathway was originally identified in Drosophila as a potent regulator of inhibition of cell growth and promotion of apoptosis. The pathway consists of a tumor suppressor kinase cascade which negatively regulates growth and results in inactivation of a transcriptional co-activator, Yorkie (Yki). The human ortholog of Yki, YAP, has a 31% sequence homology and similar biologic activity. YAP is a 65 kDa phosphoprotein, rich in proline. In biological conditions, YAP is phosphorylated by the Hpo signaling pathway, and is highly conserved with other components of this pathway, regulating the balance between cell proliferation and apoptosis to maintain the steady-state of the cellular environment.

Innovations and breakthroughs

Previous reports have found that YAP was an activator of cell death in mammalian cells. YAP was shown to activate apoptosis in response to DNA damage by interacting with p73 in several cancer cell lines. This is in direct contrast to the results of their investigation and other previous reports.

Applications

The authors investigation found that the expression of YAP and survivin in

gastric carcinoma were positively correlated. They speculate that YAP may induce a high expression of cell proliferation-related factors and apoptotic inhibitors, such as Ki67, cIAP1 and survivin. Detecting YAP and survivin together may help in early diagnosis of gastric carcinoma. Whether YAP and survivin collaborate to contribute to gastric tumorigenesis and progression requires further study.

Peer review

The investigation found that the expression of YAP and survivin in gastric carcinoma were positively correlated, and the authors speculated that YAP may induce a high expression of cell proliferation-related factors and apoptotic inhibitors, such as Ki67, cIAP1 and survivin. Survivin may participate in gastric carcinogenesis, progression and metastasis by inhibiting apoptosis of gastric carcinoma cells and regulating cellular mitosis. The study is interesting and is worth further exploration.

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BRIEF ARTICLES

Evaluation of standard liver volume formulae for Chinese adults

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Abstract

AIM: To evaluate different standard liver volume (SLV) formula and verify the applicability of the formulae for Chinese adults.

METHODS: Data from 70 cases of living donor liver transplantation (LDLT) performed at our transplantation centers between January 2008 and April 2009 were analyzed. SLV was estimated using our recently reported formula [the Chengdu formula: SLV (mL) = 11.5 × body weight (kg) + 334] and other reported formulae used for Chinese adults. Actual intraoperative liver volumes were obtained from a review of the patients' medical records.

RESULTS: The actual right liver volume was not significantly different from the estimated right liver volume determined by the Chengdu formula, but was significantly smaller than estimates using the Heinemann, Urata, Vauthey, and Lee formulae ($P < 0.01$), and significantly larger than estimates using the Fan formula ($P < 0.05$).

CONCLUSION: The Chengdu formula was demonstrated to be reliable by its application in LDLT.

Key words: Standard liver volume; Living donor liver transplantation; Chinese adult; Liver volume formula

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Shi ZR, Yan LN, Li B, Wen TF. Evaluation of standard liver volume formulae for Chinese adults. *World J Gastroenterol* 2009; 15(32): 4062-4066 Available from: URL: http://www.wjgnet.com/1007-9327/15/4062.asp DOI: http://dx.doi.org/10.3748/wjg.15.4062

INTRODUCTION

Living donor liver transplantation (LDLT) has been used to alleviate the shortage of available liver donors. Accurate estimation of the standard liver volume (SLV) of the living donor and recipient is crucial. Overestimation of the donor's SLV may result in excessive hepatic resection leading to liver failure, while underestimation of the recipient's SLV may result in small-for-size graft syndrome[1-5]. Since 2001, our transplant centers have carried out 212 LDLTs. We estimated the SLV using computed tomography (CT) or reported formulae. However, there was a difference between these estimates and the actual liver volumes (ALVs) for Chinese adults. Recently, we developed a new formula (named the Chengdu formula) to estimate SLV using data from 115 LDLTs[6]. The formula is: SLV (mL) = 11.5 × body weight (kg) + 334. Using this formula, the SLVs were evaluated in 76 cases of LDLT performed from January 2008 to April 2009. Its accuracy was compared to that of other internationally reported formulae[7-10] to assess which formula is the most accurate for Chinese adults.

MATERIALS AND METHODS

Patient selection

The data from 76 living donors were analyzed. Inclusion criteria were: (1) a healthy adult donor, aged 19-59 years; (2) right liver graft without middle hepatic vein; (3) adult-to-adult LDLT; (4) single donor; (5) no history of long term drinking. Exclusion criteria: (1) donor age < 18 or > 60 years; (2) left hepatic graft or left lateral lobe graft; (3) double donor grafts; (4) adult-to-child transplant; (5) donors who were hepatitis B or C carriers[11-14].

Clinical data

Data of preoperative donors included age, sex, height

Table 1 Reported formulae for ESLV

Author	Report date	Formula	Material used (race, number)
Urata *et al*[7]	1995	ESLV = 706.2 × BSA + 2.4	CT Volumetry (Japanese, 96)
Heinemann *et al*[8]	1999	ESLV = 1072.8 × BSA - 345.7	Autopsy (Caucasian 1332)
Vauthey *et al*[9]	2002	LV = 18.51 × BW + 191.8	CT volumetry (Western, 292)
Lee *et al*[5]	2006	ESLV = 691 × BSA + 95	LDLT (Korea, 311)
Fan *et al*[4]	2000	ESLW = 218.32+BW × 12.29 + gender × 50.74 (M = 1, F = 0)	LDLT (Chinese, 159)
Chengdu[6]	2009	ESLV = 334.024 + 11.508 × BW	LDLT (Chinese, 115)

ESLV: Estimated standard liver volume; BSA: Body surface area; BW: Body weight; CT: Computed tomography; LDLT: Living donor liver transplantation.

Table 2 Donor characteristics

Age (yr)	32.21 ± 10.07 (19-59)
Gender (Male:Female)	53:17
Body weight (kg)	62.97 ± 8.41 (42-87)
Body height (cm)	167.31 ± 8.15 (148-185)
Body mass index (kg/m^2)	22.23 ± 2.44
Body surface area (m^2) by DuBois formula	1.7082 ± 0.14
Body surface area (m^2) by Mosteller formula	1.7081 ± 0.14
Total liver volume on CT (mL)	1189.53 ± 114.75
Right lobe graft volume on CT without MHV	658.98 ± 81.14
Right lobe volume without MHV to total liver volume on CT (%)	55.4 ± 3.7
Actual right liver volume (mL)	578.58 ± 72.33

MHV: Middle hepatic vein.

(BH, measured to the nearest 1 cm), body weight (BW, measured to the nearest 0.5 kg), and body surface area (BSA) calculated using the DuBois formula: BSA (m^2) = BW (kg) 0.425 × BH (cm) 0.725 × 0.007184 or the Mosteller formula: BSA (m^2) = square root BH (cm) × BW (kg)/3600. From the diaphragm to the superior mesenteric artery plane, the entire liver image was scanned using a 7 mm thick layer. In the Leonardo workstation, the LV was measured by venous phase images[15,16]. All preoperative CT examinations of donors were performed by a single radiologist and all donor procedures were performed by the same surgical unit. The volume of the grafts was measured by a 3 L beaker using a drainage method intraoperatively and the error was less than 10 mL[17,18].

Right liver graft without middle hepatic vein reconstruction from a living donor was performed as described, with temporary occlusion of the right portal vein (PV) and right hepatic artery and use of ultrasonography to guide parenchymal transection. The right hepatic duct, right hepatic artery, right portal vein branch, and right hepatic vein were transected approximately 2-3 mm from the confluence[19,20], leaving the donor's main PV and confluence intact. The graft was flushed with University of Wisconsin solution through the PV and hepatic artery [21,22].

The volume of 70 livers was calculated using the Chengdu standard LV formula[6] as described above. The estimated right LV (ERLV) was obtained by multiplying the SLV by the proportion of the LV contributed by the right lobe on CT. The actual right LV (ARLV) was obtained by intraoperative measurement. The difference between the ERLV and ARLV was statistically evaluated.

The formulae of Heinemann *et al*[8], Urata *et al*[7], Vauthey *et al*[9], Lee *et al*[5], and Fan *et al*[4] in addition to our own formula[6] were used to determine the estimated SLV (ESLV) of our donor livers. The previously reported formulae are shown in Table 1. For each liver, we calculated the difference between the ALV and volume estimated by each formula (ELV).

Statistical analysis

After testing for normal distribution (kurtosis and skewness tests), descriptive statistics were calculated and data were expressed as means ± SD for age (year), BW (kg), BH (cm), body mass index (BMI), and BSA. The ERLV-ARLV and the ELV-ALV were compared by the 2-sided paired-samples *t*-test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the SPSS (version 13.0) program.

RESULTS

Seventy donors (all Chinese; 53 men and 17 women; mean age, 32.21 ± 10.07; range, 19-57 years) met the selection criteria. All donors were related to the recipients.

The characteristics of donors are shown in Table 2. All donors were considered healthy on the basis of BMI. All but one donor with a BMI of 17 kg/m^2 had a BMI of 18-28 kg/m^2. The mean volume of the right lobe on CT was 658.98 ± 81.14 mL and represented 55.4% ± 3.7% of the whole liver on CT.

The mean ELV and mean ERLV using the Chengdu standard formula were 1058.70 ± 96.74 mL and 586.15 ± 67.17 mL, respectively. The mean ARLV was 578.58 ± 72.33 mL. Differences for individual donors between ERLV and ARLV were not significant (t = -1.882, P = 0.064). A plot of the relationship of ARLV to the ERLV calculated using the Chengdu formula is shown in Figure 1.

The mean total LV determined preoperatively on CT was 1189.53 ± 114.75 mL. The mean RLV on CT without the middle hepatic vein was 658.98 ± 81.14 mL, and 55.4% ± 3.7% of the total LV. The ALV calculated from the volume of the graft and the ratio of the RLV to the total LV on CT (%) was 1050.10 ± 107.41 mL. The Heinemann, Urata, Vauthey, and Lee formulae significantly overestimated the LV ($P < 0.01$), while the Fan formula significantly underestimated the LV ($P <$

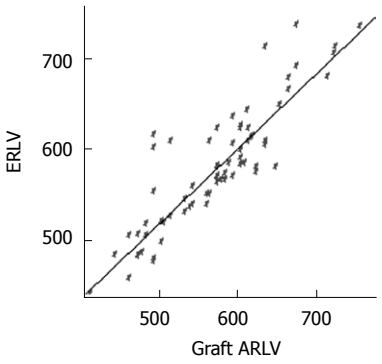

Figure 1 Correlation between actual right liver volume (ARLV) and estimated right liver volume (ERLV) by the Chengdu formula. When both were the same, a dot would be on the linear line.

Table 3 Statistical analysis of estimated LV by each formula

Formula	mean ± SD	*t*	*P*-value
Urata	1208.73 ± 99.92	-20.91	$P < 0.01$
Heinemann	1486.85 ± 151.78	-40.84	$P < 0.01$
Vauthey	1357.40 ± 155.60	-32.44	$P < 0.01$
Lee	1275.36 ± 97.77	-29.87	$P < 0.01$
Fan	1034.28 ± 111.61	2.465	$P = 0.016$
Chengdu	1058.70 ± 96.74	-1.417	$P = 0.161$
ALV	1050.10 ± 107.41	ND	ND

ALV: Actual liver volume; ND: Not determined.

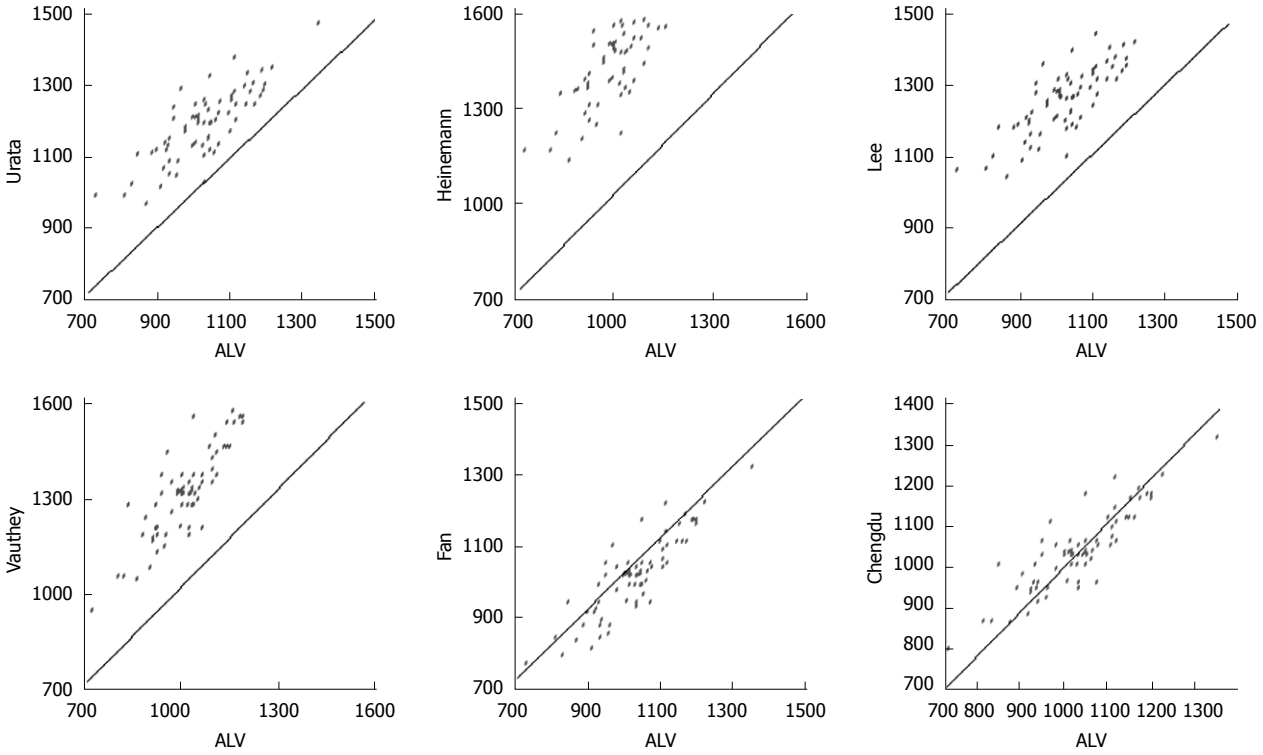

Figure 2 Correlation between actual liver volume (ALV) and estimated liver volume (ELV) by each formula. When both were the same, a dot would be on the linear line. Formulae of Urata, Heinemann, Vauthey, and Lee overestimated LV with respect to ALV. The Fan formula underestimated LV and the Chengdu formula gave a good estimate of ALV.

0.05). There was no significant difference between ALV and ELV using the Chengdu formula (Figure 2).

DISCUSSION

CT has become a standard method for assessing liver graft volume in living donors. Estimation of LV by CT (compared to actual volume) has a margin of error of 5%-25%[23,24]. In the present study, all donors had preoperative CT assessment of LV (mean total LV, 1189.53 ± 114.75 mL and mean volume of right lobe graft without middle hepatic vein, 658.98 ± 81.14 mL). The actual volume of the right liver was 578.58 ± 72.33 mL. In the present study, the LV on CT was 10%-20% higher than the ALV[25-27]. The reasons may be as follows: (1) Preoperative CT measurement is carried out under normal blood flow conditions. Perioperatively, liver resection interrupts the blood supply causing a loss of liquid volume, collapse of supporting structures, and thereby reduction in the volume of the liver. (2) Sources of error (partial volume effect, inter-observer variation, and respiratory movements) may account for this difference[28].

The difference between the ERLV (using our formula) and ARLV was compared to the difference between ERLV, calculated using the formulae of Heinemann, Urata, Vauthey, Lee, and Fan, and ARLV in our 70 donors. The Heinemann, Urata, Vauthey, and Lee formulae overestimated LV ($P < 0.01$)[29]. The reasons may include: ethnic differences (patients in Europe and the United States were Caucasian). All except the Sheung Tat Fan and Chengdu formulae were used to estimate LV from CT LV or autopsy LV. Estimates of LV by CT were 5%-25% higher than the ALV[30].

Statistical analysis showed that the Fan formula tends to underestimate LV. The weight and height of the donors in our study were higher than of those in the Hong Kong group. This may be one of the reasons both results are very close (Table 3). Above all, we believe that

the Chengdu formula was demonstrated to be reliable by its application in LDLT. We were limited to use of single center data in the present study, but we hope to improve the formula by using national multicenter data in the future[31].

COMMENTS

Background

With development of living donor liver transplantation (LDLT), especially improvement of right graft adult-to-adult LDLT, the danger of donating has been paid more and more attention. The exact liver volume is not only relevant for the recipient, but also for the donor to avoid dangerous life-threatening residual liver volumes.

Research frontiers

Scholars of different countries established several standard liver volume (SLV) formulae from clinical data. The authors estimated the SLV using computed tomography or reported formulae. However, there was a gap between these estimates and the actual liver volumes for Chinese adults. Recently, they developed a new formula (named the Chengdu formula) to estimate SLV using data from 115 LDLTs.

Innovations and breakthroughs

With the Chengdu formula, the SLVs were evaluated in 76 cases of LDLT performed from January 2008 to April 2009. Its accuracy was compared to that of other internationally reported formulae to assess which formula is the most accurate for Chinese adults.

Applications

With national multicenter data in the future, the Chengdu formula for SLV can be improved. It may then be applied to the evaluation of donors for LDLT.

Terminology

Standard liver volume: normal liver volume without disease affecting the volume of liver.

Peer review

Very interesting manuscript dealing with a very hot topic: determination of optimal size matching between graft and recipient in LDLT by means of race-adapted calculation of liver volumes. The recently published liver volume formula for Chinese people (Chengdu formula) has been demonstrated to be more reliable than others and therefore it should be adopted especially in this particular form of LT.

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CASE REPORT

Parvovirus B19 induced hepatic failure in an adult requiring liver transplantation

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Abstract

Parvovirus B19 induced acute hepatitis and hepatic failure have been previously reported, mainly in children. Very few cases of parvovirus induced hepatic failure have been reported in adults and fewer still have required liver transplantation. We report the case of a 55-year-old immunocompetent woman who developed fulminant hepatic failure after acute infection with Parvovirus B19 who subsequently underwent orthotopic liver transplantation. This is believed to be the first reported case in the literature in which an adult patient with fulminant hepatic failure associated with acute parvovirus B19 infection and without hematologic abnormalities has been identified prior to undergoing liver transplantation. This case suggests that Parvovirus B19 induced liver disease can affect adults, can occur in the absence of hematologic abnormalities and can be severe enough to require liver transplantation.

Key words: Parvovirus B19; Fulminant hepatic failure; Orthotopic liver transplant; Fulminant hepatitis

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INTRODUCTION

Parvovirus B19 is a common infection that often occurs in childhood, with 50% of adolescents having antibodies to the virus by 15 years of age[1]. The infection is transmitted by respiratory droplets and through blood products derived from viremic donors. Most acute infections in children do not result in symptoms, but the clinical presentation can range from erythema infectiosum to non-specific febrile symptoms. In adults, a polyarthropathy that can resemble rheumatoid arthritis or systemic lupus erythematosus has been reported, particularly in middle aged women[2]. In patients with hemoglobinopathies the infection can cause a clinically relevant drop in hemoglobin, which may require transfusion. In the immunocompromised patient, this infection can result in bone marrow suppression and transient mild transaminase elevations commonly occur. In children there have been several reports of severe acute hepatitis and acute liver failure, which are most often self-limited[1]. There is, however, at least one reported case of parvovirus B19 induced fulminant hepatic failure in a child that required urgent liver transplantation[3].

The majority of published cases of parvovirus B19 induced hepatitis in adults have suggested that hepatic involvement by the virus is less severe than in the pediatric population[4]. We report the case of a 55-year-old immunocompetent woman with no previous history of liver disease who developed fulminant hepatic failure secondary to acute infection with parvovirus B19 and required urgent liver transplantation.

CASE REPORT

A 55-year-old immunocompetent woman who was born in Canada presented to her family physician and subsequently a local emergency room with a two week history of fatigue, general malaise, nausea with emesis,

anorexia, dark urine and pruritus. She was noted to be jaundiced and complained of mild arthralgias in her upper extremities. One week prior to developing these symptoms, she was a passenger on a commercial airplane flight and assisted an ill jaundiced passenger who had vomited. She was a retired registered nurse with no risk factors for viral hepatitis. She denied the use of herbal remedies and acknowledged minimal alcohol consumption. On initial assessment, she was afebrile with marked icterus, no hepatosplenomegaly or ascites and mild asterixis.

Initial laboratory investigations revealed a hemoglobin 161 g/L (normal: 115-155 g/L), white blood cell count 6.6×10^9/L (normal: 4×10^9-11×10^9/L), platelet count 236×10^9/L (normal: 150×10^9-400×10^9/L), creatinine 62 μmol/L (normal: 40-95 μmol/L), aspartate aminotransferase 1838 U/L (normal: 10-38 U/L), alanine aminotransferase 1398 U/L (normal: 20-65 U/L), alkaline phosphatase 304 U/L (normal: 50-160 U/L), γ-glutamyl transpeptidase 179 U/L (normal: 10-55 U/L), direct bilirubin 166 μmol/L (normal: 0-5 μmol/L) and total bilirubin 359 μmol/L (normal: 0-18 μmol/L), international normalized ratio 1.9 (normal: 0.9-1.1), partial thromboplastin time 34 s (normal: 24-34 s), albumin 29 g/L (normal: 35-48 g/L). Hepatitis A IgG was positive and IgM was negative, hepatitis B surface antigen was negative and hepatitis C serology was negative and investigations excluded hepatitis E. Acetaminophen level was < 66 μmol/L (normal: < 66 μmol/L). An urgent abdominal ultrasound demonstrated a normal appearing liver with no focal lesions.

Two days after admission to a local hospital she was transferred to the regional liver transplantation centre for assessment. On arrival, she remained icteric and demonstrated evidence of mild hepatic encephalopathy and ascites. Her liver enzymes continued to deteriorate and her liver function tests became increasingly abnormal. Her Model for End-stage Liver Disease (MELD) score was 28[5]. Further investigations revealed a serum ceruloplasmin of 301 mg/L (normal: 215-540 mg/L), IgA 3.24 g/L (normal: 0.7-4.0 g/L), IgG 13.4 g/L (normal: 6.7-15.2 g/L), IgM 1.09 g/L (normal: 0.4-2.3 g/L). The patient's anti-nuclear antibody, anti-smooth muscle antibody and anti-mitochondrial antibody were negative and iron studies were within normal limits. Serology for human immunodeficiency virus and cytomegalovirus were negative, Epstein Barr virus IgG was positive and IgM was negative. Serology for parvovirus B19 was positive, with both IgG and IgM being detected by ELISA (Biotrin).

Five days after transfer, she was placed on the waiting list for liver transplantation. Ten days after transfer she developed worsening encephalopathy and was moved to the top of the transplant list. Twelve days after her transfer she underwent an orthotopic liver transplant. One day post-transplant she was extubated and by the following day she was transferred to the solid organ transplant ward in stable condition.

Histopathologic analysis of the explanted liver demonstrated massive hepatic necrosis consistent with viral induced fulminant hepatitis. A sample of the explanted liver was ground up with a mortar and pestle in a 2 mL volume of Minimal Essential Medium. After clarifying by centrifugation, the preparation was extracted for nucleic acid using the EasyMag platform (from bioMerieux). The extracted DNA was then tested by a parvovirus specific PCR using the forward primer CCAGGAATGACTACAAAAGGCAAATAC and the reverse primer GGTAATGCGGGGTTTCTTG. The reaction was carried out for 40 cycles, each consisting of 95℃ for 0 s, 52℃ for 30 s and 72℃ for 30 s. The PCR products were analyzed by electrophoresis on agarose gel containing ethidium bromide and visualized under UV light. A band corresponding to a 191 base pair amplicon, diagnostic for Parvovirus B19, was seen. The finding of Parvovirus B19 by PCR in the explanted liver therefore confirmed the serological diagnosis of acute Parvovirus B19 infection.

DISCUSSION

Parvovirus B19 has been proposed as a causative agent of hepatitis, hepatitis-associated anemia and acute liver failure. There are several cases reported in the literature of patients with abnormal liver biochemistry, with and without associated anemia, caused by acute infection with parvovirus B19. One small series reported detectable parvovirus B19 DNA by polymerase chain reaction in liver tissue from 4 of 6 (67%) pediatric patients with acute liver failure accompanied by hepatitis-associated anemia and in 2 of 4 (50%) of those with acute liver failure in isolation[6]. Viral DNA was not detected in any of the patients' sera. A second small series found that parvovirus DNA was detected in 5 of 6 (83%) livers from patients with idiopathic non-A-E acute liver failure with hepatitis-associated anemia, 2 of 3 (67%) livers in patients with isolated acute liver failure and 1 of 6 (17%) livers from patients with acute liver failure of known non-parvovirus etiology[7]. So *et al*[3] (2007) have recently published a case report describing an 11-year-old boy who presented with fulminant hepatic failure secondary to acute parvovirus B19 infection who required urgent liver transplantation.

The majority of the available literature regarding acute parvovirus B19 induced fulminant hepatic failure has described cases involving children. Despite this, however, there are several published reports of acute parvovirus B19 infection in adults associated with the development of acute hepatitis[8-11]. Interestingly, in virtually every case reported the patients have had a complete and spontaneous remission. This has led to speculation that the syndrome in adults is not only less common than in children, but that it has a much less severe course with better patient outcomes. The case described in this report appears to be the first reported in which an adult patient has been recognized as having acute parvovirus B19 induced fulminant hepatic failure prior to liver transplantation. There remains a

remote possibility that she acquired the infection in childhood and that this acute episode was precipitated by an immune response to viral reactivation. In any event, it demonstrates that adults may also develop fulminant hepatic failure in the absence of hematologic abnormalities and may ultimately require liver transplantation as a potential life saving intervention.

It is worth noting that the notion of parvovirus B19 as a cause of acute viral hepatitis is not universally accepted and that there is also literature published that questions this association. A small study by Wong *et al*[12] documented the presence of parvovirus B19 DNA in the liver tissue of 4 of 15 (27%) patients with acute hepatitis as compared to 3 of 22 (14%) patients with non-viral liver disease. They concluded that no difference exists in the prevalence of parvovirus B19 in liver tissue in patients with acute liver failure or hepatitis-associated anemia as compared to those with chronic hepatitis B and C infection. Despite this study's findings, evidence continues to mount in favour of parvovirus B19 as a causative agent of acute hepatitis and fulminant hepatic failure.

In conclusion, there is growing evidence that Parvovirus B19 may cause acute viral hepatitis, which can result in fulminant hepatic failure requiring liver transplantation. Although this infection is most commonly acquired in childhood, adults who become acutely infected can develop liver dysfunction as a result. The liver disease can occur independently from the often-associated hematologic abnormalities, as illustrated by the case described in this report. Fulminant hepatic failure induced as a result of acute infection with parvovirus B19 remains a rare clinical entity, however it may be underreported due to infrequent testing that results from a lack of awareness about this syndrome. A wider recognition of parvovirus B19 as a potential cause of severe liver disease is expected to augment our ability to make a definitive diagnosis of the etiology underlying such severe clinical presentations.

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CASE REPORT

"Pseudotumoral" hepatic pattern in acute alcoholic hepatitis: A case report

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Abstract

In acute alcoholic hepatitis (AAH), a "pseudotumoral" appearance of the liver parenchyma on computed tomography (CT) scan has been reported. The main findings are hypervascularized areas closely similar to those observed in large hepatocellular carcinomas. We report a case of a patient affected by AAH with an unusual appearance of these "pseudotumoral" areas on CT scan, close resembling a metastatic cancer rather than a primary hepatocellular carcinoma. In fact, in contrast with previous reports, the picture was characterized by the presence of many inhomogeneous, hypoattenuated areas highlighted during both pre- and post-contrast phases. Moreover, we report the first description of "pseudotumoral" lesions on ultrasound scan. This patient was successfully treated with corticosteroids, even if many controversies still exist regarding their efficacy in this setting.

Key words: Acute alcoholic hepatitis; Pseudotumoral hepatic lesions; Alcoholic liver disease; Computed tomography; Ultrasound

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INTRODUCTION

Alcoholic liver disease (ALD) represents a common cause of morbidity and mortality in Europe and United States, its clinical manifestations ranging from fatty liver to end-stage cirrhosis. In this context, acute alcoholic hepatitis (AAH) is a serious complication, with a short-term mortality rate exceeding 50% in most severe cases[1-4]. Liver biopsy maintains its pivotal role in diagnosing AAH, in predicting its outcome and in the selection of patients suitable for treatment: steatosis, ballooning degeneration, Mallory bodies, perivenular polymorphonuclear inflammation and "chicken-wire" fibrosis represent the most frequent findings[5].

In the setting of AAH, imaging studies do not confirm the presence of ALD, but can be used to evaluate hepatic parenchymal changes. Ultrasonography (US) scan, computed tomography (CT) scan, and magnetic resonance imaging (MRI) can be used to diagnose fatty changes, cirrhosis or neoplastic diseases of the liver.

On MRI, specific features suggestive for alcoholic cirrhosis versus virus-induced cirrhosis include a higher volume index of the caudate lobe, smaller size of regenerative nodules of the liver and more frequent visualization of the right posterior hepatic notch[6]. On US liver scan, the presence of "pseudoparallel channel signs" and of low hepatic artery resistivity index (RI) at duplex Doppler investigation have both been reported in AAH[7-9]. A "pseudotumoral" hepatic pattern at CT scan has also been reported in this setting, even if only scanty data are available[10]. Advanced fibrosis can be

determined using transient elastography[11].

The present case report refers to an AAH patient with unusual pseudotumoral US and CT scan hepatic pattern, with favourable clinical course after alcohol withdrawal and steroid treatment.

CASE REPORT

A 33-year-old man, immigrating from Bangladesh, was referred to our Gastrointestinal Unit on August 9, 2007 because of marked asthenia, nausea, vomiting, abdominal pain and weight loss of 11 kg (from 66 to 55 kg for 170 cm of height) during the previous month. Blood arterial pressure was 105/70 mmHg and heart rate 80 beats per minute. The state of consciousness was normal and the physical examination revealed a painful hepatomegaly, with the lower hepatic edge 20 cm below the right costal margin. His past history revealed heavy smoking, accounting for a lifetime packet sum of 1800, and daily alcohol intake of 60 g until 2005; alcohol intake was then denied until hospital admission. His laboratory tests are reported in Table 1 (left column). Past or current HBV, HCV and HIV infections were ruled out by determining HBsAg and anti-HBc (tested with commercial electrochemiluminescence immunoassay kits-Elecsys HBsAg, anti-HBc; Roche Diagnostics, GMBH, Manheim, Germany), anti-HCV (Innotest-HCV-Ab IV; Innogenetics, Ghent, Belgium) and anti-HIV (tested with chemiluminescence immunoassay, Ag/Ab Combo-Architect, Abbott, Chicago, Illinois, USA). Serological and stool tests for parasitic infections were negative. Anti-nuclear, anti-mitochondrial and anti-LKM antibody were searched for by indirect immunofluorescence performed on 4 μm cryostat sections from rat liver, kidney and stomach tissues, at a sera dilution of 1:40. ECG and chest X rays were negative. US liver scan revealed a severe derangement of hepatic structure, characterized by multiple micro- and macronodular hyperechoic lesions; the biliary tree was not dilated and there were no signs of portal hypertension. Color-Doppler examination showed intrahepatic arterial dilation with pseudoparallel channel sign and low hepatic artery RI (Figure 1). At total body CT scan there was a marked liver enlargement, and multiple hypoattenuated areas were noted both with and without contrast medium (Figure 2); a diagnosis of metastatic liver disease was made. Transient elastography (Fibroscan®) was also performed and the observed value of 75 kPa (normal value < 8.0) was consistent with advanced liver fibrosis. Upper gastrointestinal tract endoscopy revealed esophageal varices (F1) and portal hypertensive gastropathy. To better define hepatic lesions, US-guided liver biopsy was obtained from both hypoattenuated areas and the surrounding parenchyma, and specimens routinely stained. At histology, main findings included a diffuse fibrosis surrounding regenerating nodules, intrasinusoidal collagen deposition, perivenular polymorphonuclear infiltration, focal fatty infiltration and ballooning degeneration with Mallory bodies, all features consistent with a final diagnosis of AAH on cirrhosis, with "pseudotumoral" areas. In the meantime, careful re-evaluation of usual daily alcohol intake, extended to patient's friends, allowed to estimate an actual daily intake of 250 g for many years.

Table 1 Biochemical characteristics of the patient

Parameters (reference value)	August 9, 2007	November 22, 2007	November 12, 2008
Haemoglobin (g/dL) (13-16)	12.7	12.2	12
MCV (fL) (84-94)	86	86	82
White blood count (× 10^3) (5.5-8.5)	9.7	8	5.3
Platelets count (× 10^3) (150-350)	119	102	184
PT (%)/INR (70-100/1.0-1.2)	54/1.3	90/1.2	94/1.1
PCR (mg/dL) (< 0.5)	1.8	0.6	0.4
Total/direct bilirubin (mg/dL) (1.1/0.8)	11.9/-	4.5/2.2	1.3/0.9
AST/ALT (IU/L) (< 35)	318/73	70/36	32/28
GGT (IU/L) (< 50)	1.309	150	46
Serum iron (mcg/dL) (70-170)	212	-	141
Transferrin (mg/dL) (200-400)	183	-	293
Ferritin (ng/mL) (400-220)	2.78	665	213

Patient's main laboratory test at enrolment (left column), 2 mo after complete alcohol withdrawal (central column) and at last control 1 year later (right column). MCV: Mean corpuscular value; PT: Prothrombin time; INR: International normalized ratio; CRP: C-reactive protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase.

According to a Maddrey's discriminating factor of 63, we instituted a treatment consisting in the daily administration of 40 mg of prednisolone, rapidly followed by clinical and laboratory improvement. The regimen was slowly tapered down and at the following clinical observation in November 2007, the patient was asymptomatic, he had stopped drinking, his body weight had increased by 13 kg (from 55 to 68) and his physical examination revealed a dramatic reduction in liver size, with the lower hepatic edge at 5 cm below the right costal margin. Laboratory tests at that time are summarized in Table 1 (central column). Moreover, a new determination of the transient liver elastography indicated a value of 51 kPa, accounting for a decrease of 24 kPa as compared to the previous measurement. A further control, performed on November 11, 2008, showed the complete normalization of both physical findings and blood tests (Table 1, right column). Interestingly, at this time, the score at transient elastography was 8.5 kPa. As mean corpuscular volume of red blood cells was unusually "normal" (86 fL, with reference value of 84-94 fL) in the setting of AAH, and, also considering the ethnic origin of the patient, we investigated a possible underlying hemoglobinopathy by HPLC (high performance liquid chromatography), analysing different Hb fractions (Variant, Bio-Rad, Milan, Italy). Findings were consistent with a heterozygous state for HbE.

DISCUSSION

The present case report concerns a patient with AAH superimposed to established cirrhosis, with "pseudotumoral" hepatic areas. This unusual finding was first

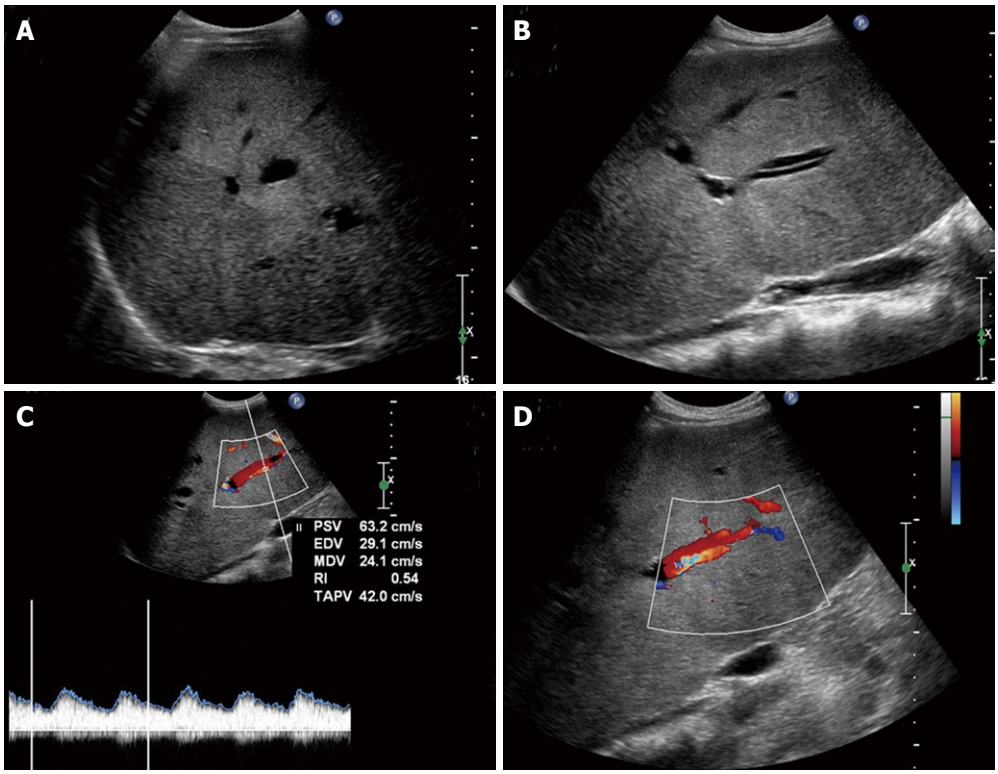

Figure 1 US scan showing a large hypoechoic area compared to surrounding parenchyma (A) and the image of "parallel channel" sign (B). Color-Doppler demonstrates the "pseudoparallel channel" sign, characterized by dilated intrahepatic arterial branch with an adjacent portal venous tract, and the low hepatic artery RI (C, D).

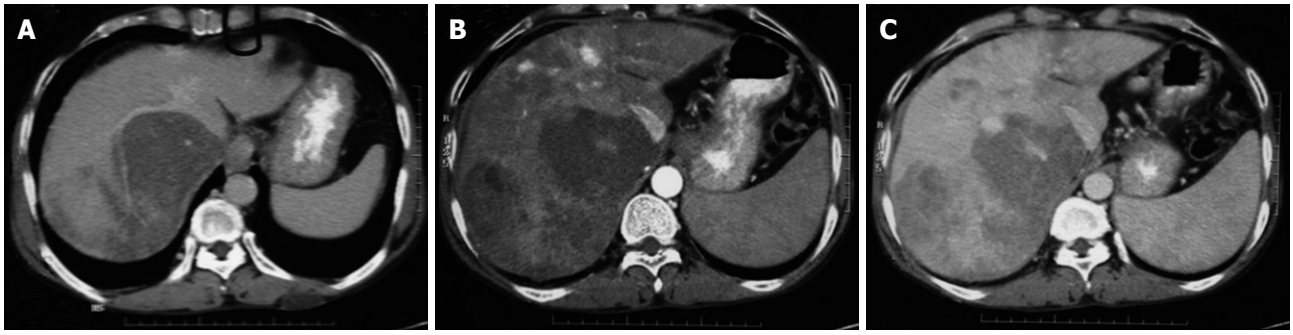

Figure 2 CT scan showing a wide hypovascularized area in the pre-contrast phase (A) that remains hypovascularized during both early (B) and late (C) arterial phases.

described in two reports[9,10], possibly involving the same single case. More recently, Colli *et al*[12] described both the CT and histological characteristics of "pseudotumoral" hepatic areas in five patients with AAH. These focal lesions were described as hypoattenuated areas when compared to the surrounding parenchyma, during the pre-contrast phase, becoming hyperattenuated during the post-contrast and late arterial phases, respectively, a pattern consistent with hypervascularized areas possibly related to a high-grade tissue regeneration. Interestingly, such lesions were closely similar to those observed in cases of large hepatocellular carcinoma[13], accounting for possible misdiagnosis. Differently from what previously reported, in this case the finding of many inhomogeneous, hypoattenuated areas highlighted during both pre- and post-contrast phases was more similar to metastatic cancer rather than to primary hepatocellular carcinoma and was responsible for the initial misdiagnosis of hepatic metastases. This led to further investigations, including a liver biopsy, which provided the correct diagnosis of AAH, ruling out any malignancy. This picture differs from the previously reported AAH CT pattern.

Occasionally, focal areas of normal parenchyma in an otherwise diffuse fatty liver may simulate mass lesions, described as "pseudolesions", that may pose a difficult diagnostic problem[14]. These areas usually present a vascularisation similar to the surrounding parenchyma: in the present case, instead, the lesions appeared clearly different in each vascular phase, as compared to the liver. Moreover, pseudo-tumoral hepatic lesions were described in a variety of other benign conditions, such as inflammatory pseudotumors, parasitic infestations, tuberculosis infection, or areas of focal sparing in diffuse processes[15-19], conditions ruled out in our patient.

An additional interesting finding, in the present case, was the presence of a typical alcohol-related duplex-Doppler image, called "pseudoparallel channel sign",

reported in patients with AAH by Sumino *et al*[7] and characterized by dilated intrahepatic arterial branch with an adjacent portal venous tract. A dilation of hepatic artery, with increased peak systolic velocity, has also been described by Han *et al*[9], who assumed that in AAH the presence of liver fibrosis increases sinusoidal resistance, blocking sinusoidal blood flow and ultimately portal blood flow in a retrograde manner. Therefore, in order to maintain hepatic perfusion, there is a dilation of hepatic artery leading to increased blood flow. A further interesting Doppler finding in this patient was the low hepatic artery RI, whose role in diagnosing AAH remains however controversial. Colli *et al*[8] reported a statistically significant decrease of hepatic artery RI in patients with AAH, as compared to both healthy and cirrhotic patients, a finding in contrast with the cirrhotic pattern observed in our patient. The possible relevance of the hepatic artery RI in AAH has also been challenged by Han *et al*[9], who reported a high variability of this sign in patients with liver disease, accounting for a lack of a clear-cut distinction between AAH and cirrhosis.

To assess the severity of underlying liver disease and to properly take care of the patient, we assessed three main prognostic models, all validated for AAH [i.e. Maddrey's discriminating factor (mDF), model for end-stage liver disease and Glasgow for acute alcohol hepatitis score (GAHS)][20-22]. Our case scored a total of 63, 14 and 9, respectively, compared to reference values of 32, 21 and 9. A mDF $\geqslant$ 32 and a GAHS score $\geqslant$ 9 identify patients with a very poor prognosis who have been reported to have had a good clinical response to corticosteroids[23]. Based on an mDF of 63 and a GAHS of 9, a corticosteroid regimen was instituted and then slowly progressive tapered down, on the basis of progressive clinical, laboratory and radiological improvement. At present, prognostic scores for AAH may orient the patient management, even if the use of steroids in this setting has recently been challenged by pertinent metanalytic data[24] while other treatments, such as anabolic steroids, pentoxifylline and infliximab, are still under investigation[25-27].

To complete the liver disease staging and to obtain data useful in the follow up, our patient underwent also a transient elastography (FibroScan®). Values obtained in this case were very high, indicating not only a possible advanced fibrosis of the liver, but also confirming recent reports suggesting that transient elastography can be influenced by other parameters, such as the degree of necroinflammatory activity, especially during acute hepatitis[28].

To summarize, in patients with AAH, "pseudotumoral" hepatic areas can appear at CT scan not only as hypervascular lesions similar to HCC, as previously described, but also as hypoattenuated lesions during all contrast phases, closely similar to liver metastases. This feature has to be carefully considered to avoid misdiagnosis.

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CASE REPORT

An adult case of celiac sprue triggered after an ileal resection for perforated Meckel's diverticulum

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Abstract

Celiac disease can be triggered by upper abdominal surgery, such as vagotomy, oesophagectomy, pancreaticoduodenectomy, and gastrojejunal anastomosis. Here we report a case of a 24 year-old woman who developed celiac disease after an ileal resection for perforated Meckel's diverticula. This is the first reported celiac case that has been triggered, not by upper abdominal surgery, but after ileal resection for Meckel's diverticula.

Key words: Celiac disease; Meckel's diverticula; Ileal resection

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INTRODUCTION

Celiac disease is an autoimmune enteropathy often seen in gluten sensitive patients[1]. It has two presentations in adults, namely the classical (diarrhea-predominant) type and the silent type[2]. The silent group includes atypical presentations. Some initiating factors, such as gluten overload, surgery, giving up smoking, and infections can trigger the disease, which can become apparent in an abrupt manner[3,4].

Meckel's diverticulum is a common congenital anomaly of the small bowel. Ulcer, hemorrhage, intussusception, intestinal obstruction, perforation, and, very rarely, vesicodiverticular fistulae and tumors are complications of these diverticula[5]. We present a case of Meckel's diverticula that was diagnosed as celiac disease after surgery. This is the first reported case of Celiac disease that has been diagnosed after an ileal resection rather than upper abdominal surgery.

CASE REPORT

A 24 year-old woman applied to the emergency service with abdominal pain, nausea, and vomiting. She did not have any bowel movements or flatus and her abdominal pain worsened after her hospitalization. There was tenderness and guarding on abdominal palpation. Her initial laboratory tests revealed a leukocytosis score of 14000/mm^3.

Due to her worsening abdominal pain and a white blood cell count that progressively increased to 16.000/mm^3, urgent surgery was performed for an acute abdomen. Perforated Meckel's diverticula, located 80 cm proximal to the ileocecal valve, were observed during the operation. Ten centimeters of small bowel segment including the Meckel's diverticulum was resected and an end-to-end anastomosis was performed. Pathological investigation of the surgical specimen revealed perforated Meckel's diverticula and segmental ileal resection. The patient was discharged 4 d after the surgery without any complications.

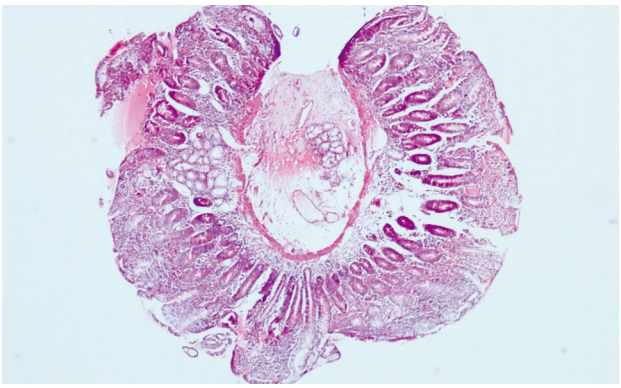

Figure 1 Diffuse villous atrophy in the duodenum (HE, × 10).

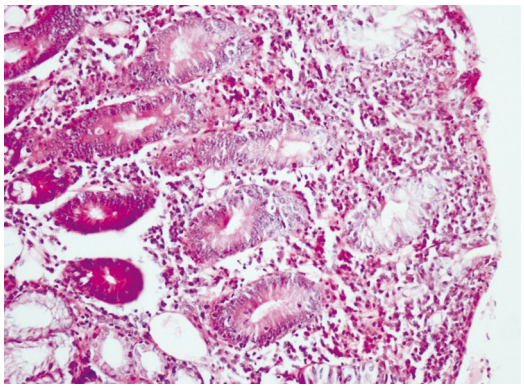

Figure 2 Increased intraepithelial lymphocytes in the duodenum (HE, × 50).

Twenty days after the discharge, the patient applied to the gastroenterology clinic with complaints of abdominal pain, flatulence and a loose stool 4-times/d. Her laboratory examination revealed Hb:10 g/dL, WBC. 5400/mm^3, Plt. 458.000/mm^3, vitamin B12: 119 ng/mL (180-914), AST: 14 U/L (5-45), ALT: 14 U/L (5-45), and ALP: 260 U/L (80-270). An upper gastrointestinal endoscopy was performed for her anemia, which showed antral gastritis and scalloping of duodenal mucosal folds. Endoscopic duodenal biopsy revealed diffuse atrophic villi with an increase in intraepithelial lymphocytes suggesting celiac disease (Figures 1 and 2). For confirmation of Celiac disease, gluten antibodies were determined as follows: anti-gliadin IgA, 88.2 U/mL (0-12); anti-gliadin IgG > 100 U/mL (0-12); anti-endomysial IgA antibody, (+++); anti tissue transglutaminase IgG, 51.2 U/mL (0-10); and anti tissue transglutaminase IgA, > 200 U/mL (0-10). Gluten was removed from the diet and thereafter her complaints of abdominal pain, flatulence, and diarrhea resolved. Her laboratory tests after a 2-mo gluten free diet were; Hb 12.2 g/dL and vitamin B12: 461 ng/mL (180-914).

DISCUSSION

Celiac disease is an autoimmune enteropathy seen in gluten sensitive patients. It is a common genetic disorder with a prevalence of 1%-2%[6]. The disease can manifest itself by different clinical presentations. There are gastrointestinal symptoms, diarrhea and weight loss in the classical type, while extra intestinal findings are most common in the atypical or subclinical form[2-4].

Our patient could have been in the silent form of the disease that became overt after the triggering effect of surgery. There are celiac disease patients in the literature that were triggered by upper gastrointestinal surgery, such as vagotomy, oesophagectomy, pancreaticoduodenectomy, and gastrojejunal anastomosis[7-10]. Our case is the first report of celiac disease being triggered by ileal surgery.

The autoimmune activation mechanism triggered by the surgery is not yet known. However, it has been postulated that raised intestinal permeability might be involved in the pathogenesis of celiac disease[11]. Andersen *et al*[12] have shown by a triple sugar test that bowel permeability is increased in ileostomy patients. Perhaps this hyperpermeability could be the triggering factor in our patient. Another possible mechanism for the emergence of post-operative Celiac disease in our patient could be antigenic overload secondary to postoperative changes[9].

Early diagnosis of Celiac disease in the postoperative period is important to prevent complications. A clinician should be aware of Celiac disease when the patient has refractory diarrhea, anemia, weight loss, and hypoalbuminemia after ileal surgery, not just after upper gastrointestinal surgery.

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CASE REPORT

Pneumobilia, chronic diarrhea, vitamin K malabsorption: A pathognomonic triad for cholecystocolonic fistulas

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Abstract

Cholecystocolonic fistula (CF) is an uncommon type of internal biliary-enteric fistulas, which comprise rare complications of cholelithiasis and acute cholecystitis, with a prevalence of about 2% of all biliary tree diseases. We report a case of a spontaneous CF in a 75-year-old diabetic male admitted to hospital for the investigation of chronic watery diarrhea and weight loss. Massive pneumobilia demonstrated on abdominal ultrasound and computerized tomography, along with chronic, bile acid-induced diarrhea and a prolonged prothrombin time due to vitamin K malabsorption, led to the clinical suspicion of the fistula. Despite further investigation with barium enema and magnetic resonance cholangio-pancreatography, diagnosis of the fistulous tract between the gallbladder and the hepatic flexure of the colon could not be established preoperatively. Open cholecystectomy with fistula resection and exploration of the common bile duct was the preferred treatment of choice, resulting in an excellent postoperative clinical course. The incidence of biliary-enteric fistulas is expected to increase due to the parallel increase of iatrogenic interventions to the biliary tree with the use of endoscopic retrograde cholangio-pancreatography and the increased rate of cholecystectomies performed. Taking into account that advanced imaging techniques fail to demonstrate the fistulas tract in half of the cases, and that CFs usually present with non-specific symptoms, our report could assist physicians to keep a high index of clinical suspicion for an early and valid diagnosis of a CF.

Key words: Cholecystocolonic fistula; Cholecystocolonic fistula; Bilioenteric fistula; Pneumobilia

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INTRODUCTION

Internal biliary-enteric fistulas (IBFs) are very rare, comprising 0.4%-1.9% of all biliary tract diseases[1-3]. IBFs are detected in only 0.2%-0.9% of all biliary tract operations[4-6]. Depending on the site of communication with the extrahepatic biliary tree, several types of fistulas are recognized (cholecystoduodenal, choledochoduodenal, cholecystogastric, cholecysto-choledochal, cholecysto-colonic, cholecystoduodenocolic)[1,2]. Peptic ulcer and malignancies of the stomach, gallbladder, pancreas, duodenum, colon and bile ducts have been identified as etiologic factors, with cholelithiasis being the predisposing factor[2]. Iatrogenesis may be responsible for an increasing incidence of IBFs in the near future[2], as the use of endoscopic retrograde cholangio-pancreatography (ERCP) and the rate of cholecystectomies performed annually increase.

A cholecystocolonic (otherwise cholectystocolonic) fistula (CF) is an uncommon type of IBF, which forms a communication between the gallbladder and the hepatic flexure of the transverse colon (Figure 1)[7]. Theoretically, fistula formation was part of the natural history of acute cholecystitis prior to the era of cholecystectomy and antibiotics[8]. Its frequency among other types of IBF

ranges between 8% and 13.6%[1,9,10]. CFs are associated with several severe complications, such as acute cholangitis, biliary peritonitis and biliary cirrhosis[3,11,12], leading to a global mortality rate between 10% and 15%[4,9,10].

Since Courvoisier first discussed spontaneous biliary fistulas in 1890[3], several authors have described interesting case-reports of CF in the literature[13-38]. However, diagnosis still remains a challenge, mainly because: (1) CF presents with varying and non-specific symptoms; (2) CF is very rare and is kept last in differential diagnosis; and (3) even advanced imaging techniques fail to demonstrate the fistulas tract in almost half of the cases[1,2,13].

In this paper, we report a case of a spontaneous CF and review the literature. The aim of this paper is to elaborate on the clinical manifestation of a CF and describe the pathophysiological mechanisms. In conclusion, we suggest that the triad of pneumobilia, chronic diarrhea and malabsorption of vitamin K could assist physicians to keep a high index of clinical suspicion for CF, leading to an early and valid diagnosis.

CASE REPORT

A 75-year-old male patient was referred to our university hospital for the investigation of chronic diarrhea and weight loss. The patient reported suffering from watery diarrhea of 3-4 bowel movements daily, lasting longer than 18 mo, and weight loss of 15 kg. The patient reported having no fever, nausea, vomiting, jaundice or abdominal pain. Medical history revealed the presence of mild diabetes mellitus type 2, which was controlled by diet, and a long-lasting smoking habit. The patient had already been subjected to colonoscopy prior to his admittance. Biopsies, including the terminal ileum, had been unrevealing. Routine laboratory tests of total white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), aminotransferase levels and electrolytes had also been found within normal.

On admission, the patient was afebrile. No pathological signs were found on physical examination. Laboratory investigation demonstrated alkaline phosphatase of 228 IU/L (normal range 35-120 IU/L), γ-glutamyl transpeptidase of 120 IU/L (normal: 10-45 IU/L), C-reactive protein of 6.51 mg/dL (normal: 0-0.5 mg/dL) and a prolonged prothrombin time (PT) of 20.3 s (normal: 12.2 s), that was returned to normal after parenteral administration of vitamin K (10 mg subcutaneously). Total and differential WBC counts, haemoglobulin, platelet count, ESR, aminotransferase levels, bilirubin, total protein, albumin and electrolytes ranged within normal values. Fecal examination included negative stool culture and negative examination of stool for ova and parasites. Stool examination for fat was negative, but fat determination of 24 h stool was unavailable.

Ultrasonographic examination of the liver and the extrahepatic biliary tree demonstrated pneumobilia and moderate dilatation of the intrahepatic bile ducts, with a normal common bile duct and a thick-walled gallbladder (Figure 2). Enhanced CT of the abdomen confirmed

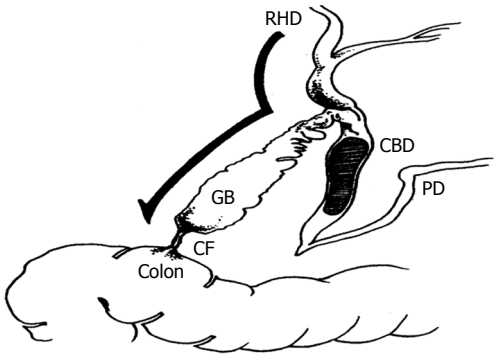

Figure 1 Schematic demonstration of a cholecystocolic fistula (modified from Benage *et al*[7]). GB: Gallbladder; CBD: Common bile duct; RHD: Right hepatic duct; PD: Pancreatic duct; CF: Cholecystocolic fistula.

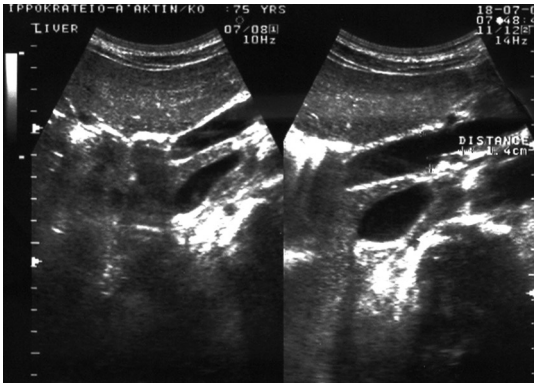

Figure 2 Ultrasonographic examination revealing pneumobilia and moderate dilatation of the intrahepatic bile ducts, with a normal common bile duct and a thick-walled gallbladder.

massive pneumobilia and absence of common bile duct dilatation (Figure 3A). Furthermore, the presence of air was detected in the gallbladder, which was neighboured to the hepatic flexure of the colon (Figure 3B). Neither the following barium enema nor the MR cholangiopancreatography were able to detect any fistulous tract between the gallbladder and the colon.

The patient was subsequently referred to surgery for diagnostic laparotomy. During surgery a fistulous tract from the fundus of the gallbladder to the hepatic flexure of the colon was detected, along with an impacted large gallstone near Vater's villa. Cholecystectomy with fistula resection and bilio-enteric anastomosis after gallstone removal was undertaken. The patient had an excellent postoperative clinical course. Diarrhea stopped, and he regained his weight within a 6 mo period.

DISCUSSION

We report a case of a spontaneous CF in a male diabetic patient, due to asymptomatic cholelithiasis. A combined triad of strong clues (pneumobilia, chronic, bile acid-induced diarrhea and malabsorption of fat-soluble vitamin K) provided high clinical suspicion for a CF, which was only confirmed during diagnostic laparotomy.

Several authors have reported their own experience of CFs[2,7,9,13-38]. Table 1 summarizes patients' demographics,

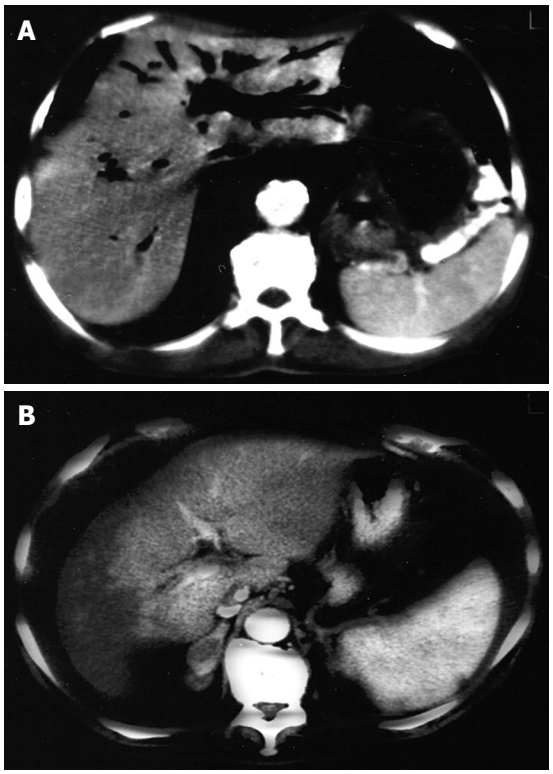

Figure 3 Computed tomography scan. A: Massive pneumobilia ("air cholangiogram"); B: Presence of air detected in the gallbladder.

etiological factors, clinical presentation, presence of pneumobilia, means of fistula visualization and treatment option preferred. CF occurs mostly in the elderly, with a female preponderance[3,9]. In our case and in more than 90% of all cases, cholelithiasis is the main etiologic factor. Cholelithiasis may cause either recurrent episodes of acute cholecystitis/cholangitis or asymptomatic chronic calculous cholecystitis. The sequence of events that lead to the formation of the fistula include gallbladder inflammation, mechanical erosion by gallstones and gangrenous changes of both the gallbladder wall and the wall of the adjacent colon[14,17,39]. The gallstone found in the common bile duct in our patient could have affected the formation of the fistula by increasing the pressure in the biliary tree and thus enhancing the mechanical erosion of the gallbladder by another gallstone or gallstones that could easily have escaped through the fistulous tract.

CF usually has a benign clinical course, remaining asymptomatic for a long time, and can only be found incidentally[24,26,28] or be suspected by the presence of unexplained pneumobilia in patients with a history of episodes of right upper quadrant (RUQ) pain[17,22]. In other cases, varying symptoms are identified like fever, nausea and vomiting, attacks of abdominal pain localized mainly in the RUQ and jaundice[15,19,23,36]. These symptoms are non-specific and cannot be directly attributable to the fistula but rather to the main etiologic factor, cholelithiasis. In these cases, clinical presentations of acute cholecystitis, cholangitis or acute/chronic pancreatitis predominate.

A more specific symptom of a CF is chronic diarrhea[2,9,13,14,21,24,27,32,35]. CFs alter the normal enterohepatic circulation of bile acids, leading to chronic watery diarrhea. This is a type 1, bile acid-induced diarrhea, which results from the stimulation of colonic mucosa by bile acids and the subsequent colonic secretion of water and electrolytes[35,40,41]. In other cases, especially when hepatic synthesis of bile acids is reduced, bypass of the distal ileum allows bile acids to escape absorption, leading to steatorrhea[7,9,35,36]. Bile salts are needed for micelle formation and subsequent absorption of fat and fat-soluble vitamins, while protein absorption is not affected. Fat malabsorption resulting from impaired micelle formation is not as severe as malabsorption resulting from pancreatic lipase deficiency, because fatty acids and monoglycerides can form lamellar structures, which to a certain extent can be absorbed[42]. Theoretically, malabsorption of fat-soluble vitamins (A, D, K and E) may be marked, because micelle formation is required for their absorption. However, malabsorption of fat-soluble vitamins A and E has never been reported in the literature, while malabsorption of vitamin D has only been described in one female patient with CF and osteoporosis/osteomalacia[7]. The deficiency of those vitamins has to be marked to provide clinical symptoms, and laboratory evaluation is difficult and outside routine clinical practice. On the other hand, vitamin K deficiency can easily be detected by a prolonged PT and can be fixed with parenteral administration.

Rare complications of CF reported in the literature are ectopic gallstone in the colon with obstruction[29,30,34,37,38] or hemorrhage of the lower gastrointestinal tract[20], and extraperitoneal abscess with sepsis due to ascending contamination by colon bacteria[18,20,43].

Barium enema[2,9,13,21,24,26,27,32], 99mTc scintigram[24,43], CT[29,44], MRCP and ERCP[7,13-15,17,19,21,22,35,36] have all been used in order to demonstrate the fistula. However, non-visualization of the fistulous tract occurs in almost half of the cases[1,2,13,44], and, subsequently, patients are subjected to exploratory laparotomy. The presence of pneumobilia, detected by plain abdominal films, abdominal ultrasound or CT, may provide presumptive evidence for the existence of a biliary-enteric fistula, but it is non-specific[2,45]. Other radiological features include a small atrophic gallbladder adherent to neighboring organs or a shrunken thick-walled gallbladder around gallstones[2,44].

Open cholecystectomy with fistula resection and exploration of the common bile duct is the treatment of choice mostly preferred in order to avoid attacks of cholecystitis and/or cholangitis. However, successful cases using the laparoscopic approach are increasingly reported in the literature[15,23,26,28,31-33]. In non-surgical patients, either conservative treatment with antibiotics and supplementation of fat-soluble vitamins[9,16], or ERCP sphincterotomy[36,46] to reduce biliary pressure are suggested.

In conclusion, especially in patients with a history of gallstone disease, the presence of chronic watery diarrhea and vitamin K malabsorption, combined with the radiological finding of pneumobilia, form a pathognomonic triad. Either internists/gastroenterologists

Table 1 Systematic review of cases of cholecystocolic fistulas reported in the literature

Author (yr)	No pts	Age (yr)	Etiology	Main clinical manifestation	Presence of pneumobilia	Means of fistula visualization	Treatment option preferred
Chatzoulis *et al*[14] (2007)	1M	52	Cholelithiasis (mirizzi syndrome type Ⅳ)	Diarrhea, RUQ pain, fever	+ (US, CT)	MRCP, ERCP	Cholecystectomy, fistula excision, Roux-en-Y bilio-enteric anastomosis
Wang *et al*[15] (2006)	1F	63	Gallbladder and CBD stone	RUQ pain	+	ERCP	Laparoscopic cholecystectomy, fistula closure
Singh *et al*[16] (2004)	1F	92	Gallstones	Melena	+	-	Conservative
Arvanitidis *et al*[17] (2003)	1M	72	Acute obstructive cholecystitis	Unexplained pneumobilia, history of RUQ pain	+ (US)	ERCP	Open cholecystectomy, fistula resection, exploration of CBD
Hussien *et al*[18] (2003)	1M	63	Xanthogra-nulomatous cholecystitis	Fever due to extraperitoneal abscesses	-	-	Cholecystectomy, fistula excision, closure of colon defect
Velayos Jiménez *et al*[9] (2003)	1F	79	Cholelithiasis	Chronic diarrhea, steatorrhea (?)	+ (US, CT)	BE	Conservative (antibiotics, vitamins)
De Keuleneer *et al*[19] (2002)	NR	NR	Cholelithiasis (Mirizzi syndrome type I)	Obstructive jaundice	-	ERCP	Cholecystectomy with Roux-en-Y hepato-enteric anastomosis
Ramos-De la Medina *et al*[20] (2002)	1F	48	Cholelithiasis	Lower gastro-intestinal bleeding	-	-	Open surgery
	1F	60	Gallbladder malignacy	Multiple hepatic abscesses	-	-	
Dutta *et al*[21] (2002)	1F	65	NR	Diarrhea, weight loss, malabsorption (?)	+	BE, ERCP	Fistulectomy, cholecystectomy
Schoeters *et al*[22] (2002)	1F	81	NR	Incidental pneumobilia, RUQ pain	+ (PAF, US)	ERCP	NR
Fujitani *et al*[23] (2001)	1F	58	Cholelithiasis, chronic cholecystitis	RUQ pain	-	Intraoperatively	Laparoscopic cholecystectomy and fistula resection
Sam *et al*[24] (2001)	1F	75	Incidental (Crohn's disease?)	Diarrhea	- (Air in gallbladder)	BE, Tc-99m chole-scintigraphy	NR
Inal *et al*[2] (1999)	1F	72	Chronic cholecystitis	Diarrhea, weight loss	+ (US)	BE	Open cholecystectomy, repair of the fistula
Kuo *et al*[25] (1999)	1F	65	Chronic cholecystitis	Diarrhea, fever, RUQ, jaundice	+ (PAF)	BE, ERCP	Cholecystectomy, fistulas division
Hida *et al*[26] (1999)	1F	61	Cholelithiasis	Incidental finding (BE screening), RUQ pain	-	BE	Laparoscopic stampling technic
Holst *et al*[27] (1999)	1F	76	Gallstone disease	Diarrhea, RUQ pain	-	BE	Open cholecystectomy, fistula resection
Reddy *et al*[28] (1998)	1F	72	History of acute cholecystitis	Incidental finding during laparoscopic cholecystectomy	-	-	Laparoscopic approach, tube caecostomy
Bornet *et al*[29] (1998)	NR	NR	Gallstone disease	Colonic gallstone obstruction	+ (CT)	CT?	None
Hession *et al*[13] (1996)	1F	70	Acute or chronic cholecystitis	Diarrhea	-	BE	NR
	1F	78		Diarrhea	-	BE	
	1F	69		Diarrhea	-	BE	
	1F	73		Diarrhea	-	BE	
	1F	77		Colon ileus	+	BE	
	1F	85		RUQ pain, diarrhea	+	BE	
	1M	43		Melena	-	ERCP	
Pérez Morera *et al*[30] (1996)	2F	NR	Gallstone disease	Colonic gallstone obstruction	+	BE (postoperatively)	Repair of the fistula at a later stage
Ibrahim *et al*[31] (1995)	1F	68	Cholelithiasis	Incidental finding during laparoscopic cholecystectomy, RUQ pain, diarrhea	-	Operative cholecystogramm	Laparoscopic approach
Gentileschi *et al*[32] (1995)	NR	NR	Gallstone disease	Severe diarrhea	-	BE	Laparoscopic approach
Prasad *et al*[33] (1994)	1F	67	NR	Incidental finding during laparoscopic cholecystectomy	-	Operative cholangiography	Laparoscopic approach
Swinnen *et al*[34] (1994)	NR	NR	Gallstone disease	Colonic gallstone obstruction	+ (US)	Colonoscopy & contrast fistulography	NR
Benage *et al*[7] (1990)	1F	78	Gallstone obstruction of CBD	Malabsorption (steatorrhea, osteomalacia, hypocalcemia)	-	ERCP	Open cholecystectomy, repair of the fistula, calcium & D3 supplementation
Sing *et al*[35] (1990)	1M	80	Cholelithiasis, CBD obstruction, (Billroth Ⅱ?)	Diarrhea, weight loss, fat malabsorption	+ (US)	ERCP	Open cholecystectomy, fistula resection, T-tube in CBD

Caroli-Bosc *et al*[36] (1990)	2		Gallstones	Steatorrhea Jaundice, fever	- -	ERCP ERCP, cholescintigram	Open surgery ERCP sphincterotomy
Patel *et al*[37] (1989)	1F	NR	Gallstone disease	Colonic gallstone obstruction	-	?	?
Anseline *et al*[38] (1981)	1F	90	Gallstone disease	Colonic gallstone obstruction	+ (X-ray contrast)	Intraoperatively	None

M: Male; F: Female; NR: Not reported; RUQ: Right upper quadrant; CBD: Common bile duct; PAF: Plain abdominal film; BE: Barium enema; US: Ultrasound examination; CT: Computed tomography; MRCP: MR cholangio-pancreatography; ERCP: Endoscopic retrograde cholangiopancreatography.

or surgeons should keep a high clinical index for a valid preoperative diagnosis of CFs.

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CASE REPORT

Suture granuloma of the abdominal wall with intra-abdominal extension 12 years after open appendectomy

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Abstract

Most complications after appendectomy occur within ten days; however, we report the unusual case of a suture granuloma 12 years after open appendectomy. The afebrile 75-year-old woman presented with a slightly painful palpable mass in the right lower abdomen. There was no nausea or vomiting and bowel movements were normal. She lost 10 kg during the 3 mo before presentation. The patient had undergone an appendectomy 12 years previously. Physical examination revealed a tender mass, 10 cm in diameter, under the appendectomy scar. The preoperative laboratory findings, tumor markers and plain abdominal radiographs were normal. Multi-slice computed tomography scanning showed an inhomogenous abdominal mass with minimal vascularization in the right lower abdomen 8.6 cm × 8 cm × 9 cm in size which communicated with the abdominal wall. The abdominal wall was thickened, weak and bulging. The abdominal wall mass did not communicate with the cecum or the ascending colon. Complete excision of the abdominal wall mass was performed *via* median laparotomy. Histopathological examination revealed a granuloma with a central abscess. This case report demonstrates that a preoperative diagnosis of abdominal wall mass after open appendectomy warrants the use of a wide spectrum of diagnostic modalities and consequently different treatment options.

Key words: Appendectomy; Differential diagnosis; Postoperative complication; Suture granuloma

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INTRODUCTION

Appendicitis was recognized as a surgical disease when Reginald Heber Fitz correctly pointed out that the frequent abscesses in the right iliac fossa were often due to perforation of the vermiform appendix, and he referred to the condition as appendicitis[1]. Since that discovery and the development of various surgical incisions and appendectomy techniques, many early and late postoperative complications and coincident conditions have become evident. One of these complications is a postoperative abdominal wall mass in the region of McBurney's muscle-splitting incision. The diagnosis and management of abdominal wall masses after open appendectomy are challenging because various conditions such as appendectomy-related, primary-local (appendectomy-unrelated) and primary-systemic could be the cause of abdominal wall masses postoperatively. This report presents the first known case of a suture granuloma with intra-abdominal extension as a cause of an abdominal wall mass after open (muscle-splitting) appendectomy.

CASE REPORT

A 75 year-old woman presented with a slightly painful palpable mass in the right lower abdomen lasting for 6 mo. The pain in the right lower quadrant was described as continuous, nonradiating, mild and non-disturbing. There was no nausea or vomiting. Body temperature

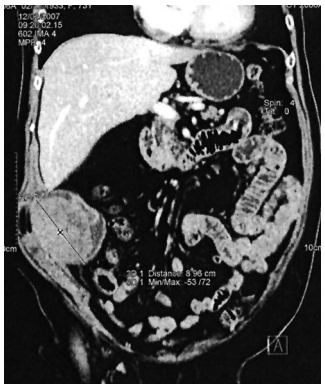

Figure 1 CT scan of the abdominal wall mass in the right lower abdomen protruding into the abdominal cavity dislocating small bowel loops.

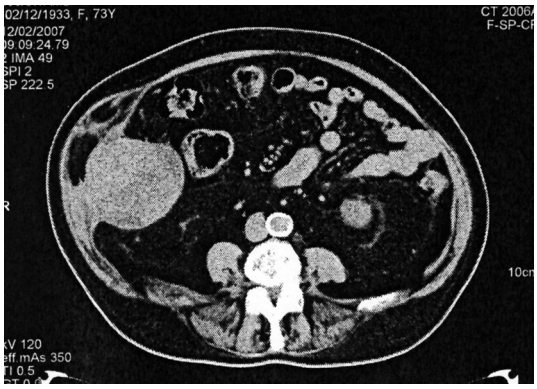

Figure 2 CT scan of the lower abdomen showing the abdominal wall mass with thickened and bulging abdominal wall and no communication with the cecum.

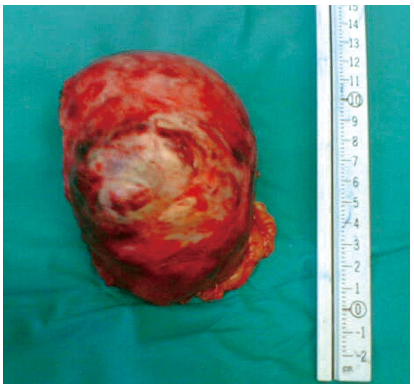

Figure 3 Excised abdominal wall mass.

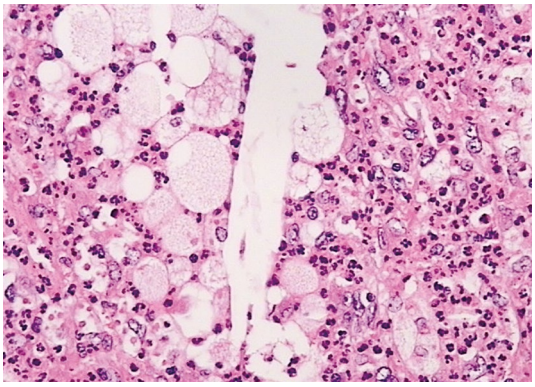

Figure 4 Histological image showing the abscess with polymorphonuclear leukocytes, histiocytes and cholesterol crystals (central part of the photograph). (HE, × 400).

was 36.8℃ and bowel movements were normal. She lost 10 kg during the 3 mo before presentation. The patient had undergone open appendectomy (muscle-splitting incision) 12 years previously. Five years ago she had undergone vaginal hysterectomy with bilateral salpingo-oophorectomy for uterine leiomyomata. Following surgery the patient was in good health and without any symptoms or complaints. She did not take any medications. Physical examination revealed a slightly tender mass, 10 cm in diameter, under the appendectomy scar in the right lower abdomen. The swelling was elastic and poor in mobility. Other resistances were not found, and the rest of the physical examination was normal.

Preoperative laboratory findings and plain abdominal radiographs were normal. Tumor markers were as follows: CEA = 2.42 μg/L; AFP 1.24 μg/L; CA19.9 = 4.89 kU/L and CA 125 = 5.80 kU/L. Abdominal ultrasonography demonstrated a low-echoic mass lesion 8 cm × 8 cm just lateral to the cecum and in communication with the lateral abdominal wall. No peristalsis or communication with the bowel lumen was observed. Esophagogastroscopy revealed chronic gastritis and colonoscopy revealed sigmoid diverticulosis and normal mucosa in the cecum with normal ileocecal valve. Multi-slice CT scanning showed an inhomogenous abdominal mass with minimal vascularization in the right lower abdomen which was 8.6 cm × 8 cm × 9 cm in size and communicated with the abdominal wall. The abdominal wall was thickened, weak and bulging (Figure 1). There was no communication between the cecum and the abdominal wall which was also confirmed by previous colonoscopy (Figure 2). From these findings an abdominal wall tumor was suspected and elective surgery was performed.

After a midline laparotomy and adhesiolysis, the greater omentum was detached from the mass in the right lower abdomen which was located intraperitoneally. There was no free intraperitoneal fluid or fibrin deposits. The elastic mass was adherent to the abdominal wall and there was no communication with the small and large bowel, retroperitoneal or vascular structures. After partial omentectomy, the mass was completely extirpated from the abdominal wall (Figure 3). Histopathological examination revealed a foreign-body granuloma with a central abscess (Figure 4). The patient's early postoperative course was uneventful and she left hospital on the 10th postoperative day. On several control examinations during the first 18 mo, the patient was completely symptomless.

DISCUSSION

This case represents an unusual complication of a suture granuloma with intra-abdominal extension as a cause of an abdominal wall mass 12 years after open appendectomy.

To our knowledge (Medline search 1962-2007) this is the third case of such a complication after appendectomy. Abdominal wall abscesses after appendectomy were diagnosed by Matsuda *et al*[2] 11 years postoperatively and by Ichimiya *et al*[3] 25 years postoperatively.

A unique feature in our case was the intra-abdominal extension of the abdominal wall suture granuloma with a central abscess which complicated definitive diagnosis.

Since the development of various surgical incisions and appendectomy techniques, many early and late postoperative complications and coincident conditions have become evident. One of these complications is a postoperative abdominal wall mass in the region of McBurney's muscle-splitting incision. Morbidity associated with appendectomy can be as high as 25% in complicated cases[4]. Morbiditiy can be divided into early and late complications. Early complications are more common and mostly include wound hematoma, seroma, abscess or intra-abdominal abscess due to persistence of cavities between the muscle layers and the subcutaneous tissue which encourages fluid collections[5]. These complications can then result in cystic formations or masses that can simulate a tumor of the abdominal wall, if they are not readily resolved. For this reason, a meticulous surgical technique, careful hemostasis and placement of suction drains in the subcutaneous tissue are recommended, principally in obese patients[6]. Late complications are rare and can include obstruction due to adhesions, postoperative hernia or progression of inflammatory bowel disease not evident at operation for suspected appendicitis. Most of these complications can present as an abdominal mass. An abdominal mass as a primary pathology or postoperative finding always makes precise preoperative diagnosis difficult. A complete list of differential diagnoses of late presenting abdominal wall masses after open appendectomy is shown in Table 1.

Table 1 Differential diagnosis of late presentation abdominal wall masses after open appendectomy

Suture granuloma
Rectus hematoma
Spontaneous
Traumatic
Postoperative
Wound hematoma (organized)
Abscess
Abdominal wall (various etiologies)
Intra-abdominal (extension)
Hernia
Incisional
Spigelian
Groin
Keloid
Traumatic neuroma
Heterotopic bone formation
Incisional
Traumatic
Abdominal wall tumors
Benign (various)
Malignant (various)
Metastatic
Hematogenous
Post-instrumentation
Port site/trocar metastases
Incisional site metastases
Percutaneous
Intra-abdominal malignancy (extension)
Urachal remnant/cyst/inflammatory mass
Uterine/extrauterine (lipo) leiomyomas
Primary
Incisional
Endometriosis
Cutaneous (primary)
Surgical scar endometriosis
Mastocytosis
Systemic juvenile xanthogranulomatosis
Lymphoproliferative disorders (congenital/acquired)
Parasitic (abscess or granuloma)
Enterobius vermicularis
Hydatid cyst
Mycetoma (endemic)
Actinomycosis
Extension from intestinal actinomycosis
Abdominal wall (hematogenous)

Several points should be stressed. Firstly, abdominal wall masses could be: (1) appendectomy-related; (2) primary; (3) posttraumatic; and (4) related to other interventions in the surrounding area for other pathologic conditions. Thus, history taking and physical examination are crucial. The time interval from appendectomy is essential because it determines the difference between early and late complications of appendectomy. Furthermore, symptoms and signs of unrelated diseases (local or systemic) should be confirmed or ruled out (abdominal wall tumors, extension of intra-abdominal malignancy, endometriosis, lymphoproliferative disorders *etc*). Confirmation of invasive interventions in the surrounding area is very important. Open/laparoscopic surgery for intra-abdominal malignancy/infectious diseases, percutaneous or laparoscopic biopsy or percutaneous fine-needle aspiration for malignant hepatobiliary disease could be the cause of abdominal wall port site or incisional metastases or abscesses.

Secondly, by delineating the peritoneal line, the intraperitoneal or extraperitoneal location of the lesion can be determined. This is important for several reasons. First, the entrance into the peritoneal cavity could be avoided during surgery if the lesion is located extraperitoneally. Also if the abscess is the cause (acute or chronic) then the extraperitoneal route avoids spillage of contents into the abdominal cavity thus eliminating the possibility of intra-abdominal abscess as a postoperative complication.

Generally, early postoperative masses are easier to diagnose and treat, while late postoperative abdominal wall masses could be of various etiologies that warrant the use of a wide spectrum of diagnostic modalities and consequently different treatment options. All these facts signify the importance of preoperative diagnosis. Thus, abdominal ultrasound, contrast-enhanced multi-slice CT and other diagnostic modalities should be used according to clinical findings. It is concluded that late postoperative abdominal wall masses after open appendectomy can be of various etiologies that warrant

the use of a wide spectrum of diagnostic modalities and consequently different treatment options.

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No evidence demonstrating hepatotoxicity associated with hydroxycitric acid

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Abstract

Although a number of cases of hepatotoxicity are associated with the use of Hydroxycut weight management products, it has been alleged that their effects are primarily due to the presence of hydroxycitric acid (HCA, as Super CitriMax) in the formulations. However, while these products contain up to 20 different ingredients, some do not contain HCA. Case studies reported to date have not considered in depth the literature on the numerous animal and human studies that have been conducted on the safety and efficacy of HCA. No HCA-associated hepatotoxicity or treatment-related adverse effects have been reported in these studies, and thus it is premature to make the assumptions presented in the recent case studies regarding Hydroxycut. If it is established in well controlled studies that the use of these formulations with and/or without HCA can result in the occurrence or progression of hepatotoxicity, additional studies should be conducted to characterize the causative factor(s).

Key words: Hydroxycitric acid; Super CitriMax; Hydroxycitric acid-SX; Garcinia cambogia; Hydroxycut; Liver failure; Hepatotoxicity; Safety and efficacy

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TO THE EDITOR

In the Letter to the Editor by Lobb[1], the author has hypothesized that the putative hepatotoxicity of the dietary supplement, Hydroxycut, may be due to one of its components, namely hydroxycitric acid (HCA)derived from *Garcinia cambogia*. However, it is important to note that, of the 14 different formulations of Hydroxycut that have been marketed, only 8 contain HCA. In general, these products are cocktails containing up to 20 different ingredients. In the case studies cited by the author[1], information is not provided regarding the specific Hydroxycut products that were used in each of the case reports. This is of concern given that of the numerous ingredients present in these products, no specific reason was given for identifying HCA as the putative hepatotoxic agent other than as the possible hepatotoxic agent in some of the previous case reports. Because of the name of this product line, it is understandable that a possible word association might be made with HCA. Can the reported hepatotoxicity of these products (assuming it is correct) be attributed to an ingredient or combination of ingredients? No information or discussion was presented regarding the potential hepatotoxicity of any of the other ingredients or combinations of ingredients, and it is distracting to make inferences without sound research support.

The importance of the issues raised above is underscored by the numerous animal[2-8] and human[5,9-12] studies on the safety and efficacy of HCA (as Super CitriMax, HCA-SX). Regrettably, none of these studies was referenced and discussed by the author of this Letter to the Editor[1]. In experimental animal studies at up to 25X the human equivalency dose of HCA, no reports are available on hepatotoxicity or other adverse effects. A HCA dose of 2500 mg/kg, equivalent to 150 000 mg in a 60 kg individual, had no adverse effect in the tested animals[2-4].

Soni *et al*[5] have summarized the results of 15 HCA human clinical studies, 14 of them were double blind and placebo controlled, and one was a single arm, open trial. No treatment-related adverse effects have been reported in any of these studies. These authors concluded that HCA at a level up to 2800 mg/d is safe for human consumption. The combined data strongly suggest that HCA itself is not the culprit with respect to the case studies reporting hepatotoxicity associated with Hydroxycut use. In the Health Hazard Report on Hydroxycut by Mozersky *et al*[13], the Board noted that it did not know what ingredient(s) present in these products can cause hepatotoxicity, assuming the products are indeed the causative agents. More studies are needed before a definitive conclusion can be made.

Interestingly, several animal studies have suggested that HCA may have hepatoprotective[14,15] and chemoprotective[16] properties. In addition, Kaats[17] has recently conducted a 60-d study on 25 human subjects using a product containing 4600 mg/d of HCA. The results showed no evidence of adverse effects and indicated hepatoprotection based on decreasing values for the hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

It should be noted that the dried fruit of *Garcinia cambogia*, a source of HCA, has been consumed for centuries throughout Southeast Asia[18] and is in the USDA' s list of Perennial Edible Fruits[19]. Consistent with the above findings, HCA (as Super CitriMax) has been generally recognized as safe (GRAS) by the Burdock Group, one of the nation's leading food ingredient safety and toxicology groups.

Finally, a key issue that was not discussed by Lobb[1] is the possibility that other co-consumed substances, such as acetaminophen, alcohol, or a wide range of prescription drugs, may have been responsible for the hepatotoxicity. The referenced case study by Shim and Saab[20] does in fact note that acetaminophen was consumed along with aspirin. Acetaminophen toxicity is the leading cause for calls to Poison Control Centers in the United States and results in almost 500 deaths annually due to acute liver failure[21].

There is no question that issues exist with respect to the regulation, quality control, and appropriate safety and efficacy studies of supplements, just as there are issues with numerous drugs, including acetaminophen, that cause extensive morbidity and mortality.

However, to point an accusatory finger at an ingredient that has been extensively studied and for which no adverse effects have been reported in animal and human studies, is counterproductive.

Given the widespread use of dietary supplements in the USA as well as in other countries, it is imperative that sound science should be used in the evaluation of the potential negative as well as the positive effects of these products. With respect to the potential negative effects of some of these products, an important step forward in this regard is the current requirement for adverse event reporting. However, it is important to note that these reports typically reflect the associations, rather than the clear-cut cases of causality. When the associations are noted, they should be rigorously examined, and if the supplements are found to be the causative factors for the pathology reported, the true agents need to be firmly identified, along with the dose at which the negative effects are induced.

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LETTERS TO THE EDITOR

Arterial embolization is the best treatment for pancreaticojejunal anastomotic bleeding after pancreatoduodenectomy

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Abstract

Massive pancreaticojejunal anastomotic bleeding, mainly from the gastroduodenal stump, is one of the most common complications of pancreatoduodenectomy. Selective angiography should be systematically the first step of investigative procedure in such situations. Pharmacoarteriography may be used if the bleeding point is not spontaneously identified, and allows safe and effective treatment with transcatheter arterial embolization compared to blind open surgical hemostasis. Coil embolization of the common or proper hepatic artery on either side of the bleeding point with "sandwich technique" is then the preferred technique to prevent retrograde filling. Surgery should be performed only as a last resort.

Key words: Pancreatoduodenectomy; Complication; Anastomotic bleeding; Hepatic artery; Transcatheter arterial embolization

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TO THE EDITOR

We read with great interest the recent article by Liu *et al*[1] published in the April issue of the "*World Journal of Gastroenterology*" comparing the results of transcatheter arterial embolization and open surgical hemostasis in the treatment of patients with massive pancreaticojejunal anastomotic hemorrhage after pancreatoduodenectomy. We have several comments. Transcatheter embolization is now accepted as the salvage treatment of choice for acute bleeding from the upper gastrointestinal tract. Many published studies have confirmed the feasibility of this approach and the high technical and clinical success rates, ranging from 91% to 100% and from 63% to 100%, respectively, in all case-series including more than 10 patients over the last decade[2,3]. First, we are surprised in this study that 6 (35.3%) of the 17 patients had no angiography prior to additional open surgical hemostasis. In our experience, arteriography plays the primary role in the initial investigation of active gastrointestinal bleeding after pancreatoduodenectomy and should be the first step of investigative procedure in such situations, even in hemodynamically unstable patients. It was reported that the gastroduodenal artery stump is one of the main sources of pancreaticojejunal anastomotic hemorrhage after pancreatoduodenectomy[3], as confirmed in this study. Selective angiography of the celiac trunk and common hepatic artery allows in the majority of cases to detect extravasation of contrast medium. However, it is usually difficult to catheterize the gastroduodenal artery stump. Then, we think that coil embolization of the common or proper hepatic artery on either side of the bleeding point ("sandwich technique") is preferable to prevent retrograde filling[4]. It seems unlikely that this technique was used by the authors, probably explaining recurrent bleeding in 2 (20%) of the 10 patients treated with transcatheter arterial embolization. Liver failure rarely occurs when hepatic artery embolization is achieved with this technique. However, verification of portal venous flow and the absence of underlying liver disease prior to embolization are required. When

the celiac trunk and common hepatic arteriograms are negative, selective catheterization of the superior mesenteric artery must be performed routinely to increase the probability of visualizing active bleeding, because the inferior pancreaticoduodenal artery sometimes supplies the pancreaticojejunal anastomosis. Furthermore, intraarterial anticoagulants, vasodilators, or fibrinolytic agents may be used during angiography to directly elicit contrast medium extravasation, thereby significantly facilitating embolization. In conclusion, we agree with the authors about the safety and efficacy of transcatheter arterial embolization for the treatment of acute hemorrhage after pancreatoduodenectomy. Angiography should be performed first in such situations. In most cases, embolization obviates the need for surgery and is associated with lower complications and mortality rates than open surgical hemostasis.

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Meetings

Events Calendar 2009

January 12-15, 2009
Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
Hong Kong Convention and Exhibition Centre, Hong Kong, China
19th Conference of the APASL
http://www.apasl2009hongkong.org/en/home.aspx

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel
Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)
http://www.easl.ch/

May 17-20, 2009
Denver, Colorado, USA
Digestive Disease Week 2009

May 29-June 2, 2009
Orange County Convention Center
Orlando, Florida
45th ASCO Annual Meeting
www.asco.org/annualmeeting

May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
McCormick Place, Chicago, IL
DDW 2009
http://www.ddw.org

June 17-19, 2009
North Bethesda, MD
Accelerating Anticancer Agent Development

June 20-26, 2009
Flims, Switzerland
Methods in Clinical Cancer Research (Europe)

June 24-27 2009
Barcelona, Spain
ESMO Conference: 11th World Congress on Gastrointestinal Cancer
www.worldgicancer.com

June 25-28, 2009
Beijing International Convention Center (BICC), Beijing, China
World Conference on Interventional Oncology
http://www.chinamed.com.cn/wcio2009/

July 5-12, 2009
Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

August 1-7, 2009
Vail Marriott Mountain Resort, Vail, CO, United States
Methods in Clinical Cancer Research

August 14-16, 2009
Bell Harbor Conference Center, Seattle, Washington, United States
Practical Solutions for Successful Management
http://www.asge.org/index.aspx?id=5040

September 23-26, 2009
Beijing International Convention Center (BICC), Beijing, China
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists(IASGO)
http://iasgo2009.org/en/index.shtml

September 27-30, 2009
Taipei, China
Asian Pacific Digestive Week
http://www.apdwcongress.org/2009/index.shtml

October 7-11, 2009
Boston Park Plaza Hotel and Towers, Boston, MA, United States
Frontiers in Basic Cancer Research

October 13-16, 2009
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009
Versailles, France
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009
Boston, MA, United States
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November 15-19, 2009
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009
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Instructions to authors

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Acknowledgments

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

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5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

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8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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