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

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Pediatric non-alcoholic fatty liver disease: Preventive and therapeutic value of lifestyle intervention

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Abstract

Nonalcoholic fatty liver disease (NAFLD), ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), and eventually cirrhosis and liver failure, is seen to be increasing amongst Western children. NAFLD rates are rising in parallel with the epidemic of childhood obesity, and in particular, fatty liver evolves more easily in NASH when poor dietary habits and sedentary lifestyle are combined. In fact, its general prevalence in the child population varies between 2.6% and 10%, but increases up to 80% in obese children. Since NASH is expected to become the most common cause of pediatric chronic liver disease in the near future, there is broad interest amongst clinical researchers to move forward, both in diagnosis and treatment. Unfortunately, to date, the expensive and invasive procedure of liver biopsy is seen as the gold standard for NASH diagnosis and few noninvasive diagnostic methods can be applied successfully. Moreover, there are still no approved pharmacological interventions for NAFLD/NASH. Therefore, current management paradigms are based upon the presence of associated risk factors and aims to improve an individual's quality of life, thus reducing NAFLD-associated morbidity and mortality. Today, lifestyle intervention (diet and exercise) is the treatment of choice for NAFLD/NASH. Thus far, no study has evaluated the potential preventive effect of lifestyle intervention on children at risk of NAFLD/NASH. Future studies will be required in this area with the perspective of developing a national program to promote nutrition education and increase physical activity as means of preventing the disease in individuals at risk. Here, we outline the clinical course, pathogenesis and management of NAFLD in children, highlighting the preventive and therapeutic value of lifestyle intervention.

Key words: Fatty liver; Children; Lifestyle; Diet; Exercise; Prevention

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD), a worldwide health problem principally affecting millions of people in Western countries, ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), and eventually cirrhosis and liver failure. A few population-based studies have reported that NAFLD/NASH prevalence has been increasing over the past three decades, both in children and adolescents, presenting a worldwide problem[1-3]. The rate of prevalence, ranging from 2.6% to 10%, increases with age and the number of risk factors associated with NAFLD. The most prominent risk factor for NAFLD/NASH is overweight/obesity and the disease is most common in male adolescents[4,5]. Studies of the prevalence of NAFLD in overweight/obese children have reported values which range from 8% to 80%[2,4]. Discrepancies in prevalence data reported in these studies depend on the methods used for diagnosis[6-11]. In fact, although definitive diagnosis of NASH and fibrosis staging and grading requires liver biopsy, most studies have been limited to the use of indirect measures, such as elevated serum alanine transaminase (ALT) and ultrasound to predict histological outcome[4,12].

The clinical course of NAFLD, as well as the management of fatty liver and steatohepatitis, is complicated by limited knowledge of the natural history and pathogenesis of the disease, and the paucity of safe and effective treatment modalities[1,13]. Most NAFLD children are asymptomatic and the few signs and symptoms that are observed are often nonspecific. NAFLD is a multifactorial disease that clinically may or may not comprise elevated serum ALT levels, hyperlipidemia, hyperglycemia and insulin resistance, associated with increased body weight and echogenic liver that is suggestive of hepatic steatosis[13-15]. Over the initial period of disease, many patients progress to the more advanced form of NAFLD, which combines the mentioned dysmetabolic pattern with severe liver injury, including steatohepatitis, necro-inflammation and fibrosis[16].

In contrast to the past, there is today, a widespread and growing recognition of the disease, but some aspects of its pathogenesis and multi-causality still remain obscure[17,18]. As a consequence, currently, it is only the presence of associated risk factors that contributes to updating the management program of pediatric NAFLD, which is essentially focused on improving the individual's quality of life, thus reducing NAFLD-associated morbidity and mortality. In this management program, lifestyle intervention (diet and exercise) is the best choice of treatment for NAFLD/NASH in children[19,20]. However, the potential preventive effect of lifestyle intervention on children at risk of NAFLD/NASH has still not been evaluated.

Here, we outline the clinical course, pathogenesis and management of pediatric NAFLD in children, and highlight the preventive and therapeutic impact of lifestyle intervention. In addition, we suggest further studies in the area of NAFLD prevention in children, but we also presume that soon a national program will be undertaken to promote nutrition education and increase the physical activity for preventing the disease in individuals at risk.

CLINICAL COURSE AND PATHOGENESIS OF PEDIATRIC NAFLD

Pediatric NAFLD, as in adults, is defined as fat accumulation in the liver that exceeds 5%-10% by wet weight, in the absence of excessive alcohol consumption[21]. Although, the natural history of NAFLD is poorly understood in children, it is a multifactorial liver disease that comprises a large spectrum of clinical features: simple fatty liver accumulation (hepatic steatosis); steatosis accompanied by inflammation and other evidence of cellular injury, including various degrees of fibrosis (NASH); and end-stage liver disease, such as rare cases of cirrhosis and hepatocellular carcinoma[12,22].

All genetic and environmental factors responsible for fatty liver and its progression to NASH are still obscure. The most widely accepted model is a "multiple hits" process (Figure 1), during which a first hit induces accumulation of fat in the liver, which causes hepatic steatosis, and renders hepatocytes more susceptible to additional cofactors (i.e. oxidative stress, mitochondrial dysfunction, overproduction and release of pro-inflammatory cytokines, adipocytokine imbalance, and stellate cell activation), which induce persistent liver injury that leads to NASH[17,23,24].

Causes of hepatic steatosis

Hepatic steatosis is caused by imbalance between the delivery of fat in the liver and its subsequent secretion or metabolism. Fat accumulates in the liver for different reasons, in particular because of: excessive intake of dietary free fatty acids (FFAs), *de novo* hepatic lipogenesis, and great liver FFA influx caused by insulin resistance[25,26]. Interestingly, Donnelly *et al*[27] have demonstrated that liver FFA accumulation in NAFLD subjects derives from non-esterified fatty acids for about 60%-80%; *de novo* lipogenesis for 26%, and originates from diet for about 15%. These findings reinforce the hypothesis that several intracellular pathways may contribute to the accumulation of hepatic fat in NAFLD. These pathways include deregulation of β oxidation, decreased hepatic lipid export *via* very low-density lipoproteins, increased lipogenesis due to insulin-resistance-dependent activation of sterol regulatory element-binding protein (SREBP-1c), and glucose-regulated activation of carbohydrate response element-binding protein (ChREBP)[28].

Steatohepatitis and fibrosis

Several factors play central roles in the second-hit progression from simple steatosis to NASH. Various mechanisms have been proposed, including increased oxidative stress, inflammation, hepatocellular apoptosis and fibrogenesis[17,29,30]. There is accumulating evidence that oxidative stress and mitochondrial dysfunction are relevant in the pathogenesis of steatohepatitis, whatever its initial cause[31,32]. Moreover, oxidative stress and mitochondrial dysfunction, with insulin resistance, form a complex network of interactions, which promotes progressive liver injury (fibrosis), which causes chronic accumulation of liver FFA, antioxidant depletion, enhanced cytokine-mediated hepatoxicity, and promotion of stellate cell activation and proliferation[33-35]. This last event ultimately results in increased inflammation, apoptosis and liver fibrosis[36].

MANAGEMENT OF PEDIATRIC NAFLD/NASH: FIRST-LINE TREATMENT AND PROMISING THERAPEUTIC AGENTS

Significantly high levels of triglycerides, glucose, insulin, serum ALT, increased body mass index (BMI) and waist circumference (central adiposity) are all possible clinical features of pediatric NAFLD, which suggests that interventions on these variables can help to cure fatty liver, as well as to prevent progression to NASH[37]. On the other hand, resolution of histological abnormalities revealed by liver biopsy, is, at this time, the main target of NASH treatment[20,38].

Several recent studies have been carried out to

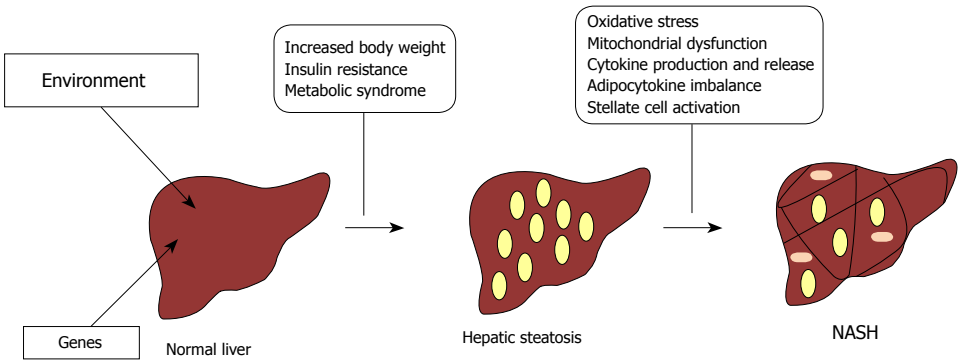

Figure 1 Nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) pathogenesis.

examine the effects of dietary composition on NAFLD/NASH in children[39-41]. However, some others have also looked at the effects of energy restriction combined with physical activity and pharmaceutical treatments (i.e. vitamin E)[42,43].

Diet and exercise

As most NAFLD children are overweight/obese, weight loss may help to reduce pediatric NAFLD prevalence. Weight loss can be achieved through diet and proper exercise, it leads to significant improvement in serum ALT and liver histology in adults with NAFLD[44,45]. Studies on pediatric subjects have shown that moderate weight loss can improve serum BMI and levels of ALT, and reduce fatty liver infiltration and necro-inflammation, although no change has been demonstrated in degree of fibrosis[43,46].

Based upon knowledge of NAFLD pathogenesis, a proper diet might be a low-glycemic index diet; in fact, a similar diet may lead to reduction in serum ALT and hepatic steatosis[47,48]. Nevertheless, a rapid and excessive caloric restriction and weight loss is not recommended because it might potentially increase dysmetabolism and liver injury[12].

Vitamin E

As oxidative stress play a pivotal role in NASH pathogenesis, the use of natural antioxidants, such as vitamin E, is under investigation as a therapeutic approach for NASH patients. Vitamin E has been shown to improve ALT and liver histology in adults with NAFLD[49]. However, only one open-label study has demonstrated that 2-4 mo treatment with vitamin E normalizes serum ALT in obese children[50]. The efficacy of vitamin E is currently under investigation in a histology-based, double-blind, randomized, placebo-controlled study conducted by NASH Clinical Research Network, and its results will be available in 2010 (ClinicalTrials.gov Identifier: NCT00063635).

Insulin sensitizers

Most pediatric NAFLD patients present with insulin resistance, therefore, another approach to decreasing dysmetabolic and histological features associated with this liver disease is treatment with insulin-sensitizing agents. Metformin, a biguanide, is the only insulin-sensitizing agent that has been evaluated for the treatment of pediatric NAFLD. Metformin seems safe and effective in treating type 2 diabetes in children[51,52]. In addition, metformin has been evaluated in several pilot studies, which have demonstrated a significant improvement in ALT and hepatic steatosis[3]. A pediatric, randomized controlled trial with metformin as a monotherapy in NAFLD is now underway (ClinicalTrials.gov Identifier: NCT00063635). Also, thiazolidinediones, such as pioglitazone and rosiglitazone, have been used successfully for improving insulin resistance and possibly liver histology in adults, but their use in children still requires an accurate control study before they can be considered for use in clinical practice[53-55].

Ursodeoxycholic acid (UDCA)

UDCA may act as a cytoprotective and antioxidant agent. It is able to reduce ALT levels and improve liver histology in adults with NAFLD[56]. However, in a randomized control trial, Vajro *et al*[57] have demonstrated by ultrasound that UDCA is ineffective in improving serum ALT or steatosis, both alone and in combination with diet. On the other hand, another randomized controlled trial has shown that UDCA in combination with vitamin E may improve serum ALT and liver histology, but also decrease hepatocellular apoptosis and restore serum levels of adiponectin[58,59].

PREVENTIVE AND THERAPEUTIC EFFECTS OF LIFESTYLE INTERVENTION ON SEVERITY AND OUTCOME OF NAFLD

Patients suffering early liver dysfunction, such as simple hepatic steatosis, or at risk of developing a severe disease, including NASH and cirrhosis, require early diagnosis and intensive treatment. As already discussed, treatment options are limited and dietary weight loss is recommended. Although diets are often difficult to adhere to, they also have an enormous preventive value. In fact, a management program (Figure 2) that incorporates and encourages an adequate diet and age-appropriate physical activities may not only promote a healthy lifestyle, but also prevent the development of NAFLD/NASH.

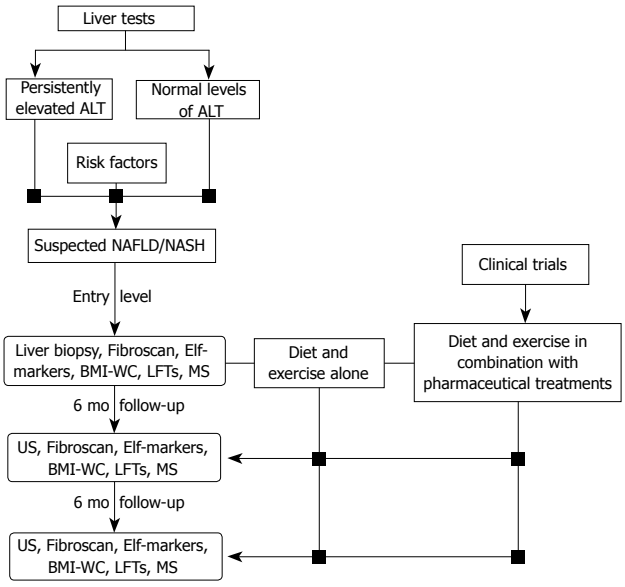

Figure 2 Management flowchart for NAFLD/NASH treatment and prevention. Elf: Enhanced liver fibrosis; BMI-WC: Body mass index-waist circumference; LFTs: Liver function tests; MS: Metabolic syndrome; US: Ultrasonography.

NAFLD-preventive effects of diet and exercise

Weight loss has been, until now, the only proven therapy for pediatric NAFLD. Thus, diet and exercise can be considered the first-line of defense for preventing the onset of NAFLD and progression to NASH in children at high risk. As demonstrated by several recent studies, the high-risk category comprises not only overweight/obese subjects, but also children with insulin resistance, metabolic syndrome, type 2 diabetes, and those with low birth weight[2,60,61].

However, recommendations for lifestyle modifications should be chosen according to the patients' general health because very rapid weight loss might expose patients to severe metabolic consequences and increased mortality risk.

We recommend a hypocaloric diet of 25-30 cal/kg per day in case of overweight/obese subjects, and isocaloric diet (40-45 cal/kg per day) for normal-body-weight children. The amount of calories prescribed also takes into account physical activity and daily routines. Diet composition consists of low gastrointestinal carbohydrate (50%-60%), fat (23%-30%), and protein (15%-20%); a fat composition of two-thirds unsaturated and one-third saturated; and a ω6/ω3 ratio of approximately 4:1, in accordance with the Italian Recommended Dietary Allowances. Diet is tailored to individual preferences to improve compliance, which may be particularly poor in children and adolescents. A moderate daily exercise program, consisting of 45 min/d of aerobic physical activity, is also recommended. At each visit, subjects or their parents fill out a 3-d dietary and physical activity recall to evaluate adherence to lifestyle recommendations. A multidisciplinary team including dieticians, hepatologists, endocrinologists, psychologists, and cardiologists evaluates and closely follows up the patients. Participants and their parents are instructed on how to exercise, and maintain adherence to the exercise program, by a skilled exercise physiologist as part of our multidisciplinary program. Every 6 mo after treatment, children with NAFLD undergo ultrasonography, laboratory analyses, dietician evaluation and psychological tests. In Table 1, we report our success and failure rates after therapeutic intervention in children with biopsy-proven NAFLD.

Table 1 Success and failure rates after therapeutic intervention in children with liver-biopsy-proven NAFLD *n* (%)

	Lifestyle intervention	Vitamin E	Metformin
n	29	25	28
Complete success (all endpoints)	11 (38)	9 (36)	2 (7)
Partial success			
Serum ALT normalization	23 (79)	13 (52)	23 (75)
HOMA-IR ⩽ 1 restoration	11 (38)	9 (36)	2 (7)
NAS amelioration	19 (65)	17 (68)	16 (57)
Complete failure	4 (14)	8 (32)	2 (7)

NAFLD: Nonalcoholic fatty liver disease; HOMA-IR: Homeostatic model assessment of insulin resistance; NAS: NAFLD activity score; ALT: Alanine transaminase.

Thus, recommendations for lifestyle intervention in children at high risk and in subjects with fatty liver should follow a program based on an integrated care model that encourages patients, as well as family members, to adopt diet and exercise goals to prevent NAFLD/NASH development.

Integrated care model

NAFLD patients require a multidisciplinary approach that involves health professionals with different areas of expertise. This is even more crucial for pediatric patients, whose care also involves their families and other care providers, such as school personnel. In this respect, it is necessary to identify clearly a case manager with a strong leadership role, who can coordinate case management. Case management is defined as the process of planning, co-ordinating, managing and reviewing the care provided in order to ensure that it responds to the appraisal needs. The challenge of this model is for different professionals to work transversally and concurrently, placing the patient at the core of the system. An effective case management system should therefore be based on different steps[62].

The first step is the strategic planning and preparation of services, which includes different activities, with the integration of skills in a team of professionals who identify and overcome individual and organizational barriers, to guarantee timely access to health care. Coordination, monitoring and evaluation of the results gained by the effort of these integrated teams should be conducted on a regular basis, because they are paramount to achieving proper implementation.

The second step is the management of information. In fact, in order to work in an integrated manner, it is essential to achieve good communication among all the professional specialties involved.

The third step of this model stems from the flow of information between the various professionals and

the patient. In this regard, there must be an educational function towards the patients and their relatives, in order that they may also become actively involved in the decisions and in the implementation of required treatments.

At the Bambino Gesù Hospital, this integrated care model is becoming the health-care model of choice. Beginning in 2003, the Liver Unit implemented the multidisciplinary outpatient clinic for the diagnosis and monitoring of patients affected by NAFLD/NASH. The hepatologist acts as case manager, establishes the individual patient's care program, and coordinates clinical activities with the goal of ensuring that all of them are conducted on the day of the outpatient visit.

In addition to the hepatologist, the multidisciplinary team includes: the endocrinologist, because these patients frequently present with metabolic syndrome, with a predisposition to hyperinsulinism, to glucose intolerance and to type 2 diabetes; the cardiologist, who takes care of cardiovascular issues, ranging from arterial hypertension to increased risk of cardiovascular diseases; the radiologist, for the monitoring of hepatic lesions; the dietician for following appropriate dietary changes and increased physical activity prescribed on an individual basis; and the psychologist to consider psychological attitudes which may have preceded, and perpetuate an unhealthy diet and sedentary life style.

As defined in the program, the results of health care and clinical outcomes are evaluated by the team, and are the object of collegial discussion in order to jointly identify obstacles and the most appropriate way to overcome them.

This organisational model, in which the case manager has a central role, guarantees the quality and efficiency of the multi-specialist care process, leading to a favorable cost/benefit ratio, both at the individual and societal level. In our hospital, this model has worked reasonably well. In fact, it has allowed us to improve considerably patient compliance and medication adherence, reaching values close to 80%[43].

CONCLUSION

NAFLD/NASH has become the leading cause of pediatric liver disease in westernized countries. Several trials are ongoing to establish pharmacological treatment of pediatric disease, but health and nutrition strategies, such as better exercise habits and comprehensive approach to weight management in the school and home surroundings, can reduce the public health impact of pediatric NAFLD. We believe that our integrated care model not only provides an efficient and effective model for the care management of NAFLD patients, who need the intervention of health-care providers with different background and expertise, but also offers a good starting point for a national program of nutritional education and exercise for preventing the disease in children at high risk (i.e. overweight/obese children).

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EDITORIAL

Genetic polymorphisms in non-alcoholic fatty liver disease: Clues to pathogenesis and disease progression

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Abstract

The spectrum of non-alcoholic fatty liver disease (NAFLD) ranges from simple steatosis through steatohepatitis to advanced fibrosis and cirrhosis. Although the reason why only a minority of patients develop progressive forms of disease still remains largely unclear, recent research has identified genetic factors as a possible basis for this variation in disease presentation. Most of the studies have been focused on finding associations between advanced disease forms and selected single nucleotide polymorphisms in genes encoding various proteins involved in disease pathogenesis. Although there are many limitations regarding the study design and interpretation of published data, further carefully planned studies together with implementation of new genetic technologies will likely bring new insights into disease pathogenesis and potential benefits to the management of patients with NAFLD.

Key words: Genetics; Liver fibrosis; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Single nucleotide polymorphisms

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common form of chronic liver disease. The spectrum of NAFLD ranges from simple steatosis through steatohepatitis (NASH) to advanced fibrosis and cirrhosis, and the minority of patients progress to end-stage liver disease requiring liver transplantation or develop hepatocellular carcinoma[1]. However, the vast majority of patients only have simple steatosis with a benign long-term prognosis. It has been observed that even when considering patients with similar environmental and metabolic NAFLD risk factors (diet, exercise, obesity and insulin resistance being the most important factors), they still differ largely in terms of disease phenotype and degree of progression[2]. This led to the research focus more recently being placed on genetic factors that may possibly have a role in NAFLD etiology, and genetic variability is now implied to be one of the most important determinants of disease phenotype and progression in individual patients.

GENETIC INFLUENCES IN NAFLD

Possible genetic risk for advanced NAFLD was initially suggested in studies which showed coexistence of NASH and/or cryptogenic cirrhosis within several kindreds, and it was not invariably associated with similar major metabolic risk factors[3,4]. Further evidence comes from reports of ethnic differences in the prevalence of steatosis, NASH and cryptogenic cirrhosis. The prevalence of all forms of NAFLD was shown to be highest in Hispanic and lowest in African American populations, and this variability did not always correlate with differences in the prevalence of major risk factors[5-7]. Furthermore, it was reported that Asian patients with NAFLD had a significantly lower body mass index (BMI) than all other racial groups[8].

As most of the common diseases today, NAFLD

is considered to be a genetically complex disorder. In complex diseases, several or many different genes interact with environmental factors in determining disease presence or its phenotype, and individual genes only have a small effect on disease risk and can therefore be very difficult to identify. Methods for detecting genes in complex disorders have included family-based linkage studies, hypothesis-based candidate gene allele association studies, genome-wide single nucleotide polymorphism (SNP) scanning and, recently, microarray and proteomic studies. Almost all of the data available on genes associated with NAFLD has so far come from the candidate gene association studies, where candidate genes are usually selected on the basis of their suggested role in disease pathogenesis, and the frequency of one or more known SNPs within or close to those genes is compared in cases and controls, in the search for a positive or negative association with the disease. Genes that are candidates for study in NAFLD have included genes influencing insulin resistance, fatty acid metabolism, oxidative stress, immune regulation and fibrosis development.

GENETIC POLYMORPHISMS

Peroxisome proliferator-activated receptor γ coactivator 1α (PPARGC1A)

PPARGC1A has been involved with different metabolic pathways, such as regulation of gene expression in glucose and lipid metabolism and transcriptional control of cellular metabolism, mainly through control of mitochondrial function and biogenesis[9,10]. Several studies have shown that *PPARGC1A* regulates several key hepatic gluconeogenic genes, is directly involved in the homeostatic control of systemic energy metabolism, and *PPARGC1A* Gly482Ser polymorphism has also been associated with the development of insulin resistance, obesity and diabetes[11-14]. *PPARGC1A* knockout mice are prone to develop hepatic steatosis due to a combination of reduced mitochondrial respiratory capacity and an increased expression of lipogenic genes[15]. Yoneda *et al*[16] therefore examined 15 SNPs in the *PPARGC1A* gene and found that the rs2290602 polymorphism was significantly associated with NAFLD (more closely with NASH than with simple steatosis), and the frequency of the T allele (allele with rs2290602 polymorphism) was significantly higher in the NASH patients than in the control subjects. They also found that intrahepatic *PPARGC1A* mRNA expression was significantly lower in the TT genotype group than in the GG or GT group. On the other hand, Hui *et al*[17] did not find any association between the Gly482Ser variant and NAFLD in Chinese Han people. However, they have reported a correlation between C161T PPAR-γ gene SNP, consequent lower plasma levels of adiponectin and increased susceptibility to NAFLD.

Microsomal triglyceride transfer protein (MTTP)

A higher incidence of -493G/T polymorphism in the MTTP gene promoter has been reported in patients with NAFLD; GG homozygosity was associated with more severe liver histology and has been considered as a risk factor for NAFLD[18]. Gambino *et al*[19] suggested that NASH patients with GG homozygosity have more atherogenic postprandial lipoprotein profiles and lipoprotein metabolism, which leads to increased peroxidative liver injury.

Leptin

Leptin is an adipocytokine whose main role is regulation of food intake. It probably has an important role in the pathogenesis of NAFLD; leptin-deficient ob/ob mice develop steatohepatitis when fed with a methionine-choline-deficient diet[20]. Common variants in the human leptin receptor (*LEPR)* gene have been associated with traits of metabolic syndrome such as obesity, insulin resistance, type 2 diabetes mellitus and altered lipid metabolism, and possibly with NAFLD[21-23]. The *LEPR* 3057 variant may link obesity to NAFLD in Chinese patients with type 2 diabetes mellitus through interference with leptin receptor signaling and regulation of lipid metabolism and insulin sensitivity[24].

Adiponectin

Adiponectin, an adipocyte-derived cytokine has an important role in mobilization, transport and muscle oxidation of free fatty acids leading to improvements in lipid profiles and insulin sensitivity[25,26]. High levels of tumor necrosis factor-α (TNF-α) mRNA in adipose tissue and high plasma TNF-α concentrations were detected in adiponectin-knockout mice, resulting in severe diet-induced insulin resistance[27]. Musso *et al*[28] reported that the adiponectin SNPs 45TT and 276GT/TT were more prevalent in Italian NAFLD patients than in the general population; these polymorphisms independently predicted the severity of liver disease in NASH and exhibited a blunted postprandial adiponectin response and higher postprandial triglyceride levels.

Hepatic lipase

Zhan *et al*[29] investigated the prevalence of the hepatic lipase gene promoter polymorphism at position -514 in Chinese patients with NAFLD. They reported a higher frequency of the CC genotype and C allele in the NAFLD group and both the CC genotype and CT genotypes were associated with higher relative risk for development of NAFLD[29].

Phosphatidylethanolamine N-methyltransferase (PEMT)

Phosphatidylcholine is required for hepatic formation and secretion of very low density lipoproteins, and it has been shown that a choline-deficient diet leads to accumulation of fat droplets in hepatocyte cytosol and the development of fatty liver[30]. PEMT catalyzes *de novo* synthesis of phosphatidylcholine and is responsible for approximately 30% of phosphatidylcholine formed in liver, the rest of it being synthesized by another pathway from dietary choline. Song *et al*[31] showed that SNP (G to A substitution in exon 8) that leads to Val to Met substitution at residue 175 of PEMT is associated

Table 1 Studies of genetic polymorphisms in non-alcoholic fatty liver disease (NAFLD) included

Gene	Polymorphism	Ref.	No. of patients with NAFLD included in the study
Peroxisome proliferator-activated receptor γ coactivator 1α (*PPARGC1A*)	rs2290602	Yoneda *et al*[16], 2008	115
	Gly482Ser	Hui *et al*[17], 2008	96
Microsomal triglyceride transfer protein (MTTP)	-493G/T	Namikawa *et al*[18], 2004	63
		Gambino *et al*[19], 2007	29
Human leptin receptor	G3057A	Lu *et al*[24], 2009	104
Adiponectin	45G/T and 276G/T	Musso *et al*[28], 2008	70
Hepatic lipase	-514C/T	Zhan *et al*[29], 2008	106
Phosphatidylethanolamine *N*-methyltransferase (PEMT)	Val175Met	Song *et al*[31], 2005	28
		Dong *et al*[32], 2007	107
Methylenetetrahydrofolate reductase (MTHFR)	C677T and A1298C	Sazci *et al*[33], 2008	57
Tumor necrosis factor-α (TNF-α)	-238 and -308	Valenti *et al*[38], 2002	99
	-1031, -863, -857, -308 and -238	Tokushige *et al*[39], 2007	102
Angiotensinogen	G-6A	Dixon *et al*[45], 2003	105
Transforming growth factor-β1 (TGF-β1)	Pro25Arg		

with significantly diminished activity of the enzyme, and determined the frequency of this polymorphism in NAFLD patients and controls. The loss of function AA genotype (Met/Met) occurred significantly more frequently in NAFLD patients than in control subjects, which led to the conclusion that genetically inherited low PEMT activity is an important risk factor for developing NAFLD. This was further proven in a Japanese study published by Dong *et al*[32]. Although the polymorphism is much rarer in the Japanese population than in Caucasians, the frequency of A allele was significantly higher in NASH patients compared with controls. NASH patients who were carriers of the Val175Met variant had significantly lower BMI and were more frequently non-obese than NASH patients who were wild-type homozygotes, further proving the role of this polymorphism as an independent risk factor for NAFLD development.

Methylenetetrahydrofolate reductase (MTHFR)

Sazci *et al*[33] investigated whether the C677T and A1298C polymorphisms of the MTHFR gene which lead to hyperhomocysteinemia and development of liver steatosis were associated with NASH. They found that the MTHFR 1298C allele was associated with increased risk for NASH in patients of both genders, C1298C genotype and C677C/C1298C compound genotype in female and C677C/A1298C compound genotype in male NASH patients.

TNF-α

TNF-α has long been proven to be one of the key cytokines in the development of all chronic liver diseases. In NAFLD, it has been shown that it may cause hepatocyte injury and apoptosis, neutrophil chemotaxis, and hepatic stellate cell activation, as well as contribute to systemic and hepatic insulin resistance[34-36]. Crespo *et al*[37] found that obese patients with NASH compared to those without NASH have significantly increased liver expression of TNF-α and its receptor p55, as well as increased expression of TNF-α in adipose tissue. Valenti *et al*[38] investigated the relationship between insulin resistance, occurrence of NAFLD and -238 and -308 TNF-α promoter polymorphisms known to be associated with an increased release of this cytokine. The prevalence of the 238 TNF-α polymorphism was higher in subjects with NAFLD than controls, and patients with these polymorphisms had higher insulin resistance indices. Tokushige *et al*[39] determined the prevalence of several TNF-α promoter region polymorphisms (positions -1031, -863, -857, -308 and -238) in a group of Japanese NAFLD patients and control subjects. There were no significant differences in the allele frequencies of any of the six polymorphisms among the group of patients with NAFLD and the control group, including the -238 polymorphism which was previously reported to be associated with NAFLD in Italian patients, but this polymorphism was much less frequent in the Japanese population[38]. However, the frequency of the -1031C polymorphism was significantly higher in the NASH group compared to the simple steatosis group, as was the frequency of the -863A polymorphism. The frequency of other polymorphisms did not differ significantly between the two groups. These two polymorphisms were also associated with higher levels of insulin resistance measured by HOMA-IR.

Transforming growth factor-β1 (TGF-β1) and angiotensin Ⅱ

TGF-β1 and angiotensin Ⅱ are two molecules that have been extensively studied in models of liver fibrogenesis. TGF-β1 has a major role in development of liver fibrosis by activation of hepatic stellate cells and stimulation of production of extracellular matrix proteins[40]. Besides its well-known effects in the cardiovascular and renal systems, angiotensin Ⅱ also has an established role in liver fibrogenesis, and based on those observations, studies with angiotensin Ⅱ receptor antagonists have been performed in patients with NASH[41,42]. There have been several suggestions that profibrotic effects of angiotensin Ⅱ in heart and kidney are mediated by induction of transcription of TGF-β1[43,44]. Considering these data, and based on their previous study in hepatitis C patients, Dixon *et al*[45] investigated the relationship between the

presence of advanced fibrosis and angiotensinogen G-6A polymorphism or TGF-β1 Pro25Arg polymorphism in a group of severely obese patients. There was no correlation between either high angiotensin or TGF-β1 producing genotypes alone and hepatic fibrosis. However, patients who inherited both high angiotensin and TGF-β1 producing polymorphisms had a higher risk of advanced fibrosis. These data also support the hypothesis that angiotensin Ⅱ stimulated TGF-β1 production promotes hepatic fibrosis.

A comprehensive list of the above-mentioned polymorphism studies is shown in Table 1.

CONCLUSION

While all this and other evidence clearly indicates that genetic factors have a key role in determining susceptibility to advanced forms of NAFLD and its progression, the majority of studies mentioned here had small sample sizes and therefore limited statistical power, which makes it rather difficult to draw definitive conclusions. However, we believe that the development and wider availability of high throughput genetic technologies together with careful design and performance of large multicenter studies with adequate statistical power will soon provide new insights in this vast and very interesting area. Further study and new data on genetic effects have many potential benefits - advancement in understanding the pathogenesis of NAFLD, identification of new potential treatment targets, and, eventually, categorization of patients with respect to disease prognosis, leading to a change in management approach in specific subgroups of patients. Despite the currently limited data on genetic influences in NAFLD and all the difficulties in studying them, we believe that most of the variability in NAFLD presentation will eventually be attributed to and explained by variations in SNP frequencies and their effects on the function of factors involved in the pathogenesis of the disease.

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EDITORIAL

Indian task force for celiac disease: Current status

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Abstract

There are limited data on celiac disease (CD) from India. The limited knowledge about CD in India might be attributed to several factors. The first meeting of the Indian Task Force for Celiac Disease was held in the Asian Institute of Gastroenterology, Hyderabad, India in December 2008. The objectives of the meeting were to focus research on prevalence of CD in the wheat-eating Northern *vs* the rice-eating Southern Indian population, low-budget serological assays to study the underprivileged population, to involve other medical subspecialties in CD, to suggest proper legislation regarding wheat food labeling, and to organize affordable food substitutes for patients with celiac disease.

Key words: Celiac disease; Food labeling; Gluten-free diet; India; Legislation; Malnutrition; Rice; Wheat

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INTRODUCTION

Celiac disease (CD) is an autoimmune disease that is caused by interaction of gluten in genetically predisposed individuals[1]. The diagnosis is based upon European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)/United European Gastroenterology Foundation (UEGF) criteria[2].

CD in India is submerged in an ocean of malnutrition. The limited research on CD in India can be attributed to several factors: (1) a common belief that CD is uncommon in India; (2) recognition of tropical sprue and gastrointestinal tuberculosis as major causes of chronic diarrhea and malabsorption syndromes; (3) non-realization that partial villous atrophy (PVA) may be a feature of CD; (4) more pressing problems of malnutrition; (5) lack of awareness regarding non-diarrheal manifestations of CD[3-5].

The first meeting of Indian Task Force for Celiac Disease was held in the Asian Institute of

Gastroenterology, Hyderabad, India on December 6, 2008. The main objectives of this meeting were to evaluate the Indian data on CD and discuss future research. A panel of experts from different parts of India, who have special interest in CD, took part in this Task Force. Professor Mulder CJJ, VU University Medical Center, Amsterdam, also participated in the meeting as an international expert. All the participants addressed issues specific to CD and discussed the area of future research and the strategies to carry out these objectives. In addition, legal issues related to food labeling and availability of gluten-free food items in India were also discussed.

EPIDEMIOLOGY OF CD IN INDIA

Based on epidemiological studies from Europe and the United States, 90% of CD remains undiagnosed. There are limited data on prevalence of CD from India[6-9]. The majority of data are from Northern India. The incidence of CD is increasing[10]. The prevalence of CD in India is probably not different from that in western Caucasian populations[11]. In a field study conducted among school children in Punjab, the estimated frequency of disease was 1 in 310 (0.3%)[12]. This prevalence is probably an underestimation. The siblings of CD patients have a high prevalence of CD (22%). In other studies, the prevalence of CD among the first-degree relatives has been reported to be 8%-25%[13-15]. There are regional variations in the prevalence of CD due to genetic and dietary factors, that is, the wheat-rice shift from the North to the South in India, which will be discussed in the next section.

REGIONAL DIFFERENCES AND CHANGING EPIDEMIOLOGY IN INDIA

CD has a strong genetic predisposition. The main genetic factors are *HLA-DQ* genes, that is, the genes encoding DQ2 or DQ8 in the HLA complex. In the West, approximately 95% of CD patients have a DQ2 heterodimer comprised of DQB1*02 and DQA1*05, and most of the remaining 5% have a DQ8 heterodimer comprised of DQB1*302 and DQA1*03. Adequate data about DQ2 and DQ8 distribution in India are lacking[16-19].

Regional differences in CD can be explained by genetic, dietary and immunological factors. High prevalence areas of CD such as Saharawis (North Africa, up to 5%) and Europe (1%) have a very high carrier rate of DQ2 and DQ8. On the other hand, Japan and Burkina Faso, which have very low prevalence of CD, have low or absent DQ2 and DQ8 carriage.

Dietary patterns also contribute to geographical differences in CD. Wheat consumption broadly parallels CD prevalence, being particularly low in the Far East and Sub-Saharan Africa. In India also, CD is reported frequently in high wheat-consuming states in Northern India.

Immune conditioning might also influence the development of CD. Dose of gluten in early childhood may be an important determinant of lifelong susceptibility. Breast feeding during gluten induction probably reduces susceptibility[20]. Increased exposure to enteric infections in infancy confers modest increase (1.5 RR) in susceptibility to CD[21] .

CASE FINDING IN HIGH-RISK GROUPS

A wide gap exists in India between the CD prevalence in the population (1%) and the prevalence based on diagnosis (0.02%-0.27%). Thus 90%-95% of CD remains undetected[22]. Several complications might occur among untreated CD subjects. Among non-diarrheal adult cases with gastrointestinal symptoms, diagnosis of CD and treatment with gluten-free diet results in a significant improvement in symptoms of abdominal pain, bloating and lactose intolerance. A twofold increase in standard mortality ratio has been reported in adult CD[23]. Excess mortality occurs in the first 5-10 years after diagnosis among subgroups of patients with malabsorption, delayed diagnosis and poor compliance. Enteropathy-associated T-cell lymphoma (EATL) is an important mortality risk in patients diagnosed above 50 years of age. There is a higher frequency of so-called associated disorders in CD in comparison to controls, such as endocrine disorders, type 1 diabetes mellitus and connective tissue disorders. Higher risk of malignancy in adult CD is known. Over-presentation of cancer occurs in the small bowel, esophagus and T-cell lymphoma. After diagnosis, despite dietary compliance, an increased risk was observed for EATL[24]. On the contrary, a protective role of gluten-free diet has been reported for these so-called associated malignancies[25]. The risk of breast cancer seems lower in CD. A higher frequency of CD occurs with autoimmune disorders. The incidence of autoimmune disorders in CD seems to be related to duration of gluten exposure[26].

Targeted screening of CD might be important[27]. Among children, screening for CD in India is not indicated before age 1-3 years. Compliance with gluten-free diet and giving consent for small bowel biopsy are problems, because the subjects usually are not convinced about investigations and treatment in the absence of severe symptoms. Serological tests like tissue transglutaminase (tTGA) have a positive predictive value of 75%-80%, however, seronegative CD is well-recognized in milder degrees of villous atrophy[28]. In future, we have to define how to interpret serology positivity when biopsies are normal. There is no consensus regarding treating subjects with silent disease with positive serology.

CD IN CHILDREN IN INDIA

CD was first reported in India in 1966[29]. The triad of symptoms of chronic diarrhea/malabsorption, failure to thrive and anemia were common until 2000 in India. However, the presentation of disease seems to have changed over the past few years. An upsurge has been observed by clinicians from North-West India. The so-called typical presentation is now below 50%[30-32].

Symptomatic disease is just the tip of the iceberg but, because of the availability of new serological tests,

we are exploring the hidden CD groups in India. The demographic profile of CD in children in India is different from that in the West[33]. In one Indian study, the male/female ratio was 3:2. The sole atypical presentations were short stature in 20%, anemia in 14%, constipation in 5%, family history of CD in 5%, and rickets in 1.5% of patients. Common associations observed in children were IgA deficiency in 6%, asthma in 2%, type 1 diabetes in 1.5%, autoimmune hepatitis in 1.5%, seizures in 1.5%, juvenile rheumatoid arthritis in 0.7%, Down syndrome in 0.7%, and nephrotic syndrome in 0.7% of patients. The recent upsurge is due to factors like improved awareness among pediatricians, cost-effectiveness of serological tests, and increasing pediatric endoscopic facilities.

CAPSULE ENDOSCOPY IN CD

Video capsule endoscopy (VCE) provides high resolution views of the small intestinal mucosa in a noninvasive manner. Characteristic mucosal abnormalities are seen on capsule endoscopy in CD, which include scalloping of mucosal folds, fissures or grooves, mosaic pattern, and absent or reduced mucosal folds[34]. Although esophagogastroduodenoscopy (EGD) and multiple duodenal biopsies continue to remain the gold standard, VCE may be used for initial diagnosis and follow-up of CD patients. VCE may be a reasonable alternative to upper gastrointestinal endoscopy in those patients who are strongly positive for tTGA or endomysial antibodies (EMAs), who are unwilling to undergo EGD. In patients with positive serology for CD and negative histology, VCE might be of help. VCE is useful in follow-up of patients of CD who remain symptomatic despite being on a gluten-free diet[35].

MAGNIFICATION ENDOSCOPY IN CD

The role of conventional endoscopy in the diagnosis of CD has been limited because of low and varying sensitivity and specificity. The small bowel mucosal damage associated with CD can be distributed unevenly and present as patchy villous atrophy, with some parts appearing normal and others severely diseased[36]. Endoscopic markers are not adequate to target biopsy sampling to sites of villous damage in the duodenum .

In the past few years, newly developed procedures and technologies have improved endoscopic recognition of the duodenum. These new technologies include the water immersion technique, chromoendoscopy, high-resolution magnification endoscopy, narrow band imaging, and optimal band imaging[37]. These new endoscopic techniques have increased the accuracy of CD diagnosis in patients with patchy villous atrophy, and achieve optimal accuracy for the recognition of severe villous atrophy[38].

HISTOLOGICAL FEATURES AND PROBLEMS IN INTERPRETATION

Diagnosis of CD is confirmed by biopsy, with a characteristic mucosal injury in association with a clinical response to a gluten-free diet. Biopsy of the small bowel remains the gold standard for the diagnosis of CD[39]. Normal small intestinal mucosa contains long villi, varying in length depending on orientation and depth of biopsy. Histological features of CD comprise small intestinal mucosal injury, surface enterocyte damage, increased intraepithelial lymphocytes, crypt hyperplasia and villous blunting or flattening. A reliable histological diagnosis of CD requires lifelong adherence to a gluten-free diet, which is expensive, socially limiting and difficult on a contemporary diet with manufactured food stuffs. Pathologists should avoid overdiagnosis based on minimal nonspecific histological changes. The uniform classification according to Marsh and its modification as described by Rostami should be applied, which includes Marsh I lesion (lymphocytic enteritis); Marsh II (lymphocytic enteritis with crypt hyperplasia; Marsh IIIA in addition shows partial villous atrophy; Marsh IIIB, subtotal villous atrophy; and Marsh IIIC, total villous atrophy.

Jejunal biopsies are not necessary anymore if adequate duodenal biopsies are taken. Numerous intestinal disorders can present with a CD-like histology but are not responsive to a gluten-free diet, and therefore, are not CD cases. Villous atrophy is noted in various infections such as giardiasis, tropical sprue, HIV, Whipple's disease, and immune-mediated diseases. In the same way, increased intraepithelial lymphocytes (IELs) are seen in tropical sprue, after nonsteroidal anti-inflammatory use, Crohn's disease, and bacterial overgrowth. In cases of histological features suggestive of CD, the diagnosis should be based on ESPGHAN criteria.

Diagnosis of refractory CD, ulcerative jejunitis and EATL requires multiple biopsies. Identification of the two categories of refractory CD (RCD), Marsh type I without aberrant T cells and type II with aberrant T cells requires correlation with T-cell immunophenotyping by flow cytometric analysis and immunohistology. An increase in IELs in uncomplicated CD shows a phenotype of sCD3+, CD8+, γ^+ population of T cells, which contrasts with RCD II, which shows an aberrant immunophenotype of sCD3- cCD3+, CD8-. Immunostaining methods using anti-CD3 and anti-CD8 antibodies distinguish active CD from RCD.

Pathologists should be attentive to recognize the less severe histopathological abnormalities of Marsh type I and II CD, and must be aware of the pitfalls in the assessment of mucosal biopsy specimens[40]. In general, we do not advise a gluten-free diet for Marsh type I lesions, unless serology (tTGA and EMA) is positive and the patients are symptomatic for CD.

ATYPICAL CD

Atypical presentations of CD are on the rise in children and adults[41]. Patients may present with CD-related symptoms in other specialties, such as cardiology, hematology, ENT, endocrinology, dermatology and dental services. Clinicians should be aware of CD. Screening for CD should be considered in unexplained anemia, unexplained gastrointestinal symptoms, idiopathic

osteoporosis, unexplained infertility, first-degree relatives of CD patients, and autoimmune diseases.

DIETARY COMPLIANCE IN CD

CD is well recognized in most parts of the world where wheat is the staple diet. Irrespective of the manifestations of CD, the mainstay of treatment is a gluten-free diet. Proper dietary compliance leads to alleviation of symptoms, improvement of anthropometry, improvement in quality of life, and prevention of EATL and osteoporosis. It is important to determine factors that affect dietary compliance. Non-compliance to any dietary modification is multifactorial and is determined by several socioeconomic and cultural factors. Dietary compliance can be assessed by questionnaires, serology or histology, or a combination of these methods.

In a study to determine factors to assess gluten-free dietary compliance, strict compliance was seen in 45%, 50% and 35% in pediatric, adolescent and adult populations, respectively. Temptation was the main reason for default in children. Ignorance combined with temptation were major problems in adolescents, whereas digression in adults was mainly due to sociocultural and economic factors. Overall compliance rates to GFD vary from 45% to 80%. tTGA normalizes in 75% of the compliant patients at 1 year and serves as a useful marker for medium-term compliance and beyond. Histological improvement lags behind serological response. Overall non-compliance was seen in 58% at 2 years[42].

MANAGEMENT PROBLEMS OF CD IN INDIA

The only treatment available for CD is strict adherence to a gluten-free diet for life. Data suggest that diagnosed but untreated patients with CD have significantly higher morbidity and mortality.

Gluten-free diet

A gluten-free diet is defined as one that excludes wheat, rye and barley. Even small quantities of gluten may be harmful. The strict definition of a gluten-free diet remains controversial because of the lack of an accurate method to detect gluten in food, and the lack of evidence for what constitutes a safe amount of gluten ingestion. The patients and their relatives should be counseled by a trained dietician. Vitamin and mineral deficiencies, including iron, calcium, phosphorus, folate, B12, and fat-soluble vitamins should be looked for. It is important to have a team-based approach to management. In addition to treatment by a physician and participation in a local support group, consultation with a skilled dietitian is essential (Table 1).

Dietary counseling of the patient and the family is the cornerstone of the treatment of CD. In India, it is common practice for families to purchase whole grain and have the flour processed at a small neighborhood flourmill, where other cereals like corn and rice are ground separately at a different time slot after cleaning the grinding machine. Despite cleaning of the flour-making machine, there may be mixing during grinding of cereals. The mixing occurs in the initial part of cereal grinding, therefore, initial flour should not be used by the patients with CD. These measures are inadequate, and some quantity of wheat becomes mixed with other cereals and may be a factor for non-response in a strictly compliant patient. It might make sense for patients to use solely home grinding for gluten-free flour. The major problem is faced by the patients and families on certain occasions: birthday cakes, chocolates, ice creams, biscuits, social functions, and traveling (Table 2).

Table 1 Key elements in the management of celiac disease (CD)

Consultation with a skilled dietitian
Education about the disease
Lifelong adherence to a gluten-free diet
Identification and treatment of nutritional deficiencies
Access to a support group
Continuous long-term follow-up by a multidisciplinary team

Table 2 Factors to improve compliance

Learning about CD
Identify gluten-containing products
Improved self-management
Trust in physicians and dietitians
Proactive follow-up measures
Understanding the risk factors and serious complications
Ability to reinforce positive changes internally
Positive coping skills
Participation in a support groups

CD has come to attention of physicians in the past two decades. The number of patients diagnosed with CD is also limited. Therefore, the market value of gluten-free products and food items has not been properly realized. With time, the exact number of patients with CD is going to rise and there will be a requirement for commercially available food items. Besides, there is no legislation for gluten labeling in India, therefore, a patient with CD will not be able to know if any of the food items is safe.

MANAGEMENT OF RCD

A small subset of CD patients fails to respond to a gluten-free diet. This condition is referred to as RCD, which can be either primary or secondary. There is no standard definition of RCD. Currently, RCD is defined as persisting or recurrent villous atrophy with crypt hyperplasia and increased IELs, in spite of a strict gluten-free diet for more than 12 mo[43].

Before making a diagnosis of RCD, the following causes must be ruled out: (1) dietary non-compliance; (2) ubiquitous gluten source (pill capsules); (3) wrong initial diagnosis; and (4) associated disease, such as collagenous colitis, lactose intolerance, or bacterial overgrowth syndrome.

There are no clear clinical or biological markers that

predict the development of RCD. This disorder usually manifests in patients diagnosed in adulthood, and all reported cases have been patients diagnosed over the age of 40-50 years. The exact incidence of RCD amongst CD is not known. However, in a small subgroup of patients, the clinical and histological abnormalities persist or recur while taking a gluten-free diet. This non-responsiveness leaves a poorly understood syndrome known as RCD[4,38,40]. RCD may appear in a subgroup of CD patients with persistent histological abnormalities. In all patients screened for RCD, DQ2 and DQ8 need to be checked. In non-DQ2/DQ8 patients, the diagnosis of CD has to be reconsidered and differentiated from diseases such as autoimmune enteropathy. Most of the patients referred for RCD are affected by other diseases. Probably, the commonest cause of non-responsiveness is continued gluten intake. Exocrine pancreas insufficiency, hyperthyroid disease and collagenous colitis are other common explanations. Immunosuppressive treatment might moderate this. We suggest azathioprine and steroids in RCD- I (without aberrant T lymphocytes). However, in RCD-Ⅱ (with aberrant T lymphocytes), we suggest chemotherapy. As the prognosis of EATL is extremely poor, the early detection of CD is crucial[44].

CONCLUSION

The spectrum of CD in India is changing. There is a need to start studies to estimate prevalence of CD all over India. This task can be accomplished by establishing nodal centers in different parts of the country. In addition, competent authorities must be approached with specific recommendations to make food labeling regarding gluten content legally mandatory. Based on available data and discussion, the following recommendations are made. (1) Common questionnaire to collect data at different centers needs to be developed. (2) Studies to estimate community prevalence of CD must be started. (3) Prevalence of CD in high-risk groups should be studied. (4) Simple and low-budget serological assays should be developed for studies in underprivileged individuals. (5) Genetic studies to identify HLA typing of CD patients in India can be taken up in a small sub set of patients. (6) Subspecialties like endocrinology and neurology must be approached and involved in the Indian Task Force. (7) Rapid assays for CD serology need studies among populations suffering from parasitic infections to look for interference with CD. (8) Proper legislation about wheat food labeling should be framed.

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ORIGINAL ARTICLE

Reduced normogastric electrical activity associated with emesis: A telemetric study in ferrets

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Abstract

AIM: To characterize the gastric myoelectric activity (GMA) and intra-abdominal pressure changes induced by emetic stimuli (apomorphine and cisplatin) in the ferret.

METHODS: GMA and intra-abdominal pressure were recorded in conscious, unrestrained ferrets surgically implanted with radiotelemetry transmitters. Animals were challenged with apomorphine (0.25 mg/kg *sc*) and cisplatin (10 mg/kg ip), and the emetic response was quantified *via* direct observation and intra-abdominal pressure recording for 1 and 4 h, respectively. The GMA was analyzed by spectral analysis; the parameters used to characterize the GMA were the dominant frequency (DF) and the repartition of spectral power in the bradygastric, normogastric and tachygastric frequency ranges.

RESULTS: Retches were identified on the intra-abdominal pressure trace as peaks 0.30 ± 1.01 s in duration and 59.57 ± 2.74 mmHg in amplitude, vomit peaks were longer (0.82 ± 0.06 s, $P < 0.01$) and reached a higher pressure (87.73 ± 8.12 mmHg, $P < 0.001$). The number of retches and vomits quantified *via* direct observation [apomorphine: 65.5 ± 11.8 retches + vomits (R+V), cisplatin: 202.6 ± 64.1 R+V] and intra-abdominal pressure (apomorphine: 68.3 ± 13.7 R+V, $n = 8$; cisplatin: 219.0 ± 69.2 R+V, $n = 8$) were correlated ($r = 0.97$, $P < 0.0001$) and the timing of emesis was consistent between the 2 methods. Apomorphine induced a decrease in normogastria from 45.48% ± 4.35% to 36.70 ± 4.34% ($n = 8$, $P < 0.05$) but the DF of the slow waves was not changed [8.95 ± 0.25 counts/min (cpm) *vs* 8.68 ± 0.35 cpm, $n = 8$, $P > 0.05$]. Cisplatin induced a decrease in normogastria from 55.83% ± 4.30% to 29.22% ± 5.16% and an increase in bradygastria from 14.28% ± 2.32% to 31.19% ± 8.33% ($n = 8$, $P < 0.001$) but the DF (9.14 ± 0.13 cpm) remained unchanged ($P > 0.05$). The GMA changes induced by cisplatin preceded the emetic response as normogastria was reduced for 1 h before the onset of emesis (57.61% ± 5.66% to 39.91% ± 5.74%, $n = 6$, $P < 0.05$). Peri-emesis analysis revealed that the GMA was significantly disturbed during and immediately after, but not immediately before, the emetic episodes.

CONCLUSION: The induction of emesis is reliably associated with a disrupted GMA, but changes may also occur prior to and following the emetic response.

Key words: Emesis; Nausea; Stomach; Ferret; Cisplatin; Apomorphine; Electromyography

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INTRODUCTION

Nausea and vomiting are components of the body's

defense response against toxins either ingested (e.g. plant alkaloids: nicotine, veratridine, emetine), or released following bacterial (e.g. *Staphylococcus aureus*, *Salmonella enterica*, *Vibrio cholerae*) or viral infection (e.g. norovirus, rotavirus)[1]. However, in the clinic, the emetic response can also be triggered inappropriately, for example, as a sequelae of anesthesia with surgery (postoperative nausea and vomiting)[2], or as a side effect of cancer chemotherapy (e.g. cisplatin)[3]. In addition, nausea and vomiting are also commonly encountered during the pre-clinical and early clinical development of novel chemical entities (NCE) for a variety of therapeutic indications. Indeed, nausea and vomiting has been ranked second, after abuse liability, in the side effects negatively impacting on the development of NCE[4].

Pre-clinical studies using species capable of emesis such as the ferret (*Mustela putorius furo L.*) or the least shrew (*Cryptotis parva*) correctly identified an emetic liability for several NCE. For example, emetic side effects were observed for phosphodiesterase-IV inhibitors[5], a nicotinic receptor agonist[6], and a cannabinoid CB1 receptor antagonist[7], considered for the treatment of asthma, pain and obesity , respectively. Studies in the ferret also identified the anti-emetic potential of 5-hydroxytryptamine-3 (5-HT_3) and tachykinin NK_1 receptor antagonists against cancer chemotherapy agents (see[8] for review), however, their more limited efficacy against nausea[9] could not be predicted from pre-clinical studies. Emesis can be divided into 2 components: the pre-ejection (or prodromal) phase and the ejection phase. The prodromal phase is characterized by sympathetic outputs such as cold sweating, skin vasoconstriction and pupil dilation. Additionally, under vagal control, the proximal stomach relaxes, delaying gastric emptying, and the retrograde giant contraction carries to the stomach intestinal contents. This phase is usually temporally correlated with the sensation of nausea[10]. Retching and emesis (vomiting), which constitute the ejection phase, are therefore end-stages in activation of the emetic reflex and there would be considerable utility in the identification of biomarkers induced at sub-emetic doses of a compound, which could be used to better identify the separation between a therapeutic dose and one with emetic liability (therapeutic index). Such biomarkers may provide insights into the potential for induction or detection and reduction of nausea, which remains controversial in studies using laboratory animals[4].

In humans, correlates of nausea include an increase in plasma levels of vasopressin and changes in the electrogastrogram (EGG) frequency, rhythm and power[11]. The EGG reflects gastric myoelectric activity (GMA), or gastric slow waves; it is usually recorded from cutaneous abdominal electrodes to investigate the frequency of pacemaker activity, which underlies the genesis of gastric contractile activity[12]. However, the precise relationship of the EGG to motility itself is unclear[13] but tachygastria has been linked to gastric motor quiescence[14]. Of the 2 markers, only the recording of the gastric slow waves provides a method amenable to telemetry with an adequate temporal resolution to examine relationships to emesis in unrestrained animals.

This paper reports the use of radiotelemetry in the conscious unrestrained ferret to record gastric slow wave activity and investigate the effect of the emetic challenges, apomorphine and cisplatin. Data are also included on telemetric recordings of intra-abdominal pressure following administration of emetic agents in this species. The results show that both apomorphine and cisplatin are associated with a reduction of normal gastric rhythm, and demonstrate the potential applicability of telemetric recording techniques to the study of emetic mechanisms and the identification and understanding of emetic liability of NCE. This provides insights into the changes in gastric function occurring prior to the onset of emesis and which, in humans, have been associated with the occurrence of nausea.

MATERIALS AND METHODS

Animals

Castrated male fitch (pigmented) ferrets (*Mustela putorius furo L.*) (1.17-1.65 kg) were obtained from Southland Ferrets (Invercargill, New Zealand). Prior to the experiments, they were housed communally in a temperature-controlled room at 24 ± 1℃, under artificial lighting with lights on between 06:00 am and 18:00 pm. Water and pelleted cat food (Tri Pro Feline Formula Cat Food, American Nutrition®, Utah, USA) were available *ad libitum* until the start of the experiments. All animals were then housed individually from the day of surgery to the end of the experiment. The experiments were conducted under the authority of a license provided by the Government of the Hong Kong SAR and approval from the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

Surgical techniques

Telemetry transmitter implantation for GMA and abdominal pressure recording: Anesthesia was induced with ketamine (20 mg/kg im; Alfasan, Holland) and maintained with isoflurane (Halocarbon Products Corporation, USA) about 1.5%, in a 3:1 O_2 to N_2O ratio using a custom-made face mask and an anesthetic machine (Narkomed 2C, Drager, USA). Animals were placed on a heating pad (UCI#390 Analog moist heating pad, Rebirth Medical & Design, Inc., Taiwan) and the level of anesthesia was assessed and monitored throughout the surgery by the pedal withdrawal reflex. Following a midline abdominal incision, the antrum was exposed and the biopotential wires of the telemetry transmitter (C50-PTX, DSI, USA) were inserted in the muscle and secured in place by suturing the serosa. The body of the transmitter (with the pressure catheter) was inserted in the peritoneal cavity and sutured to the muscle layer on the side. The abdominal cavity was treated with antibiotic (Nebacetin®, Altana Pharma, Germany), sutured closed

in layers and covered with a permeable spray dressing (Opsite®, Smith and Nephew, UK).

Analgesia and post-operative recovery: Buprenorphine (0.05 mg/kg *sc*; Temgesic®, Schering Plough, UK) was given as a preoperative analgesic 15 min before the induction of anesthesia, and 12 h post surgery. Recovery was unremarkable and the wound healed within a week.

Experimental design

Following surgery, animals were housed individually in observation cages (W49 cm × L61 cm × H49.5 cm). They were allowed to recover for at least 7 d prior to further experimentation. Some ferrets were administered apomorphine (0.25 mg/kg *sc*) at least 7 d prior to the administration of cisplatin (10 mg/kg ip). These doses of apomorphine and cisplatin have been shown to induce a reliable emetic response in the ferret[15,16]. At the end of the observation period, animals were killed with an overdose (> 100 mg/kg ip) of pentobarbital sodium (Dorminal®, Alfasan, Woerden, Holland).

Baseline telemetry recordings (GMA and abdominal pressure) were made for a least 1 h prior to presenting the animals with food, or an emetic challenge. Recordings then continued for 1 h in studies assessing the effect of food alone or apomorphine (0.25 mg/kg *sc*), or for 4 h in experiments assessing the action of cisplatin (10 mg/kg ip). Emesis was characterized by rhythmic abdominal contractions that were either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting), or not associated with the passage of material (i.e. retching movements). An episode of retching and/or vomiting was considered separate when the animal changed its location in the observation cage, or when the interval between retches and/or vomits exceeded 5 s[17]. The latency was defined as the time between the administration of the drug and the first emetic episode.

Effect of feeding on GMA: Food was withdrawn for 12-14 h before the start of the studies. The ferrets were then presented with 20 g of pelleted cat food, and all uneaten food was withdrawn 10 min later. This design was chosen to mimic human studies describing the effect of a meal on GMA in humans[12]. GMA data were analyzed during the 5 min period prior to presentation of food, and during a 5 min post-prandial period, starting 5 min after the uneaten food was withdrawn.

Effect of apomorphine (0.25 mg/kg *sc*) and cisplatin (10 mg/kg ip) on GMA and abdominal pressure: On the day of the experiment water was freely available but food was withdrawn for approximate 2 h before the start of the studies. Ferrets were presented with pelleted cat food and 30 min later animals were injected subcutaneously with apomorphine (0.25 mg/kg) or saline (0.5 mL/kg NaCl 154 mmol/L); or injected intraperitoneally with cisplatin (10 mg/kg) or saline (10 mL/kg NaCl 154 mmol/L).

Drugs: Cisplatin [cis-diamminedichloroplatinum(Ⅱ), David Bull Laboratories, Victoria, Australia] was purchased as a sterile saline solution at an active concentration of 1 mg/mL. Apomorphine hydrochloride (Sigma-Aldrich, St. Louis, USA) was dissolved in sodium metabisulphite (526 μmol/L, Riedel-de Haën, Germany) and injected at a concentration of 0.5 mg/mL and a volume of 0.5 mL/kg *sc*. Doses are expressed as the free base.

Telemetry system and analysis of the data

A DSI Dataquest® A.R.T. telemetry system (Data Science International, Minnesota, USA) was used. The GMA and intra-abdominal pressure were recorded using PhysioTel® C50-PXT Small Animal Transmitters. Telemetric signals were recorded *via* 2 receiver plates (PhysioTel® RPC-1) placed under the cages. The receivers were connected to a PC desktop computer *via* a matrix (Dataquest ART Data Exchange Matrix). An ambient pressure reference monitor (APR-1) was connected to the exchange matrix. Data was recorded with the Dataquest Acquisition software (DQ ART 4.0). Analysis of telemetry recordings was carried out using Spike2® (version 6.06, Cambridge Electronic Design, UK).

Quantification of the retches and vomits *via* intra-abdominal pressure: The abdominal pressure signal was recorded with a sampling frequency of 500 Hz. Retches and vomits were quantified from the intra-abdominal pressure recordings in a semi-automated manner. Thus, the traces of each ferret were inspected visually and then a detection threshold was set and pressure profiles corresponding to retches and vomits were isolated manually. To test the validity of this method, the number of retches + vomits (R+V) detected was compared to the number obtained *via* direct observation using a Spearman test for non-parametric correlation.

GMA recordings: The GMA signal was recorded with a sampling frequency of 1000 Hz; selected steps of the analysis procedure are presented in Figure 1. Briefly, a low pass finite impulse response (FIR) filter with a cut-off frequency of 2.5 Hz (transition gap: 10 Hz) was used to remove any signal with a frequency higher than 150 counts/min (cpm). The traces were then interpolated to a sampling frequency of 10.24 Hz and a second low pass FIR filter with a cut-off frequency of 0.3 Hz (18 cpm, transition gap: 0.1 Hz) was applied. This cut-off was chosen to filter out signals of probable cardiac (about 200 cpm in the ferret) and respiratory (33-36 cpm) origins[18].

The following parameters were used to characterize the GMA (Figure 1): (1) the dominant power (DP, the highest power in the 0 to 15 cpm range); (2) the dominant frequency (DF, frequency bin with the highest power in the 0 to 15 cpm range); (3) the repartition of power in the bradygastric, normal and tachygastric ranges (i.e. bradygastria, normogastria and tachygastria). The DF

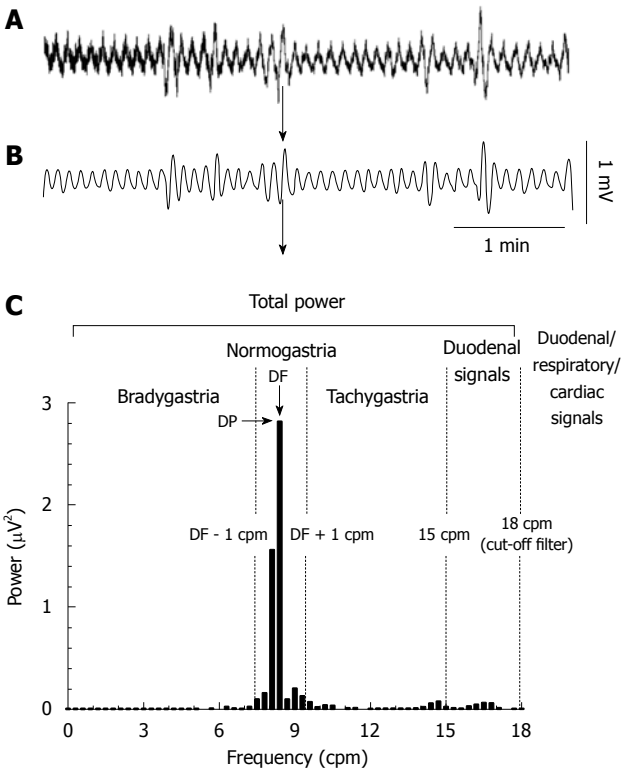

Figure 1 Telemetric recordings of the ferret gastric myoelectric activity and spectral analysis. A: The raw trace sampled at 1 kHz; B: The same trace after filtering (low pass filter, cut-off: 0.3 Hz; sampling frequency 10 Hz); C: The fast Fourier transform of the same 5 min of data (Hann window, 2048). DF: Dominant frequency; DP: Dominant power; cpm: Counts/min; Total power: Total power contained in all the frequency bins between 0 and 18 cpm.

during a 1 h baseline was used to define the normal range in each animal, the limits of each range were then defined as follow: bradygastria: 0 to (DF - 1) cpm, normogastria: DF ± 1 cpm, tachygastria: (DF + 1) to 15 cpm. To investigate the general effect of apomorphine and cisplatin on the GMA, fast Fourier transforms (FFT, bin size: 0.3 cpm) were computed on successive 10 min epochs to construct the profiles of GMA repartition and the data were averaged in 1 h blocks for statistical analysis.

Peri-emesis analyses were carried out following cisplatin; FFTs (bin size: 0.6 cpm) were used on 2 min sections of data. Percentages of bradygastria, normogastria and tachygastria were computed from 2 min long sections divided as follows: (1) before cisplatin (mean of 5 successive 2 min sections immediately before the drug was injected); (2) before episodes (mean of all 2 min sections, free of emesis, immediately preceding an emetic episode); (3) during episodes (mean of all the 2 min sections containing emetic episodes); (4) after episodes (mean of all the 2 min sections, free of emesis, immediately after an episode).

Statistical analysis

Prior to any statistical comparisons, the normality of the data was assessed with a Kolmogorov-Smirnov test. Differences between the abdominal pressure correlates of retches and vomits were assessed with unpaired *t*-tests, the number of retches and vomits quantified by direct observation and abdominal pressure recordings were compared with paired *t*-tests and the correlation between the 2 methods was assessed with a Spearman test. For the overall effect of apomorphine and cisplatin on the GMA, differences between treatment groups were compared using repeated measures two-way ANOVAs (factors: treatment and time) followed by Bonferroni post-tests. In the peri-emetic analysis, the differences in GMA repartition between the different time-points were computed using repeated measures one-way ANOVA followed by Bonferroni post-tests.

RESULTS

Apomorphine and cisplatin-induced emesis and the intra-abdominal pressure changes

Figure 2 shows specific patterns on the intra-abdominal pressure recordings that are correlates of retching and vomiting. Retches were identified as round-ended peaks, 0.30 ± 0.01 s in duration and reaching a pressure of 59.57 ± 2.74 mmHg (mean ± SE of 40 measures, 5 retches × 8 animals). Vomits reached a higher pressure than retches (87.73 ± 8.12 mmHg; mean ± SE of 8 measures, 1 vomit × 8 animals, $P = 0.0002$, unpaired *t*-test) and they lasted longer (0.82 ± 0.06 s; mean ± SE of 8 measures, $P = 0.0056$, unpaired *t*-test); typically, an oscillation in pressure was observed during the peak (Figure 2).

Apomorphine (0.25 mg/kg *sc*) induced emesis with a latency of 7.17 ± 0.74 min ($n = 8$), 65.5 ± 11.8 R+V (59.6 ± 11.1 retches and 5.8 ± 0.8 vomits) and 68.3 ± 13.7 R+V (61.6 ± 12.9 retches and 6.6 ± 0.9 vomits) were quantified *via* observation and pressure, respectively; these values were not different ($P = 0.34$, paired *t*-test).

Cisplatin (10 mg/kg ip) induced emesis with a latency of 1.70 ± 0.23 h ($n = 8$). One ferret had an episode of retching immediately (20 s) after the intraperitoneal injection of cisplatin. This case seems more likely to be a result of the effect of the injection/handling rather than an effect of cisplatin itself; for this ferret, the latency of its second episode (46 min and 50 s), after which a sustained emetic response was initiated, was taken as the latency. Overall, 202.6 ± 64.1 R+V (185.0 ± 60.1 retches and 17. 6 ± 4.6 vomits) were calculated by direct observation and this number was increased by 8.1% ± 1.0% to 219.0 ± 69.2 R+V (199.1 ± 64.5 retches and 19.9 ± 5.2 vomits) using the pressure traces, a statistically significant difference ($P = 0.0189$, paired *t*-test).

The linear correlation ($r = 0.9728$) between the values obtained *via* observation and pressure was extremely significant (Figure 2C, $P < 0.0001$). The time of occurrence of emetic episodes was consistent between the 2 methods.

Effect of feeding on the GMA

After being deprived of food overnight, the animals displayed a GMA characterized by a DF of 9.63 ± 0.23 cpm and a DP of $4.80 \times 10^{-4} \pm 1.15 \times 10^{-4}$ mV^2 ($n = 10$). Five minutes after food ingestion, there was a trend for the DF to be reduced to 9.24 ± 0.34 cpm and the DP was

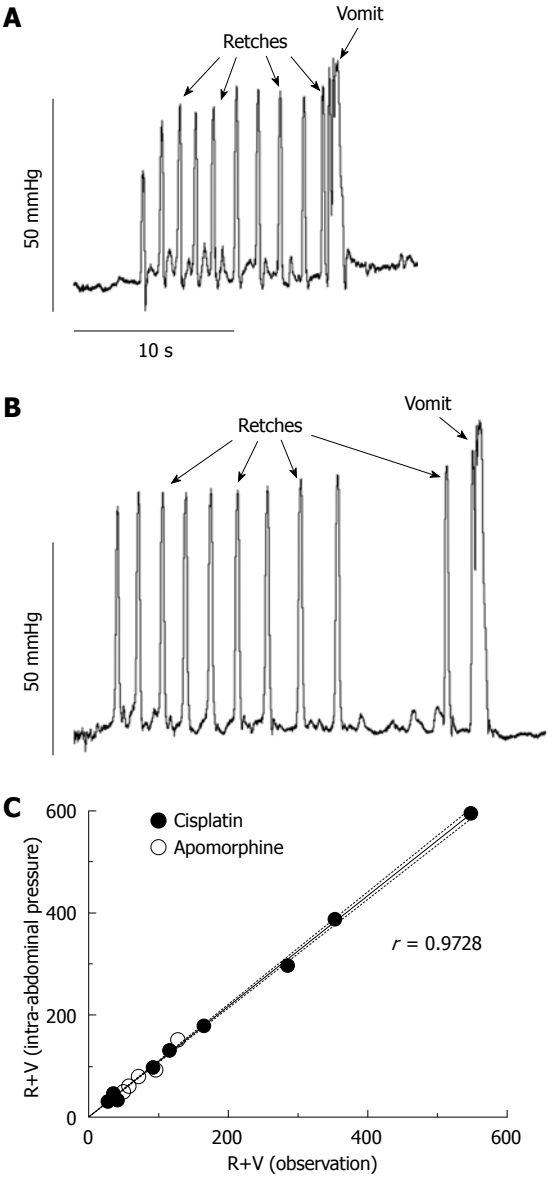

Figure 2 Intra-abdominal pressure recordings. Intra-abdominal pressure was recorded *via* a telemetry transmitter in the abdominal cavity (C50-PTX, DSI®), showing emetic episodes-each composed of several retches and one vomit-induced by apomorphine (0.25 mg/kg *sc*) (A) and cisplatin (10 mg/kg ip) (B). Both recordings were obtained from the same animal. C: Correlation between the number of retches + vomits (R+V) induced by cisplatin (10 mg/kg ip) and apomorphine (0.25 mg/kg *sc*), quantified by direct observation and from the intra-abdominal pressure traces. Results plotted as individual values, n = 16 (data obtained from 11 animals). Spearman correlation coefficient (r), the linear regression (plain line) and 95% confidence interval (dotted line) are indicated on the graph.

increased by a ratio of 6.80 ± 3.61, to 9.45 × 10^{-4} ± 2.84 × 10^{-4} mV^2. These changes were not statistically significant, P = 0.38 and P = 0.13 for the DF and DP respectively (paired t-tests, n = 10).

Effect of apomorphine and cisplatin on the GMA

Effect of apomorphine: During the 1 h that preceded the injection of saline (0.5 mL/kg *sc*), baseline bradygastria, normogastria and tachygastria were 13.57% ± 3.53%, 63.31% ± 9.99% and 16.26% ± 5.82% of the total power, respectively. Baseline DF was 9.54 ± 0.26 cpm (n = 4). Following saline injection, the GMA repartition was 15.14% ± 1.28%, 57.40% ± 8.03% and 18.46% ± 6.13% in the bradygastric, normal and tachygastric ranges respectively; the DF was 9.73 ± 0.24 cpm. None of these values were significantly different from the baseline and no differences were detected between the saline and apomorphine groups during baseline (P > 0.05). During the 1 h that preceded the injection of apomorphine, the GMA was repartitioned as follows: bradygastria: 16.57% ± 3.17%, normogastria: 45.48% ± 4.35% and tachygastria: 25.66% ± 2.72%; DF: 8.95 ± 0.25 cpm (n = 8). As shown in Figures 3 and 4, following the administration of apomorphine, the percentage of power in the normal range decreased to 36.70% ± 4.34% (P < 0.01), whereas the percentage of power in the bradygastric and the tachygastric ranges was not significantly altered: 25.76% ± 4.65% and 22.77% ± 4.67%, respectively (P > 0.05). The DF did not change, and was 8.68 ± 0.35 cpm following apomorphine [P > 0.05, two-way ANOVAs followed by Bonferroni post-tests, n = 8 (apomorphine) and n = 4 (saline)].

Effect of cisplatin: During the 1 h baseline recordings prior to the injection of saline (10 mL/kg ip), baseline bradygastria, normogastria and tachygastria were 17.11% ± 5.35%, 43.32% ± 6.37% and 25.85% ± 3.83%, respectively; baseline DF was 8.44 ± 0.61 cpm (n = 4). The saline treatment had no significant effect on the GMA up to 3 h following intraperitoneal injection, however 4 h post injection the percentage of power in the tachygastric range was significantly increased compared to baseline to 40.40% ± 8.31% (P < 0.05). During the 1 h baseline prior to the injection of cisplatin, the percentages in the bradygastric, normogastric and tachygastric ranges were 14.28% ± 2.32%, 55.83% ± 4.30% and 19.17% ± 3.08%, respectively. The DF was 9.14 ± 0.13 cpm (n = 8). As shown in Figures 3 and 4, following the administration of cisplatin the percentage of power in the normogastric range decreased and reached a nadir in the second hour post-injection (29.22% ± 5.16%), whereas the percentage of bradygastria and tachygastria increased. Bradygastria reached a peak during the third hour after the injection (31.19% ± 8.33%) and tachygastria reached a peak during the second hour post-injection (29.56% ± 6.01%); the effects on normogastria and bradygastria were statistically significant during the entire observation period whereas the increase in tachygastria were only statistically significant 2 h post-injection (P < 0.05, Bonferroni post-tests compared to the 1 h baseline). The DF was not significantly altered after cisplatin administration (P < 0.05). No differences could be detected at any time points between saline and cisplatin [P > 0.05, repeated measures two-way ANOVA followed by Bonferroni post-tests, n = 8 (cisplatin), n = 4 (saline)].

A secondary analysis was carried out only on the animals with a latency to the onset of emesis greater than 1 h (n = 6). In these animals, the percentage of power in the normogastric range was significantly reduced in

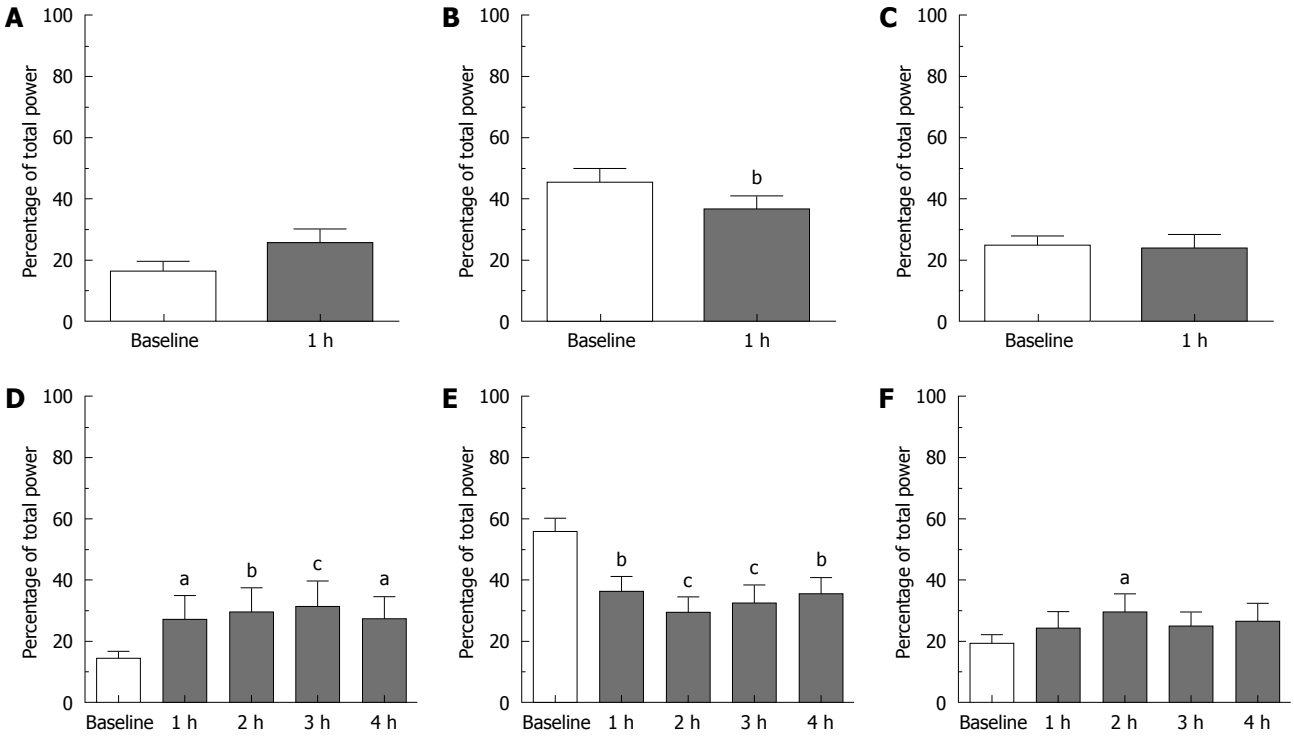

Figure 3 Effect of apomorphine (0.25 mg/kg *sc*, A-C) and cisplatin (10 mg/kg ip, D-F) on the gastric myoelectric activity (GMA) in ferrets. The graphs represent the percentage of total (0-18 cpm) power in the bradygastric range [0 - (DF - 1 cpm), A and D], in the normogastric range [(DF - 1 cpm) - (DF + 1 cpm), B and E] and in the tachygastric range [(DF + 1 cpm) - 15 cpm, C and F]. Results are plotted as mean ± SE (n = 8). Differences compared to the effect of a dose of saline (*sc* or ip as appropriate, data not shown on this graph) were computed using two-way ANOVA (factors: treatment and time) and Bonferroni post-tests, Differences with baseline are indicated as $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

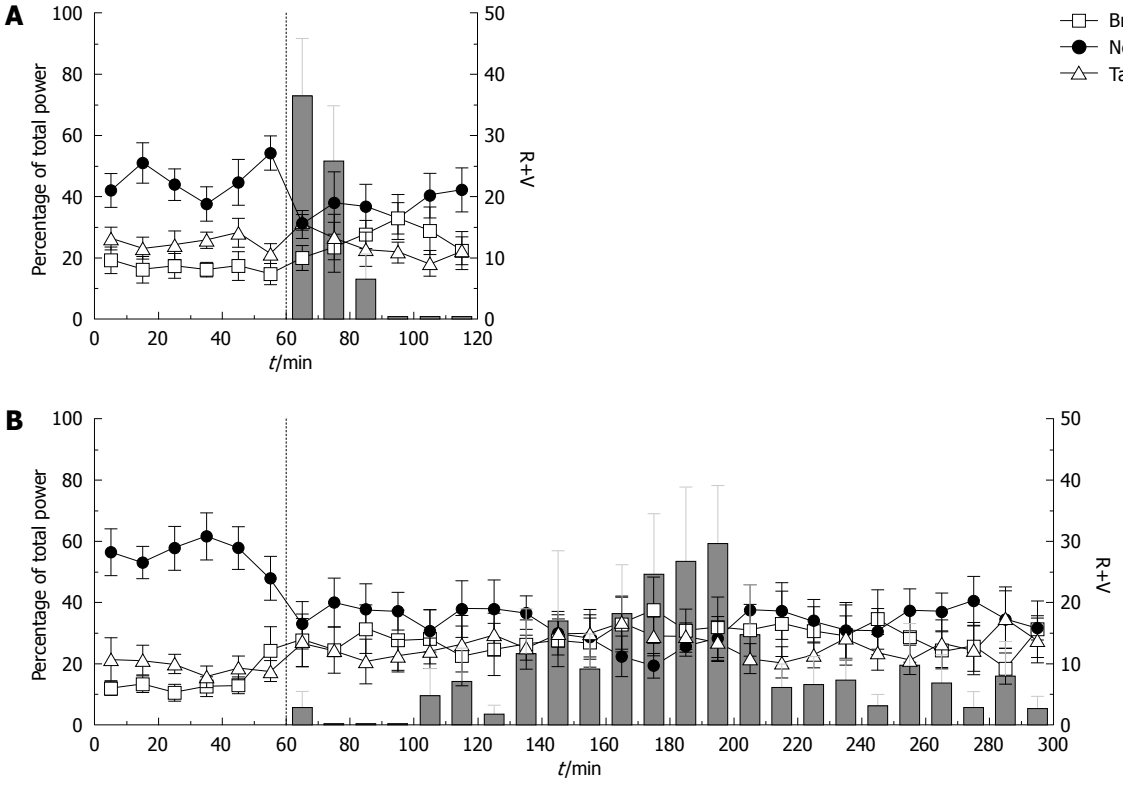

Figure 4 Profile of emesis and GMA repartition in the bradygastric, normogastric and tachygastric ranges following the administration of apomorphine (0.25 mg/kg *sc*) (A) and cisplatin (10 mg/kg ip) (B) in the ferret. Data plotted as mean ± SE per 10 min, n = 8.

the first hour post-cisplatin injection from 57.61% ± 5.66% to 39.91% ± 5.74% ($P < 0.05$) even though no emesis was observed during that period. Percentages of power in the bradygastric and tachygastric ranges

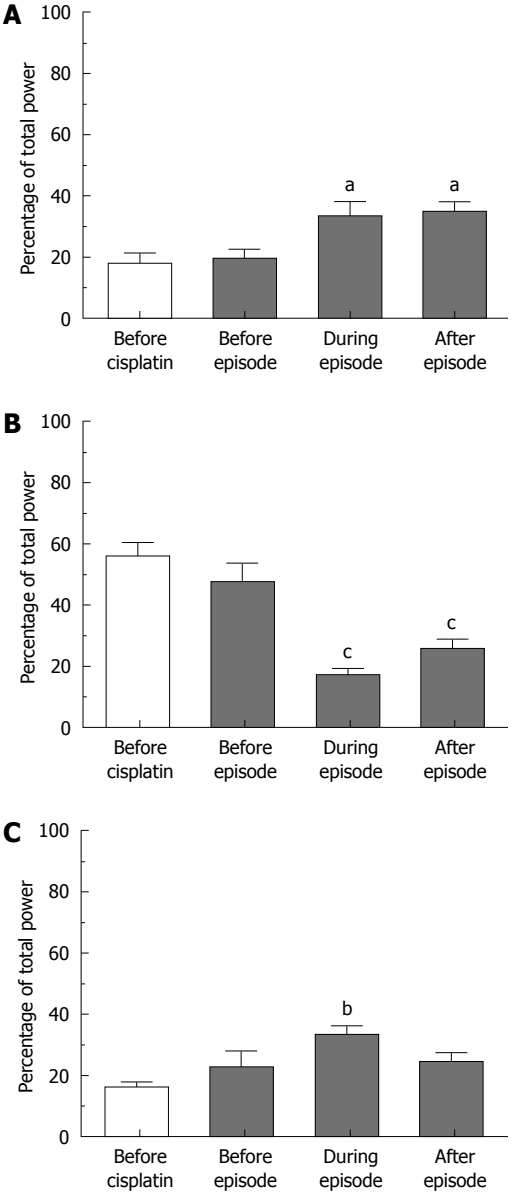

Figure 5 Repartition of the GMA in the bradygastric (A), normogastric (B) and tachygastric (C) ranges before the injection of cisplatin, immediately before emetic episodes, during emetic episodes and immediately after emetic episodes induced by cisplatin (10 mg/kg ip) in the ferret. Results are plotted as mean ± SE, n = 8. Differences compared to baseline are calculated using repetitive measures one-way ANOVA followed by Bonferroni-post tests. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.

were unchanged [$P > 0.05$, repeated measures two-way ANOVA followed by Bonferroni post-tests, $n = 6$ (cisplatin), $n = 4$ (saline)].

We investigated the GMA repartition surrounding and during emetic episodes; altogether, 40, 41, 62 and 41 determinations were used to characterize the GMA repartition before cisplatin and before, during and after emetic episodes, respectively. Bradygastria, normogastria and tachygastria values during the 10 min preceding the injection of cisplatin were 17.67% ± 3.50%, 56.00% ± 4.41% and 16.15% ± 1.66% (mean ± SE for 8 animals), respectively. Peri-emesis, the percentage repartition in all 3 ranges was significantly altered ($P = 0.0085$, $P < 0.0001$ and $P = 0.0261$ for bradygastria, normogastria and tachygastria respectively, repeated measures one-way ANOVAs, Figure 5). Bonferroni post-tests revealed that these values did not change significantly immediately before an episode (bradygastria: 19.36% ± 3.02%, normogastria: 47.56% ± 6.18 % and tachygastria: 22.87% ± 5.20%, $P > 0.05$), but were altered during (bradygastria: 33.08% ± 4.90%, normogastria: 17.23% ± 2.06% and tachygastria: 33.27% ± 3.00%, $P < 0.05$) and immediately after emetic episodes (bradygastria: 34.68% ± 3.33%, normogastria: 25.57% ± 3.27% and tachygastria: 24.38% ± 3.12%, $P < 0.05$).

DISCUSSION

Recording and analysis of GMA in the ferret

The present study is the first report of ambulatory gastric myoelectric recordings in conscious unrestrained ferrets. The frequency of the gastric slow waves was 9.12 ± 0.23 cpm (mean ± SE of 12 animals), which is in accordance with post-prandial frequency of antral contractions (8.8 ± 0.5 cpm)[19], and the frequency of the antral slow waves (9.5 cpm) reported by Diamant *et al*[20] in abstract form; both were measured in conscious but restrained ferrets. In contrast to common protocols used to investigate the GMA in humans[21-23] and other animals (dog)[24,25], which used a fixed normogastric range (typically 2.5-3 to 3.7-4 cpm in humans and 4-6 cpm in dogs)[12,26,27], normogastria was defined according to each animal's intrinsic slow waves frequency during baseline. This was done for 2 reasons: (1) because of a relative paucity of literature, not enough data on the ferret's GMA was available to determine with confidence what the normogastric range should be (DF range 7.8-9.2 cpm in 12 animals in the present study) or how wide it should be; (2) the purpose of the present study was to focus on the changes induced by an experimental stimulus; setting the normogastric range relative to each individual animal reduced the influence of inter-animal variability and rendered the analysis more powerful to detect the effect of an emetic stimulus on the GMA in a limited number of animals.

Regarding the GMA analysis parameters, we chose to report the DF and compute the repartition of power in the normogastric, bradygastric and tachygastric ranges. An alternative analysis, which has been used in most preclinical studies[27-29] and some studies in humans[30], consists of calculating the percentage of time during which the DF falls within each frequency range. The analysis parameters used in the present study were chosen as the power repartition encompass all the data present in the GMA signal and does not solely focus on the time at which the DF is included within a range of interest[12].

GMA changes induced by apomorphine and cisplatin

Similar GMA changes were observed following apomorphine and cisplatin; both stimuli induced a decrease in the percentage of power in the normogastric range, which was accounted for in the case of cisplatin by a clear increase in power in the bradygastric range,

without changing the DF. Our findings on the effects of apomorphine are partially supported by a number of studies in conscious, restrained dogs, which also reported a transient disruption of the gastric antral rhythm following the administration of apomorphine[31-33]. To the best of our knowledge, the present study represents the first clear evidence that apomorphine is associated with a reduction of normogastria for up to 1 h, which outlasts the emetic response as the last emetic episode was observed 21.12 ± 1.76 min (n = 8) post-apomorphine.

Regarding the effect of cisplatin, GMA changes were temporally correlated with the occurrence of emesis and maximal changes in the GMA repartition were observed when the emetic response was the most intense (2-3 h post cisplatin). However, GMA changes preceded the emetic response as evidenced by a decrease in normogastria during the first hour post-cisplatin in animals, which had not yet developed emesis during that period. Consistent with our findings, a recent study in the dog showed that cisplatin reduced the percentage of normal gastric slow waves in the hours preceding the onset of emesis and during the emetic response[34]. In the present study, a peri-emesis analysis revealed that normogastria was decreased and bradygastria and tachygastria were increased during and immediately after- but not immediately before-an emetic episode, which is in accordance with the short periods of dysrhythmia associated with emetic episodes have been reported in human patients during the administration of cisplatin[35]. Our findings are partly supported by human studies, which reported dysrhythmias in a few patients treated with anti-cancer chemotherapy. However, such events appeared to be transient rather than an overall change in slow wave activity[30,35]. The apparent differences in the effect of chemotherapy in human patients and in the ferret model could have four explanations:

(1) In the present study, the ferret model of acute cisplatin-induced emesis was used. This model uses a high bolus dose of cisplatin (10 mg/kg ip)-a highly emetic chemotherapeutic agent-to provoke an intense, reliable emetic response in all the animals, typically quantifiable over 4 h[36]. In human patients however, chemotherapy (platinum-based or not) is infused over hours and the emetic response is less reliable. Correspondingly, the GMA disturbance may be more severe in the ferret model than it is in human patients.

(2) Additionally, cancer patients treated with chemotherapy are not healthy subjects and their GMA may already be altered before they receive chemotherapy, rendering it less likely to detect an additional effect of the anti-cancer treatment. It is interesting that following a chemotherapy session, Riezzo *et al*[30] reported a higher percentage of tachygastria in cancer patients compared to healthy volunteers. However, they did not compare the EGG repartition in the cancer patients prior to chemotherapy, the measurements were collected 7 d post-chemotherapy and the chemotherapy regimens were not reported, precluding any direct comparison with the present study.

(3) The use of anti-emetic prophylaxis may also have an influence on GMA, and 5-HT_3 receptor antagonists-commonly administered with chemotherapy and used in the 2 above-mentioned human studies-have been reported to reduce vection-induced dysrhythmias[37].

(4) The analysis of the data is an important factor to consider; as discussed above, in the present study, the DF and the percentage repartition of the power in the bradygastric, tachygastric and normogastric ranges were computed. In contrast, Samsom *et al*[35] identified bradygastria and tachygastria as intervals of at least 2 min, during which the DF was < 2.4 cpm or > 3.6 cpm and no overall assessment of normogastria was made, apart from the mean DF. Also, Riezzo *et al*[30] calculated the percentage of successive spectra in which the DF falls within one of the 3 ranges, or used visual inspection of the waveform traces or the regular spiking activity (RSA).

Intra-abdominal pressure recordings

On the intra-abdominal pressure traces, retches were identified as brief peaks whereas vomits were more prolonged. These findings in the freely moving, conscious ferret are consistent with what McCarthy *et al*[38] described in the decerebrate cat. Our technique represents a great advantage in that quantifying the retches and vomits from the intra-abdominal pressure traces is more accurate than from a video or even direct observation; the distinction between retches and vomits is unequivocal and abdominal pressure recordings enable precise analysis regarding the timing and the frequency of the retches. Further information regarding the central neuronal circuitry involved in the mechanism of various emetics and anti-emetics can be gained from, for example, the interval between retches, the pattern of retching preceding a vomit or the characteristics of episodes including a vomit and those of episodes of retches. The major disadvantage is that this is an invasive technique, which requires abdominal surgery.

CONCLUSION

The use of telemetry enables the recording of gastric slow waves in freely moving, conscious animals; additionally, analysis of the emetic response *via* intra-abdominal pressure permits a more rigorous data collection and the investigation of the precise temporal correlation between gastric changes and emesis. Using an EGG-like analysis of GMA recordings, disruption of the gastric rhythm was detected in ferrets challenged with apomorphine and cisplatin. Both emetic stimuli were associated with a reduction of normogastria, and cisplatin increased bradygastria. The GMA changes were subtle and not detectable by a simple DF analysis but comparison of the power repartition of the gastric signal, before and after the emetic challenges, indicated a clear change, which was temporally correlated with the development of the emetic response but not restricted to the immediate peri-emetic period. In the ferret, recording and analysis of the GMA, which is a physiological correlate of nausea in

humans, will improve the understanding of the changes in gastric function associated with emesis.

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COMMENTS

Background

Nausea is a subjective sensation, therefore impossible to study directly in animals. However physiological correlates of nausea have been identified in humans and include disruption of the gastric electrical rhythm. As nausea usually precedes the onset of emesis, recording the gastric electrical rhythm in animals potentially enables the detection of emetic pathway activation before the emetic threshold is reached.

Research frontiers

The application of human biomarkers of nausea to an animal model enables the refinement of this model as it improves its translational potential. The research hotspot is the correlation of physiological events occurring around the time of vomiting, which may give insight into nausea.

Innovations and breakthroughs

Recent *in vivo* research studies investigating the relationship between emesis and gastric myoelectric activity (GMA) have used either restrained or anesthetized animals (mainly dogs) with wires connected to the serosa and externalized through the skin. The use of telemetry represents a novel approach and the present study is the first report of GMA recording in freely moving animals, which represents an indisputable advantage in terms of animal welfare.

Applications

The ferret is a species commonly used in emesis research and our approach could be integrated to standard study designs, therefore refining this animal model by enabling the detection of emetic pathway(s) activation before the emetic threshold is reached.

Terminology

The GMA consists of electrical pacemaker signals, which trigger contractions of the stomach. Gastric dysrhythmia refers to a departure from the normal gastric electrical rhythm, the term encompasses bradygastria, tachygastria and gastric arrhythmia.

Peer review

This is a well written manuscript from an established group expert in this field using a novel relevant approach to assess GMA and intra-abdominal pressure in the conscious ferret implanted with radiotelemetry transmitters.

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ORIGINAL ARTICLE

MRI *versus* 64-row MDCT for diagnosis of hepatocellular carcinoma

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Abstract

AIM: To compare the diagnostic capability of multidetector computed tomography (MDCT) and magnetic resonance imaging (MRI) for the detection of hepatocellular carcinoma (HCC) tumour nodules and their effect on patient management.

METHODS: A total of 28 patients (25 male, 3 female, mean age 67 ± 10.8 years) with biopsy-proven HCC were investigated with 64-row MDCT (slice 3 mm native, arterial and portal-venous phase, 120 mL Iomeprol, 4 mL/s, delay by bolus trigger) and MRI (T1fs fl2d TE/TR 2.72/129 ms, T2tse TE/TR 102/4000 ms, 5-phase dynamic contrast-enhanced T1fs fl3d TE/TR 1.56/4.6, Gadolinium-DTPA, slice 4 mm). Consensus reading of both modalities was used as reference. Tumour nodules were analyzed with respect to number, size, and location.

RESULTS: In total, 162 tumour nodules were detected by consensus reading. MRI detected significantly more tumour nodules (159 *vs* 123, $P < 0.001$) compared to MDCT, with the best sensitivity for early arterial phase MRI. False-negative CT findings included nodules ≤ 5 mm ($n = 5$), ≤ 10 mm ($n = 17$), ≤ 15 mm ($n = 12$), ≤ 20 mm ($n = 4$), and 1 nodule > 20 mm. MRI missed 2 nodules ≤ 10 mm and 1 nodule ≤ 15 mm. On MRI, nodule diameters were greater than on CT (29.2 ± 25.1 mm, range 5-140 mm *vs* 24.1 ± 22.7 mm, range 4-129 mm, $P < 0.005$). In 2 patients, MDCT showed only unilobar tumour spread, whereas MRI revealed additional nodules in the contralateral lobe. Detection of these nodules could have changed the therapeutic strategy.

CONCLUSION: Contrast-enhanced MRI is superior to 64-row MDCT for the detection of HCC nodules. Patients should be allocated to interventional or operative treatment according to a dedicated MRI-protocol.

Key words: American Association for the Study of Liver Diseases; European Association for the Study of the Liver; Hepatocellular carcinoma; Multidetector computed tomography; Magnetic resonance imaging

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer death, with 600 000 to 1 million new cases being diagnosed each year[1,2]. In North America and Europe, the most common risk factors are alcoholic cirrhosis and chronic hepatitis C and hepatitis B infection[3-6]. Patient survival has not significantly improved in the last 30 years because most cases are still not diagnosed until the disease is already in an advanced stage, which limits the most effective therapeutic options[7]. Therefore, early tumour detection is one of the most important issues in HCC therapy[8]. Currently, magnetic

resonance imaging (MRI) and multidetector computed tomography (MDCT) are both equally used for HCC diagnosis, although in the past MRI has been reported to produce significantly higher detection rates[9-11]. State-of-the-art MRI provides fast imaging techniques with dynamic contrast-enhanced sequences to detect the mostly hypervascularized HCC tumour nodules with a high sensitivity and specificity. However, current developments in MDCT techniques provide better spatial resolution than MRI and quick scan times potentially cause fewer motion artifacts and improve the accuracy of MDCT. The exact number and the distribution of tumour nodules is crucial for allocating these patients to adequate treatment regimens; however, it is well known that particularly small nodules often remain undetected using radiological methods[8,12,13]. The purpose of this study was to compare the diagnostic capability of 64-row MDCT and MRI for the detection of hypervascularized tumour nodules in the cirrhotic liver to allow for adequate treatment.

MATERIALS AND METHODS

Between July 2006 and July 2007, 28 patients with a suspected diagnosis of HCC on ultrasound or CT (25 male, 3 female, mean age 67.0 ± 10.8 years, range 46-89 years) were included in the study protocol. Diagnosis was confirmed by liver biopsy of at least one of the tumour nodules in 25 cases (Table 1). According to the guidelines of the European Association for the Study of the Liver (EASL), the other 3 patients were diagnosed with two different imaging techniques by arterial hypervascularization of nodules > 2 cm and/or a corresponding increase in serum levels of alpha-feto-protein[14]. For final diagnosis, all patients were included in this comparative imaging protocol that comprised contrast-enhanced MDCT and MRI in order to evaluate number, size, and location of HCC tumours for subsequent treatment allocation. The study protocol was approved by the local ethical review board. All patients gave their informed consent before entering the study.

The CT diagnosis was based on a triphasic contrast-enhanced protocol using a 64-row MDCT scanner (Brilliance 64®, Philips Medical Systems, Eindhoven, Netherlands, 120 kV, 200 mAs, collimation 64 mm × 0.6 mm, pitch 0.625, reconstruction interval 1.172 mm, slice thickness 5 mm native and 3 mm in contrast-enhanced phases). 1 mm slices were reconstructed for CT-angiography of liver arteries if the patients were considered for surgery. The contrast bolus consisted of 120 mL Iomeprol (Imeron 300®, Altana Pharma, Konstanz, Germany) administered at a flow rate of 4 mL/s using a bolus trigger technique (positioning of the respective region of interest (ROI) in the abdominal aorta just above the celiac trunk, threshold 150 Hounsfield Units (HU), start delay 10 s). The portal phase started with a delay of 50 s after reaching the threshold.

MRI was performed using a 1.5-Tesla MR scanner (Magnetom Vision®, Siemens Medical Solutions, Erlangen, Germany; software: syngo MR 2004A 4VA25A) with two body coils (CP Body Array Flex®). The study protocol covered (1) T1w-2D-Flash fatsat (TE/TR 2.72/129 ms, flip 70° , slice 6 mm, matrix 256*), (2) T2w TSE (TE/TR 102/4000 ms, flip 150° slice thickness 6 mm, matrix 256*), (3) in phase and out of phase (TE/TR 2.36/4.76/108 ms, flip 70°, slice 6 mm, matrix 256*), and (4) five dynamic contrast-enhanced T1w-3D-Flash fat sat sequences (TE/TR 1.56/4.6 ms, flip 15°, slice 4 mm, matrix 256*) with 0, 20, 45, 90, and 300 s start delay after contrast material injection (0.1 mmol/kg Gadolinium-DTPA (Magnevist®, Bayer Schering Pharma AG, Berlin, Germany), 2 mL/s by power injector (Spectris®, Medrad, Dusseldorf, Germany).

All phases of the MDCT and MRI scans were independently analyzed by two independent investigators with respect to the number, size, and location of the tumours. Both investigators had at least 10 years experience in evaluating HCC in daily practice. In order to gain the highest diagnostic sensitivity, each nodule was rated positive whenever CT or MRI or both modalities were equivocally positive by both investigators in consensus. Positive diagnosis was based on the EASL and American Association for the Study of Liver Diseases (AASLD) guidelines which require hypervascularization in

Table 1 Demographics, aetiology of liver cirrhosis and clinical condition of the patients (*n* = 28)

	n
Gender	
Male	25
Female	3
Mean age (yr)	67.0 ± 10.8 (range 46-89)
Aetiology of liver cirrhosis	
Ethanol	13
Hepatitis B	2
Hepatitis C	7
Cryptogenetic	6
Clinical stage	
BCLC stage	
A	6
B	22
C	0
D	0
Child Pugh	
A	24
B	4
C	0
Okuda	
I	25
II	3
III	0
ECOG	
0	26
I	2
II	0
III	0
IV	0
Histological tumour grading	
Well	16
Moderate	4
Poor	2
Unknown	3
No biopsy	3

BCLC: Barcelona Clinic Liver Cancer; ECOG: Eastern Cooperative Oncology Group.

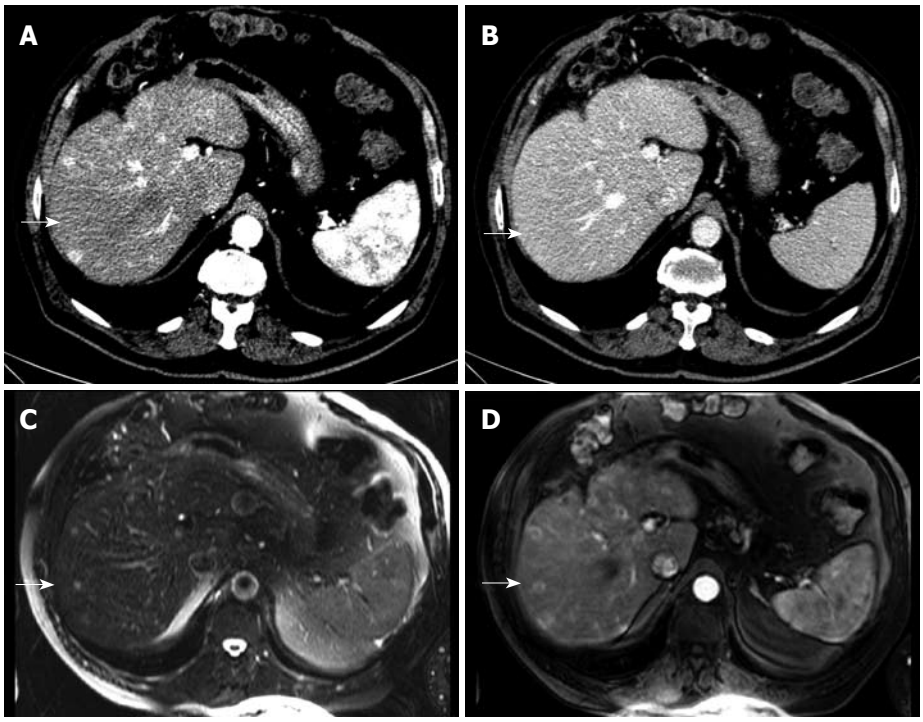

Figure 1 71-year-old man with biopsy-proven hepatocellular carcinoma (HCC). Detection of an additional tumor nodule by magnetic resonance imaging (MRI), size 12 mm (size category ⩽ 15 mm). Multi-detector computed tomography (MDCT) demonstrates two hypervascularized tumor nodules in the contrast-enhanced arterial phase (A, arrow) but not in the portal venous phase (B, arrow). MRI arterial phase depicts one more tumor nodule (arrows) in the T2w (C) and the T1w contrast-enhanced early arterial phase (D).

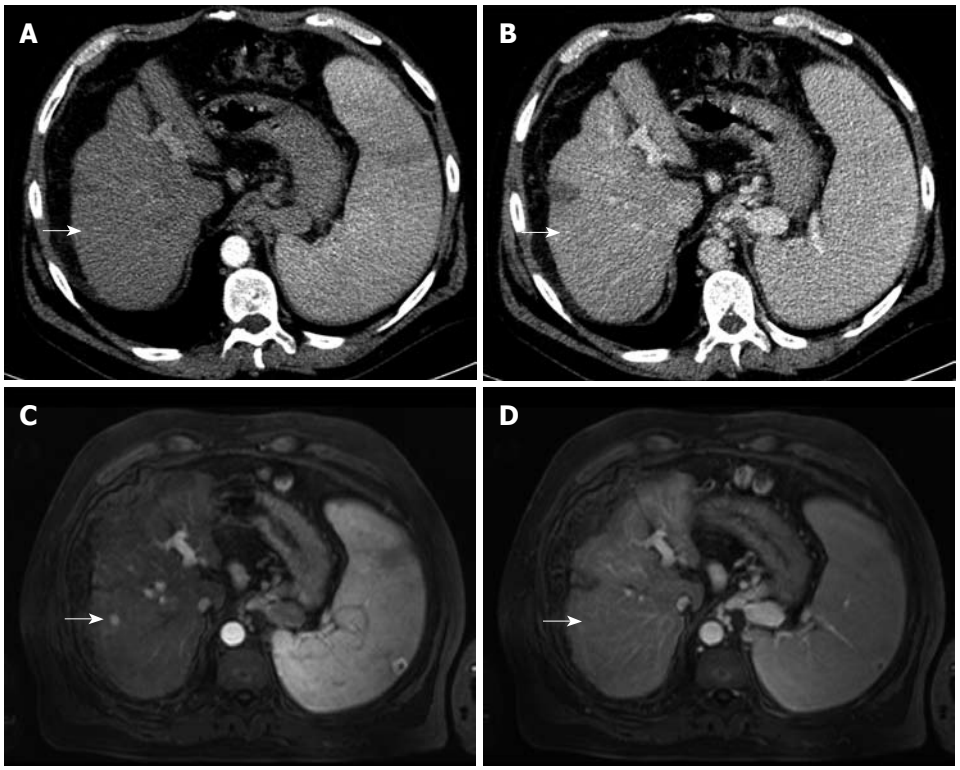

Figure 2 70-year-old man with biopsy-proven HCC. Detection of an additional tumour nodule by MRI, size 10 mm (size category ⩽ 10 mm). MDCT does not show any contrast enhancement in the arterial (A, arrow) or portal venous phase (B, arrow). MRI arterial phase depicts one more tumour nodule (C, arrow) which is hypo- to isointense on the portal venous phase (D, arrow).

arterial phase and contrast washout in the early or delayed venous phase[15]. All but three cases were histologically proven by biopsy from one representative nodule. Biopsy proof from each nodule, however, is not feasible *in vivo* due to ethical reasons. The other three cases were not histologically proven because of poor coagulation status and inappropriate subcapsular location for biopsy in order to avoid tumor cell spreading. However, these cases fulfilled the diagnosis criteria according to EASL (hypervascularized nodules > 2 cm in two imaging modalities).

All images were analyzed on a separate workstation with magnification. Tumour diameters were sized with a measuring tool integrated in the workstation software. All nodules visible in both modalities were compared in size. Additionally, the influence of HCC aetiology on tumour detection was analysed. Explanted liver specimens from patients who underwent liver transplantation (3 ×) or hemihepatectomy (1 ×) were analyzed pathologically. The specimens were cut in 4 mm slices in the same orientation as in CT and MRI in order to compare the findings. All nodules found by the

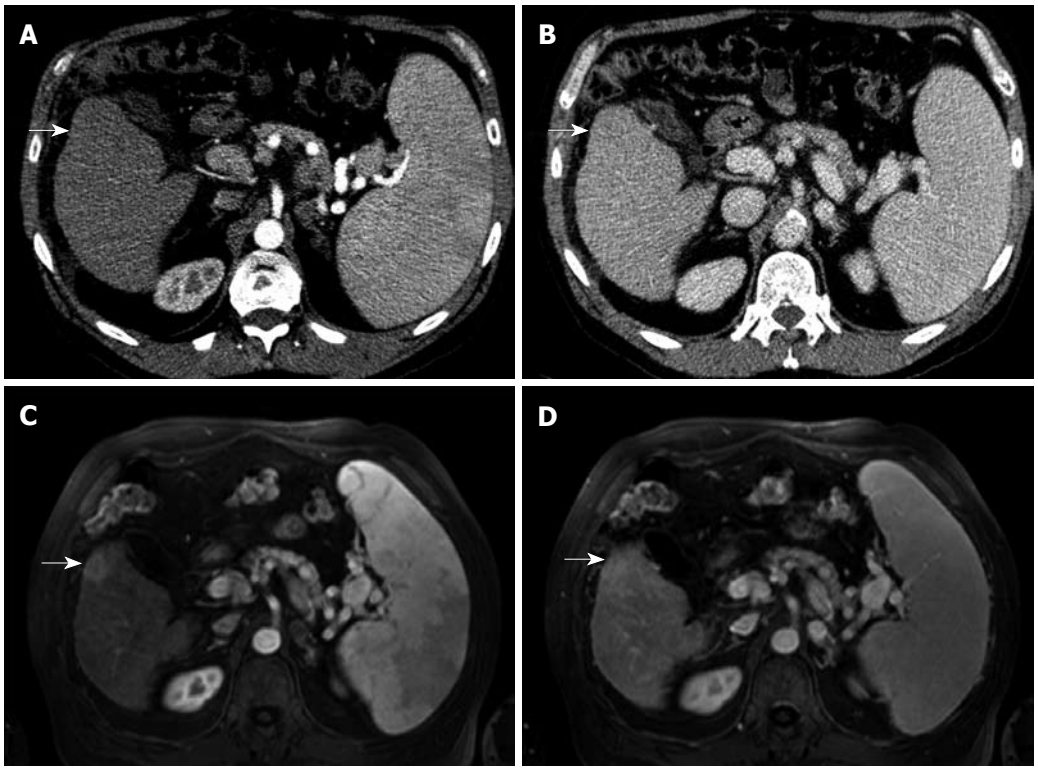

Figure 3 70-year-old man with biopsy-proven HCC. Detection of an additional tumour nodule by MRI, size 19 mm (size category ≤ 20 mm). MDCT demonstrates no hypervascular enhancement in the contrast-enhanced arterial phase (A, arrow) or the portal venous phase (B, arrow). MRI arterial phase depicts a hypervascularized area in the T1w phase (C, arrow) which became isointense in the portal venous phase (D, arrow).

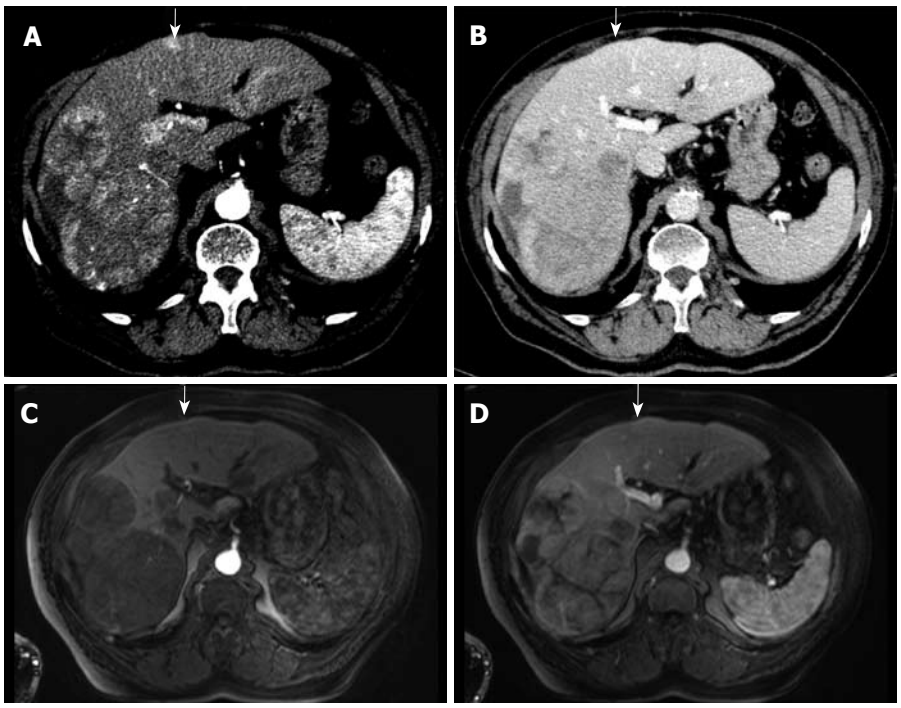

Figure 4 82-year-old man with biopsy-proven HCC. Detection of an additional tumour nodule by MDCT. The contrast-enhanced arterial phase MDCT demonstrates large tumours in the right liver lobe and one additional hypervascularized nodule in segment 4 (A, arrow) but not in the portal venous phase (B, arrow). Contrast-enhanced MRI depicts the large tumours in the right liver lobe but not in segment 4 (arrows) in early arterial phase (C) and portal venous phase (D).

pathologist were correlated to the CT and MRI data and investigated histologically.

Statistical analysis

Sensitivity for tumour detection was calculated with "R" (R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Version 2.5.0, Vienna, Austria, 2007), including Geepack (Generalized Estimating Equations). *P* values less than 0.05 were considered statistically significant. Statistical testing was performed by an independent statistician to avoid review bias.

RESULTS

Consensus reading of MRI and MDCT depicted a total of 162 nodules. On a per nodule basis, MRI detected

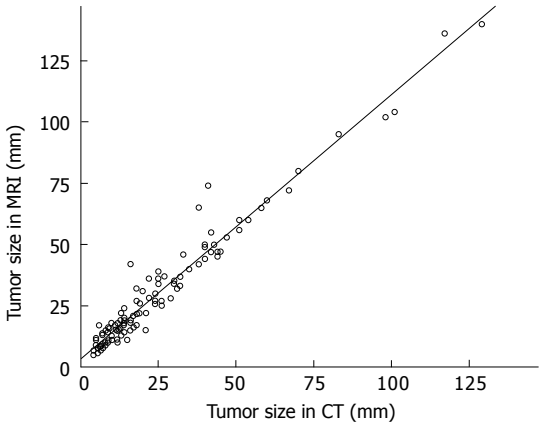

Figure 5 Correlation of tumor sizes measured with MDCT and MRI using a scatterplot. There is a tendency towards greater diameters on MRI compared to MDCT (y = 1.08x + 3.2).

Table 2 Diagnostic impact of imaging protocols on tumour detection *n* (%)

Imaging protocols of MDCT and MRI	Tumour nodules detected
MRI, T1w 3D-Flash, arterial phase (20 s start delay)	158 (97.5)
MRI, T1w 3D-Flash, portal-venous phase (45 s start delay)	145 (89.5)
MRI, T1w 3D-Flash, equilibrium phase (90 s start delay)	127 (78.4)
MDCT, arterial phase (bolus trigger for start delay)	119 (73.5)
MRI, T1w 3D-Flash, delayed phase (300 s start delay)	115 (71.0)
MRI, T1w 3D-Flash, dynamic phase Phase (T1 native)	109 (67.3)
MRI, T1w 2D Flash native	104 (64.2)
MRI, Dual-GRE in-phase	98 (60.5)
MRI, Dual-GRE out-phase	96 (59.3)
MDCT, portal-venous phase (55 s start delay)	84 (51.9)
MRI, T2w TSE	72 (44.4)
MDCT, native phase	56 (34.6)

MDCT: Multidetector computed tomography; MRI: Magnetic resonance imaging.

significantly more nodules than MDCT (159 *vs* 123, $P \leqslant 0.001$, Figures 1-3), resulting in an overall sensitivity of 0.98 for MRI and 0.76 for MDCT. The best diagnostic sensitivity was ascertained for the early arterial phase MRI, followed by portal venous phase MRI, equilibrium phase MRI, arterial phase MDCT, and late phase contrast enhanced MRI (Table 2). For native MRI phases (T1w, T2w, Dual-GRE in/out phase) and native and portal venous MDCT phases, sensitivities were low. Negative MDCT findings included 5 nodules $\leqslant$ 5 mm, 17 nodules $\leqslant$ 10 mm, 12 nodules $\leqslant$ 15 mm, 4 nodules $\leqslant$ 20 mm, and 1 nodule greater than 20 mm. In contrast, MRI missed two nodules $\leqslant$ 10 mm and one nodule $\leqslant$ 15 mm. With respect to unilobular and bilobular tumour dissemination, MRI detected 7 MDCT-negative nodules in 2 patients which were located in the contralateral liver lobe and could have changed the therapeutic strategy if not detected. In contrast, the three nodules missed in MRI (Figure 4) had no influence on the treatment regimen because the patient had multinodular disease in both lobes.

Figure 5 shows the sizes of the nodules positive in both, CT and MRI. Compared to MDCT, the diameters of the tumour nodules were slightly greater in MRI (29.2 $\pm$ 25.1 mm *vs* 24.1 $\pm$ 22.7 mm, $P < 0.005$, Figure 5). The median lesion diameter was 15.5 mm (range 5 mm to 140 mm) in MRI compared to 12 mm (range 4 mm to 129 mm). Irrespective of the false-negative MDCT findings, tumour diameters were underestimated with MDCT in 43 nodules compared to MRI. In contrast, MRI underrated tumour diameter in only one case compared to MDCT (Table 3).

During the study period, three patients underwent liver transplantation and one was allocated to hemihepatectomy. The explanted specimens (three complete organs and one right liver lobe) were transected in 4 mm slices in transverse orientation for a comparative correlation with the respective MDCT and MRI slices (Figure 6). These four specimens revealed a total number of 20 tumour nodules, 16 of which were depicted by MRI (80%) and only 13 by MDCT (65%).

DISCUSSION

The main reason for the poor survival of HCC patients is the fact that most cases are not diagnosed until disease has reached an advanced stage, which limits the most effective therapeutic options[7]. HCC cases that fulfil the Milan criteria (one nodule < 5 cm or three nodules < 3 cm) might be indicated for liver transplantation with curative intention because it not only completely removes the tumour but also the critical precancerous liver cirrhosis[8]. However, a significant number of additional intrahepatic tumours have been missed in comparative radiological studies, particularly small nodules < 20 mm[8,13,16], calling the decision-making process into question. Moreover, resection and local tumour ablation with percutaneous ethanol injection (PEI) or radiofrequency ablation (RFA) might have curative potential if the tumour nodules are not multilocular and do not exceed defined nodule diameters[17]. Thus, for optimal treatment allocation, current efforts in diagnostic work-up focus on increasing the correctness of preoperative diagnosis with respect to number, size, and location of tumour nodules.

The purpose of the present study was to evaluate the diagnostic potential of 64-row MDCT and MRI for the detection of hypervascularized tumour nodules in the cirrhotic liver for adequate treatment allocation of HCC patients. MDCT has advantages compared to MRI, such as fewer motion artifacts due to much shorter scanning time (3 to 5 s *vs* 18-25 s) and higher spatial in-plane resolution (512* *vs* 256* Matrix). However, overall sensitivity of state-of-the-art 64-row MDCT has been demonstrated to be significantly inferior compared to contrast-enhanced MRI and thereby confirms respective findings from older studies with less sophisticated CT technology[9-11,18,19]. Recent data have reported slightly higher detection rates for MDCT compared to MRI[20]. In our study, however, the outstanding contrast resolution of MRI scored much better with greater sensitivity particularly in small lesions of $\leqslant$ 10 mm compared to MDCT (0.95 *vs* 0.48, $P < 0.001$) which is in concordance

Table 3 Results of consensus reading of MDCT and MRI: No. of detected tumour nodules by MDCT and MRI depending on tumour size scaling

		MRI						
		Negative	≤ 5 mm	≤ 10 mm	≤ 15 mm	≤ 20 mm	> 20 mm	Total
MDCT	Negative		5	17	12	4	1	39
	≤ 5 mm		2	2[1]	2[1]			6
	≤ 10 mm	2		13	11[1]	4[1]		30
	≤ 15 mm	1		1[2]	12	11[1]	4[1]	29
	≤ 20 mm					3	9[1]	12
	> 20 mm						46	46
	Total	3	7	33	37	22	60	162

[1]Nodules which appeared greater in MRI compared to CT; [2]Only the single nodule was bigger in CT compared to MRI.

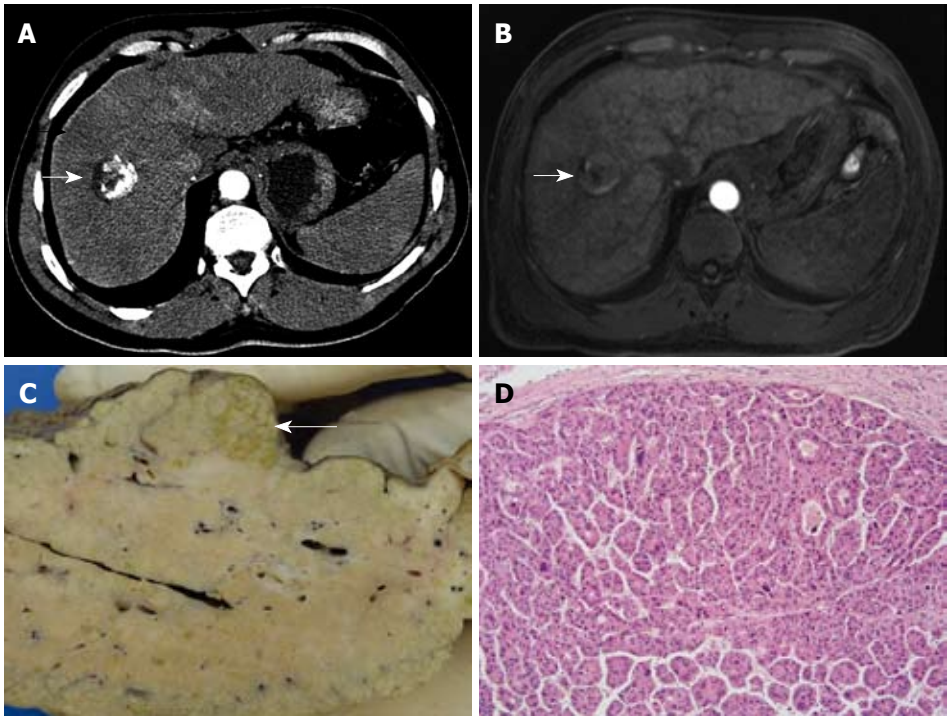

Figure 6 54-year-old man with biopsy-proven HCC. False-negative finding in the two modalities. Contrast-enhanced early arterial and portal venous phase MDCT (A) and arterial and portal venous phase MRI (B) detected a 3 cm tumour in the right liver lobe (A, B, arrows) but failed to detect another tumour nodule at the posterior surface of the left liver lobe. The explanted liver specimen clearly depicts this additional 2 cm tumour nodule on gross-sectional pathology (C, arrow) and histology (D, 10 × magnification, HE staining).

with recent reports from the literature[18,21].

Our study has some limitations. First, there was an obvious bias in patient recruitment since prior imaging results had suggested HCC in all patients before they entered the study protocol. Only 4 patients were diagnosed at an early stage with tumour nodules of small diameters so that they could be allocated to either local-ablative treatment with radiofrequency ablation or liver transplantation if they complied with the Milan criteria[8]. The majority, however, was diagnosed in an intermediate stage with multilocular disease and sometimes large tumour nodules which might have biased sensitivity. Second, the start delay for the arterial phase CT was 10 s after reaching the trigger threshold. This might have been slightly too short in light of recent reports[22] and could possibly explain the great difference between the sensitivity rates of MDCT and MRI. Third, for histological confirmation of HCC diagnosis, in most cases only one representative lesion was examined pathologically, meaning that the diagnosis of additional tumour nodules relied on imaging only. Fourth, the reference diagnosis might not actually represent the final pathological diagnosis in all cases. For example, the explanted specimen of four cases (3 × liver transplantation, 1 × hemihepatectomy) revealed a total of 20 tumour nodules, whereas MRI and MDCT had depicted only 16 and 13, respectively. So far, we have to concede that even the dedicated MRI protocol used, underrated the intrahepatic tumour spread compared to histological examination. This is consistent with the findings of previous studies which demonstrated an even worse overall tumour detection rate of around 50%-70%[8,13,16]. Fifth, the consensus reading of MDCT and MRI could have potentially overestimated nodules in MDCT and MRI by mistaking a benign hypervascularized lesion for a malignant nodule due to the lack of an absolute standard of reference. However, in light of the specimens mentioned above, the underrating of the nodule numbers

and tumour spread is still a major issue for HCC diagnosis with both modalities.

Despite these limitations, the data demonstrate that diagnostic results depend considerably on the multiphasic imaging protocols. Although the early arterial phase in MRI depicts the greatest numbers of tumour nodules[23], it potentially underestimates the real tumour spread in particular cases and might result in incorrect treatment allocation with respect to the Barcelona Clinic Liver Cancer (BCLC) classification[8,12,24-28]. Substantial efforts are required to improve the diagnostic correctness, and MRI seems to have better pre-requisites and a greater potential for future developments, either by improving MRI sequences or by employing more specific contrast materials[9,29-31], the double contrast technique[32,33] or special imaging techniques[34]. Since MDCT failed to demonstrate equivalence with MRI, triphasic contrast-enhanced MDCT protocols might only be used in the first instance. However, if CT suggests local-ablative treatment, resection, or allocation to liver transplantation, dynamic multiphasic contrast-enhanced MRI should be used in order to exclude additional tumour nodules which would probably change the initial strategy.

In conclusion, dynamic contrast-enhanced MRI is superior to triphasic 64-row MDCT for detecting numbers, sizes, and distribution of HCC tumour nodules. HCC patients should be assigned to operative or interventional treatment according to a dedicated MRI protocol.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer death with 600 000 to 1 million new cases being diagnosed each year. Due to the lack of specific early symptoms, most cases are not diagnosed until the disease is already in an advanced stage, which limits the most effective therapeutic options. That is why, although new treatments are available, patient survival has not significantly improved in the last 30 years. Therefore, early tumor detection is one of the most important issues in HCC therapy. The study was to compare the diagnostic capability of two imaging systems, computed tomography (CT) and magnetic resonance imaging (MRI), for tumor detection.

Research frontiers

In recent years, an enormous improvement has been taking place in the field of imaging systems. With new generations of CT and MRI scanners available, the question arised, which one is the better technique to depict this tumor.

Innovations and breakthroughs

Previous studies used older equipment, e.g. single slice CT. The advance in this study is the evaluation of a new scanner generation (64-row CT) which allows faster acquisition and therefore fewer motion artifacts with a lower slice thickness.

Applications

This article should help radiologists, gastroenterologists and other physicians dealing with HCC patients in daily practice to use the correct method of imaging in the right patient.

Peer review

The study aimed at determination of the nodule detection sensitivity of MDCT compared with MRI. The presentation of the data should be more concise.

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BRIEF ARTICLE

Different faces of gastroparesis

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Abstract

AIM: To test the hypothesis that pain and affect rather than impaired emptying determine symptom severity in patients with gastroparesis.

METHODS: Adult patients with documented gastroparesis were enrolled prospectively in a single center and asked to complete the Gastroparesis Cardinal Symptom Index (GCSI), Hospital Anxiety and Depression Scale (HADS), the Short Form 12 (SF-12) as quality of life index, rate pain severity and answer 10 open-ended questions.

RESULTS: A total of 55 patients (44 women) participated. Idiopathic (n = 29) or diabetic (n = 11) gastroparesis and connective tissue disease (n = 8) were the most common underlying causes. Antiemetics (n = 30) and prokinetics (n = 32) were most often prescribed. Seventeen patients used opioids on a daily basis. Nausea and/or vomiting (n = 28), pain (n = 24) and bloating (n = 14) were most commonly listed as dominant symptoms. Patients subjectively attributed symptom improvement to nutritional and dietary therapy (n = 11), prokinetics (n = 11), antiemetics (n = 10) or analgesic agents (n = 3). In univariate analyses, the physical subscore of the SF-12 and HADS, but not gastric emptying delay or symptom duration significantly correlated with disease severity as measured by the GCSI. In multivariate analyses, the combination of vomiting, bloating and depression best predicted the overall impact on quality of life.

CONCLUSION: The study confirms the importance of pain and affect in gastroparesis, which requires novel approaches to improve more effectively the quality of life in patients with this disorder.

Key words: Pain; Depression; Gastroparesis; Quality of life

Peer reviewer: Volker F Eckardt, MD, Professor, Chief, Department of Gastroenterology, Deutsche Klinik für Diagnostik, Aukammallee 33, 65191 Wiesbaden, Germany

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INTRODUCTION

Gastroparesis is an increasingly diagnosed functional disorder, characterized by delayed emptying of gastric contents. Considering the defining abnormality, prokinetics continue to play a primary role in managing this disorder[1-3]. However, cross-sectional and longitudinal studies have demonstrated a poor correlation between the documented delay in gastric emptying and symptom severity[4-6]. Moreover, motilin agonists do not improve symptoms even though they significantly accelerate gastric emptying[5,7-10]. Thus, other mechanisms must contribute to the clinical manifestation of gastroparesis, such as impaired gastric accommodation, altered distribution of ingested material within the stomach, and peripheral and/or central hyperalgesia[1-4]. The relative importance of any of these potential mechanisms remains unclear. More importantly, there is no evidence that medication that targets these abnormalities is indeed beneficial when used clinically. As we typically cannot change the underlying processes that lead to gastroparesis, treatment remains largely symptomatic with the primary goal of improving the overall quality of life of patients suffering from this, at times, disabling disorder.

We performed a cross-sectional study in patients with confirmed gastroparesis to define better the relative importance of key symptoms of gastroparesis, their

impact on the patients' functional status, and the potential differences between patient subgroups defined by the underlying etiology. The main hypotheses were: that pain significantly contributes to the impaired quality of life in patients with gastroparesis, and that affect plays an important role in determining symptom severity in patients with gastroparesis.

MATERIALS AND METHODS

Patient recruitment

Adult patients with confirmed gastroparesis seen by one investigator (Bielefeldt K) in the Digestive Disorders Clinic of the University of Pittsburgh Medical Center were invited to participate. Patients with gastroparesis had to be symptomatic for at least 6 wk and have objective evidence of impaired gastric function with delayed gastric emptying by scintigraphy, or documentation of retained food within the stomach after a 12-h fasting period obtained by endoscopy or contrast study. Structural abnormalities, such as gastric outlet obstruction or mechanical obstruction in the more distal gastrointestinal tract had to be excluded. The protocol was approved by the Institutional Review Board of the University of Pittsburgh (PRO8020059).

Surveys

After obtaining informed consent, all patients were asked to provide basic demographic information, complete the Gastroparesis Cardinal Symptom Index (GCSI), the Hospital Anxiety and Depression Scale (HADS), and the Medical Outcomes Study Short Form 12 (SF-12).

The GCSI is a self-administered assessment tool developed for patients with gastroparesis. The original survey comprises three subscales: postprandial fullness/early satiation (4 items); nausea/vomiting (3 items), and bloating (2 items). Considering the potential importance of pain, pain rating was added to the scale and scored similarly to the other items. Internal consistency and test-retest reliability were 0.84 and 0.76, respectively[11].

The HADS is a 14-item self-report questionnaire that asks patients to rate symptoms using statements that are converted into a numeric score between 0 and 3. The total score of can range between 0 and 21 for anxiety and depression. Extensive studies support an internal consistency exceeding 0.8. When used in different populations, scores of > 8 have a sensitivity around 80% for depressive or anxiety disorders[12].

The SF-12 is a self-report measure of perceived health, which consists of 12 questions. Two summary scores are generated: a physical health factor score (PHS) and a mental health factor score (MHS). The scores have been constructed with the population norm for each score being 50. Compared with the more extensive Short Form 36 (SF-36), the measure has been shown to be valid with correlations of 0.905 for the SF-36 Physical Component Summary and 0.938 for the SF-36 Mental Component Summary[13].

Open ended questions

Patients were also asked a series of open-ended questions (Table 1) that focused on the impact of gastroparesis on professional and private spheres of their lives. These questions included verbal assessments of the most significant symptoms, perceived treatment effects, and concerns related to the disease.

Data abstraction

Clinical data about nature and duration of symptoms, severity, character, location of pain, weight changes, documented delay in gastric emptying, prior and ongoing treatment were abstracted after the clinical encounter, de-identified and linked to the survey data.

Statistical analysis

Responses to open-ended questions were analyzed by two independent coders who were aware of the underlying diagnosis of gastroparesis, but were blinded to patient identity, survey data as well as duration and etiology of the disorder. The qualitative analysis focused on frequency rather than expressions of severity of factors. A codebook was generated to record responses. Statements supporting the code were highlighted and added to a master file that contained all qualitative data.

Dichotomous variables were analyzed using χ^2 statistics. Continuous and categorical variables are given as mean ± SE. Group comparisons were performed using analysis of variance. The GCSI summary score and quality of life as measured by the SF-12 were defined as the main endpoints. In an initial univariate analysis, correlations between the main endpoints and between different measures and the main endpoints were analyzed using Spearman correlation. Only variables with $P < 0.1$ were entered into the multilinear regression model.

RESULTS

Patients

Between June 2008 and February 2009, a total of 55 patients (mean age: 42.4 ± 1.9 years; 81% women) agreed to participate (Table 2). Gastric emptying studies had documented delayed emptying in all but five patients, who could not tolerate or complete studies because of emesis. In these individuals, retained food ($n = 4$) or a bezoar ($n = 1$) had been demonstrated endoscopically prior to enrollment. Thus, all patients met the entry criterion of objectively documented impairment in gastric emptying. However, studies were not performed in a single center with differences in test meals, test duration and data reporting. In 26 patients, the half-emptying time for solid-phase gastric emptying had been calculated and given. One additional patient only underwent a gastric emptying study for liquids that showed a significant delay. In five patients, only gastric retention at 90 or 120 min was quantified and compared with institutional norms. In the remaining patients, only a qualitative comment described delayed gastric emptying without further quantification by radiologists in outside institutions. The mean half-emptying time for the available studies was 319 ± 50 min.

The most common causes of gastroparesis were diabetes mellitus ($n = 11$), connective tissue disease (n

Table 1 Open-ended questions

How does the stomach problem affect your life?
How has gastroparesis affected your relationships with family and friends?
How has gastroparesis affected your ability to work and your professional life?
What do you worry about when you think about your stomach problem?
What symptom or problem related to your gastroparesis disturbs you the most?
What helped you the most in the treatment of your stomach problem?
What do you know and understand about your stomach problem?
Patients are often left with questions about their condition. What do you want to know about your disease?
What were your experiences as a patient with gastroparesis when you met physicians, nurses and other healthcare providers?
What can we do to better serve patients with your condition?

Table 2 Baseline patient characteristics *n* (%)

Variable	All patients	Idiopathic gastroparesis	Diabetic gastroparesis	Connective tissue disease
Women	55 (80)	29 (90)	11 (55)	8 (100)
Age (yr)	42.4 ± 1.9	38.1 ± 2.8[b]	46.0 ± 2.9	57 ± 2.2
Education				
High school	38	20	8	5
Bachelor	9	5	1	1
Graduate	8	4	2	2
Employed	15 (27)	11 (38)	3 (27)	0
Annual household income ($)				
< 20000	20	10	7	0
20000-39000	13	8	2	2
40000-59000	8	5	0	2
60000-79000	7	3	0	2
> 80000	7	3	2	2
Symptom duration (mo)	32 ± 4	24.2 ± 4.2	44 ± 15	25.8 ± 6.7
Weight loss (pounds)	3.7 ± 1.6	4.5 ± 2.6	2.9 ± 2.7	7.1 ± 3.6

[b]$P < 0.01$ *vs* patients with gastroparesis due to connective tissue disease.

Table 3 Treatment of gastroparesis

Variable	All patients	Idiopathic gastroparesis	Diabetic gastroparesis	Connective tissue disease
Prokinetics				
Metoclopramide	25	11	7	4
Erythromycin	7	4	2	1
Other	4	1		3
Antiemetics				
Phenothiazine	21	10	5	2
Ondansetron	20	12	4	1
Scopolamine	6	3	2	
Meclizine	2	1	1	
Dronabinol	5	3		
Antidepressives				
TCA	3	1	1	
SSRI	15	4	5	4
Benzodiazepines	16	10	3	1
Opioids (daily)	17	7	5	1
Nutritional support				
Jejunostomy	8	1	4	
TPN	3	2		1

TCA: Tricyclic antidepressant; SSRI: Selective serotonin reuptake inhibitor; TPN: Total parenteral nutrition.

= 8), abdominal surgery or trauma (n = 4), osteogenesis imperfecta (n = 1), mitochondrial myopathy (n = 1) and Marfan syndrome (n = 1). In the remaining 29 patients, no underlying cause could be identified. Four of the patients with idiopathic gastroparesis recalled an acute illness prior to the onset of the disease, which suggested a post-infectious form of gastroparesis. Patients with idiopathic gastroparesis were significantly younger than those in the other two major groups (Table 2). Gastroparesis caused by connective tissue disease showed an expected female predominance, considering the preferential manifestation of systemic sclerosis in women[14]. Similarly, 90% of patients with idiopathic gastroparesis were women, while nearly half of the patients with diabetic gastroparesis were men ($P < 0.05$).

Symptoms of gastroparesis had been present for 32 ± 4 mo. Ten patients reported a significant weight loss that exceeded 5% of their body weight within the preceding 6 mo. The average weight loss for the entire group was 1.7 ± 0.7 kg. All but four patients (93%) complained about nausea. A sense of bloating, fullness and/or early satiation was present in 47 (87%) patients. At least mild pain was mentioned by 44 (81%) patients. The pain was primarily postprandial in 11 patients, with the remaining 34 participants complaining about constant discomfort. The pain was typically located in the epigastrium and described as pressure (n = 18), sharp (n = 12) or burning sensation (n = 7). Three patients mentioned generalized abdominal pain. In one patient, the pain radiated to the right upper quadrant and in another to the chest.

Seven patients (6 idiopathic gastroparesis; 1 diabetic gastroparesis) reported undergoing a cholecystectomy for biliary dyskinesia after symptom onset, which led to transient improvement in five patients. However, symptoms recurred within 3 mo in all but one of these patients, who continued to do well for 4 years before experiencing recurrent problems. Within the month prior to enrollment, 32 (60%) patients were using prokinetic agents, most commonly metoclopramide (Table 3). Ten of these patients experienced at least moderate side effects with extrapyramidal motor disorders (n = 3) worsening fatigue (n = 5) or depression (n = 1), which led to discontinuation of the agent. One patient was switched to domperidone. Three patients with systemic sclerosis and gastroparesis complicated by chronic intestinal pseudo-obstruction were using octreotide (n = 2) or pyridostigmine (n = 1). A total of 30 (55%) patients required daily antiemetic medication, most commonly phenothiazines and/or ondansetron. Eighteen patients (33%) received chronic antidepressant medication; mostly serotonin reuptake inhibitors. In four

Table 4 Survey data

Variable	All patients	Idiopathic gastroparesis	Diabetic gastroparesis	Connective tissue disease
GCSI	25.7 ± 1.4	24.6 ± 2.0	30.7 ± 2.3	20.8 ± 2.5
HADS				
Anxiety	8.3 ± 0.6	8.6 ± 0.9	9.3 ± 1.1	6.6 ± 1.7
Depression	7.7 ± 0.7	7.1 ± 1.1	9.9 ± 1.2	6.3 ± 1.5
SF-12				
PHS	31.5 ± 1.4	33.3 ± 2.0	28.7 ± 2.8	31.7 ± 3.6
MHS	41.7 ± 1.6	41.4 ± 2.1	37.7 ± 3.5	47.7 ± 4.1

GCSI: Gastroparesis Cardinal Symptom Index; HADS: Hospital Anxiety and Depression Scale; SF-12: Short Form 12; PHS: Physical health factor score; MHS: Mental health factor score.

Table 5 Univariate correlation analysis

Variable	Spearman coefficient	*P*
GCSI		
SF-12 PHS	-0.56	0.0001
SF-12 MHS	-0.212	0.12
HADS-anxiety	0.17	0.22
HADS-depression	0.33	0.02
T-1/2	0.05	0.82
Symptom duration	-0.08	0.56
Age	-0.08	0.55
PHS		
GCSI-sub score nausea	-0.52	0.0001
GCSI-sub score fullness	-0.4	0.003
GCSI-sub score bloating	-0.4	0.003
GCSI-sub score pain	-0.35	0.01
HADS-depression	-0.44	0.001

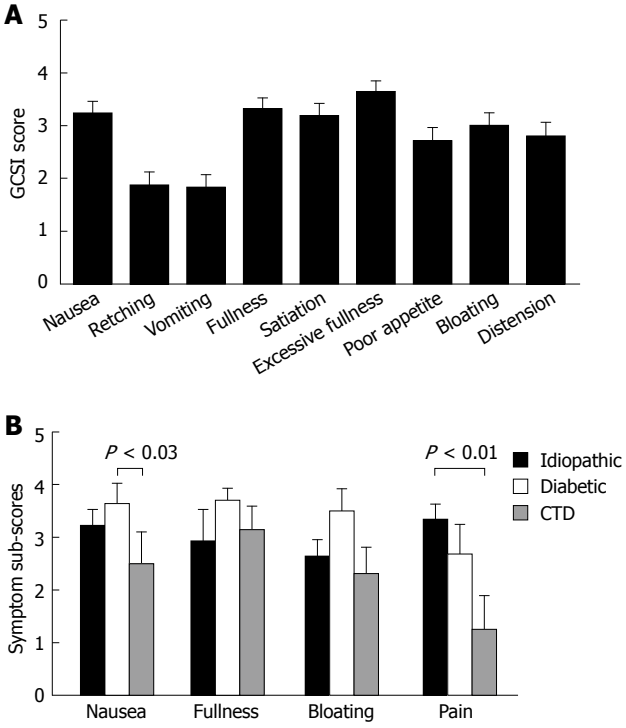

Figure 1 Symptom severity scores in patients with gastroparesis. The individual GCSI scores are shown for the entire group (A). Component subscores for the GCSI and pain ratings are summarized for the three major patient groups, to allow comparison between the different etiologies of gastroparesis (B). Significant differences were seen for nausea (diabetes *vs* connective tissue disease) and pain (idiopathic *vs* connective tissue disease). CTD: Connective tissue disease; GCSI: Gastroparesis Cardinal Symptom Index.

patients, tricyclic antidepressants or duloxetine were given to improve pain control. Regular use of benzodiazepines was reported by 16 (29%) patients. A total of 17 patients (31%) received chronic opioids for pain management. In two patients, narcotics were given for painful diabetic neuropathy. One patient had sickle cell anemia with severe bone pain. An additional patient with severe joint and muscle pain caused by systemic sclerosis and polymyositis used a fentanyl patch, which left 13 (24%) patients who were taking opioids daily for control of their abdominal pain. As opioids can impair gastric emptying, assessment of gastric function was performed after transient discontinuation of narcotics in all but the two patients who suffered from painful diabetic neuropathy. A total of 11 (20%) patients received nutritional support *via* enteral ($n = 8$) or parenteral ($n = 3$) nutrition.

Survey data

As shown in Table 4, the GCSI summary score demonstrated a moderate symptom severity for the entire group. Group comparisons only showed a trend for a lower overall score in patients with connective tissue disorders as the underlying etiology ($P = 0.075$). Figure 1A summarizes the individual symptom scores for the entire group. When asked to rate abdominal pain using the GCSI coding scale, the mean pain severity was 2.97 ± 0.24, which was similar to the subjectively rated severity of other symptoms. The pain rating correlated with the pain scale of the SF-12 that assessed the impact of pain over the preceding month ($R^2 = 0.22$, $P < 0.01$), and the routinely assessed patient pain rating that addressed pain presence on the day of the clinic visit ($R^2 = 0.36$, $P < 0.001$). A group comparison of component scores revealed more significant nausea for patients with diabetic gastroparesis and higher pain scores for patients with idiopathic gastroparesis (Figure 1B).

Patients in all groups had moderately elevated scores for both anxiety and depression, without significant differences between the groups. Using the proposed cutoff score of > 8[12], 40 participants (74%) met screening criteria for anxiety or depression. In 16 patients (29%), anxiety and depression were both above the proposed threshold for clinically relevant affective spectrum disorders.

Compared to a population norm of 50, the mental and the physical component of the SF-12 showed a significant impairment of health-related functional status. There were no significant differences between the groups.

The GCSI summary score showed a significant inverse correlation with the physical but not mental score of the SF-12. It was correlated positively with depression scores, but not HADS anxiety scores, age, symptom duration or degree of gastric emptying delay (Table 5). In order to identify the relative contribution of symptoms, we performed univariate analyses, correlating the physical health scale of the SF-12 with GCSI subscores and pain ratings. Based on these results, we performed

multiple linear regressions to identify the most important predictors of poor health function. A combination of GCSI subscores for nausea and bloating and the HADS score for depression best predicted the PHS subscore with an adjusted R^2 of 0.44.

Qualitative data

When asked to describe the impact of gastroparesis on their lives, most patients focused on the effects of interactions with family, friends or colleagues (n = 37). Three main topics emerged in the more detailed analyses of responses. First, eating out, dinners or other social functions were seen as causing difficulties because of the limited ability to tolerate food (n = 26). One patient explained these problems: "[With] every- and anything that goes on socially there is food. When you can't eat, it is really hard to cope with. People feel funny asking you out to dinner or invite you to something where there will be food." The second problem was related to fatigue, which made it difficult for patients to meet expectations (n = 14) as exemplified by statements such as "I push myself to get out of bed every morning" or "I can barely make it through the day". The third major topic revolved around the frustration and emotional impact of the disease as patients experienced difficulties interacting with others (n = 16). One patient summarized her experience: "…enjoying life has become difficult because my mind is always on my stomach".

Participants cited a significant strain on their relationships with partners and/or family members (n = 13). Such problems could be a simple consequence of limitations imposed by their disease, as this woman explained: "… having to sleep sitting up doesn't contribute to closeness with your partner". A young mother with idiopathic gastroparesis reported that her disease "…has prevented me from caring for my children and participating in their activities". In other cases, it was more anxieties and concerns in response to the physical evidence of illness, such as frequent vomiting: "…children - always frightened I will never get well or I will die". Some patients reported a gradual withdrawal of others in the face of ongoing illness: "My friends don't really call anymore because they know I am always feeling sick. … I also broke off a wedding engagement with someone I was with for 9 years because he couldn't stand how sick I was and felt I was holding him back".

When asked about the impact of gastroparesis on their professional activity, three patients described themselves as retired and seven responded that they were on disability (in 2 cases, for reasons other than gastroparesis). An additional eight patients mentioned that they felt forced to quit jobs or school because of their disease. One patient who had successfully completed college education explained his difficulties: "…nausea is consuming… [making it] difficult to concentrate on anything else…" Another participant described the impact as being "… unable to work… therefore, I feel worthless. I feel like my stomach problems wasted everything I achieved".

Patients were also asked to explain their most bothersome symptoms and their concerns related to gastroparesis. Nausea or vomiting were listed by 28 participants, followed by pain (n = 24), bloating (n = 14) and emotional difficulties (n = 6). Nearly half (n = 26) of the patients mentioned the fear of an unrelenting chronic disease as their main concern, as shown by the following quote: "I worry that I'll never feel like a normal person". These concerns expressed themselves in some instances through anxiety that a more serious, perhaps even lethal disease caused the problems. Despite prior and extensive investigations and explanations, five patients were afraid that they had cancer.

When describing the perceived impact of treatment they had received for gastroparesis, patients most commonly listed the benefits of dietary and nutritional therapy (n = 11), prokinetics (n = 11) and antiemetics (n = 10). However, 10 patients mentioned that no treatment had led to any improvement. Others reported benefits of acid suppression (n = 5), relaxation techniques (n = 3) and analgesics (n = 3).

Patients also described their experiences with healthcare providers, with 15 (27%) expressing frustration or dissatisfaction. They primarily focused on a perceived lack of knowledge or understanding, which may have contributed to a perceived diagnostic delay and limited symptomatic improvement. One patient summarized her experiences: "I've had horrifying experiences from the medical community. Most physicians just say what you can [eat] or drink [like] Ensure".

DISCUSSION

Our results provide a cross-sectional picture of gastroparesis and its impact on patients seen in a tertiary referral center. Consistent with prior studies, most patients were women in their thirties and early forties, with the majority suffering from idiopathic forms of gastroparesis[11,15-18]. Overall disease severity as judged by the GCSI score, other symptom severity scores or the physical and mental component of the SF-12 were comparable to those reported in previously published studies[6,11,15,16]. Similarly, the frequency of different symptoms, such as nausea, vomiting, early satiation and pain, were consistent with prior reports[6,16,18-21]. About 10% of patients with idiopathic gastroparesis recalled an acute symptom onset after a flu-like illness, which suggested a post-infectious form of the disease. These results are slightly lower than previously reported[15,22]. We also did not see differences in the apparent clinical course based on patient recall, as all individuals with post-infectious forms of gastroparesis had symptoms for more than 1 year and complained about ongoing and at least moderately severe symptoms. However, the cross-sectional design and relatively small sample size do not allow definitive conclusions to be drawn. While the symptom pattern of patients with diabetic and idiopathic gastroparesis did not differ, patients with systemic sclerosis complained less about pain compared to the other groups, which may be important in management decisions[18].

Impact of gastroparesis

When asked to rate their overall health, more than two thirds used descriptors of fair or poor, which demonstrated the significant impact of gastroparesis, which is also shown by the low score in the physical functioning domain of the SF-12. We included a qualitative analysis of open-ended questions to capture important details that led to impairment of quality of life. Most of the comments focused on social interactions with family, friends or colleagues rather than symptoms. A striking finding was the high number of patients who reported significant problems in their professional activities that may have contributed to unemployment or disability. Despite a mean age below 45 years, only about one quarter of study participants was fully employed. The number of individuals not working and/or on disability benefit was higher than reported for inflammatory bowel disease[23,24]. Consistent with the impact of the disease on professional lives, more than half of the patients reported household incomes well below the national average, which also has been observed in other disorders associated with chronic pain[25]. Chronic illness certainly increases work absenteeism and may even result in permanent disability[26-29]. Our findings suggest a disproportionate impact in patients with gastroparesis, which corresponded with patient statements about their own disease experience and functional health status. Additional studies with larger patients groups and longitudinal design are needed to better define the effect of impaired gastric function on professional productivity.

Not surprisingly, our results also highlighted the importance of food intake. Dietary management and nutritional therapy primarily and appropriately target the caloric and nutritional value of ingested materials, which relies on changes in meal volume and frequency, food consistency, and in more severe cases, even enteral or parenteral alimentation[2,30]. Although successful in preventing the development of nutritional deficiencies, these measures do not address the hedonic and social aspects related to eating. Aversive reactions to the previously pleasant experience of food intake, the inability to fully participate in daily routines, such as dinners, fully meet professional obligations associated with eating or drinking, or enjoy dining out with friends reminded patients on a daily basis how the illness had changed their lives. The answers often hinted at resentments, when healthcare providers did not appreciate this fact and briefly told them that simple dietary adjustments would solve their problems, without acknowledging the tremendous impact on quality of life.

Gastroparesis and affect

The high scores for anxiety and depression and patient statements certainly demonstrated the association between mood and impaired gastric function. Our design did not allow us to determine whether and how these factors are causally related. Interestingly, in the multivariate model, depression but not anxiety significantly contributed to the overall impairment in health status. Only one study has described the prevalence of depression in a large group of patients with gastroparesis, with nearly one quarter carrying the diagnosis of depressive illness[15]. This figure corresponds with the reported chronic use of antidepressant medications in our patient group. Our findings also fall in line with previous results obtained in patients with functional dyspepsia, in whom depression significantly correlated with the physical domain of quality of life[31,32]. Recent evidence suggests that depression is the key determinant of symptom severity in functional dyspepsia, which is primarily mediated through somatization[33]. Considering the overlap between functional dyspepsia and gastroparesis, additional studies will be needed to better define the role of psychological mechanisms in disease severity and manifestations.

Pain and gastroparesis

The recent consensus statement of the American Gastroenterological Association comments on pain in gastroparesis as being relatively common, but typically not the primary symptom or concern[3]. Our findings, especially the patient comments, argue against this statement, when one considers the high prevalence of pain and subjective pain ratings, which has been emphasized previously by Hoogerwerf *et al*[18]. The relevance of pain was also reflected in the treatments received by patients. Despite concerns about the use of opioids in patients with gastroparesis, about 30% of the patients regularly used narcotics. Only one study has mentioned specifically narcotic use in patients with gastroparesis, and has reported an even higher number of close to 50%, which may have been caused partly by selection bias, because all patients underwent implantation of a gastric electrical stimulator[16]. The interaction between narcotic use and impaired gastric emptying is admittedly complex, as opioids affect gastrointestinal motility. However, virtually all assessments of gastric function were performed when patients did not receive narcotics. It is thus unlikely that the documented delay in gastric emptying seen in our patients was solely a consequence of medication.

Beyond its prevalence, pain remains a significant challenge in managing gastroparesis. The conventional approaches with dietary and/or prokinetic therapy do not provide significant benefit[18]. Tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) or serotonin/norepinephrine reuptake inhibitors have been used in functional dyspepsia and other visceral pain syndromes[34-36]. Although they may acutely lower pain thresholds in healthy volunteers[37-39], efficacy in patients has not been demonstrated consistently[32,40,41]. Gabapentin or pregabalin similarly may decrease sensory thresholds acutely[42], but have not been shown to possess true analgesic properties in patients[43]. Considering the negative impact of opioids on gastric emptying and their addictive potential, kappa opioids agonists have been tried in gastroparesis or functional dyspepsia, but were not superior to placebo[44,45]. In view of recent advances in our understanding of visceral sensory mechanisms, we will have to see whether other potential targets for pharmacological interventions, such as purinergic receptors, TRPV1 or TRPA1, will provide greater benefit for these patients[46,47].

Gastroparesis and gastric emptying

Although gastroparesis is defined by delayed emptying, our study did not show a correlation between symptom severity and half-emptying time as an objective and quantitative measure of this impairment. Only minor differences emerged in comparisons between symptomatic individuals with (gastroparesis) and without (functional dyspepsia) delayed gastric emptying, which did not allow differentiation between the groups based on symptoms only[6,48]. In addition, potent prokinetics have a limited or no impact on symptoms, which further argues against a primary role of transit delay as a determinant of disease severity[8,9]. Consistent with this conclusion, only a small fraction of patients reported subjectively perceived benefit when taking prokinetics. This relatively low response rate contrasts starkly with the relatively frequent adverse effects. More than one third of the patients who received metoclopramide had to discontinue the medication because of side effects; a rate that is in line with previous reports[1,2]. These results are likely caused by the complex pathophysiology of gastroparesis, which includes impaired accommodation, delayed emptying, hypersensitivity and (preexisting or secondarily evolving) affective spectrum disorders, all of which contribute to the clinical manifestation of the disorder[4,30].

Study limitations

As is true for most clinical studies, our investigation was conducted in a tertiary referral center, which biases and skews findings as a result of the likely higher proportion of difficult-to-treat patients. However, patient age, sex distribution, the high proportion of patients with idiopathic gastroparesis and self-reported symptom severity were comparable with prior studies that used similar scoring systems[4,15,16,49]. The relatively high number of patients with systemic sclerosis and the slightly smaller group of diabetic patients likely reflects institutional idiosyncrasies. A cross-sectional study design certainly comes with limitations. It enabled us to identify association between symptom severity and its potential determinants, from underlying etiology to treatment. Although we noticed an important impact of depressive symptoms, longitudinal studies are needed to better define this relationship and correlate time- and/or treatment-dependent changes in the various indices. Moreover, our data cannot identify psychological or physiological mechanisms that mediate the interactions between affect and symptoms, which will require more detailed investigations. Similarly, our study was not designed to measure systematically the effects of different treatments in patients with gastroparesis. More than half of the patients were enrolled during their initial encounter, with data thus reflecting the approaches of many referring physicians. We also used previously obtained data on gastric emptying, which provided less standardized but still objective evidence of impaired gastric function in all patients. Although appropriate for reducing increasing healthcare costs and typical for clinical practice[11], it limited our ability to correlate fully this disease-defining variable with symptom severity.

Taken together, our data raise questions about some of the key premises that we as clinicians use when approaching patients with gastroparesis. Delayed gastric emptying still remains the defining and only routinely available diagnostic tool to identify this disorder. However, it may detract from the much more complex pathophysiology and may even misguide our treatment. As we cannot cure the illness, our treatment has to focus on improving the overall quality of life. A paradigm shift may be needed to take into account the important influence of under-recognized and under-treated problems, such as pain or affect. Pain assessment should be included in the GCSI. Gastric electrical stimulation was initially thought to alter gastric motility, with investigators now increasingly speculating on its effect on visceral sensation and affect[20,50,51]. Some data are promising, especially in diabetic patients[16,20,50,52]. However, the high cost, frequent need of reoperation, and the limited benefit in patients with significant pain and/or idiopathic gastroparesis clearly force us to look for alternatives. Our results may be seen as initial, yet circumstantial evidence that supports psychologically based interventions, which have been quite successful in functional bowel disease and have shown promise in a small case series[53].

COMMENTS

Background

Gastroparesis is a chronic disorder that is characterized by significant dyspeptic symptoms and delayed gastric emptying. Despite the defining abnormality in gastric motor function, prokinetics typically have limited efficacy, which leaves patients with persistent symptoms and poor quality of life.

Research frontiers

Anxiety and depression play an important role in the clinical manifestation of many functional disorders, which range from irritable bowel syndrome to fibromyalgia. We prospectively enrolled patients with gastroparesis to better understand the relative importance of different gastrointestinal symptoms and associated or coexisting emotional factors on their quality of life.

Innovations and breakthroughs

Our data highlight that depression plays a significant role in the overall impact of gastroparesis on quality of life. The findings also emphasize the importance of pain as a symptom of gastroparesis, which is under-appreciated and under-treated.

Applications

The results stress the need to shift treatment strategies for gastroparesis away from approaches that simply try to accelerate gastric emptying. In our clinical assessment, we need to assess the different symptoms including pain, and design a therapy that takes into account the primary problems the individual patients experience. Because affect, especially depression, significantly impairs quality of life, diagnostic approaches and treatment should address emotional factors. In future research, we will have to define better the interrelationship between altered gastric function and emotion to understand underlying mechanisms. Interactions will likely be reciprocal, which raises the question of how much psychologically or psychiatrically oriented treatment may be beneficial in patients with gastroparesis.

Terminology

Gastroparesis is a chronic disorder that is characterized by symptoms of nausea, vomiting, fullness and bloating after meals, and a delay in gastric emptying, which is typically determined using standardized scintigraphic tests.

Peer review

The article fits into the increasing evidence that structural and especially functional disorders of the gastrointestinal tract should be seen as biopsychocial phenomena that require more holistic diagnostic and therapeutic approaches.

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BRIEF ARTICLE

Association of the *GNAS1* T393C polymorphism with tumor stage and survival in gastric cancer

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Abstract

AIM: To analyze the impact of the *GNAS1* T393C polymorphism on prognosis and histopathology of gastric cancer.

METHODS: Genomic DNA was extracted from paraffin-embedded tissues of 122 patients with primary gastric carcinoma and from the blood of 820 healthy white individuals. Allelic discrimination was performed by quantitative real-time polymerase chain reaction. Genotyping was correlated with histopathologic parameters and with overall survival according to the Kaplan-Meier approach and with multivariate analysis by multiple stepwise regression.

RESULTS: Thirty-nine (32%) patients displayed a CC genotype, 57 (46.7%) a CT genotype and 26 (21.3%) a TT genotype. The frequency of the C allele (fC) in the patient group was 0.55, which was not significantly different from that of healthy blood donors. The distribution was compatible with the Hardy-Weinberg equilibrium. Analysis of clinicopathological parameters did not show any significant correlation of the T393C genotype with gender (P = 0.50), differentiation (P = 0.29), pT-category (P = 0.19), pN-category (P = 0.30), pM-category (P = 0.25), R-category (P = 0.95), the classifications according to WHO (P = 0.34), Laurén (P = 0.16), Goseki (P = 1.00) and Ming (P = 0.74). Dichotomization between C+ (CC+CT) and C-genotypes (TT), however, revealed significantly more advanced tumor stages (P = 0.023) and lower survival rates (P = 0.043) for C allele carriers.

CONCLUSION: The present study provides strong evidence to suggest that the *GNAS1* T393C allele carrier status influences tumor progression and survival in gastric cancer with higher tumor stages and a worse outcome for C allele carriers.

Key words: Gastric cancer; G Protein; Polymorphism; Prognosis; Tumor stage

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INTRODUCTION

Gastric cancer has substantially decreased in incidence over the past decades, but it still remains one of the most common cancers in the world and the second most frequent cause of cancer-related death after lung cancer[1]. Most patients are diagnosed with advanced gastric cancer, and overall survival remains poor[2,3]. The 5-year survival rate for gastric cancer is still only at 40%[4,5].

Of particular interest are prognostic factors, as they give the basis to identify gastric cancer patients with high-risk and poor prognosis. The identification of patients with poor outcome can help to set up novel treatment strategies at the beginning of treatment and may lead to better and more individualized therapy strategies with better survival[2]. Current efforts in research are therefore focused on the detection and validation of biomarkers and genetic markers that give additional information about prognosis to classical prognostic factors such as the TNM classification. The majority of new detected markers are related to properties of the tumor itself, e.g. somatic mutations or differential expression of genes or proteins. However, difficulties in standardization of such markers often prevent their routine application in clinical practice[6].

In recent years, studies have focused on the detection of single nucleotide polymorphisms (SNPs) that have a prognostic impact in cancer. One major advantage of SNPs as prognostic markers is that they can be determined independently from the availability and quality of tumor material as they can be easily evaluated from a blood sample from individual patients.

The T393C polymorphism of the gene *GNAS1* is one such polymorphism. This SNP is located in exon 5 of the gene *GNAS1*, which encodes the ubiquitously expressed Gαs subunit of heterotrimeric G proteins. Previous studies indicate that increased expression of Gαs enhances apoptosis[7,8] and that Gαs mRNA expression is different between T393C genotypes[9]. For various solid tumors, previous studies demonstrated that patient survival and tumor progression depended on T393C genotype[10-17].

Until now, nothing has been published about the impact of the *GNAS1* T393C polymorphism on gastric cancer. Thus, the aim of the present study was to determine the influence of this polymorphism on prognosis in gastric cancer. Furthermore, we looked for possible correlations between the *GNAS1* T393C polymorphism and clinicopathological parameters.

MATERIALS AND METHODS

Patients

Of 159 patients, who were treated surgically between May 1996 and January 2005 for primary gastric carcinoma at the Department of General, Visceral and Cancer Surgery of the University of Cologne, 13 (8.2%) patients with a second tumor, a previous operation of the upper digestive tract or missing paraffin-embedded tissue from normal cells, and 24 (15.1%) patients with neoadjuvant treatment received before surgery were excluded. Excluded patients did not differ in age and gender from the remaining patients.

All of the included 122 patients [median age 67.6 years, range 33-87 years; 78 (63.9%) male, 44 (36.1%) female] were initially treated by operation with curative intention. Gastroscopic examination, endoscopic ultrasound and computed tomography (CT) of the chest and abdomen were performed before surgery on all patients for clinical staging.

One hundred and six (86.9%) of the 122 patients underwent a gastrectomy with D2-lymphadenectomy (compartment I and II) and in 16 (13.1%) cases, a subtotal gastrectomy with D2-lymphadenectomy was performed. The median number of resected lymph nodes was 36.0 (range 15-80).

The present study was performed according to the guidelines of the local Research Ethics Commission.

Histopathology

The specimens were removed *en bloc* and the lymph nodes of the specimens were dissected with the cooperation of surgeons and pathologists according to a standardized protocol. The resected specimens were routinely fixed in 5% phosphate-buffered formalin and embedded in paraffin. Histopathologic examination of all resected specimens consisted of a thorough and standardized evaluation of the tumor stage, residual tumour (R) category, grading and the number of resected and infiltrated lymph nodes. The gastric lymph nodes were documented according to the classification of the Japanese Research Society of Gastric Cancer (JRSGC) with lymph node groups 1 to 13[18]. The tumor localization was defined according to the International Classification of Diseases for Oncology. The lesions were further classified and graded in accordance with WHO recommendations, the Laurén-classification and tumor differentiation. Postoperative staging was performed according to the 6th edition of the TNM-classification of malignant tumors[19].

Genotyping

DNA was extracted from paraffin-embedded tissues from resection boundaries containing exclusively normal cells using a DNA extraction kit (QIAamp, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping was performed in 96-well plates by 5'nuclease assay (TaqMan) using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Darmstadt, Germany).

The pre-developed TaqMan assay ID C_9901536_10 (Applied Biosystems, Darmstadt, Germany) was used for genotyping of *GNAS1* T393C polymorphism (dbSNP rs7121). Polymerase chain reaction (PCR) reactions contained 10 ng DNA, 200 μmol/L dNTPs and 900 nmol/L primers (Figure 1).

PCR conditions were: 95℃ for 10 min followed by 40 cycles of 15 s at 92℃ and 60 s at 60℃.

Reference group

The Caucasian control sample consisted of 820 healthy

Table 1 Allele frequencies and genotype distribution of *GNAS1* T393C polymorphism in 122 gastric cancer patients and in the reference group (n = 820) n (%)

T393C	Patients (n = 122)			Reference group (n = 820)			χ^2	P	Odds ratio	95% CI
Allele	C 135 (55.3)		T 109 (44.7)	C 873 (53.2)		T 767 (46.7)	0.38	0.54	0.92	0.70-1.20
Genotype	CC 39 (32.0)	CT 57 (46.7)	TT 26 (21.3)	CC 235 (28.7)	CT 403 (49.1)	TT 182 (22.2)	0.37	0.54	0.92	

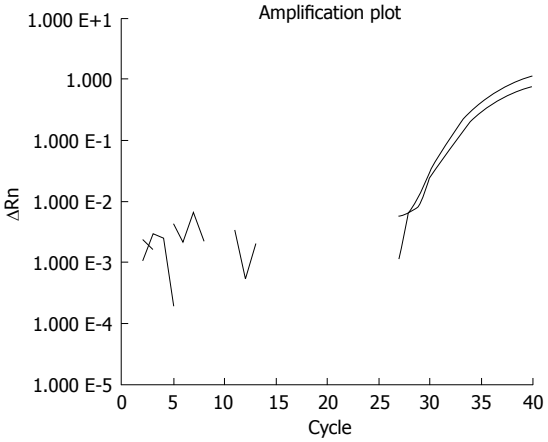

Figure 1 Amplification plot of one heterozygous *GNAS1* T393C (CT) by two allele specific TaqMan probes.

white individuals who were recruited at the local Department for Transfusion Medicine, University Hospital, Essen. All samples were collected at random from subjects donating blood. The details of this sample have been published previously[12].

Statistical analysis

Associations between T393C genotype and clinicopathological parameters were evaluated using the χ^2 test. Pearson's χ^2 was used for Hardy-Weinberg analysis and to examine differences in allele frequencies between our patient group and the reference group. Relations to overall survival were evaluated with univariate analysis according to the Kaplan-Meier approach using the log-rank test to assess statistical differences between groups. Prognostic factors were determined by multiple stepwise regression analysis using the Cox model. Only potential prognostic factors were included in the multivariate analysis. The level of significance was set at $P < 0.05$ and P values were for 2-sided testing. All statistical tests were performed using the Software Package SPSS for Windows, Version 17.0 (Chicago, IL, USA).

RESULTS

Genotype distribution and reference group

Thirty-nine (32.0%) patients displayed a CC genotype, 57 (46.7%) a CT genotype and 26 (21.3%) a TT genotype. The frequency of the C allele (fC) in the entire patient group was 0.55, which is not significantly different from that of healthy blood donors (Table 1). The distribution was compatible with the Hardy-Weinberg equilibrium.

Clinicopathological characteristics

Clinicopathological characteristics of the whole patient group with genotype distribution are displayed in Table 2. Thirty (24.6%) patients showed an early gastric carcinoma (pT1). In 73 (59.8%) cases, lymph node metastasis (pN+) was detected. An M1 category was found in 23 (18.9%) patients with localized peritoneal carcinosis, distant lymph node metastasis (M1 lymph) or single liver metastasis (M1 Hep). Patients with diffuse peritoneal or multiple liver metastasis had been treated non-surgically and were excluded from the study.

Analysis of clinicopathological parameters did not show any significant correlation of the T393C genotype with gender (P = 0.50), differentiation (P = 0.29), pT-category (P = 0.19), pN-category (P = 0.30), pM-category (P = 0.25), R-category (P = 0.95), or the classifications according to WHO (P = 0.34), Laurén (P = 0.16), Goseki (P = 1.0), Ming (P = 0.74) and UICC (P = 0.15).

When genotypes were dichotomized in C+ (CC+CT) and C-genotypes (TT), a significantly higher rate of advanced tumor stages (stage Ⅲ and Ⅳ), according to the UICC classification, was seen for C allele carriers (P = 0.023). Only 6 (23.1%) of 26 patients with TT genotype were diagnosed with a tumor stage of Ⅲ or Ⅳ. In contrast, an advanced tumor stage was detected in 50 (52.1%) of 96 C allele carriers.

Univariate survival analysis

Overall survival dependent on T393C genotypes is displayed in Figure 2. The 5-year survival rate for patients with a TT genotype was 56.9% (SE ± 10.4%), followed by patients with CC genotype with a 5-year survival rate of 42.6% (SE ± 8.3%). Heterozygous CT patients showed a 5-year survival rate of 32.7% (SE ± 6.3%). Survival was not significantly associated with the T393C genotype when the three genotypes were compared (P = 0.082). However, dichotomization between C+ (CC+CT) and TT demonstrated a significantly (P = 0.043) lower survival rate for C allele carriers (Figure 3) with a 5-year survival rate for the C+ group of only 36.7% (SE ± 5.1%) *vs* 56.9% (SE ± 10.4%) for the TT group.

Multivariate survival analysis

In the multivariate Cox regression analysis, known prognostic factors for gastric cancer (pT, pN, pM and R-category) and T393C genotype with dichotomization between C+ (CC+CT) and TT were included. pT-category (P < 0.001), R-category (P = 0.022) and pM-category (P = 0.027) maintained their prognostic independence (Table 3). pN-category (P = 0.55), and the T393C genotype (P = 0.33) lost their prognostic independence.

DISCUSSION

Gastric cancer is the fourth most common cancer with

Table 2 Clinicopathological characteristics of 122 patients with gastric cancer *n* (%)

	All	T393C genotypes			*P*
		CC	CT	TT	
n (%)	122 (100)	39 (32)	57 (46.7)	26 (21.3)	
Gender					
Male	78 (63.9)	25 (32.1)	34 (43.6)	19 (24.4)	
Female	44 (36.1)	14 (31.8)	23 (52.3)	7 (15.9)	0.274
WHO					
Papillary/Tubular/Mucinous	76 (62.3)	23 (30.3)	34 (44.7)	19 (25)	
Signet-ring cancer	38 (31.1)	12 (31.6)	19 (50)	7 (18.4)	
Other	8 (6.6)	4 (50)	4 (50)	0	0.340
Differentiation					
Well/Moderate (G1-G2)	42 (34.4)	12 (28.6)	22 (52.4)	8 (19)	
Poor (G3-G4)	80 (65.6)	27 (33.8)	35 (43.8)	18 (22.5)	0.805
Laurén					
Intestinal	52 (42.6)	16 (30.8)	25 (48.1)	11 (21.2)	
Diffuse	55 (45.1)	17 (30.9)	29 (52.7)	9 (16.4)	
Mixed	15 (12.3)	6 (40)	3 (20)	6 (40)	0.171
Ming					
Expanding	47 (38.5)	14 (29.8)	24 (51.1)	9 (19.1)	
Infiltrative	75 (61.5)	25 (33.3)	33 (44)	17 (22.7)	0.620
pT-category					
T1	30 (24.6)	7 (23.3)	13 (43.3)	10 (33.3)	
T2	44 (36.1)	12 (27.3)	22 (50)	10 (22.7)	
T3	38 (31.1)	14 (36.8)	18 (47.4)	6 (15.8)	
T4	10 (8.2)	6 (60)	4 (40)	0	0.110
pN-category					
N0	49 (40.2)	11 (22.4)	24 (49)	14 (28.6)	
N1	34 (27.9)	13 (38.2)	13 (38.2)	8 (23.5)	
N2	14 (11.5)	6 (42.9)	6 (42.9)	2 (14.3)	
N3	25 (20.5)	9 (36)	14 (56)	2 (8)	0.196
pM-category					
M0	99 (81.1)	30 (30.3)	45 (45.5)	24 (24.2)	
M1	23 (18.9)	9 (39.1)	12 (52.2)	2 (8.7)	0.101
R-category					
R0	118 (96.7)	38 (32.5)	54 (46.2)	25 (21.4)	
R1/R2	4 (3.3)	1 (25)	2 (50)	1 (25)	0.950
UICC stage					
Ⅰa	26 (21.3)	5 (19.2)	11 (42.3)	10 (38.5)	
Ⅰb	22 (18)	7 (31.8)	12 (54.5)	3 (13.6)	
Ⅱ	18 (14.8)	4 (22.2)	7 (38.9)	7 (38.9)	
Ⅲa	11 (9)	4 (36.4)	5 (45.5)	2 (18.2)	
Ⅲb	4 (3.3)	2 (50)	2 (50)	0	
Ⅳ	41 (33.6)	17 (41.5)	20 (48.8)	4 (9.8)	0.023

P values are given for dichotomization between C+ (CC+CT) and C- (TT) genotypes.

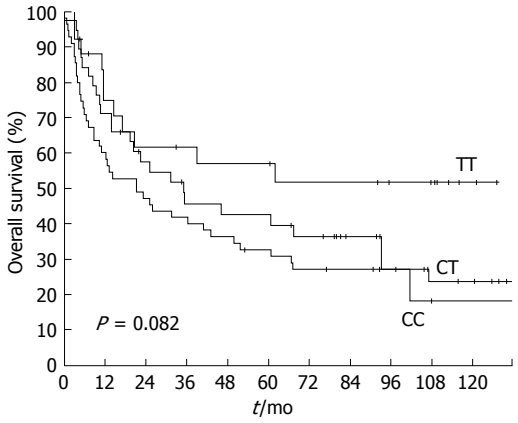

Figure 2 Overall survival of 122 resected gastric cancer patients based on *GNAS1* T393C genotype (Kaplan-Meier analysis), *P* = 0.082 (Mantel-Cox log-rank test).

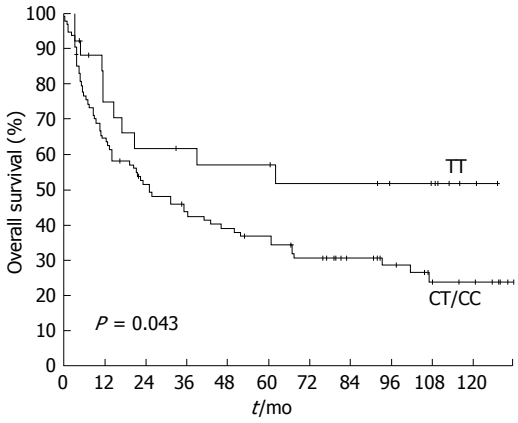

Figure 3 Overall survival of 122 resected gastric cancer patients based on *GNAS1* T393C genotype with dichotomization between C+ and C- genotypes, *P* = 0.043.

Table 3 Univariate and multivariate survival analysis of 122 gastric cancer patients

Covariate	*n*	Univariate analysis			Multivariate analysis		
		P value	5-yr-SR (%)	SE (±%)	*P* value	HR	95% CI
pT-category		< 0.001			< 0.001		
pT1	30		85.4	6.8		1	
pT2	44		44.5	7.8	< 0.001	6.212	2.31-16.70
pT3	38		5.4	3.7	< 0.001	13.026	4.44-38.23
pT4	10		33.3	15.7	0.001	7.838	2.24-27.46
pN-category		< 0.001			0.549		
pN0	49		61.6	7.4		1	
pN1	34		47.1	8.6	0.226	0.663	0.34-1.29
pN2	14		16.9	10.9	0.986	0.993	0.43-2.27
pN3	25		8.0	5.4	0.814	0.905	0.40-2.07
T393C SNP		0.043			0.333		
CC/CT	96		36.7	5.1		1	
TT	26		56.9	10.4		0.712	0.36-1.42
pM-category		< 0.001			0.027		
M0	99		48.1	5.2		1	
M1	23		9.2	6.2		2.087	1.09-4.01
R-category		< 0.001			0.022		
R0	118		42.3	4.7		1	
R+	4		0	0		3.128	1.18-8.27

SNP: Single nucleotide polymorphism; 5-yr-SR: 5-yr-survival; HR: Hazard ratio.

Table 4 Summary of the effect of the *GNAS1* T393C polymorphism on various carcinomas

Cancer type	Yr	*n*	Effect	Benefit (survival)
Gastric cancer	2009	122	The present study demonstrates a significant survival benefit for the TT genotype with a 5-yr-survival rate of 56.9% *vs* the CC/CT group with a 5-yr-survival rate of only 36.7% ($P = 0.043$)	TT-genotype
Squamous cell cancer of larynx[15]	2008	157	Survival was significantly dependent on the T393C genotype in advanced American Joint Committee on Cancer (AJCC) stages (Ⅲ-Ⅳ) with higher 5-yr survival rates for TT, followed by TC and CC ($P = 0.0437$)	TT-genotype
Oro- and hypo-pharyngeal squamous cell carcinoma[16]	2008	202	C homozygous patients displayed a higher risk for disease progression than T homozygous patients ($P = 0.019$) and a higher risk for death ($P = 0.015$). In multivariate analysis, besides cancer stage and tumor localization, the T393C polymorphism was an independent prognostic factor for disease progression and death	TT-genotype
Clear cell renal cell carcinoma[11]	2006	150	Tumor progression, development of metastasis and tumor-related death was significantly associated with the T393C polymorphism. In multivariate analysis CC patients were at highest risk for progression or tumor-related death compared with T-allele carriers ($P = 0.018$)	TT-genotype
Chronic lymphocytic leukemia[17]	2006	144	Median progression-free survival was significantly higher for T-allele carriers ($P = 0.007$). In multivariate analysis, the T393C polymorphism kept its prognostic independence ($P = 0.01$) besides of ZAP-70 ($P = 0.005$) and Binet stage ($P < 0.001$). Regarding overall survival, CC genotypes were significantly at highest risk for death compared to T-alleles both in univariate ($P < 0.001$) and multivariate analysis ($P = 0.002$)	TT-genotype
Bladder cancer[10]	2005	254	Progression-free survival ($P = 0.011$), metastasis-free survival ($P = 0.001$) and cancer-specific survival ($P = 0.014$) were significantly increased in TT genotypes compared with CC genotypes. In multivariate analysis, the T393C polymorphism kept its prognostic independence	TT-genotype
Sporadic colorectal cancer[12]	2005	151	In UICC stages Ⅰ to Ⅱ, the 5-yr survival rate was significantly ($P = 0.009$) higher in TT genotypes (88%) compared with TC (71%) and CC genotypes (50%). In multivariate analysis, the T393C polymorphism was also an independent prognostic factor. No significant effect could be seen for UICC stages Ⅲ to Ⅳ	TT-genotype
Cholangio-carcinoma[14]	2007	87	Disease-specific overall survival was significantly dependent on the T393C genotype ($P = 0.02$), with TT genotypes showing reduced survival compared to patients carrying at least one C allele. In multivariate analysis (TT/C+) the T393C genotype kept its prognostic independence ($P = 0.04$)	CC-genotype
Breast carcinoma[13]	2007	279	Overall survival was significantly ($P = 0.033$) associated with the T393C polymorphism with lowest survival rates for the TT-genotype and highest survival rate for the CC-genotype. In multivariate analysis, the TT-genotype still had a significant survival benefit compared to the CC genotype ($P = 0.045$)	CC-genotype
Esophageal cancer[28]	2009	51	T393C polymorphism was significantly associated with tumor response to Cisplatin/5-FU-based radiochemotherapy. 63% of the T allele carriers had a minor histopathologic response (MiHR) with more than 10% residual vital tumor cells in resection specimens. For the CC genotype MiHR was seen only in 20%. In binary logistic regression analysis, the T393C genotype kept its independence ($P < 0.05$)	CC-genotype

approximately 800 000 new cases per year and the second leading cause of cancer-related death worldwide[20]. Many patients have advanced disease at the time of diagnosis, resulting in poor prognosis and high mortality[2,21,22]. Pretreatment staging of the disease is of high importance as it provides the basis for selecting the most appropriate therapeutic strategy[23]. Based on the preoperative staging, patients with early stage tumors are treated by endoscopic mucosal resection, while patients with advanced tumors are treated by partial or total gastrectomy[5]. Accurate staging is also the basis for selecting patients for neoadjuvant, adjuvant or palliative treatment[24]. By identifying patients with poor outcome, novel treatment strategies could be set up at the beginning of treatment which can lead to better and more individualized therapy strategies with superior survival[2].

The present study demonstrated that besides the known prognostic factors pT, pM, pN and R-category, the T393C polymorphism was also a significant prognostic factor in the univariate analysis with a survival benefit for homozygous TT patients. In addition, it demonstrated that compared to C allele carriers, homozygous TT patients were diagnosed with significantly less advanced tumor stages according to UICC, which is possibly the main reason why the T393C genotype lost its independence in the multivariate analysis.

The gene *GNAS1* is mapped to chromosome 20q13 and consists of 13 exons. Somatic activating mutations of *GNAS1* have been implicated in the etiology of McCune Albright Syndrome[25] and sporadic, isolated endocrine tumors[26,27] which supports a role of *GNAS1* in tumor initiation and progression.

Recent studies have shown that genotypes of the T393C polymorphism are significantly associated with survival of patients suffering from colorectal cancer, bladder cancer, clear cell renal carcinoma, intrahepatic cholangiocarcinoma, invasive breast carcinoma and squamous cell carcinoma of the larynx, oropharynx and hypopharynx (Table 4)[10-14].

Comparable to previous results in bladder cancer, clear cell renal carcinoma and colorectal cancer, the present study also demonstrated significantly higher survival rates for TT genotypes in gastric cancer (Figure 3). Patients with the TT genotype showed a 5-year survival rate of 57%, whereas the 5-year survival rate for C allele carriers was only at 37%.

In contrast to our findings in gastric cancer and previous findings in the above-mentioned tumor types, an unfavourable clinical course for T allele carriers has been described in studies of invasive breast cancer and intrahepatic cholangiocarcinoma, suggesting that the biological effect of the T393C polymorphism may be different in different tumor types. In a recent study, we demonstrated that this polymorphism is a predictive molecular marker for tumor response to cisplatin/5-FU-based radiochemotherapy in esophageal cancer, with CC genotypes mostly showing a major response[28].

In vitro experiments suggest that expression of Gαs is associated with enhanced apoptosis[7,8]. The second messenger, cyclic AMP, which is generated by activated Gαs, seems to play a major role in this proapoptotic process. An increased concentration of the intracellular second messenger, cyclic AMP promotes apoptosis in several cell types including leukemic cells[29], ovarian cancer cells[30], and lymphoma cells[25]. Increased Gαs expression in tissues of patients with TT genotypes may therefore confer enhanced apoptosis in 393T allele carriers. Hypothetically, this mechanism may contribute to the described more favorable clinical course and the less advanced tumor stages of homozygous TT patients. This hypothesis remains to be supported by additional functional studies which were beyond the scope of the present study. The T393C polymorphism as a risk factor for gastric cancer could not be established in the present study.

In conclusion, this study demonstrated for the first time that in primary gastric cancer, homozygous *GNAS1* 393T patients have less advanced tumor stages and higher survival rates than C allele carriers. These findings further support the concept of a general role for the *GNAS1* T393C polymorphism in tumor progression.

COMMENTS

Background

Identification of gastric cancer patients with poor outcome can help to set up novel treatment strategies at the beginning of treatment and may lead to better and more individualized therapy strategies with better survival. In recent years, studies have focused on the detection of single nucleotide polymorphisms (SNPs) as prognostic molecular markers in cancer.

Research frontiers

The *GNAS1* T393C polymorphism is located in exon 5 of the gene *GNAS1*. In this study the authors describe, for the first time, the impact of this SNP in gastric cancer. The study demonstrates that the *GNAS1* T393C polymorphism affects tumor stage and prognosis in gastric cancer.

Innovations and breakthroughs

For various solid tumors, previous studies have demonstrated that patient survival and tumor progression depend on the *GNAS1* T393C genotype. In the present study, the authors have described for the first time that the *GNAS1* T393C polymorphism affects tumor stage and prognosis in gastric cancer.

Applications

The *GNAS1* T393C polymorphism will contribute to identifying high-risk patients with gastric cancer and might help to establish a more individualized treatment strategy for gastric cancer.

Terminology

A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide - A, T, C, or G - in the genome differs between members of a species. The *GNAS1* T393C is located in exon 5 of the gene *GNAS1*. For several cancer types, studies have demonstrated that patient survival is affected by this SNP.

Peer review

Overall, this paper provides information on *GNAS1* T393C allele carrier status which influences tumor progression and survival in gastric cancer, with higher tumor stages and worse outcome for C allele carriers.

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BRIEF ARTICLE

Elevated pro-inflammatory and lipotoxic mucosal lipids characterise irritable bowel syndrome

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Abstract

AIM: To investigate the pathophysiology of irritable bowel syndrome (IBS) by comparing the global mucosal metabolic profiles of IBS patients with those of healthy controls.

METHODS: Fifteen IBS patients fulfilling the Rome Ⅱ criteria, and nine healthy volunteers were included in the study. A combined lipidomics (UPLC/MS) and metabolomics (GC × GC-TOF) approach was used to achieve global metabolic profiles of mucosal biopsies from the ascending colon.

RESULTS: Overall, lipid levels were elevated in patients with IBS. The most significant upregulation was seen for pro-inflammatory lysophosphatidylcholines. Other lipid groups that were significantly upregulated in IBS patients were lipotoxic ceramides, glycosphingolipids, and di- and triacylglycerols. Among the metabolites, the cyclic ester 2(3H)-furanone was almost 14-fold upregulated in IBS patients compared to healthy subjects (P = 0.03).

CONCLUSION: IBS mucosa is characterised by a distinct pro-inflammatory and lipotoxic metabolic profile. Especially, there was an increase in several lipid species such as lysophospholipids and ceramides.

Key words: Functional gastrointestinal diseases; Irritable bowel syndrome; Histopathology

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INTRODUCTION

Irritable bowel syndrome (IBS) is a functional bowel disorder characterised by abdominal pain or discomfort and an irregular bowel habit[1]. The prevalence is up to 20% in Western adults, which makes IBS the most common diagnosis in gastroenterology. The precise aetiology and pathophysiology of IBS are incompletely understood, despite extensive interest and investigation. The current knowledge does, however, suggest that altered gut motility, visceral hyperalgesia, and dysregulation of the brain-gut axis are central to IBS.

IBS is diagnosed by the presence of symptoms according to the Rome criteria[2], with concomitant exclusion of organic diseases; hence, there is no specific biological,

radiological, endoscopic, or physiological marker for IBS. Medical treatment options for IBS are limited, possibly due to the lack of biomarkers and data about the pathophysiology of the condition. Different immune markers are among the most studied putative biomarkers in IBS. Increased mast cell counts, mast cells in close proximity to nerves, and mast cell mediators that are able to stimulate murine visceral sensory nerves appear to be characteristic of IBS[3-6]. Elevated plasma levels of pro-inflammatory interleukin (IL)-6 and IL-8 have been observed in IBS[7], and peripheral blood mononuclear cells obtained from IBS patients produce higher amounts of tumour necrosis factor (TNF)-α, IL-1β, IL-6 and IL-12 than cells from healthy controls[8,9]. Furthermore, an increased number of immunocytes have frequently been observed in mucosa from IBS patients[10,11]. In addition to immune markers, other markers, such as 5-hydroxytryptamine (5-HT, serotonin) and gut hormones, have also been associated with IBS. Decreased expression of 5-HT has been associated with constipation-predominant IBS[12]. Elevated plasma 5-HT concentrations have been observed in a mixed IBS population[13] and in post-infectious IBS[14], while the opposite was demonstrated in constipation IBS[14]. Moreover, IBS patients have also been demonstrated to have decreased 5-HT levels and turnover, and lower 5-HT transporter mRNA concentrations[14,15].

The recent technological development of analytical instruments combined with rapid progress in bioinformatics has opened novel opportunities to quickly and simultaneously measure and model huge numbers of molecular metabolites in biological samples[16,17]. This metabolomic approach is considered a powerful tool for characterising complex phenotypes and developing biological markers for specific physiological states. Thus, metabolomics provides an interesting platform to investigate the pathophysiology of a complex syndrome like IBS at the molecular level. Studies on the molecular abnormalities in IBS are needed to understand the mechanisms behind the emergence of symptoms, and to enable the development of novel therapies.

The aim of the current study was to compare the global metabolic profiles of mucosal biopsies from IBS patients with those from healthy subjects using a high-throughput approach comprising lipidomics and metabolomics.

MATERIALS AND METHODS

Subjects

Sixteen adult IBS patients fulfilling the Rome Ⅱ criteria[18] and without organic intestinal diseases were recruited to participate in the study. One statistical outlier IBS patient was left out of the analyses after an initial quality check of the results, and thus a total of 15 patients were analysed (Table 1). Nine healthy subjects (mean age 49 years, SD 14; 4 male) without organic intestinal diseases or gastrointestinal symptoms consistent with IBS and undergoing colonoscopy for clinical reasons were recruited as controls. The healthy subjects were either polyp control patients having a minimum of 3 years since the previous polyp finding or anaemic patients. Inclusion criteria for all subjects were: an age between 20 and 65 years; normal blood count (erythrocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, platelets, leukocytes); serum creatinine, alanine aminotransferase (ALT) and alkaline phosphatase (ALP) within reference values; and normal gut histology as evaluated by an experienced pathologist (PS). Subjects were excluded if they had a history of major or complicated gastrointestinal surgery, severe endometriosis, complicated abdominal adhesions, malignant tumours, were pregnant or lactating, or had received antimicrobials during the previous month. Patients with lactose intolerance were allowed to participate if they followed a continuous low-lactose or lactose-free diet.

Table 1 Sociodemographic and clinical characteristics of IBS patients (n = 15) (mean ± SD)

Age (yr)	42 ± 16
Sex (n): F/M	11/4
BMI (kg/m^2)	23.3 ± 5.0
Predominant bowel habit[1]: (n)	
Diarrhoea	2
Constipation	3
Alternating	10

[1]According to the Rome Ⅱ criteria; BMI: Body mass index.

The Human Ethics Committee of the Hospital District of Pirkanmaa, Finland, approved the study protocol. All subjects provided written informed consent.

Sample collection and preparation

Mucosal biopsies (mean weight 5.2 mg/sample; SD 1.5) from the ascending colon were obtained from each subject during colonoscopy after bowel cleansing. The samples were immediately frozen at -20℃, and stored at -70℃ until required for analysis. The samples for lipidomic analysis were weighed into Eppendorf tubes, and 10 μL of 0.9% sodium chloride and 10 μL of an internal standard mixture (10 lipid compounds, 0.1 μg each) were added. The samples were extracted with 100 μL of chloroform:methanol (2:1; two min vortexing, one h extraction time) and centrifuged (7800 *g*, 3 min). Of the lower organic phase, 60 μL aliquots were transferred into vial inserts and 10 μL of a standard mixture containing three labelled lipid compounds was added. The internal standard mixture contained the following lipid compounds with heptadecanoic acid (C17:0) as the esterified fatty acid: D-*erythro*-Sphingosine-1-Phosphate (C17 Base, Avanti Polar Lipids, Alabaster, AL, USA), LysoPC (Avanti Polar Lipids), MG (17:0)[rac] (Larodan Fine Chemicals, Malmö, Sweden), PG (17:0/17:0)[rac] (Avanti Polar Lipids), Cer (d18:1/17:0) (Avanti Polar Lipids), PS (17:0/17:0) (Avanti Polar Lipids), PC (17:0/17:0) (Avanti Polar Lipids), PA (17:0/17:0) (Avanti Polar Lipids), PE (17:0/17:0) (Avanti Polar Lipids), DG (17:0/17:0)[rac] (Larodan Fine Chemicals), and TG (17:0/17:0/17:0) (Larodan Fine Chemicals). The labeled standard mixture consisted of LysoPC (16:0-D3) (Larodan Fine Chemicals), PC (16:0/16:0-D6) (Larodan Fine Chemicals), and TG (16:0/16:0/16:0-13C3) (Larodan Fine Chemicals).

For water-soluble compounds, the samples were weighed into Eppendorf tubes and 10℃ of 1000 ppm (mg/mL) labelled palmitic acid (16:0-16, 16, 16D3) was added as an internal standard. The samples were extracted with 500 μL methanol (two min vortexing, 0.5 h extraction time) and centrifuged (7800 *g*, 3 min). The separated supernatants were evaporated to dryness under nitrogen, and the residues were derivatised with 2% methoxyamine HCl in pyridine (MOX; 25 μL, 90 min at 30℃) and N-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA; 50 μL, 30 min at 37℃). All samples were run in duplicate.

Analysis of lipids by UPLC/MS

Characterisation of lipid molecular species in colonic mucosa was performed by a lipidomics strategy using ultra performance liquid chromatography coupled to mass spectrometry (UPLC/MS, Waters Micromass Q-Tof Premier). The column (50℃) was a Waters Acquity UPLC™ BEH C18 (Waters Inc., Milford, MA, USA) 1 mm × 50 mm with 1.7 μm particles. The solvent system included: (A) ultra pure water (1% 1 mol/L NH_4Ac, 0.1% HCOOH) and (B) LC/MS grade acetonitrile:isopropanol (5:2, 1% 1 mol/L NH4Ac, 0.1% HCOOH). The gradient started from 65% A and 35% B, reached 100% B in 6 min and remained there for the next 7 min. There was a 5 min re-equilibration step before the next run. The flow rate was 0.200 mL/min and the injected amount 1.0 μL. Lipid profiling was carried out using ESI+ mode, and the data were collected at a mass range of m/z 300-2000 with a scan duration of 0.08 s.

Lipids were identified using an internal spectral library or with tandem mass spectrometry. The normalisation of lipidomics data was performed as follows: all monoacyl lipids, except cholesterol esters (such as monoacylglycerols and monoacylglycerophospholipids), were calibrated with lysophosphatidylcholine lysoPC (17:0) internal standard; all diacyl lipids, except ethanolamine phospholipids, were normalised with phosphatidylcholine PC (17:0); the diacyl ethanolamine phospholipids were calibrated with phosphatidylethanolamine PE (17:0); and the triacylglycerols and cholesterol esters were calibrated with triacylglycerol TG (17:0). Other molecular species were normalised by lysoPC (17:0) for retention time < 310 s, PC (17:0) for retention time between 310 and 450 s, and TG (17:0) for higher retention times. Data was processed using the MZmine software, version 0.60[19], and metabolites were identified using an internal spectral library or with tandem mass spectrometry (UPLC/MS/MS).

Analysis of water-soluble metabolites by GC × GC-TOF

A broad screening of water-soluble metabolites was conducted by a comprehensive two-dimensional gas chromatography coupled to a high speed time-of-flight mass spectrometry (GC × GC-TOF)[20]. The instrument used was a Leco Pegasus 4D GC × GC-TOF with Agilent 6890N GC from Agilent Technologies, USA and CombiPAL autosampler from CTC Analytics AG, Switzerland. Modulator, secondary oven, and time-of flight mass spectrometer were from Leco Inc., USA. The GC was operated in split mode (1:20) using helium as carrier gas at 1.5 mL/min constant flow. The first GC column was a relatively non-polar RTX-5 column, 10 m × 0.18 mm × 0.20 μm, and the second was a polar BPX-50, 1.10 m × 0.10 mm × 0.10 μm. The temperature programme was as follows: initial 50℃, 1 min -> 280℃, 7℃/min, one min. The secondary oven was set to +30℃ above the primary oven temperature. The second dimension separation time was set to 3 s. The mass range used was 40 to 600 amu and the data collection speed was 100 spectra/s. A commercial mass spectral library, Palisade Complete 600K, was used for identifying metabolites.

Statistical analysis

Partial least squares discriminant analysis (PLS/DA) was utilized as a supervised modelling method using the SIMPLS algorithm to calculate the model. The contiguous-blocks cross-validation method and Q^2 scores were used to develop the models. Top loadings for latent variables associated with drug-specific effects were reported. The VIP (variable importance in the projection) values were calculated to identify the most important molecular species for clustering of specific groups. Multivariate analyses were performed using Matlab, version 7.2 (Mathworks Inc., Natick, MA, USA), and the PLS Toolbox, version 4.0, for the Matlab package (Eigenvector Research Inc., Wenatchee, WA, USA). One statistical outlier IBS patient was left out of the analyses after an initial quality check of the results. Univariate comparisons for individual metabolites between the groups were performed using the Wilcoxon rank-sum test. A P value < 0.05 was considered statistically significant. To account for multiple comparisons, the False Discovery Rate (FDR) Q-value is also reported[21].

RESULTS

Lipidomic analysis

By applying UPLC/MS, a total of 651 lipid peaks were found, and 75 of them were identified using the internal spectral library, as described by Yetukuri *et al*[22], or with tandem mass spectrometry using UPLC/MS/MS. PLS/DA analysis of lipidomic data revealed significant differences in the mucosal lipid profiles of IBS patients and healthy controls. Overall, lipid species were upregulated in biopsies from IBS patients compared to those from healthy subjects. The 20 lipids with the largest differences between the groups by fold change appear in Table 2. A significant upregulation in the concentrations of typical cell membrane metabolites, lysophospholipids, in IBS patients was the most obvious finding (Figure 1A). Other lipid groups with a significant contribution to the distinction between IBS patients and healthy controls were ceramides (Figure 1B), glycosphingolipids, and di- and triacylglycerols. All of these showed upregulation in the IBS group.

Metabolomic analysis

Broad metabolite screening by GC × GC-TOF resulted in several hundred mucosal metabolites, of which 107 were

Table 2 The 15 lipids with the largest and most significant differences between patients and controls

Lipid name	Fold (IBS/healthy control)	*P* value[1]	FDR *Q* value
Cer (d18:1/24:1)	1.3	0.001	0.022
Cer (d18:1/24:2)	1.4	0.00004	0.0018
DG (36:2)	1.9	0.000001	0.0003
GlycoSL (m/z = 1199.805)	1.9	0.001	0.018
GlycoSL (m/z = 1195.851)	2.0	0.0003	0.0069
lysoPC (16:0)	2.1	0.00006	0.0020
lysoPC (18:0)	1.9	0.0002	0.0049
lysoPC (18:1)	2.8	0.00002	0.0013
lysoPE (18:1e)	2.4	0.00002	0.0013
7TG (46:5)	1.4	0.04	0.19
TG (48:5)	1.6	0.03	0.17
TG (48:6)	1.7	0.03	0.18
TG (49:3)	2.1	0.009	0.089
TG (51:4)	1.6	0.04	0.19
TG (51:5)	1.8	0.02	0.12

[1]Wilcoxon rank sum test. Cer: Ceramide; DG: Diacylglycerol; GlycoSL: Glycosphingolipid; lysoPC: Lysophosphatidylcholine; lysoPE: Lysophosphatidylethanolamine; TG: Triacylglycerol.

Table 3 Major water soluble metabolites contributing to differentiation between patients and controls[1]

Metabolite	Fold (IBS/healthy control)	*P* value[2]	FDR *Q* value
2(3H)-furanone	13.7	0.03	0.25
Ribitol	3.6	NS	0.25
Heptan	2.9	0.02	0.25
L-Mannose	2.8	NS	0.25
Creatinine	1.7	0.04	0.25
Dodecane	1.5	NS	0.29
Decanoic acid	1.3	NS	0.52
Dodecanoic acid	-1.5	NS	0.25
n-Butylamine	-1.5	0.01	0.25
D-ribose	-1.5	NS	0.25
Glucopyranose	-1.6	NS	0.29
Azelaic acid	-1.8	0.02	0.25
Adipic acid	-2.7	0.0008	0.05

[1]Separation is based on a variable importance projection (VIP) analysis with a cut-off value of 2; [2]Wilcoxon rank sum test. NS: Not significant.

identified and kept in analyses. Based on PLS/DA analysis, a clear distinction between IBS cases and controls was obtained (Figure 2). Both upregulation and downregulation of metabolites were observed in IBS patients *vs* controls. The top ranked metabolites contributing to the distinction between the groups appear in Table 3.

The metabolite contributing most to the distinction was 2(3H)-furanone, a cyclic ester commonly produced in biochemical pathways, which was almost 14-fold upregulated in IBS patients compared to healthy subjects (P = 0.03). The fold changes for other top ranked metabolites were clearly lower (a 3.7 to -2.7 fold change). Other basic metabolites frequently found in biochemical pathways, such as the second messenger, D-ribose, were also among the major factors contributing to the distinction between cases and controls. Organic, carboxylic acids were found to be both slightly downregulated (dodecanoic, azelaic, and adipic acid) as well as slightly upregulated (decanoic acid) in IBS patients compared to healthy controls.

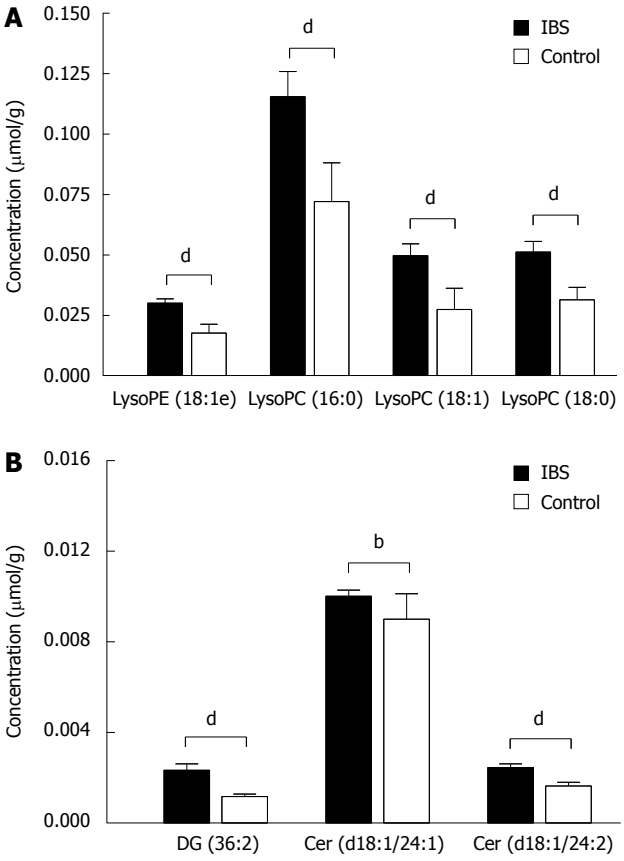

Figure 1 The concentrations (mean ± SE) of selected lysophospholipids in mucosal biopsies from irritable bowel syndrome (IBS) patients (n = 15) and healthy controls (n = 9) as measured by UPLC/MS. (A) Patients and controls differ significantly from each other for all presented lysophospholipids, as well as for (B) diacylglycerol and ceramides. LysoPE: Lysophosphatidylethanolamine; LysoPC: Lysophosphatidylcholine. *P* values are based on Wilcoxon rank sum test with $^{b}P < 0.01$ and $^{d}P < 0.001$.

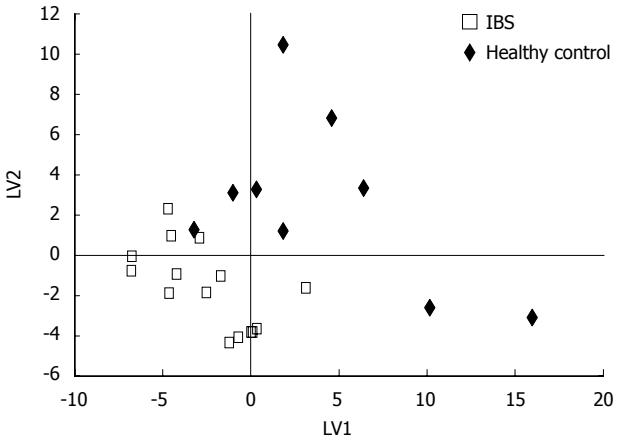

Figure 2 Partial least squares discriminant analysis (PLS/DA) of GC × GC-Tof-based metabolic profiles for IBS patients (n = 15) and healthy controls (n = 9). Two latent variables (LVs) were used (Q^2 = 61%).

DISCUSSION

Data on the molecular abnormalities in IBS are urgently

required, as the pathophysiology of the condition is largely unknown and current therapies are therefore also limited. In this study, the differences between colonic mucosa from IBS patients and healthy controls were investigated by employing two high-throughput metabolomic platforms, UPLC/MS based lipidomics and GC × GC-TOF based metabolomics. Data indicated multiple differences between IBS mucosa and healthy mucosa, including an increase in the IBS group of several lipid species, such as lysophospholipids and ceramides.

The present study is the first to utilise a metabolomic approach to investigate molecular differences between IBS patients and healthy controls. Metabolomics can be seen as an optimal tool for studying diseases with unknown or complex pathophysiology, as a global study of the metabolome is a non-targeted approach that requires no preselection of markers, in contrast to the traditional way of limiting the analysis to a predefined set of known compounds[23]. Recently, metabolomics has been utilised in the investigation of multiple diseases, e.g. inflammatory bowel disease[24], obesity[25], and cancer[26].

In the present study, lipids - particularly lysophosphatidylcholines (lysoPCs) and ceramides - were the most upregulated molecules in IBS patients. Mounting data suggest that certain lipids, including phospholipid derivatives and ceramides, play a role in modulating and enhancing pain sensitivity[27,28], which could be one explanation for their involvement in IBS pathophysiology. LysoPCs have not previously been associated with IBS, but studies indicate elevated levels of lysoPCs or phospholipase A2, an enzyme involved in lysoPC formation, in inflammatory bowel disease (IBD)[29,30]. A high lysoPC concentration has been suggested to impair mucosal barrier function and increase gastrointestinal permeability *in vivo* and *in vitro*[31-34]. The role of permeability defects in IBS is not fully elucidated, but a recent review by Camilleri and Gorman concludes that there appears to be at least one IBS subgroup with increased gut permeability[35]. Interestingly, lysoPCs have also been associated with vascular inflammation, endothelial dysfunction, and coronary atherosclerosis[36], implying that lysoPCs might also play a role in the subtle type of mucosal inflammation present in IBS.

Lipids of the ceramide/sphingomyelin pathway are another lipid type that we observed to be altered in IBS. Previous studies indicate that ceramides might be involved in the pathology of IBD[37,38], whereas no reports on ceramides in IBS are available. Ceramides have, however, been shown to be toxic in several cell types. Current data reveal a possible role for ceramides in the damage of cells and tissues, and ultimately in the development of chronic metabolic diseases, such as diabetes and cardiovascular disease[39]. The toxic effects of ceramides might be partly mediated *via* the production of reactive oxygen species in cells[40]. It has been proposed that, similar to lysophosphatidylcholines, epithelial oxidative stress might also contribute to gut barrier dysfunction[41]. Our results thus suggest that the molecular mechanism behind increased permeability could, to some extent, be similar in IBS and IBD.

Based on a global analysis of water-soluble metabolites, IBS cases and healthy controls were rather well separated into two distinct groups. The physiological relevance of the main molecules contributing to the separation is, on the other hand, less evident than in the case of lipid species. Differences were seen in basic metabolites, such as 2(3H)-furanone (also known as lactone) and D-ribose, both of which are produced in common biochemical pathways in cells. A recent study investigating a *Trichinella spiralis* infected mouse model of post-infectious IBS and utilizing metabolomics, demonstrated an increase in molecules involved in energy metabolism (lactate, citrate, and alanine) in the IBS group[42]. The authors suggest that this might reflect a muscular hypercontractility possibly present in IBS, though it should be underlined that this was an experimental model. Concerning organic acids, our results are in line with the study by Martin *et al*[42], in that we found that organic acids contributed to the separation between cases and controls. Specifically, we did observe increased concentrations of alanine in the IBS group, but the difference between IBS and control groups was not significant (fold = 1.3, $P = 0.14$). Organic acids are known to be produced by the intestinal microbiota, and a disruption in the acid profile could reflect a possible deviance in microbiota previously reported in IBS[43,44]. In further support of findings by Martin *et al*[42], we detected elevated levels of creatinine in the IBS group. Creatine and creatinine are tightly interlinked with the energy metabolism in smooth muscle, and a raised creatinine concentration might be a sign of increased energy consumption and muscle contractility[45].

The field of metabolomics is evolving rapidly, and it is already considered a sensitive analytical tool for investigating the health-disease continuum[17]. Like any method, however, it has its own limitations. As large numbers of metabolites are included in the studies, caution is necessary in the interpretation of results[16]. The relevance of a single identified biomarker might not be high, but it could be that systematic up- or down-regulation in specific groups of molecules (such as certain lipids in the current study) indicates a biologically relevant metabolite type. Another drawback of metabolomics is that a large proportion of spectral peaks are still unknown, and consequently more effort has to be placed on the compilation of standardised metabolite libraries[16,23]. Considering the current study setting, one obvious weakness is the small number of subjects. On the other hand, it is highly encouraging to see that IBS patients and healthy controls were fairly well differentiated, even with this limited sample size. Moreover, it would have been interesting to investigate whether differences between IBS and healthy subjects could also be observed in metabolic profiles from non-invasive tissues, such as faecal material or blood, as these are more easily obtained in clinical settings.

Taken together, our results suggest significant differences in the global mucosal metabolic profile between IBS patients and healthy controls. The current study is the first to attempt to identify colonic mucosal metabolites typical for IBS using a high-throughput

metabolomic approach. In this study, IBS was particularly characterised by an upregulation of specific lipid groups, such as lysophosphatidylcholines and ceramides. These lipid species have been associated with the modulation of pain sensitivity and gut permeability, and our data thus indicate that these molecules might be involved in the pathophysiology of visceral pain and gut barrier dysfunction associated with IBS.

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COMMENTS

Background

Irritable bowel syndrome (IBS) is the most common diagnosis in gastroenterology. The syndrome is classified as functional, and no biological marker exists for IBS. The precise aetiology and pathophysiology is not fully known, and this might partly explain why pharmacotherapy is considered rather ineffective.

Research frontiers

More data on the molecular abnormalities in IBS are required to better understand the mechanism behind the emergence of symptoms, and to be able to treat the patients in a safe and efficient way.

Innovations and breakthroughs

This study characterises the differences between colonic mucosa from IBS patients and healthy controls using two high-throughput metabolomic platforms, UPLC/MS based lipidomics and GC × GC-TOF based metabolomics. Metabolomics is a useful tool for investigating diseases with complex or unknown backgrounds, because it is possible to simultaneously measure and model a huge number of metabolites. Data indicated multiple differences between IBS mucosa and healthy mucosa, thus providing novel information about the pathophysiology of IBS. An increase in the IBS group of several lipid species, such as lysophospholipids and ceramides, was the major difference observed.

Applications

By better understanding the mucosal abnormalities behind IBS, it might be possible to improve the diagnosis and therapy of patients.

Peer review

This article is surely innovative, not only in the hypothesis, but also in the methodology. This could be a hot article if could be popularized appropriately. A review of the current literature indicates that the present article is a pioneer in its field.

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BRIEF ARTICLE

Wireless capsule endoscopy in detecting small-intestinal polyps in familial adenomatous polyposis

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Abstract

AIM: To detect the prevalence of small bowel polyps by wireless capsule endoscopy (WCE) in patients with familial adenomatous polyposis (FAP).

METHODS: We examined prospectively 14 patients with FAP to assess the location, size and number of small-intestinal polyps. Patients' age, sex, years of observation after surgery, type of surgery, duodenal polyps and colorectal cancer at surgery were analyzed.

RESULTS: During WCE, polyps were detected in 9/14 (64.3%) patients. Duodenal adenomatous polyps were found in nine (64.3%) patients, and jejunal and ileal polyps in seven (50%) and eight (57.1%), respectively. The Spigelman stage of duodenal polyposis was associated with the presence of jejunal and ileal polyps. Identification of the ampulla of Vater was not achieved with WCE. Importantly, the findings of WCE had no immediate impact on the further clinical management of FAP patients. No procedure-related complications were observed in the patients.

CONCLUSION: WCE is a promising noninvasive new method for the detection of small-intestinal polyps. Further investigation is required to determine which phenotype of FAP is needed for surveillance with WCE.

INTRODUCTION

Familial adenomatous polyposis (FAP) is a dominant inherited syndrome with an incidence of 1 in 11 000. It is caused by an alteration of the FAP (APC) gene that is located on chromosome 5q21, which causes multiple disorders of the development of the ecto-, endo- and mesoderm. The syndrome is characterized by the presence of adenomatous polyps in the gastrointestinal tract, mainly in the colon, rectum and duodenum, with a demonstrated adenoma-carcinoma sequence[1-3]. The duodenum is characterized by the presence of adenomas in 80% of patients with FAP and the development of periampullary cancer in 4%[4,5]. In patients who have undergone colectomy, periampullary cancer is the main cause of death. Between five and 10% of FAP patients die from upper gastrointestinal cancer, which is frequently periampullary in origin. In an attempt to prevent malignancy, a screening program appears to be mandatory to detect particularly those patients most at risk of developing the disease. Therefore, endoscopic surveillance of the second part of the duodenum with side-viewing endoscopy is advised. Since it was introduced in 2000, wireless capsule endoscopy (WCE) has opened the way for the noninvasive and painless test of the entire small intestine, thereby becoming the gold standard for endoscopic evaluation of the small bowel

in several clinical situations, including surveillance of polyposis syndromes. There have been only a few studies that have evaluated the utility of WCE in detecting small-intestinal polyps in patients with FAP[6-12].

The aim of our prospective study was to investigate the diagnostic yield of WCE in being able to detect adenomatous polyps in a Greek FAP cohort,and to establish potential risk factors for small-bowel polyp development for a more targeted surveillance with WCE.

MATERIALS AND METHODS

We performed an open prospective, non-randomized clinical trial from September 2007 to September 2008, which evaluated the use of WCE in FAP patients. The study was conducted in accordance with good clinical practice, as set forth by the Helsinki agreements and their later amendments. The study was approved by our hospital Ethics Committee and informed consent was obtained from all patients.

We included male and female patients, aged 18-70 years, who were referred to our clinic. Patients excluded were those with severe swallowing disorders, implanted cardiac pacemaker or other electronic devices, pregnant women, patients with a clinical suspicion of small-bowel obstruction/pseudo-obstruction, strictures or fistulas, and children under 10 years old[13,14].

The following information was gathered from patients' records: age, sex, diagnostic [endoscopy, small-bowel radiography, computed tomography (CT)] and surgical procedures (type of colectomy and time of surgery) before WCE. All the procedures were performed on an outpatient basis, in the morning, after an overnight fast. Bowel preparation was performed with 4 L polyethylene glycol solution given 15 h before the procedure. Patients were allowed to drink clear fluids 2 h after capsule ingestion. Furthermore, the patients were able to maintain their normal activities while the capsule was passing through the digestive tract. Patients returned to the hospital 8 h after capsule ingestion. The registration device and the antennae were disconnected from the patient and a questionnaire about symptom occurrence and overall satisfaction with the procedure was completed. On each of the 2 d following the procedure, a telephone call was made to inquire about any symptoms and to confirm that the capsule had been expelled. In view that the major risk from WCE is capsule retention or impaction, all patients were instructed to contact the study staff should they develop any gastrointestinal symptoms during or after WCE.

Capsule videorecordings were reviewed by a single experienced endoscopist (Katsinelos P) who previously had performed more than 200 WCE procedures. A polyp was defined as a discrete mass of tissue that protruded into the bowel lumen. The location of small-bowel polyps was approximately estimated as duodenal (Figure 1A), jejunal, or ileal (Figure 1B), according to the timing of polyp appearance after entrance of the capsule to the duodenum, the total small-bowel passage time, and the endoscopic appearance of the small-intestinal mucosa. Entrance to the duodenum is easy to detect because it begins just after the pylorus, which can be identified easily. The location of small-bowel polyps was estimated by analyzing the WCE transit time between pylorus passage and ileocecal valve or pouch ileostoma. The duodenum was designated to be the small bowel that was visualized during the first 15 min after the capsule exited the pylorus, while the jejunum and ileum were designated to be the small bowel that was visualized after < 50% and > 50% of small-bowel transit time, respectively. Moreover, the prominent folds and high narrow villi characterized the jejunum, while fewer folds and shorter villi were observed in the ileum. WCE allows only an approximate estimation of the size of polyps, therefore, based on previous experience[11], we classified polyps as small or large, using an open pylorus orifice (diameter 10 mm) as a reference for polyp size estimation. Small and large polyps were classified with a diameter < 10 mm and > 10 mm, respectively.

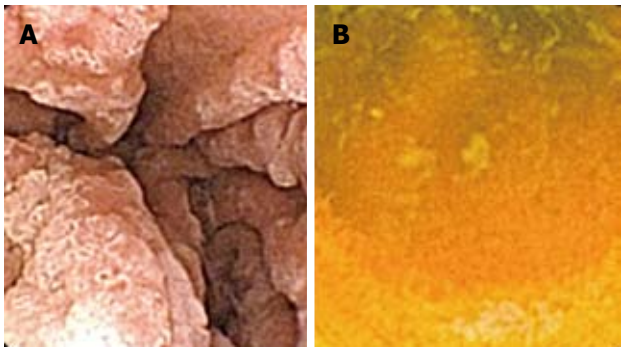

Figure 1 Wireless capsule endoscopy (WCE) view. A: Large and small mushroom-shaped adenomas in the distal duodenum; B: Small ileal polyp.

Following WCE, conventional endoscopy was performed within 2 wk in all patients. Standard duodenoscopy up to the second part of the duodenum was performed with a forward-looking gastroscope and a side-viewing duodenoscope, on an outpatient basis. To reduce motility artifacts, 20 mg butylscopolamine were administered intravenously. Biopsies and polypectomies were performed for staging of duodenal disease according to the Spigelman classification (Table 1)[15].

The primary end point of the study was to identify the number and size of small-bowel polyps in each patient, and the secondary end point was the impact of WCE findings on the management of the patients.

RESULTS

Fourteen patients (9 men, median age 34 years, range: 22-56 years) with FAP were recruited. Eight patients had undergone total proctocolectomy with ileal-pouch-anal anastomosis, four had undergone ileorectal anastomosis, and two were examined before colectomy (Table 2).

Endoscopic investigation of the entire length of the small bowel was achieved in all patients. The quality was considered as good except for one case in which food debris in the duodenum, jejunum and ileum made reading the film very difficult; in the last case, the procedure

Table 1 Spigelman classification of duodenal polyposis (adenomas in FAP)[15]

	Number of points		
	1P	2P	3P
Number of polyps	1-4	5-20	> 20
Polyp size (mm)	1-4	5-20	> 10
Histology	Tubulous	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe
Stage		Spigelman score	
0		0	
I		1-4	
II		5-6	
III		7-8	
IV		9-12	

Table 2 Clinical characteristics of the 14 patients with FAP studied with WCE

Patient No.	Sex	Age (yr)	Time of surgery before WCE (yr)	Type of surgery	No. of colon polyps	Colon cancer
1	M	54	4	IPAA	> 1000	No
2	F	23	BS	BS	> 100	No
3	M	53	5	IPAA	> 100	No
4	F	27	5	IPAA	> 100	No
5	M	28	4	IPAA	> 100	No
6	M	22	BS	BS	> 100	No
7	M	53	2	IRA	> 1000	No
8	F	26	3	IPAA	> 100	No
9	M	56	18	IRA	> 1000	No
10	F	29	10	IPAA	> 100	No
11	M	54	4	IPAA	> 1000	Yes
12	M	36	15	IRA	> 100	No
13	F	41	17	IRA	> 100	No
14	M	32	7	IPAA	> 100	No

FAP: Familial adenomatous polyposis; WCE: Wireless capsule endoscopy; IPAA: Ileal pouch-anal anastomosis; IRA: Ileorectal anastomosis; BS: Before surgery.

was repeated and the patient was asked to avoid food intake prior to the examination. Mean gastric and small-bowel transit time were 36 (range: 12-58) and 256 (range: 128-360) min, respectively. No abnormal additional findings were identified. Overall, 81 polyps, mainly small (96.3%), were detected by WCE. The presence, size and location of duodenal, jejunal and ileal polyps were related to the Spigelman stage of duodenal polyposis and age of the patient, but not sex (Table 3). None of the five young FAP subjects with Spigelman stage 0 had small-bowel polyps detected. Three large sessile polyps were found in the duodenum in one patient with Spigelman stage IV disease. All other polyps detected were small (Table 3). WCE was inferior diagnostically to standard duodenoscopy and gastroscopy regarding the second part of the duodenum and especially the ampullary region. The capsule technique could not identify the papilla of Vater in any of our patients and four small ampullary adenomas were missed by WCE as compared with duodenoscopy. Endoscopic polypectomy of duodenal adenomas was performed in five patients, and biopsies were taken from the rest of the patients. Histological

Table 3 Distribution and size of polyps according to Spigelman stage as assessed by WCE

Patient No.	Sex	Spigelman stage	Duodenal	Jejunal	Ileal	Rectal stump
1	M	IV	13	3	5	No
2	M	0				
3	F	II	3	2	4	No
4	M	I	3		2	No
5	F	I	2	1	3	No
6	M	0				No
7	M	III	4	4	3	Yes
8	F	0				Yes
9	M	III	7	3	2	Yes
10	M	0				No
11	M	II	4	1	2	Yes
12	M	I	3			No
13	F	I	4	1	2	No
14	M	0				No

examination of the specimens confirmed the diagnosis of tubular or tubulovillous adenomas with low-grade dysplasia in one case with large polyps. We detected small ileal white-colored polyps with a normally appearing mucosal surface in two young patients (both Spigelman stage 0), and we classified these lesions as lymphoid hyperplasia, which occurs commonly in the terminal ileum and rectum associated with FAP, especially in young patients[16].

All the patients described the procedure as comfortable and were willing to repeat it had it been deemed necessary. Difficulty/inability to swallow the capsule or clinically significant complications, including symptomatic capsule retention and aspiration, did not occur during the procedure. Five patients previously had undergone enteroclysis, and although a comparison of the two methods was not within the scope of this study, as there was a significant time lapse between them, all the patients preferred WCE when they were asked to compare it with enteroclysis. All patients reported no pain or discomfort when contacted 1 wk after the WCE examination.

DISCUSSION

Small-intestinal adenomas can occur in FAP patients, but their prevalence varies, depending of the modality used for their detection[17]. The advent of WCE in 2000 has changed noticeably the diagnosis and management of numerous diseases of the small intestine, including polyps associated with FAP[14]. Our study shows that WCE is able to detect even small polyps in the entire small intestine in subjects with FAP. We found jejunal and ileal polyps to be common. The frequency and number of polyps and the length of small bowel involvement was found to increase with Spigelman classification (Table 3). All polyps were small except for three in the duodenum. These findings were similar to those previously reported by other studies[6-11], although Iaquino *et al*[12] have found the presence of duodenal adenomas to be the only clinical feature predictive of small-intestinal adenoma, but not associated with Spigelman

stage. We cannot define the true sensitivity of WCE for detection of small-bowel adenomas because the lack of visualization of the entire small-bowel mucosa by WCE leads to underestimation of polyp burden. To achieve this, we would have to compare the performance of WCE with that of the criterion standard of surgical enteroscopy. However, given the invasiveness and the high morbidity rate of the latter procedure, such a study would be extremely difficult to perform. The advent of double or single balloon enteroscopy of the small bowel may have opened a new avenue to gain less invasive access, even to polyps located in the distal small bowel. Double balloon enteroscopy appears to be equivalent to an intraoperative enteroscopy for scrutiny of small-intestinal polyps in FAP[17]. The region around the papilla of Vater was not visualized in any of our patients, which calls for the mandatory use of side-view duodenoscopy for staging duodenal disease.

Magnetic resonance enteroclysis combines the advantages of cross-sectional resonance with those of the volume challenge of conventional enteroclysis in the recognition and characterization of small-bowel-wall abnormalities, including initial tumors. There are few promising reports about the role of magnetic resonance enteroclysis and CT enteroclysis in the diagnostic algorithm of small-bowel neoplasms[18]. Whether the use of WCE in combination with these new diagnostic techniques will lead to earlier diagnosis of small-intestinal polyps in FAP patients remains to be elucidated in the future.

We observed no complications from WCE in our study. Other reports of WCE performed in individuals with FAP also have failed to detect any complications[6-12].

Forward and side-viewing endoscopic surveillance for gastric and duodenal/periampullary neoplasia is recommended for all individuals with FAP[19,20]. The frequency of surveillance should be based on the Spigelman classification of duodenal polyposis[19,20]. However, the implication of jejunal and ileal adenomas in FAP is unknown. The risk of cancer distal to the duodenum in FAP has been reported much more rarely than that of duodenal and periampullary carcinoma[21]. The lack of data may rely on the fact that patients with FAP usually are not studied because of the low incidence of non-duodenal small-bowel cancer[21]. Therefore, should a search for small bowel adenomas with WCE be performed in all patients with FAP? Keeping in mind the high cost of WCE, identification of a subset of FAP patients who might be at the highest risk for developing small bowel tumor is desired. The analysis of germline APC gene mutation was not available in our patients, to compare with WCE findings. However, as reported by other investigators, the incidence of small-intestinal adenomas is correlated with mutations found in exon 15[22]. Mutations in this exon traditionally have been associated with a more aggressive phenotype[22,23]. The identification of genotypic factors that predict the phenotype of small-bowel adenomas is important. It has been suggested that WCE should be performed only in patients with exon 15 mutations[12], thereby requiring relative WCE surveillance. This approach may allow for a more cost-effective evaluation of FAP patients. Obviously, the current genotype-phenotype correlation must be confirmed in a larger cohort of FAP patients.

The frequency of WCE surveillance of jejunal and ileal adenomatous polyps in patients with FAP remains unknown. The detection of these small polyps in our study and previous studies had no immediate impact on the clinical management, other than establishing further surveillance intervals in these patients[6-12]. The tendency is for WCE to become the standard imaging modality for small-bowel surveillance, since Spigelman stage Ⅲ and Ⅳ patients have a high burden of small-intestinal adenomas on WCE (Table 3). With the potential exception of the mentioned high risk of FAP patients developing small-bowel cancer, we recommend surveillance every 3-5 years in these patients; despite more data on the prevalence of small-bowel polyps in patients with advanced stage (Ⅲ or Ⅳ) duodenal polyposis being needed to understand the utility of WCE in these groups. The small number of polyps observed in our FAP patients with Spigelman stage 0-Ⅱ disease (Table 3) is in accordance with other studies[6-8]. We agree with other investigators'[6-10] recommendations that WCE is not useful for routine small-bowel surveillance in these patients. Although management of jejunal and ileal polyps has not as yet been well defined considering the adenomatous nature of polyps in FAP, it seems reasonable to remove these polyps that are easily accessible by endoscopy. Whenever endoscopic polypectomy cannot be performed, although there is not enough evidence to propose surgical resection, surveillance with WCE seems advisable.

In conclusion, WCE is noninvasive, safe and comfortable, and can be performed on an ambulatory basis in FAP patients. It is effective for the detection of small-bowel polyps, but larger studies are needed to define better the impact of WCE on the clinical outcome of FAP patients with small-intestinal polyps, to elaborate which mutant gene carries the highest prevalence of small-intestinal adenomas, and to decide the timing of surveillance and polypectomy treatment by double or single balloon enteroscopy.

COMMENTS

Background

Endoscopic surveillance of the duodenum and periampullary area is recommended in patients with familiar adenomatous polyposis (FAP), because 4% of patients develop cancer. However, the significance of the presence of jejunal and ileal polyps in patients with FAP is unknown.

Research frontiers

FAP is a dominant inherited syndrome characterized by the presence of adenomatous polyps in the gastrointestinal tract, with a demonstrated adenoma-carcinoma sequence. In the present study, the authors investigated the diagnostic utility of wireless capsule endoscopy (WCE) in detecting small intestine polyps in a Greek FAP cohort.

Innovations and breakthroughs

Few studies have evaluated the utility of WCE in detecting small-intestinal polyps and their clinical significance in patients with FAP. The rate of detection of small polyps in our patients was high but had no immediate impact on clinical management, other than establishing further surveillance intervals in these patients.

Applications

This study represents a new role for WCE in the examination of the small intestine in FAP patients and emphasizes the need for a highly targeted surveillance based on Spigleman classification.

Terminology

WCE is a technology that uses a swallowed video capsule to take photographs of the inside of the esophagus, stomach, and small intestine. Since it was introduced in 2000, it has become the gold standard for endoscopic evaluation of the small bowel in several clinical situations, including surveillance of polyposis syndromes.

Peer review

This is an interesting observational study of the role of WCE in screening for small-intestinal polyps in a small cohort of patients with established FAP.

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BRIEF ARTICLE

Sphincter of Oddi dysfunction: Psychosocial distress correlates with manometric dyskinesia but not stenosis

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Abstract

AIM: To compare postcholecystectomy patients with Sphincter of Oddi (SO) dyskinesia and those with normal SO motility to determine the psychosocial distress, gender and objective clinical correlates of dyskinesia, and contrast these findings with comparisons between SO stenosis and normal SO motility.

METHODS: Within a cohort of seventy-two consecutive postcholecystectomy patients with suspected SO dysfunction, manometric assessment identified subgroups with SO dyskinesia (n = 33), SO stenosis (n = 18) and normal SO motility (n = 21). Each patient was categorized in terms of Milwaukee Type, sociodemographic status and the severity of stress-coping experiences.

RESULTS: Logistic regression revealed that *in combination* certain psychological, sociodemographic and clinical variables significantly differentiated SO dyskinesia, but not SO stenosis, from normal SO function. Levels of psychosocial stress and of coping with this stress (i.e. anger suppressed more frequently and the use of significantly more psychological coping strategies) were highest among patients with SO dyskinesia, especially women. Higher levels of neuroticism (the tendency to stress-proneness) further increased the likelihood of SO dyskinesia.

CONCLUSION: A motility disturbance related to psychosocial distress may help to explain the finding of SO dyskinesia in some postcholecystectomy patients.

Key words: Gender; Functional gastrointestinal disorders; Psychosocial distress; Sphincter of Oddi dyskinesia

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INTRODUCTION

Sphincter of Oddi (SO) dysfunction (SOD), characterized by recurring episodes of severe biliary-like abdominal pain following cholecystectomy, is one of a number of functional disorders of the biliary tract[1]. However, based on SO manometry, which is the "gold standard" for the diagnosis of SOD, the condition is a heterogenous one, currently considered within the context of the functional disorders, but known to include both stenotic and non-stenotic (functional) groups. Thus, SO dyskinesia can be differentiated from SO stenosis, a structural defect of the sphincter orifice.

Unlike other functional gastrointestinal disorders (FGIDs)[2], the psychosocial and gender concomitants of SOD have received little attention. In particular, psychosocial issues specifically relating to SO dyskinesia have not been addressed. Although two previous studies have implicated psychosocial factors in the genesis of symptoms in SOD, patients were not differentiated manometrically[3,4]. A more recent study found that health-related quality of life is impaired, and abuse histories are common, in SOD patients, but again patients were not differentiated manometrically[5].

Given the strength of the relations between psychosocial disturbance and alterations in gastrointestinal transit, motor activity and sensitivity previously documented

in irritable bowel syndrome (IBS)[6-12], our central hypothesis was that a similar association may exist with respect to SO dyskinesia. While the term stress or chronic stress can be viewed as a generic or umbrella term to include all features of a social stressor situation including psychophysiological effects, we have applied the term psychosocial distress to represent a person's experience of and transactions with a stressor situation including on-going attempts to gain or regain emotional and psychological equilibrium/control.

The aims of the present study, therefore, were to determine, among subgroups of postcholecystectomy patients exhibiting SO dyskinesia, SO stenosis (± dyskinesia) or normal SO motor activity: (1) whether levels of psychosocial distress are higher in patients with manometrically proven SO dyskinesia than in those with normal SO motor activity, and (2) whether higher levels of psychosocial distress are specific to SO dyskinesia (that is, relative to patients with SO stenosis), and (3) whether the presence of objective clinical criteria, according to the Milwaukee classification[13], influences these associations. We chose specific psychological assessments of emotional state, personality and coping behaviour based on previously documented associations in other FGIDs[6-12]. Thus, during an individual's exposure to high levels of threat, powerful emotions such as fear, anger, anxiety and frustration are aroused and coping strategies, strongly influenced by personality and past experiences, are employed in an attempt to reduce the impact of both the emotions and the situation. A high level on any cluster of these psychological dimensions suggests high levels of psychosocial distress.

MATERIALS AND METHODS

Patients

The study population comprised seventy-two consecutive postcholecystectomy patients with biliary-like pain consistent with SOD [64 women, mean age 45 (SD ± 12) years], referred to the Gastrointestinal Investigation Unit for SO manometry. The presence of recurrent biliary-like pain fulfilling the criteria for SOD was confirmed from responses on the Bowel Disease Questionnaire (BDQ)[14] and defined according to the Rome criteria[1], namely - episodes of pain in the right upper quadrant or epigastrium, rated by the patients as severe or very severe, steady and lasting from 30 min to 6 h. In all patients, organic disease had been excluded on the basis of normal physical examination, negative screening blood tests, negative gastroduodenoscopy and upper abdominal ultrasound or computed tomography scan, and the absence of calculi and strictures as demonstrated by endoscopic retrograde cholangiopancreatography (ERCP). Approval for the procedures was given by the Medical Research Ethics Committee of the Royal North Shore Hospital, and all subjects gave written informed consent.

Clinical assessment

SO Milwaukee Type was determined according to Hogan *et al*[13], with patients sub-grouped into patients with objective clinical criteria (Types I & II), including abnormalities of the biliary tree at cholangiography or abnormalities in hepatic biochemistry associated with episodes of pain, and patients without objective criteria (Type III) but with recurrent episodes of biliary-like pain. Thus, biliary-type I patients exhibited elevated liver biochemistries documented on two or more occasions, delayed contrast drainage, and a dilated common bile duct with a corrected diameter equal to or greater than 12 mm at ERCP; biliary-type II patients exhibited only one or two of the above criteria; while biliary-type III patients exhibited none of the above criteria[1].

SO manometry

Manometry of the SO was performed in standard fashion according to the technique of Toouli *et al*[15], using an Olympus JFIT10 duodenoscope and a triple-lumen catheter with inner lumen diameters each of 0.5 mm, an outer diameter of 1.7 mm and side holes radially orientated 2 mm apart (Wilson-Cook Medical, Winston-Salem, NC, USA). SO manometric tracings were analyzed by two experienced observers, blinded to the results of the symptom questionnaire. The following parameters[15,16] were determined: basal sphincter pressure, peak sphincter pressure, and phasic wave contractile frequency and propagation. Abnormalities of these parameters were defined as values outside normal ranges established previously using an identical recording technique[15]: basal pressure < 40 mmHg, contractile frequency < 8/min, and proportion of retrograde contractions < 50%. Complete inhibition of phasic contractions following cholecystokinin (CCK) was considered a normal CCK response. Failure of such a response, including a "paradoxical response" of the sphincter to CCK (i.e. increase in either the basal pressure and/or phasic contractions)[15], was considered an abnormal response. SO manometric recordings were classified[15,17,18] as either: (1) sphincter dyskinesia, defined as an abnormally high basal pressure resolving after CCK, or an abnormally high phasic contractile frequency, and/or an elevated proportion of retrograde contractions, and/or an abnormal response to CCK in the absence of sphincter stenosis or (2) sphincter stenosis, defined as an abnormally high basal pressure persisting after CCK irrespective of the presence of some features of dyskinesia or (3) normal sphincter motor function. The presence of SO stenosis had hierarchical importance over any other feature of dyskinesia in stratifying patients.

Psychosocial assessments

Psychometric measures assessed sociodemographic and psychological factors. The following data were collected prior to the SO manometry.

Sociodemographic data: Sex, age, marital status, highest education level, current employment status (i.e. whether working full-time, part-time or unemployed) and highest occupation level of self and father.

Emotional distress/mood state: Depression - in particular the affective component of a depressed mood state - was assessed using The Centre of Epidemiological Studies

Depression Scale[19] and state anxiety using the Spielberger State and Trait Anxiety Inventory (STAI)[20]. Responses on these scales reflect complementary dimensions of psychosocial distress arising from stressful life situations.

Personality traits: Trait anxiety (the tendency to anxious states) was also assessed from responses on the STAI[20], and neuroticism (high scores reflect a tendency to stress-proneness and to excessive worry) and extroversion (orientation to things external or internal) were assessed using the Eysenck Personality Inventory[21].

Coping style: The Defense Style Questionnaire (DSQ 40) measured the tendency to use emotion-focused coping defenses or strategies to reduce emotional distress classified as mature (e.g. using humor, suppression, anticipation, sublimation), immature (e.g. using denial, acting out, being passive aggressive) and/or neurotic [e.g. using pretence (pseudo-altruism), idealization, undoing, reaction formation][22]. Normative and reliability data from patient and non-patient groups show these factors to have the internal consistency and the temporal stability of a trait measure - the mature dimension proving to be the primary discriminating factor[23].

Emotional suppression/expression: The Courtauld Emotional Control Scale[24] assessed the tendency to suppress unwanted emotions such as anger and anxiety and the Anger Expression Scale[25] assessed the tendency to hold in anger (anger-in) to express anger (anger-out) and to control and/or resist becoming angry (anger-control). Anger-in and anger-out are empirically independent, factorially orthogonal dimensions[26]. Differences in the physiological effects of suppression *vs* expression of powerful emotions on autonomic, neuroendocrine and digestive functioning underlie the inclusion of these scales.

Locus of control of behavior: This scale[27] assessed the extent to which a person believes that personal efforts more than external factors can achieve a positive outcome.

Each of the above measures has established reliability and validity and relevance with respect to the investigation of the hypothesized links between psychosocial distress and the development of SO dyskinesia.

Statistical analysis

Univariate and multivariate analyses were performed to compare the SO dyskinesia subgroup, and for comparison purposes the SO stenosis subgroup, with the normal SO motility subgroup, on a range of clinical, sociodemographic and psychological factors[28]. The relation of individual continuous variables such as age, sociodemographic and psychosocial factors was assessed by logistic regression, while χ^2 analyses were performed to determine any sex or clinical differences with respect to SO subgroups. Using a Stepwise regression - a model-building procedure by which sample data (not the investigator) determines order of entry into the model - an optimal subset of clinical, sociodemographic and psychological factors that had independent, statistically significant effects in relation to SO subgroups was then identified.

Table 1 Clinical features of the post-cholecystectomy patient subgroups of SO dyskinesia, SO stenosis with or without dyskinesia and normal SO motility (mean ± SD) *n* (%)

	SO dyskinesia (*n* = 33)	SO stenosis (*n* = 18)	Normal SO motility (*n* = 21)
Age (yr)	45 ± 10	44 ± 13	48 ± 16
Gender: % female	91	89	86
SOD			
Type Ⅰ	2 (6)	2 (11)	0 (0)
Type Ⅱ	19 (58)	12 (67)	14 (67)
Type Ⅲ	12 (36)	4 (22)	7 (33)

$P > 0.05$ for all comparisons. SO: Sphincter of Oddi; SOD: SO dysfunction.

Table 2 Clinical, psychosocial and demographic variables which significantly differentiated between SO dyskinesia and normal SO motility in postcholecystectomy patients: logistic regression model of best fit

	Effect size and significance		
	B	SE	*P*-value
Female sex	5.023	1.981	0.01
Frequent suppression of anger[1]	2.399	1.239	0.05
Frequent use of mature coping strategies[1]	0.232	0.085	0.006
Frequent use of immature coping strategies[1]	0.03	0.057	0.026
Infrequent use of neurotic coping strategies[1]	0.004	0.266	0.091
Higher occupational status of father	1.475	0.588	0.01
Neuroticism (stress-proneness)	4.901	4.901	0.02

[1]See text for further details; Model variance explained = 36.3%; B: Regression coefficient.

RESULTS

SO manometry revealed evidence of SO dyskinesia (with no stenosis) (n = 33), SO stenosis [n = 18, some with concurrent SO dyskinesia features (n = 8)], or normal SO motility (i.e. no SO dyskinesia or SO stenosis (n = 21). The three groups did not differ with respect to age, gender, or any of the independent clinical variables (Table 1), sociodemographic or psychological variables assessed.

When logistic regression was used to determine the effect of combinations of independent variables in relation to manometric outcome, however, a particular subset of clinical demographic and psychological variables significantly differentiated SO dyskinesia from normal SO motility (Table 2). In this model, variables positively associated with SO dyskinesia were being female, and the psychological variables of frequently suppressing anger, frequently using stress coping strategies, and neuroticism (the propensity to an overly anxious response to stressors). The negative association with abnormal motility (or positive association with normal motility) was related to the sociodemographic background, namely a lower occupational status of the patient's father. The variables of anxiety and depression were not associated with SO dyskinesia, nor were there positive associations with objective clinical criteria.

In contrast, duplicate analyses revealed no significant differences between patients with SO stenosis and those with normal SO motor activity, on psychosocial distress and gender and also on objective clinical criteria (data

not shown). This was the case whether the SO stenosis group included patients with (n = 18) or without SO dyskinesia features (n = 10).

DISCUSSION

The novel finding in this study was the identification of a cluster of psychosocial and gender factors which together differentiate postcholecystectomy patients with manometric SO dyskinesia - but not those with SO stenosis - from those with normal SO motor activity. In comparison with the normal motor activity group, patients with SO dyskinesia, especially women, used significantly more stress-management strategies that were problem-focused (i.e. they suppressed anger more frequently) and emotion-focused (i.e. they frequently used mature and immature but not neurotic coping strategies), findings which implicitly represent on-going attempts to reduce psychosocial stress. While the prominence of the emotion anger (but not depression or anxiety) in the stress-coping profile reveals the potent nature of the psychosocial distress, for each individual the effectiveness of the particular range of coping strategies used to reduce emotional distress (and the associated physiological responses) is unclear. Thus, although patients with SO dyskinesia displayed a preference for strategies such as anticipation, humor, suppression, and sublimation (especially adaptive in the short term), other less effective (immature) strategies such as passive aggression and denial were also employed from time to time. Neurotic coping was rare in this group.

Our other major finding was that the biopsychosocial model of SO dyskinesia described above was, in essence, independent of objective clinical non-manometric criteria. This was despite the fact that the distribution of our patients with manometric evidence of SOD according to the Milwaukee classification was generally in keeping with that of other published reports: half of our patients with Type I exhibited stenosis, 61% of Type Ⅱ exhibited sphincter dyskinesia, and 75% of Type Ⅲ exhibited sphincter dyskinesia. The fact that our patients with Type Ⅲ SOD exhibited a higher overall proportion of manometric dysfunction than published reports may reflect the fact that we employed CCK provocation, which is not now routinely undertaken in Units performing SO manometry. Moreover, our use of CCK considerably strengthens the distinction between SO hypertension due to sphincter hypertonicity and that due to a true fixed stenosis.

The significant association between psychosocial distress and sphincter dyskinesia is a new finding with respect to the sphincter of Oddi. It is, however, conceptually consistent with reports of similar links between stress and alterations in gut motility and sensation in patients with FGID categorized as IBS[29,30]. Studies using measures of stress-coping behavior similar to those used in the present study, suggest that higher levels of stress correspond with an increasing degree of dysmotility (and heightened perceptual sensitivity to mechanical distension) in the jejunum of women with IBS[11], and with the severity[9,12] and the extent (number of regions) of gut stasis[12], especially gastric stasis, in mixed gender groups. Similar relations have been reported with respect to functional gut symptomatology. For example, higher levels of distress, assessed as outlined above, and also an objective measure of life stress, namely chronic stressor threat, are associated with a larger number of FGID syndromes[31], with the overall intensity of FGID symptoms, and with the direction and extent of change in symptom intensity over time[7]. Also for patients with FGID, anger provoked in real life situations is the emotion which most strongly contributes to the net severity and extent of symptoms[31] and sensorimotor dysfunction[9,11], while anger provoked in the laboratory inhibits antral motor activity in patients with these disorders in contrast to its enhancing effects on antral motor activity in healthy control subjects[32]. Consistent with all of these findings, the psychosocial distress model which described postcholecystectomy patients with SO dyskinesia in this study also included anger and female gender; these findings suggest both the potency of the stressful input on the one hand, and perhaps the more subtle and discriminating influence of sex hormones in SO motility on the other.

There is only very limited data available relating to psychosocial associations with SOD. Psychological disturbance has been implicated in one study[3] in the recurrence of biliary-type pain in some patients following cholecystectomy. In comparison to healthy controls, psychological factors assessed indirectly in terms of the number of concurrent multisystem gastrointestinal and non-gastrointestinal symptoms, was higher in patients with a diagnosis of SOD. However, patients were not differentiated manometrically in this study. In another report, psychological disturbance (anxiety, somatization, depression and obsessive-compulsive behavior) was found to be higher in patients with SOD Type Ⅲ[4] than in other types. Interestingly, our findings did not confirm significantly higher levels of anxiety and depression in patients evaluated according to their manometric findings and not their Milwaukee criteria alone. In a longitudinal study, Jørgensen *et al*[33] reported that psychological vulnerability (assessment prior to cholecystectomy in terms of the severity of multisystem somatic and neurotic symptoms) predicted failure to achieve a full recovery post-operatively, after controlling for age, sex, pre-operative pain characteristics, history of disease, type of surgery, histology and complications. The contribution of the present study is the notion that a stress-related sensorimotor dysfunction may help to explain the presence and the persistence of the syndrome for some patients with a diagnosis of SO dyskinesia, while high rates of recovery following endoscopic sphincterotomy in patients with manometric features of SO stenosis[16,34] suggest that fixed structural or anatomical defects may explain the syndrome in others. Indeed, an important feature of the stress-related sensorimotor dysfunction in this study is that it was determined in the absence of confounding influences arising from the presence of SO stenosis.

We are aware of the potential limitations of our

findings, especially the potential selection bias because patients with certain personality or mood-state characteristics may be more likely to seek medical attention than other patients, and also that recurrent pain may have influenced some of their responses to the various questionnaires. However, we sought to limit any such bias by also including patients with the same symptoms who had presented for medical care but were found to have sphincter stenosis. Moreover, as all patients reported intermittent episodes of pain as severe or very severe it was not feasible to relate the psychosocial measures to pain scores. Further studies will be required to confirm and extend these findings, as they are of potential clinical importance given that the psychological distress levels including clinical levels of anxiety and depression may be eminently treatable (e.g. with medication and/or psychotherapy). Although individual psychotherapies (e.g. biofeedback, cognitive-behavioral, psychodynamic, hypnotherapy), achieve reductions in emotional distress and symptom severity in patients with IBS, an integrated psychophysiological approach to the management of these disorders that is sensitive to the unique nature of each stressor situation would seem most likely to be helpful long term.

In summary, the close association found, for the first time, in this study between psychosocial distress and SO dyskinesia, but not between psychosocial distress and SO stenosis or normal SO motor activity, suggests that, for some patients with a diagnosis of SO dyskinesia, a stress-related motor dysfunction may help to explain the recurrence of their biliary-like symptoms following cholecystectomy. This is consistent with pathophysiological models of the FGIDs in general[35-37].

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COMMENTS

Background

Sphincter of Oddi (SO) dysfunction (SOD) is one of a number of functional disorders of the biliary tract. It is known to include both stenotic and non-stenotic (functional) groups. Thus, SO dyskinesia can be differentiated from SO stenosis, a structural defect of the sphincter orifice. Unlike other functional gastrointestinal disorders, the psychosocial and gender concomitants of SOD have received little attention. In particular, psychosocial issues specifically relating to SO dyskinesia have not been addressed.

Research frontiers

Because of the strong relationships between psychosocial disturbance and alterations in gastrointestinal transit, motor activity and sensitivity previously documented in irritable bowel syndrome, we hypothesized that a similar association may exist with respect to SO dyskinesia.

Innovations and breakthroughs

This is the first study to identify a cluster of psychosocial and gender factors which together differentiate postcholecystectomy patients with manometric SO dyskinesia - but not those with SO stenosis - from those with normal SO motor activity. The findings suggest that a stress-related motor dysfunction may help to explain the recurrence of biliary-like symptoms following cholecystectomy. This is consistent with pathophysiological models of the functional gastrointestinal disorders in general.

Applications

These findings are of potential clinical importance given that psychological distress levels including clinical levels of anxiety and depression may be eminently treatable (e.g. with medication and/or psychotherapy).

Terminology

Sphincter of Oddi dysfunction: a disorder characterized by recurring episodes of severe biliary-like abdominal pain following cholecystectomy. Sphincter dyskinesia: an abnormally high basal pressure resolving after cholecystokinin (CCK), or an abnormally high phasic sphincter contractile frequency, and/or an elevated proportion of retrograde contractions, and/or an abnormal response to CCK in the absence of sphincter stenosis. Sphincter stenosis: an abnormally high basal pressure persisting after CCK irrespective of the presence of some features of dyskinesia.

Peer review

It is a solid research, well-written paper with reasonable conclusion. Although there are limitations to the study, these are nicely outlined and discussed.

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BRIEF ARTICLE

Balloon overtube-guided colorectal endoscopic submucosal dissection

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Abstract

AIM: To evaluate the usefulness of a balloon overtube to assist colorectal endoscopic submucosal dissection (ESD) using a gastroscope.

METHODS: The results of 45 consecutive patients who underwent colorectal ESD were analyzed in a single tertiary endoscopy center. In preoperative evaluation of access to the lesion, difficulties were experienced in the positioning and stabilization of a gastroscope in 15 patients who were thus assigned to the balloon-guided ESD group. A balloon overtube was placed with a gastroscope to provide an endoscopic channel to the lesion in cases with preoperatively identified difficulties related to accessibility. Colorectal ESD was performed following standard procedures. A submucosal fluid bleb was created with hyaluronic acid solution. A circumferential mucosal incision was made to marginate the lesion. The isolated lesion was finally excised from the deeper layers with repetitive electrosurgical dissections with needle knives. The success of colorectal ESD, procedural feasibility, and procedure-related complications were the main outcomes and measurements.

RESULTS: The overall *en bloc* excision rate of colorectal ESD during this study at our institution was 95.6%. *En bloc* excision of the lesion was successfully achieved in 13 of the 15 patients (86.7%) in the balloon overtube-guided colorectal ESD group, which was comparable to the results of the standard ESD group with better accessibility to the lesion (30/30, 100%, not statistically significant).

CONCLUSION: Use of a balloon overtube can improve access to the lesion and facilitate scope manipulation for colorectal ESD.

Key words: Balloon overtube; Colorectal neoplasm; Early colorectal cancer; *En bloc* tumor excision; Endoscopic submucosal dissection; Laterally spreading tumor

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INTRODUCTION

Endoscopic submucosal dissection (ESD) has evolved to become one of the therapeutic options for the treatment of early stage gastric cancers in Asian countries[1,2]. In Japan, ESD has been increasingly applied to various levels of the gastrointestinal tract and results of initial experiences in a few high volume endoscopy centers have demonstrated the technical feasibility of this unique and aggressive approach, even in the colon. However, the

number of institutions allowed to perform this procedure is still restricted because colorectal ESD is technically more challenging, and may carry a higher risk of perforation and the most common sequela, bacterial peritonitis[3-6]. Inherited anatomic variability in the colon such as a long tubal structure, folds, or looping in mobile segments of the colon attached to the mesentery may hinder any endoscopic intervention in the colon. A further challenge arises from the need for a longer colonoscope; this can increase procedural workloads due to the need for careful, intuitive manipulation of the tip of the scope during needle knife dissections of the paper thin submucosal tissue plane[7,8]. At our institution, the gastroscope is therefore often preferentially used even for colorectal ESD, despite the shorter scope length, for deep scope intubation.

Use of a balloon overtube in enteroscopy provides optimal traction on the intestinal wall, thereby facilitating scope intubation. By inflation and withdrawal of a balloon attached to the tip of the overtube, the intestinal wall can be pleated on the overtube. This balloon overtube-guided technique has enabled a standardized total enteroscopy and has also provided a shorter direct access to the innermost locations in the gastrointestinal tract[9-11]. In addition, this approach has facilitated various interventions in the small intestine including biopsy, hemostasis and most recently polypectomy and endoscopic mucosal resection (EMR). Based on these results in the small intestine, we postulated that the a balloon overtube could form an ideal platform for colorectal ESD[12,13].

We used a balloon overtube as an endoscopic channel and platform for colorectal ESD in cases in which access to the lesion with a gastroscope was difficult. Here we review the results of colorectal ESD in our institution to evaluate whether the balloon overtube-guided technique could improve access and scope manipulation during colorectal ESD.

MATERIALS AND METHODS

Patients

From October 2008 to March 2009 we performed colorectal ESD in 45 patients. The mean age of the patients was 70.7 years (range, 58 to 83 years). Indications for colorectal ESD were: 1. laterally spreading tumors (LST) over 20 mm in size, 2. lesions evaluated as being difficult to remove *en bloc* regardless of lesion size, e.g. local residual recurrent tumors after endoscopic removal and flat or depressed mucosal lesions. Histopathology of the lesions was preoperatively confirmed as adenoma or cancer with magnifying endoscopy or biopsy.

In all cases, access to the lesion was preoperatively evaluated using a therapeutic gastroscope (GIF Q260-J, Olympus, Tokyo, Japan). When circumferential access to the lesion with the tip of the endoscope was difficult, the access was considered difficult with a standard gastroscopic approach and the lesion was indicated for balloon overtube-guided ESD.

Instrumentation and ESD procedure

ESD procedure with a gastroscope: A transparent cap (D201-11802; 2 mm Olympus, Tokyo, Japan) fitted therapeutic gastroscope (GIF-Q260-J, Olympus, Tokyo, Japan) was used for the conventional ESD group. The gastroscope is equipped with a water jet system, which supplies a continuous jet of high-pressure water to wash out blood and mucous during the endoscopic dissection. Two types of needle knives specially designed for ESD with minor modifications to the diathermy wire tip (Flex knife, KD-630L, Olympus, Tokyo Japan or Dual knife, KD-650Q, Olympus, Tokyo Japan) were used for the standard ESD procedure with a VIO300D high-frequency generator (ERBE, Elektromedizin, Tubingen, Germany). Ten percent sodium hyaluronate solution mixed with a small amount of indigo carmine and epinephrine hydrochloride was used as the injection solution to create a submucosal safety bleb[8]. A circumferential mucosal incision was made using one of the needle knives on endocut I (effect 2, interval 2, duration 2) mode. After the horizontal margination of the lesion from the surrounding normal mucosa, the electrosurgical dissection of the submucosal tissue plane was continuously performed with repetitive electrosurgical needle knife dissections. When bleeding and vascular structures were encountered, hemostasis were performed with point cauteries with the diathermy tip of the needle knife with Swift Coag mode 45W (effect 3) or a coagulation forceps (Coagrasper FD411-QR, Olympus, Tokyo, Japan) with Swift Coag mode 45W (effect 3). Patient posture rotations were carried out as needed to improve access to the lesion and deflect the overlying mucosa away from the dissection plane by gravity.

Single balloon overtube-guided ESD: Balloon overtube-guided ESD was performed with a standard diagnostic gastroscope (GIF-Q260, Olympus, Tokyo, Japan) with a 9.2-mm outer diameter. A balloon overtube with a 13.2-mm outer diameter, 11-mm inner diameter over-tube (ST-SB1, total length 1400 mm Olympus, Tokyo, Japan) designed for enteroscopy was shortened to 70 cm in length from the distal end leaving the balloon inflation tube intact. A gastroscope preloaded into the length-adjusted overtube was then inserted into the colon and the lesion was accessed using techniques similar to balloon enteroscopy (Figure 1). A transparent hood (D201-10704; 4 mm, Olympus, Tokyo, Japan) was attached to the tip of the endoscope. The procedural processes for ESD and the tool set used in the balloon overtube-guided procedure were the same for the ESD procedure performed without the overtube.

The study protocol was approved by the Institutional Ethical Committee of Kanto Medical Center NTT. Written informed consent was obtained from each patient before the ESD procedure.

Statistical analysis

The significance of differences between patient characteristics and clinicopathological features was determined using χ^2 test, the Mann-Whitney *U* test, or Student *t* test as appropriate. *P* values < 0.05 were considered statistically significant.

RESULTS

In the preoperative evaluation of accessibility with a

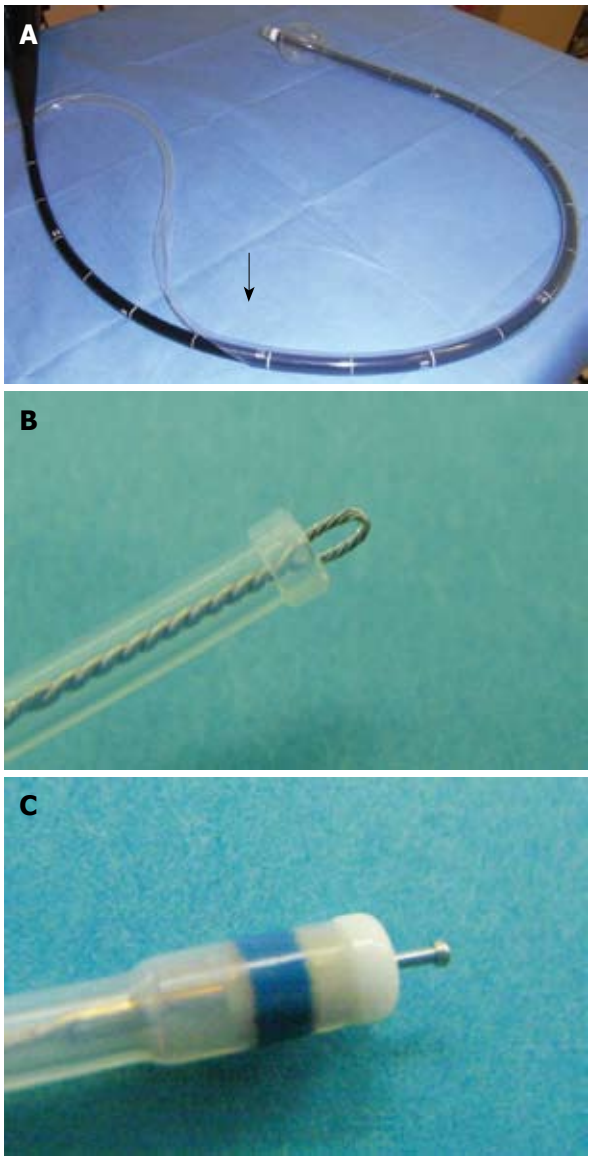

Figure 1 Balloon over-tube and endoscopic submucosal dissection (ESD) devices. A: Single balloon over-tube shortened to 70 cm in length (arrow) from the distal end leaving the balloon inflation tube intact. A standard diagnostic gastroscope (GIF Q260, Olympus, Tokyo, Japan) is preloaded into the shortened over-tube; B: Flex knife (KD-630L, Olympus, Tokyo, Japan) used for ESD procedure; C: Dual knife (KD-650Q, Olympus, Tokyo, Japan) used for ESD procedure.

gastroscope, fifteen patients were identified as difficult, and were enrolled in the balloon overtube-guided ESD group. Thirty patients, who met the circumferential access criteria, were treated with the standard ESD method using a gastroscope without the overtube.

The overall *en bloc* excision rate of colorectal ESD was 95.6%. In the patients treated with the standard ESD method with a gastroscope, *en bloc* excision of the lesion was performed successfully in all 30 patients (100%). The lesions were located in the cecum in 2 patients, in the ascending colon in 10 patients, in the transverse colon in 2 patients, in the descending colon in 2 patients, in the sigmoid colon in 6 patients, and in the rectum in 8 patients. The median procedure time was 60 min (12-200 min). The median size of the lesion was 35 mm (SD: 13-98), and the median resected specimen size was 43 mm (17-112 mm).

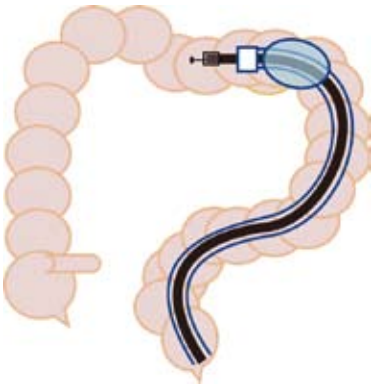

Figure 2 Scheme shows anchoring of the transverse colon with the single balloon over-tube.

There were no perforations, however, post-ESD bleeding occurred in one case, which required repeated endoscopic hemostasis.

En bloc excision of the lesion was successfully achieved in 13 of the 15 patients (86.7%) in the balloon overtube-guided colorectal ESD group. In 2 cases of failure in the single balloon overtube-guided group, the endoscope did not reach the lesion due to elongation of the sigmoid colon. These two patients were eventually treated by piecemeal snare EMR using a colonoscope (CF-Q240L, Olympus, Japan). One lesion was located in the ascending colon and the other was in the transverse colon. Lesions in the balloon overtube-guided group were located in the transverse colon in 10 patients, the descending colon in 3 patients, the ascending colon in 1 patient and the sigmoid colon in 1 patient. The median procedure time was 80 min (30-160 min). The median size of the tumors was 27 mm (10-46 mm), and the median resected specimen size was 38 mm (18-57 mm). There were no severe complications such as perforation or bleeding.

There were no significant differences in the age (P = 0.352), sex (P = 0.292), lesion size (P = 0.472), or resected specimen size (P = 0.597) between the two groups. Lesions were more frequently located in the transverse colon in the balloon overtube-guided ESD group (10 *vs* 2, P < 0.001). Operation time was longer in the balloon overtube-guided group (P = 0.050).

On pathology, twenty lesions were diagnosed as tubular adenomas [44.4%, 15 in the ESD without overtube group, 5 in the ESD with overtube group; P = NS (not statistically significant)], and 25 were diagnosed as adenocarcinomas (55.6%, 15 in the ESD without overtube group, 10 in the ESD with overtube group; P = NS). Four patients had submucosal invasion (3 in the ESD without overtube group, 1 in the ESD with overtube group, P = NS) and one patient also had venous involvement. None of the patients had lymphatic involvement.

DISCUSSION

The development of endoscopic snare polypectomy represents one of the most important achievements in the history of flexible endoscopy. This approach benefits patients enormously by reducing the physical burden associated with colonic polyp removal compared to traditional

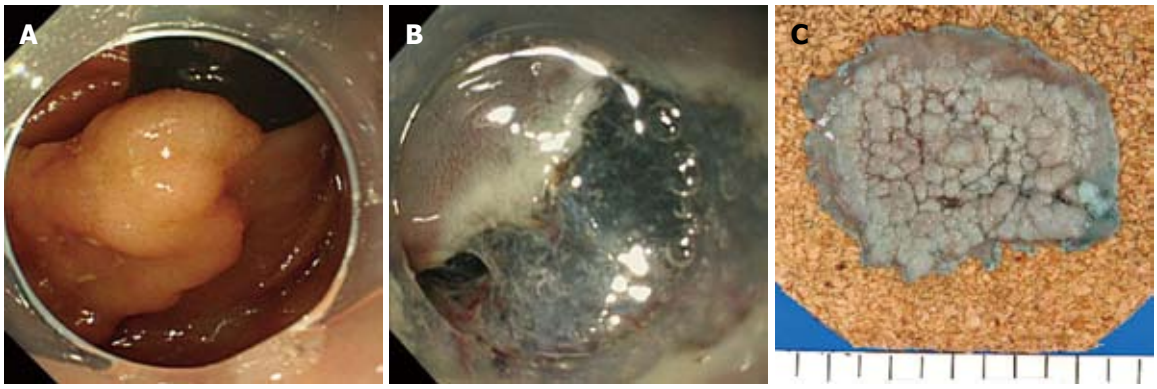

Figure 3 A colorectal laterally spreading tumor (LST) in the transverse colon that was difficult to approach with a standard endoscope. A: Retroflex view of the sessile granular-type lesion in the hepatic flexure of the transverse colon. The sharp angle of the colon made it difficult for a stabilized scope maneuver; B: Submucosal dissection plane with good elevation using hyaluronic acid injection; C: Gross specimen, showing the sessile, raised lesion resected *en bloc*.

surgical colectomy. Due to the limited size of the snare, removal of large polyps by snare polypectomy requires a piecemeal resection, which may lead to incomplete tumor removal. Local residual recurrence may occur after piecemeal colonic polyp excision in between 3% to 27% of cases[4]. In general, the majority of recurrent colorectal lesions are not clinically significant and can be managed with repeated endoscopic interventions. However, since some endoscopically removed lesions require additional surgical resection due to invasion into the deeper layers and vasculature, *en bloc* tumor excision is of interest not only for minimization of local tumor recurrence, but also for ensuring the precise histopathological evaluation of the sampled specimen[14]. In this study, three patients had shallow submucosal invasions and one patient had a solid submucosal invasion with vascular involvement that required additional surgical treatment. If it were possible to overcome the major technical difficulties associated with colorectal ESD, we believe this treatment could be an appropriate therapeutic option for colonic lesions that are difficult to remove *en bloc*, and this approach may be better accepted in western countries with a higher incidence of colonic polyps and cancers[15].

In this study, preoperative evaluation showed that the majority of lesions in the transverse colon were in a difficult location and were assigned to the balloon overtube-guided group (10 *vs* 2). The transverse colon is the mobile segment most distant from the anus, and hence could be embarrassing due to the situation of the distal side of the colon. Mid-transverse colon may present as a sharp bend in patients with a redundant and drooping transverse colon. Reformation of sigmoid looping may generate friction for scope passage interfering with scope manipulation. Use of the balloon overtube provided an anchor on the colon wall giving optimal traction to maintain a shorter, straighter and more stabilized access to the lesion during ESD (Figure 2). Lesions that were preoperatively identified as being in a difficult location in the transverse colon could be accessed repeatedly using a diagnostic gastroscope by guidance of the balloon overtube, with the exception of one case with a surprisingly elongated sigmoid and severe adhesion. In addition, stabilized access *via* the overtube allowed direct and intuitive scope manipulation and *en bloc* tumor excision could be completed with the standard ESD technique in all attempts (Figure 3). Furthermore, a thin diagnostic gastroscope used in the balloon overtube-guided group could be more smoothly inserted into the submucosal tissue plane following a minimal mucosal isolation of the surgical margin. Once the cap fitted tip of the endoscope was inserted into the submucosal space thus created, electrosurgical dissection of the submucosa, the most error prone procedural process in ESD, could be safely performed with a clear visualization of the working field by deflecting the overlying isolated mucosa from the dissection plane. Although the single balloon-guided technique seems to be a promising approach to reduce the technical challenges of colorectal ESD, some important questions still remain unanswered. Two lesions, one located in the ascending colon and the other in the transverse colon were still difficult to access even with use of the balloon overtube. Both lesions were eventually treated with a colonoscope in a piecemeal fashion. In order to conclusively demonstrate that use of the balloon overtube can reduce technical difficulties in colorectal ESD, this novel approach should be directly compared with the standard ESD techniques using a colonoscope. Additionally, development of an overtube specially designed for the colorectal ESD of larger diameter to enable passage of a colonoscope could potentially reduce procedural difficulties and operation time further.

In conclusion, our preliminary experience suggests that the combined use of the balloon overtube and a thin diagnostic gastroscope is an effective and useful platform for colorectal ESD, especially in cases with difficult to access target lesions. Further studies are needed in which this novel technique is compared to the existing ESD techniques.

COMMENTS

Background

Endoscopic submucosal dissection (ESD) is technically more challenging for colorectal lesions than other locations in the gastrointestinal tract due to the anatomic characteristics of the colon and difficulties establishing stabilized manipulation of a long colonoscope.

Research frontiers

Use of a balloon overtube in enteroscopy provides optimal traction on the intestinal wall thereby facilitating scope intubation. This balloon overtube-guided

approach has facilitated various interventions in the small intestine including biopsy, hemostasis and most recently polypectomy and endoscopic mucosal resection (EMR). Based on these results in the small intestine, the authors postulated that the balloon overtube could form an ideal platform for colorectal ESD. In this study, the authors reviewed the results of the balloon overtube-guided colorectal ESD technique.

Innovations and breakthroughs

Colorectal ESD for the treatment of large superficial colorectal tumors is technically feasible, can improve *en bloc* resection rates, and is also less invasive compared to surgical treatment. However, colorectal ESD is technically more difficult and carries a higher risk of perforation than ESD at other levels of the gastrointestinal tract. Use of a balloon overtube improved access to the lesion and scope manipulation during colorectal ESD by shortening and straightening the access.

Applications

If it were possible to overcome the major technical difficulties associated with colorectal ESD, the authors believe colorectal ESD could be an appropriate therapeutic option for colonic lesions that are difficult to remove *en bloc*, and this approach may be better accepted in Western countries with a higher incidence of colonic polyps and cancers.

Terminology

ESD has evolved to become one of the therapeutic options for treatment of early stage gastric cancers in Asian countries. In Japan, ESD has been increasingly applied to various levels of the gastrointestinal tract and results of initial experiences in a few high volume endoscopy centers have demonstrated the technical feasibility of this unique and aggressive approach, even in the colon.

Peer review

ESD for colorectal tumors is not generally recommended because of the technical difficulties and complications, including perforation. The authors performed ESD in 45 cases using gastroscope with a low perforation rate.

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BRIEF ARTICLE

Stereotactic body radiotherapy for isolated paraaortic lymph node recurrence from colorectal cancer

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Abstract

AIM: To evaluate the efficacy and complications of stereotactic body radiotherapy in localized paraaortic lymph node recurrence from colorectal cancer.

METHODS: From 2003 to 2009, 7 patients with paraaortic lymph node recurrence (1-3 lesions) from colorectal cancer were treated with stereotactic body radiotherapy. Total gross tumor volumes ranged from 4 to 40 mL. The doses were escalated from 36 Gy/patient to 51 Gy/patient and were delivered in 3 fractions.

RESULTS: One and 3 year overall survival rates were 100% and 71.4%, respectively, and median survival was 37 mo. Grade Ⅳ intestinal obstruction was reported in 1 of 7 patients. This patient received 48 Gy in 3 fractions with a maximum point dose to the intestine of 53 Gy and V_{45Gy} = 3.6 mL. However, 6 patients received an intestinal maximum point dose of < 51 Gy and V_{45Gy} of < 1 mL, and did not develop any severe complications.

CONCLUSION: This pilot study suggests selected paraaortic lymph node recurrence (1-3 closed lesions) that failed to respond to chemotherapy can be potentially salvaged by stereotactic body radiotherapy.

Key words: Stereotactic body radiotherapy; Paraaortic lymph node recurrence; Colorectal cancer

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INTRODUCTION

Metastatic hepatic and pulmonary lesions from colorectal cancer (CRC) are commonly resected, and if tumorectomy for liver and lung recurrence from CRC is performed successfully, about 20% of patents are expected to achieve a long-term cure. On the other hand, isolated paraaortic lymph node (PALN) recurrences are rarely encountered from CRC. Moreover, surgical treatment in these cases is not widely accepted even when lesions are localized, due to their relative rarity, high postoperative morbidity, and poor prognosis. However, if such patients are left untreated, the survival rates are 31% at 1 year, 7.9% at 2 years, and 0.9% at 4 years[1,2]. Therefore, this patient subset is considered for various chemotherapy regimens. However, despite optimal treatment and initial response, overall survival approaches only 20 mo[3]. Furthermore, radiotherapy usually provides only temporary symptom relief in most cases.

On the other hand, stereotactic body radiotherapy (SBRT) is an emerging technology in the radiation oncology field. This technique utilizes stereotactic principles for localization and delivers multiple beams to well defined targets in a few fractions, and therefore, SBRT can deliver higher doses to tumors due to reduced mechanical error margins, and thus, causes less normal tissue damage. To our knowledge, no previous report has described the role of radiation, including conventional RT, intensity-modulated RT, and SBRT, for PALN recurrence from CRC, although several reports have been issued on the treatment of cervical cancer with isolated PALN recurrence, which responds well to both chemo- and radiation therapy[4-6]. Therefore, the aim of this study was to evaluate the feasibility and efficacy and

the complications associated with SBRT in patients with isolated PALN recurrence from CRC.

MATERIALS AND METHODS

Patients

From May 2003 to March 2009, we reviewed retrospectively 7 patients with isolated PALN recurrence from rectal cancer after curative resection who were treated with SBRT using a CyberKnife (Accuray Inc., Sunnyvale, CA). Isolated PALN recurrence was initially detected by computed tomography (CT) or by an elevated serum carcinoembryonic antigen (CEA) level during a routine check-up, and was confirmed by elevated standardized uptake values of paraaortic lesions by positron emission tomography (PET) or PET/CT. According to our hospital's protocol, patient eligibility criteria for curative SBRT for paraaortic lymph node recurrence from CRC were as follows: (1) resection of CRC after diagnosis; (2) PALN recurrence after primary cancer resection; (3) progression after chemotherapy for recurrence; (4) a single conglomerate recurrent node or 2-3 recurrent nodes in close proximity (< 1 cm); (5) a greatest tumor diameter of < 8 cm; and (6) an Eastern Cooperative Oncology Group (ECOG) performance score of 1 or 2. Exclusion criteria were as follows: (1) a tumor attached to the stomach or intestine by CT; (2) extra-lymphatic active lesion by CT or PET/CT; (3) more than three separate lymph nodes affected (4) time from primary operation to recurrence of < 6 mo; or (5) previous radiation therapy applied to the treatment site. Patients' characteristics are summarized in Table 1. Ages ranged from 47 to 73 years (median 59 years) and the male:female ratio was 5:2. All patients underwent primary tumor resection. Liver resection was also performed in 2 patients with liver metastasis. Adjuvant chemotherapy was performed in all patients. Initial pathologic stages were stage Ⅱ in 2, stage Ⅲ in 3, and stage Ⅳ in 2. Pathologic diagnoses were adenocarcinoma in all 7. Times between operation and first relapse ranged from 7 to 44 mo (median 21 mo). Three patients had a conglomerated LN and the other four had 2 or 3 separate enlarged lymph nodes on a paraaortic lesion. Greatest tumor diameters and heights were calculated using measurements taken from CT scans during planning, and are itemized in Table 1. Total gross tumor volumes (GTV) ranged from 4 to 40 mL (median 22 mL). After recurrence had been detected, all patients received chemotherapy based on 5-FU before SBRT. Chemotherapy regimens were variable because of the different initial adjuvant chemotherapy regimens used. All 7 patients demonstrated disease progression despite chemotherapy, and thus, were defined as non-responders. After performing SBRT, patients were followed up every 2 or 3 mo. When recurrence was detected after SBRT, salvage or palliative treatment was performed according to the status of recurrence. All patients provided written informed consent for SBRT. This study was approved by Institutional Review Board-approved protocol for SBRT at the Korea Institute of Radiological and Medical Sciences (KIRAMS).

SBRT technique

In accord with our hospital protocol, gold fiducials (4 mm long and 0.8 mm in diameter) were used as markers for tumor localization. Six fiducials were placed percutaneously on transverse processes of the spine located nearest tumors using an 18 gauge spinal needle under fluoroscopic guidance. Patients were immobilized using an Alpha Cradle (Smithers Medical products, North Canton, OH) 5-7 d after fiducial placement. Panning CT scans were performed with patients in the planned treatment position, and these images were then processed for the CyberKnife planning system. GTV were determined based on CT tumor visualizations. To better delineate tumor volumes, PET/CT images were used as a reference. Clinical target volumes (CTV) were considered to be identical to GTV. Planning target volumes (PTV) were CTV plus a 2-3 mm margin. Radiation doses were prescribed to the 76%-83% isodose line of the maximum dose covering the PTV (Table 1). Critical structures, such as the esophagus and spinal cord, were contoured. Treatment plans involved the use of hundreds of pencil beams shaped using a single 20, 25, or 30 mm diameter circular collimator. The method used to increase SBRT dose is described in detail in our previous report[7]. Briefly, the protocol adopted was follows; if at least 5 patients who received SBRT due to PALN from variable primary tumors (cervical cancer, CRC, or gastric cancer) did not develop Grade Ⅳ or Ⅴ complications for 3-4 mo after radiation was administered, escalations of 1 Gy/fraction (to a total increase of 3 Gy/fraction) were administered for the next cohort. According to this protocol, total SBRT doses ranged from 36 to 51 Gy (median 48 Gy), and were delivered in 3 fractions. Maximum point dose limits were applied for critical organs, i.e. 18 Gy for the spinal cord and 24 Gy for the esophagus. No constraints were applied to limit intestinal or colon exposure. Table 1 summarizes SBRT dosage details.

Survival and complications

Overall survival was calculated from the commencement of SBRT using the Kaplan-Meier method. Disease progression free survival was also measured from the commencement of SBRT to the date of local progression, distant metastasis, or both. All statistical calculations were performed using SPSS, version 13.0 (SPSS, Inc., Chicago, IL).

Tumor response during follow up was assessed using Response Evaluation and Criteria for Solid Tumors (RECIST)[8]. Local progression was defined as an increase in tumor size *vs* the previous CT image or the development of a new lesion in the radiation field. Regional failure was defined as the development of a new lesion in the PALN region.

Acute and late toxicities were defined as symptoms that developed within or after 3 mo of treatment completion, respectively. Toxicities were graded using the National Cancer Institute Common Toxicity Criteria version 3.0[9]. To identify factors related to complications, total CTV, intestinal maximum point dose (D_{max}) and intestinal volume administered ≥ 45 Gy (V_{45}) were calculated retrospectively (Table 1). Total CTV was defined as sum of the CTV of affected lymph node as determined by the CyberKnife planning system. V_{pre} was defined as the total volume administered the prescribed dose or more.

Table 1 Demographic data of 7 patients

Patient No.	Age (yr)/ Sex	Latent time (mo)	GTV (mL)	Dose (Gy)	Prescribed isodose (%)	Dmax of intestine (Gy)	V45 (mL)	Failure pattern	F/U (mo)	Final status
1	63/M	9	20	36	83	33	0	Lt SCLN (23) Lung (32)	70	AWD
2	64/F	44	4	41	82	38	0	-	37	Died due to lymphoma
3	52/F	29	24	45	78	48	0.2	Spine (26)	41	DOD
4	59/M	21	22	48	80	51	0.7	Lung (7)	22	DOD
5	56/M	10	9	48	80	40	0	PALN(9)	21	AWD
6	47/M	18	40	48	76	53	3.6	Rectum (7) Lt SCLN (7) Peritoneal seeding (25) Local recur (13)	25	DOD
7	73/M	7	29	51	78	50	0.9	-	26	CDF

GTV: Gross tumor volume; AWD: Alive with disease; CDF: Continuously disease-free; DOD: Died of disease; Latent time: Disease free interval from operation to first relapse; F/U: Follow-up from commencement of SBRT to last follow-up or death; Lt SCLN: Left supraclavicular lymph node; Dmax: Maximum point dose of intestine; V45: Intestinal volume receiving 45 Gy or more; Vpre: Total volume receiving the prescribed dose or more; Parenthesis (mo) means the period from SBRT to detection of complication.

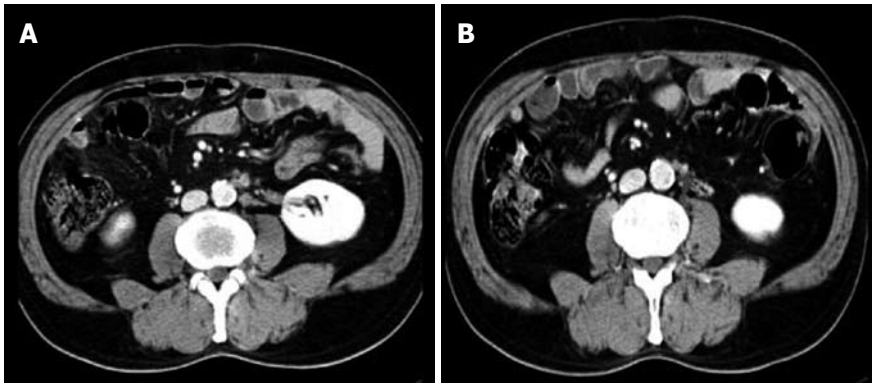

Figure 1 Computed tomography (CT) images obtained in a patient with paraaortic lymph node recurrence before (A) and 2 mo after (B) stereotactic body radiotherapy (SBRT).

RESULTS

Follow up durations ranged from 15 to 70 mo (median; 26 mo). Final outcomes were as follows: 1 patient remained alive without evidence of disease; 2 patients remained alive with disease; 3 patients died of disease; and 1 patient died of an unrelated disease without recurrence (Table 1). During follow-up, 3 patients achieved a complete response and 4 patients achieved a partial response (Figure 1). One- and 3-year overall survival rates were 100% and 71.4%, respectively, and median survival was 37 mo. Local recurrence was observed at 13 mo after SBRT in patient No 6 (Table 1). Regional recurrence in the PALN region was observed in patient No. 5. However, 4 patients experienced distant failure with/without primary rectal recurrence (Table 1).

Grade I acute toxicity (nausea and vomiting) occurred in 2 patients at first date of SBRT, and these resolved spontaneously without medication. Grade 4 toxicity occurred in 1 patient (patient No. 6; Table 1). This patient had one conglomerated lymph node and another lymph node and had received 48 Gy in 3 fractions. Abdominal pain developed at 4 mo post-SBRT, and at 5 mo an obstruction was observed during a colon study. He underwent bypass surgery and recovered completely. A photograph of the excised obstructive lesion is shown in Figure 2. This patient had a larger CTV than CTV of the other patients. In this patient, the maximum point intestine dose was 53 Gy and V_{45Gy} was 3.6 mL. No late complications occurred in any patients.

DISCUSSION

The management of patients with locally recurrent rectal cancer is challenging. For technically resectable recurrent rumors, complete resection can be achieved by limited surgery and outcomes are relatively favorable. Vassilopoulos *et al*[10] and Pihl *et al*[11] reported 5-year survival rates of 49% and 42%, respectively, after resection for anastomotic recurrence. However, the treatment of isolated PALN recurrence from CRC is not well established. Recently, Min *et al*[12] categorized PALN recurrence as a retroperitoneal recurrence, which is a type of locoregional recurrence. Furthermore, several studies[13-16] have investigated the therapeutic efficacies of surgery for retroperitoneal, intraabdominal, and PALN recurrences, and several reported outstanding survival rates, which appear to have resulted from the selection of patients with a resectable mass at time of recurrence. In these studies, reported 5-year survival rates approached a maximum of 56% after complete resection, whereas they ranged from 0% to 7% after incomplete resection (Table 2). Because radical surgery is rarely feasible for PALN recurrence, traditionally, those affected have been considered for chemotherapy. However, despite optimal treatment and the achievement of initial response, patients invariably become non-responsive and achieve overall survivals approaching 20 mo. On the other hand, conventional radiotherapy has played a limited palliative role in the treatment of recurrent CRC involving locoregional recurrence, especially PALN

Table 2 Comparison of survival for recurrence from CRC treated by surgery or other treatment method

Study	Failure site	Treatment	*n*	Median survival (mo)	Survival rate (yr)
Gwin *et al*[15], 1993	Non-hepatic intra-abdominal	R0[1]	15	25.5	60 (2), 0 (3)
		R1[2]	6	8	34 (2), 34 (3)
		R2[3]	7	3.5	15 (2)
Shibata *et al*[13], 2002	Retroperitoneum	R0	15	81	56 (5)
		R1	5	29	0 (5)
		R2	4	3	0 (5)
Bowne *et al*[14], 2005	Retroperitoneum	R0	8	44	NA
		R1	8		
Min *et al*[12], 2008	PALN	R0	6	34	80 (3), 0 (5)
		Chemotherapy[4]	33	12	18 (3), 7 (5)
Present study	PALN	SBRT	7	41	71.4 (3)

[1]Curative resection; [2]Marginal resection; [3]Incomplete, bypass or colostomy; [4]Resection is not planned. CRC: Colorectal cancer; PALN: Paraaortic lymph node; SBRT: Stereotactic body radiotherapy; NA: Not assessed.

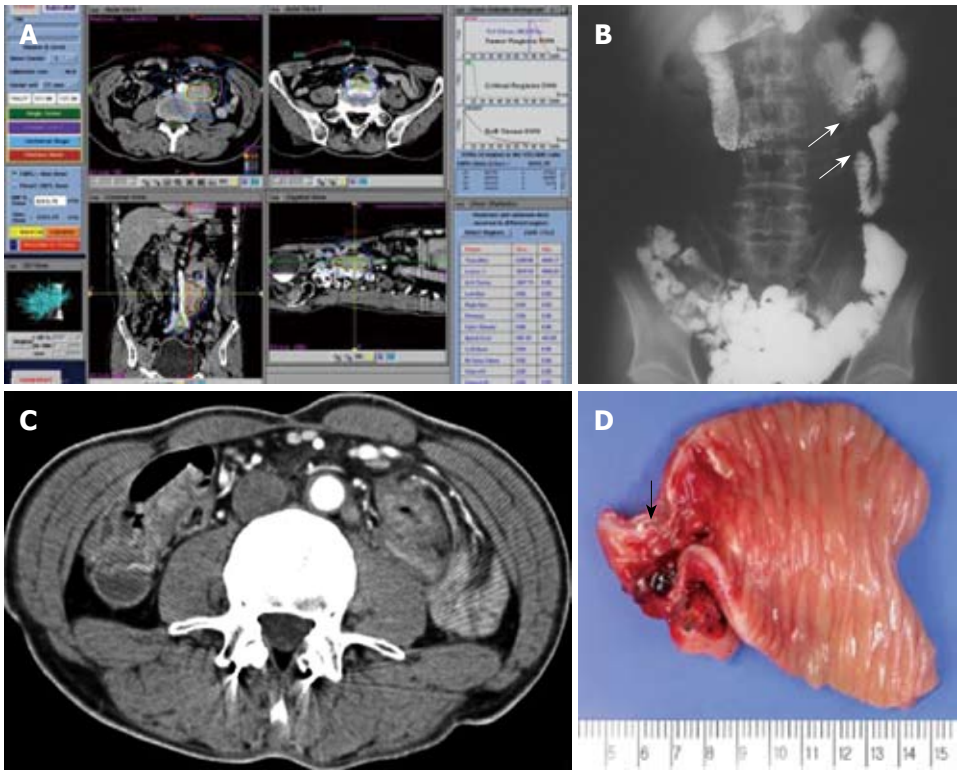

Figure 2 A case with complications. A: Isodose distribution and target coverage with SBRT to a dose of 48 Gy in 3 fractions prescribed to planning target volume; B: Small bowel series 5 mo after SBRT. Near total obstruction (arrows) in left side of abdomen; C: Computed tomography at 4 mo after SBRT. Wall thickening of proximal jejunal loop was observed; D: 2.5 cm × 1.5 cm ulceration & stricture lesion (arrow) in operation specimen. Eventually, he underwent bypass surgery and recovered completely.

recurrence. The proximities of involved lymph nodes and critical organs, such as the spinal cord, intestine, and colon, often prevent the delivery of sufficient radiation to achieve local control when conventional radiation modalities are used. However, SBRT can deliver higher doses to tumor and cause less tissue damage. Furthermore, it can have three times the biological effect of fractionated radiation therapy. However, the SBRT field is usually directed at the tumor burden, and thus, the prophylactic effect of SBRT in peritumoral regions is limited which in turn means the incidence of regional failure after SBRT seems higher than that after conventional radiation therapy. Fortunately, in the present study, only 1 regional failure pattern was observed.

At our institute, SBRT has been utilized for isolated PALN from gastric or cervical cancer, and 3-year survival rates of 43%[17] and 63%[18] have been achieved, respectively. In addition, an excellent 3-year overall survival rate of 71.4% was achieved in the present study. Accordingly, the findings of studies on PALN recurrence from variable primary cancers treated by SBRT appear to support our hypothesis that a subset of isolated PALN recurrence cases exist that are likely to pursue an indolent disease course and be salvaged by adjustable treatments.

Theoretically, rectal cancer is classified as a slow-growing tumor that is likely to respond better to hypofractionation. However, no recommended optimal doses, fraction numbers, or planning constraints for SBRT of PALN recurrence are available in the literature. SBRT doses and fractions for PALN recurrence from variable tumors were started at our hospital from 33 Gy in 3 fractions. In the

present study, converted 58 Gy in normalized total doses of 2 Gy was used, and escalated step by step[18]. Although, 1 of our patients developed grade 4 toxicity due to an intestinal obstruction and required surgery, this patient recovered after surgery. Specifically, this patient received 48 Gy in 3 fractions, and had a larger GTV than the other 6 patients. Based on our experience, we consider the factors that most contribute to severe complications are maximum point dose delivered to normal tissue and the volume of normal tissue administered a high dose. Therefore, we tentatively consider maximum intestinal point dose of 51 Gy or V_{45Gy} < 1 mL as constraints of the intestine for SBRT.

Summarizing, the findings of this preliminary study suggest that selected isolated PALN recurrence patients that fail to respond to chemotherapy with 1-3 closely located attached lymph nodes may be salvaged by SBRT. However, a further larger-scale study is required to define optimal dose, intestinal constraints, and adequate indications for SBRT in recurrent CRC.

COMMENTS

Background

Isolated paraaortic lymph node (PALN) recurrences are rarely encountered from colorectal cancer (CRC). Moreover, surgical treatment in these cases is not widely accepted even when lesions are localized, due to their relative rarity, high postoperative morbidity, and poor prognosis. This patient subset is considered for various chemotherapy regimens. However, despite optimal treatment and initial response, overall survival approaches only 20 mo.

Research frontiers

Stereotactic body radiotherapy (SBRT) is an emerging technology in the radiation oncology field. This technique utilizes stereotactic principles for localization and delivers multiple beams to well defined targets in a few fractions. SBRT can deliver higher doses to tumors due to reduced mechanical error margins, and thus, causes less normal tissue damage.

Innovations and breakthroughs

Delivery of a therapeutic dose of radiation to the PALNs is limited by the sensitivity of the surrounding normal tissues, such as those in the gastrointestinal tract, liver, spinal cord, and kidneys. However recent technologies such as intensity-modulated RT, image-guided RT, and SBRT have allowed higher doses to be delivered to tumor and caused less normal tissue damage. SBRT could lead to better local control through delivery of a higher radiation dose to the tumor and this could ultimately translate into survival gain. Furthermore, SBRT requires complex planning and relatively long treatment times (30-45 min) but generally is completed in 3-5 treatments. SBRT is associated with few side effects because the treatment field is generally very small and treatment is precisely delivered.

Applications

This pilot study suggests selected paraaortic lymph node recurrence (1-3 closed lesions) that failed to respond to chemotherapy can be potentially salvaged by stereotactic body radiotherapy.

Terminology

Isolated PALN metastasis is defined as metastasis only to the PALNs. SBRT is an image-guided radiation method. SBRT is directed to extremely well-defined targets within the body. SBRT has evolved from the intracranial experience of stereotactic radiosurgery (single fraction treatment) or stereotactic radiotherapy (multiple fractions of treatment).

Peer review

The authors evaluate efficacy and complications of stereotactic body radiotherapy in localized paraaortic lymph node recurrence from colorectal cancer. it is well written.

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BRIEF ARTICLE

First endoscopic procedure for diagnosis and staging of mediastinal lymphadenopathy

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Khoo KL, Ho KY, Khor CJL, Nilsson B, Lim TK. First endoscopic procedure for diagnosis and staging of mediastinal lymphadenopathy. *World J Gastroenterol* 2009; 15(48): 6096-6101 Available from: URL: http://www.wjgnet.com/1007-9327/15/6096.asp DOI: http://dx.doi.org/10.3748/wjg.15.6096

Abstract

AIM: To compare a first diagnostic procedure of transbronchial needle aspiration (TBNA) with selection of endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) or TBNA for mediastinal lymphadenopathy.

METHODS: Sixty-eight consecutive patients with mediastinal lymphadenopathy on computed tomography (CT), who required cytopathological diagnosis, were recruited. The first 34 underwent a sequential approach in which TBNA was performed first, followed by EUS-FNA if TBNA was unrevealing. The next 34 underwent a selective approach where either TBNA or EUS-FNA was selected as the first procedure based on the CT findings.

RESULTS: The diagnostic yield of TBNA as the first diagnostic procedure in the sequential approach was 62%. In the selective approach, the diagnostic yield of the first procedure was 71%. There was no significant difference in the overall diagnostic yield, but there were significantly fewer combined procedures with the selective approach.

CONCLUSION: Selecting either EUS-FNA or TBNA as the first diagnostic procedure achieved a comparable diagnostic yield with significantly fewer procedures than performing TBNA first in all patients.

INTRODUCTION

Lung cancer is the commonest cause of mediastinal lymphadenopathy. For non-small cell lung cancer (NSCLC), which accounts for about 80% of lung cancers, mediastinal lymph node enlargement occurs in up to 38% of cases at diagnosis[1]. As surgical resection of NSCLC offers the best chance of cure in patients without distant metastases, the pathological confirmation of cancer spread to enlarged mediastinal lymph nodes is crucial to staging because this excludes curative surgical resection.

In the approach to suspected lung cancer without distant metastases, the lung mass is the initial target for cytopathological diagnosis. Following a diagnosis of NSCLC, mediastinal staging is the next step. In patients with mediastinal lymphadenopathy however, the mediastinum may be targeted first, even when a lung mass is present. This might achieve simultaneous diagnosis and mediastinal staging of lung cancer with a single procedure.

The esophagus and tracheobronchial tree offer endoluminal access to mediastinal lymph nodes, therefore endoscopic techniques such as endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) and transbronchial needle aspiration (TBNA) offer minimally invasive approaches for diagnosis of mediastinal lymphadenopathy.

Although EUS-FNA has a higher accuracy than TBNA, the transbronchial approach is preferred for anterior and right paratracheal lymph nodes. Real-time endobronchial ultrasound-guided TBNA (EBUS-TBNA) is now available but requires expensive specialized equipment and operator training. TBNA does not require specialized equipment and can be performed during the initial diagnostic bronchoscopy[2-4]. When we evaluated patients with mediastinal lymphadenopathy with bronchoscopy and TBNA, the diagnostic yield for mediastinal lymphadenopathy was 65%[3].

We have also used EUS-FNA for cases in which TBNA was unrevealing or non-diagnostic, given its higher accuracy[4]. However, this resulted in subjecting these patients to two diagnostic procedures even though both procedures could be performed in the same outpatient session[4,5].

We then hypothesized that bronchoscopy with TBNA need not be performed as the first procedure in all cases of mediastinal lymphadenopathy, and that by selecting the appropriate endoscopic procedure based on anatomical access, a higher diagnostic yield could be obtained after the first procedure. This could also result in subjecting the patient to fewer diagnostic procedures. Therefore, in this study, we compared an approach utilizing TBNA as the first diagnostic procedure with one utilizing selection of either EUS-FNA or TBNA.

MATERIALS AND METHODS

Between December 2003 and June 2006, consecutive patients with mediastinal lymphadenopathy on thoracic computed tomography (CT) who presented to, or were referred to our respiratory division for cytopathological diagnosis were recruited for the study. Mediastinal lymphadenopathy was defined as a node larger than 1 cm in its short axis. The institutional review board of our hospital approved the study and informed consent was obtained for all the procedures.

Sequential approach

During the first 16 mo of the study period, we employed a sequential approach for which bronchoscopy with TBNA was performed as the first diagnostic procedure, with or without other conventional bronchoscopic techniques. If TBNA was unrevealing on rapid on-site cytopathological evaluation (ROSE), EUS-FNA was performed immediately after TBNA, during the same session. Details of this approach and the results of the first 20 patients have been described when we explored the one-stop approach to mediastinal lymphadenopathy[4,5].

Selective approach

From April 2005, we employed a selective approach for which either EUS-FNA or TBNA was performed as the first diagnostic procedure. This was selected based on the predominant location of the lymphadenopathy on CT. If either the esophageal or transbronchial approach could access the nodes, the pulmonologist was left to decide which procedure he deemed most appropriate. In general, TBNA was selected mainly for patients with right paratracheal lymphadenopathy, whereas EUS-FNA was the preferred option for left paratracheal lymphadenopathy. Subcarinal lymph nodes could be approached by either procedure. If TBNA was selected as the first diagnostic procedure, EUS-FNA remained a subsequent option.

TBNA, EUS-FNA and ROSE

Bronchoscopy was performed by experienced pulmonologists using standard flexible videobronchoscopes (Olympus Optical Co. Ltd., Tokyo, Japan). Premedication with pethidine and atropine and sedation with midazolam were optional, while all patients received topical anesthesia with xylocaine. TBNA was performed blind with a Wang 22-gauge (MW 222) cytology needle (Bard Endoscopic Technologies, Billerica, MA, USA) at sites of mediastinal lymph node enlargement based on review of the CT scan. TBNA was performed before other conventional bronchoscopic procedures to avoid contamination.

EUS-FNA was performed as previously described using the curved linear array echoendoscope (GF-UC30P; Olympus) by experienced gastroenterologists[6]. Patients received topical anesthesia with xylocaine and sedation with a combination of midazolam and pethidine.

ROSE was employed to determine the adequacy of the needle aspirates. The aspirated material was blown onto a slide using the direct smear technique[7]. The smears were either air-dried and stained with Diff-Quik (American Scientific Products, McGraw Park, IL, USA) or fixed immediately in 95% ethanol and stained with Papanicolaou stain. Solid particles were fixed in formalin, routinely processed, and made into cell blocks for histological examination. The air-dried smears for Diff-Quik staining were reviewed immediately by an experienced cytotechnician. Endoscopists were then advised as to the need for additional needle aspirates (up to a maximum of six passes).

Diagnostic yield

The final cytopathological diagnoses were made based upon analysis of the aspirated material by experienced cytopathologists. The diagnostic yield of TBNA was the number of patients in whom a definite diagnosis was made by TBNA over the total number of patients subjected to TBNA. The diagnostic yield after the first procedure was the number of patients in whom a definite diagnosis was made after the first procedure over the total number of patients. The overall diagnostic yield for each approach was the number of patients in whom a definitive diagnosis was made by needle aspiration over the total number of patients. When a diagnosis could not be made by either procedure, the final diagnostic categories were determined by review of further tests and clinical assessments.

Statistical analysis

Descriptive statistics are presented as mean ± SD. Discrete

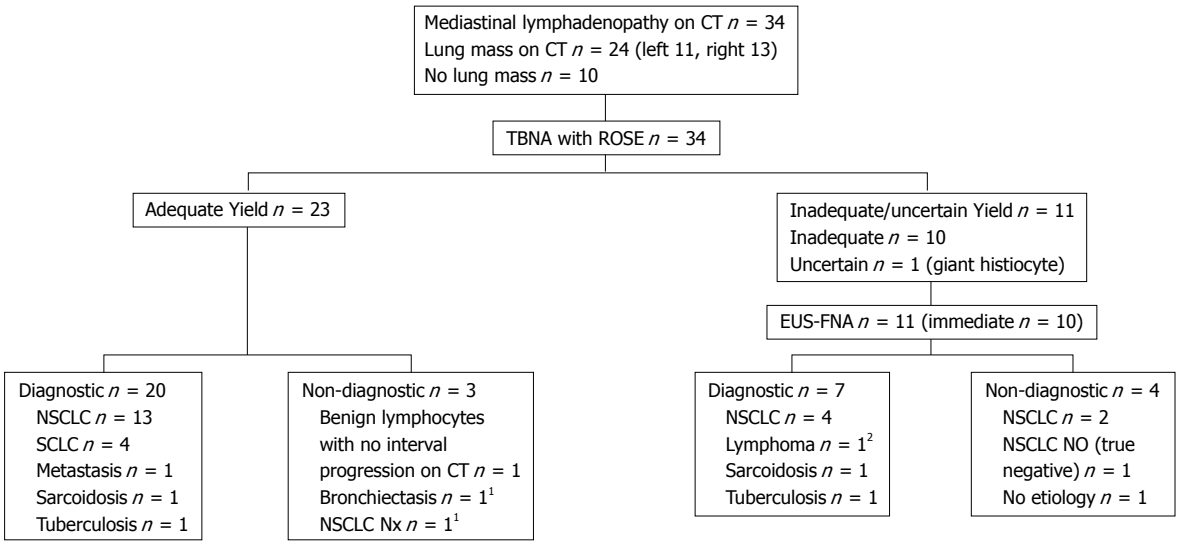

Figure 1 The sequential approach. [1]False positive TBNA with ROSE; [2]False negative TBNA with ROSE. CT: Computed tomography; TBNA: Transbronchial needle aspiration; ROSE: Rapid on-site cytopathological evaluation; NSCLC: Non-small cell lung cancer; EUS-FNA: Endoscopic ultrasound-guided fine-needle aspiration.

variables were analyzed with χ^2 test and $P < 0.05$ was defined as statistically significant.

RESULTS

Sixty-eight consecutive patients with mediastinal lymphadenopathy on CT were recruited during the study period. The main indication for CT was suspected malignancy ($n = 58$). Other indications included suspected pulmonary embolism ($n = 4$), pyrexia of unknown origin ($n = 2$), suspected aortic dissection ($n = 1$), investigation of weight loss ($n = 1$), suspected sarcoidosis ($n = 1$), and follow-up of non-Hodgkin's lymphoma ($n = 1$).

The baseline characteristics and diagnostic categories of the sequential group ($n = 34$) and the selective group ($n = 34$) were similar (Table 1).

Results of the sequential approach are shown in Figure 1. TBNA was performed at the following mediastinal sites according to regional lymph node map definitions as described by Mountain *et al*[8]: 4R in 10 patients, 7 in 24 patients, and 4L in 7 patients. The TBNA obtained adequate specimens in 23 of the 34 patients. In the remaining 11 patients, TBNA with ROSE showed the specimens to be inadequate or unrevealing, thus, EUS-FNA was performed immediately after bronchoscopy, at lymph node stations 7 (seven patients), 4L (10 patients) and 4R (one patient). Some patients had TBNA or EUS-FNA performed at more than one lymph node station. When the final cytopathological results were analyzed, TBNA with ROSE was falsely negative in one patient. In another patient, TBNA with ROSE showed a giant histiocyte and a decision was made to proceed with EUS-FNA. The final cytopathological diagnosis for both specimens returned as granulomatous inflammation. Results of the first 20 patients with this approach have been described previously[4].

Results of the selective approach are shown in Figure 2. TBNA was performed in 22 patients in the following mediastinal sites: 4R (six patients) and 7 (19 patients).

Table 1 Baseline characteristics and diagnostic categories of study population *n* (%)

Variables	Sequential approach	Selective approach	*P* value
No. of patients	34	34	
Male/female	24/10	25/9	
Age (mean ± SD, yr)	64.7 ± 11.2	65.1 ± 12.7	
Mass on CT	24	23	
Right-sided/left-sided,	13/11	12/11	
No. of patients undergoing			
TBNA	34 (100)	22 (65)	< 0.001
TBNA and EUS-FNA	11 (32)	2 (6)	< 0.05
Diagnostic yield (%)			
First procedure	62	71	0.6
TBNA	62	73	0.6
Overall	79	73	0.8
Diagnostic categories			
Malignancy	26	28	
NSCLC/SCLC	22/3	21/7	
Benign tumor	8	6	
Sarcoid/tuberculosis	2/2	1/1	

CT: Computed tomography; TBNA: Transbronchial needle aspiration; NSCLC: Non-small cell lung cancer; EUS-FNA: Endoscopic ultrasound-guided fine-needle aspiration.

EUS-FNA was performed as a first diagnostic procedure in 12 patients at lymph node stations 7 (12 patients), 4L (five patients) and 2R (one patient, Figure 3). In contrast to the sequential approach for which all 34 patients had TBNA performed first, 35% (12/34) of patients in the selective approach had EUS-FNA performed first, while the remaining 65% (22/34) had TBNA performed first. In the selective approach, TBNA was performed only for right paratracheal and subcarinal stations, whereas EUS was performed predominantly in the left paratracheal and subcarinal stations.

The diagnostic yield of TBNA as the first diagnostic test was 62% in the sequential approach, while the diagnostic yield of the first diagnostic procedure in the selective approach was 71%. The diagnostic yield of

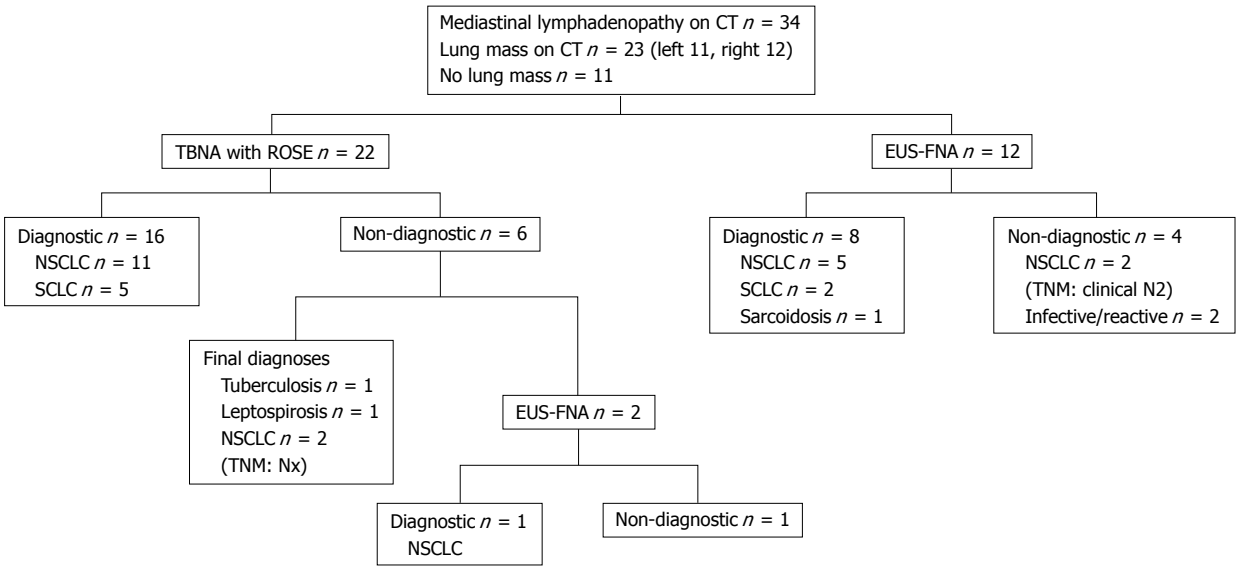

Figure 2 The selective approach.

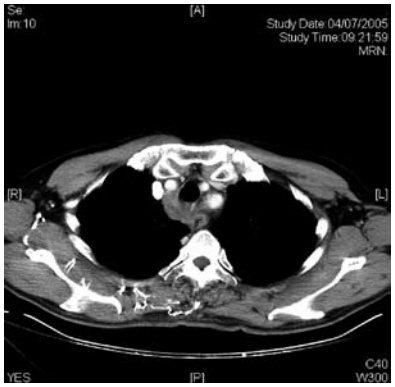

Figure 3 CT showing right paratracheal lymphadenopathy that was sampled by EUS-FNA.

EUS-FNA was 67% (8/12). The overall diagnostic yield of the sequential approach was 79% (27/34) and that of the selective approach was 74% (25/34). There was no significant difference in the overall diagnostic yields. Significantly fewer combined diagnostic procedures (6% *vs* 32%, $P < 0.05$) were required with the selective approach. The yield of TBNA was higher with the selective approach (73%) as compared to the sequential approach (62%). There were no complications with either TBNA or EUS-FNA, or damage to the bronchoscopes or endoscopes.

DISCUSSION

The present study compared a diagnostic approach utilizing TBNA as the first diagnostic procedure with one in which EUS-FNA or TBNA was selected as the first procedure. The selection was based on whether the optimal anatomical approach was transesophageal or transbronchial. We found a higher diagnostic yield after the first diagnostic procedure with the selective approach, and this translated to a significant reduction in the number of diagnostic procedures performed.

The transesophageal and transbronchial routes to the mediastinum are complementary. The transesophageal approach has limited access to the right paratracheal nodes, therefore, the endobronchial route offers better access, as shown by Herth *et al*[9]. Therefore, the procedure of choice for right paratracheal lymphadenopathy with the selective approach was TBNA, unless CT showed a peri-esophageal location of these nodes that was easily accessed by EUS-FNA (Figure 3). Harrow *et al*[10] also have shown that the right paratracheal and subcarinal locations are predictors of a positive aspirate with TBNA. With the selective approach, TBNA was limited to these two locations, and the yield of TBNA improved from 62% to 73%.

Although mediastinoscopy remains the diagnostic standard for the mediastinal staging of lung cancer, with a sensitivity of 80%-85%, this invasive surgical procedure requires general anesthesia and has a morbidity and mortality rate of 2% and 0.08%, respectively[11]. In contrast, both TBNA and EUS-FNA are minimally invasive and can be performed in the outpatient setting under local anesthesia and sedation. EUS-FNA permits real-time visualization of needle sampling and has been shown to be highly accurate in the mediastinal staging of lung cancer, as well as in the diagnosis of mediastinal lymphadenopathy of unknown etiology[6,12-18].

The development of EBUS-TBNA for mediastinal lymph nodes has lagged behind EUS-FNA by more than a decade[19,20]. As such, the new convex-probe EBUS is still not as widely available as EUS. Wallace *et al*[21] have suggested that the use of ultrasound-guided needle sampling of mediastinal lymph nodes in patients with suspected lung cancer, whether by EUS or EBUS, is superior to conventional TBNA. By combining EUS-FNA and EBUS-TBNA, they have achieved a near-complete medical mediastinoscopy, thus reinforcing the complementary nature these procedures[22].

Our aim was not to achieve comprehensive staging of the mediastinum in the setting of lung cancer, but

rather, to demonstrate that, with appropriate selection of the first endoscopic procedure, a higher diagnostic yield could be obtained. This would mean that EUS-FNA could be selected as the first procedure, rather than routinely subjecting all patients to bronchoscopy. Indeed, a recent meta-analysis has suggested that EUS-FNA is the diagnostic test of choice for mediastinal lymphadenopathy[23]. In addition, the transesophageal route may be better tolerated as compared to the transbronchial route, with less coughing and the absence of obstruction of the needle by cartilaginous rings.

Most studies with EUS-FNA for mediastinal evaluation have been performed in patients only after confirmation of the diagnosis of NSCLC. Singh *et al*[24], however, have demonstrated that EUS-FNA may be performed as the first diagnostic procedure for suspected lung cancer. In the setting of mediastinal lymphadenopathy in NSCLC, this diagnostic procedure also has enabled simultaneous mediastinal staging. Thus, besides showing that bronchoscopy need not be the first diagnostic procedure in patients with suspected lung cancer, they also have demonstrated that diagnosis and staging of lung cancer need not be performed sequentially or require multiple procedures. This highlights a paradigm shift where mediastinal staging is no longer performed only after confirming the diagnosis of NSCLC.

We believe that, in the diagnostic approach to the mediastinum, the transesophageal and transbronchial routes are complementary rather than competing. Instead of pitting TBNA against EUS-FNA, this study emphasizes that the complementary value of these endoscopic approaches is best exploited by appropriate procedure selection. Thus, when either EUS-FNA or TBNA was selected as the first procedure, the diagnostic yield increased from 62% to 71%, thereby significantly reducing the need for additional procedures. Targeting the mediastinum first to enable simultaneous diagnosis and staging, and optimizing the yield of the first diagnostic procedure may lead to fewer delays in the treatment of lung cancer patients. Devbhandari *et al*[25] have reported that a negative initial bronchoscopy in suspected lung cancer resulted in significant delays in diagnosis and treatment. In that study, initial bronchoscopy was diagnostic in less than 50% of cases.

The present study had several limitations. Firstly, this was not a randomized trial and the patient population was small. However, they were consecutive patients with similar baseline characteristics and diagnostic categories (Table 1). Secondly, a definitive diagnosis could not be made in all cases because some patients and their referring physicians declined further invasive surgical sampling. However, the aim of this study was to determine the diagnostic yield of the sequential and selective approaches rather than the accuracy of either endoscopic procedure. Thirdly, conventional TBNA was employed rather than EBUS-TBNA. This was because at the time of the study, EBUS-TBNA was not available at our center.

Three practical clinical points are highlighted here. Firstly, the cytopathological diagnosis of mediastinal lymphadenopathy may be achieved in the majority of patients utilizing widely available endoscopic techniques. Secondly, targeting the mediastinum first may establish simultaneously diagnosis as well as mediastinal staging for patients with NSCLC. Finally, appropriate selection of the first diagnostic procedure may optimize the yield and minimize the number of procedures required for the diagnosis and/or staging of mediastinal lymphadenopathy. Thus, with the availability of EUS-FNA, bronchoscopy may no longer be required in selected patients with suspected lung cancer.

Endoscopic techniques are becoming essential high-utility tools in the investigative approach to the mediastinum. With the rapid evolution of newer endoscopic techniques, the physician's diagnostic armamentarium is likely to expand. The question of which is the most appropriate initial diagnostic procedure for mediastinal lymphadenopathy, given what is available, will become even more important. While awaiting further studies comparing the different emerging endoscopic techniques and combination of techniques, we suggest that the optimal diagnostic approach for mediastinal lymphadenopathy depends on selection of the most appropriate initial diagnostic procedure.

COMMENTS

Background

In the absence of distant metastasis, mediastinal staging remains crucial for determining prognosis and therapy of non-small cell lung cancer. An approach to patients with mediastinal lymphadenopathy regardless of whether a lung mass in present, is to target the mediastinum first. This may achieve simultaneous diagnosis and mediastinal staging with a single procedure, in the event of diagnosis of lung cancer, which is the commonest cause of mediastinal lymphadenopathy.

Research frontiers

The transbronchial and transesophageal routes allow minimally invasive endoscopic needle sampling of mediastinal lymph nodes. These endoscopic procedures may be done under sedation in contrast to the gold standard mediastinoscopy, which requires general anesthesia.

Innovations and breakthroughs

Minimally invasive mediastinal staging with endoscopic ultrasound-guided fine-needle aspiration and blind or endobronchial ultrasound guided transbronchial-needle aspiration may be a substitute for mediastinoscopy.

Applications

Not all patients require all three procedures, therefore, appropriate initial procedure selection may be important in the diagnostic approach to mediastinal lymphadenopathy, because only a single procedure may be diagnostic in the majority of cases.

Peer review

This is a very interesting topic for the readers of *World Journal of Gastroenterology.*

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BRIEF ARTICLE

Azithromycin-containing *versus* standard triple therapy for *Helicobacter pylori* eradication: A meta-analysis

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Abstract

AIM: To evaluate whether adding azithromycin to first-line *Helicobacter pylori* (*H pylori*) eradication improved eradication and reduced side effects.

METHODS: Eligible articles were identified by searches of electronic databases. We included all randomized trials that compared azithromycin-containing with standard triple-therapy regimens for first-line treatment of *H pylori* infection. Statistical analysis was performed with Review Manager 5.0.10. Sub-analyses were also performed.

RESULTS: We identified 14 randomized trials (1431 patients). Pooled *H pylori* eradication rates were 72.01% (95% CI: 58.09%-85.93%) and 69.78% (95% CI: 66.47%-73.09%) for patients with or without azithromycin by intention-to-treat analysis, and the odds ratio (OR) was 1.17 (95% CI: 0.64-2.14). The occurrence of side effects differed significantly and was 15.81% (95% CI: 12.50%-19.12%) and 25.20% (95% CI: 21.44%-28.96%) for treatment with or without azithromycin, respectively, and the summary OR was 0.58 (95% CI: 0.41-0.82). Furthermore, the azithromycin-containing group had a lower occurrence of diarrhea, nausea and taste disturbance.

CONCLUSION: Our review suggests that azithromycin-containing triple-therapy regimens could be equally effective in eradication of *H pylori* compared with standard first-line triple-therapy regimens.

INTRODUCTION

Infection caused by *Helicobacter pylori* (*H pylori*), one of the most common pathogens worldwide, causes chronic gastritis and increases the risk of peptic ulcer and gastric cancer. Although some *H pylori*-positive individuals are asymptomatic, many experience symptoms such as dyspepsia. It is increasingly common to screen patients, even those with mild symptoms, for *H pylori* infection, and to treat them actively. The first-line treatment for *H pylori* infection, as recommended by the Maastricht III Consensus Report, is 7-d triple therapy that includes clarithromycin, amoxicillin and a proton-pump inhibitor (PPI)[1]. Even though this triple therapy is effective and its short duration helps maintain patient compliance, a considerable number of patients experience undesirable side effects.

In first-line therapy, eradication rates using combinations of PPI-based triple therapies range from 75% to 98%, with most of them near 80%[2]. This signifies that up to 20% of patients are expected to be treatment failures, a value which could be even higher in areas with a high prevalence of resistant *H pylori* strains. The recommended second-line therapy is a quadruple regimen composed of tetracycline, metronidazole, bismuth salts and a PPI; however, the efficacy of this regimen is limited by poor compliance, treatment duration, number and dose of the prescribed drugs, and bacterial antibiotic resistance.

Gastroenterologists and microbiologists continue the search for new therapies because of the increasing number of target subjects for *H pylori* and the physiological and pharmacoeconomic burden of a second course of therapy.

Among the new options against *H pylori* brought to light recently, azithromycin has attracted substantial interest. Azithromycin is a macrolide antibiotic that has been shown to reach high concentrations in gastric tissue after oral administration; furthermore, these high concentrations are maintained for several days, which make it potentially useful in the eradication of *H pylori*[3]. Clinical trials with triple therapy regimens that contain azithromycin have reported eradication rates of approximately 60%-80%, depending on the regimen and azithromycin dose used[4,5]. However, results from some other available trials utilizing azithromycin have yielded conflicting results. The primary aim of the present meta-analysis was to evaluate whether adding azithromycin to *H pylori* eradication regimens could improve eradication and reduce side effects.

MATERIALS AND METHODS

Selection of studies

Studies evaluating azithromycin-containing triple therapy for the eradication of *H pylori* were considered. For the meta-analysis, the selection criteria were as follows: (1) articles that reported comparative randomized controlled trials (RCTs); (2) studies had to include at least two branches of treatment that consisted of (a) triple first-line therapy (one PPI and two antibiotics) and (b) azithromycin-containing triple regimen; (3) study population consisted of subjects who had never been treated for *H pylori* infection previously; and (4) data for successful eradication and/or side effects were available.

Search strategy for identification of studies

Trials were identified by searching the Cochrane Controlled Trials Register (Issue 2, 2009), PubMed (1966 to May 2009), Embase (1980 to May 2009), Science Citation Index (1945 to May 2009) and the Chinese Biomedical Database (1981 to May 2009). A search strategy was constructed by using a combination of the following words: (*Helicobacter pylori* OR *H pylori*) AND (azithromycin). Articles published in any language were included. Reference lists from the trials selected by electronic searching were hand-searched to identify further relevant trials. We also conducted a manual search of abstracts from 1995 to May 2009 from the following congresses: International Workshop of the European Helicobacter Study Group, American Digestive Disease Week (DDW), and United European Gastroenterology Week (UEGW). Abstracts of the articles selected in each of these multiple searches were reviewed and those meeting the inclusion criteria were recorded. References of reviews on *H pylori* treatment with azithromycin, and from the articles selected for the study, were also examined for articles that met the inclusion criteria. Authors of some identified trials were asked whether they knew of additional studies, including unpublished randomized ones. In case of duplicate reports, or studies obviously reporting results from the same study population, only the latest published results were used.

Data extraction

Standardized data abstraction sheets were prepared.

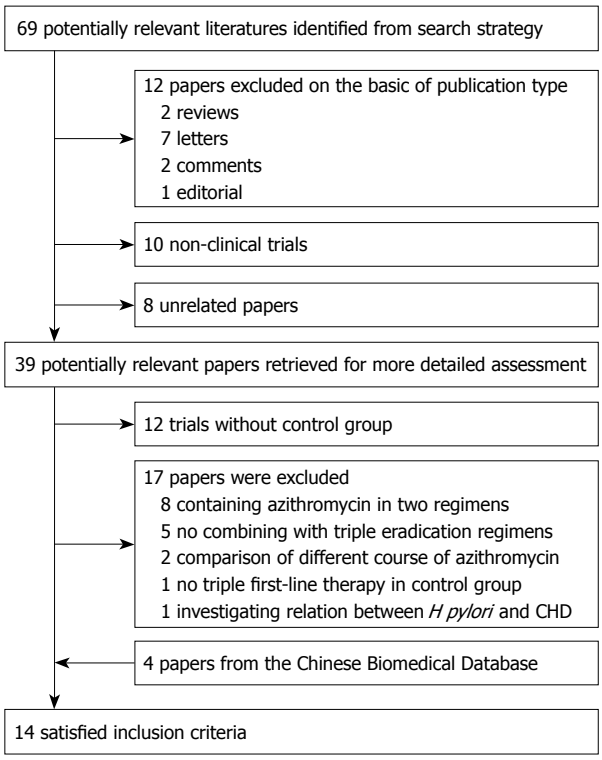


Figure 1 Flowchart of study selection. *H pylori*: *Helicobacter pylori*.

Data were exacted for study quality, dose and duration of azithromycin treatment, anti-*H pylori* regimens, and the number, sex and age of enrolled subjects, diagnostic methods of testing *H pylori* infection before enrolling and after completing the study, and scoring systems for assessing side effects. Key outcome data, such as eradication rates, occurrence of diarrhea, nausea, taste disturbance and abdominal pain were abstracted from all included studies. All articles were examined independently for eligibility by two reviewers. Disagreements were resolved by consulting a third reviewer. Quality was assessed using the Jadad score system based on three items, randomization, double blinding and description of withdrawals/dropouts. We considered that they were low quality when scores were < 3.

Data synthesis

Data were entered into the Cochrane Collaboration review manager programme RevMan 5.0.10 (released on May 16, 2008). The outcome measure examined was the OR of improving *H pylori* eradication rates and reducing side effects with azithromycin compared to without azithromycin-containing triple regimens. Categorical variables were compared with the χ^2 test, and $P < 0.05$ was considered statistically significant. Eradication rates and side effects were analyzed based on a fixed-effects model using the methods of Mantel-Haenszel[6], both by intention-to-treat and per-protocol. Heterogeneity between the studies was assessed by χ^2 test. Statistical significance of heterogeneity was set at 0.10. If significant heterogeneity existed, it would have been inappropriate to combine the data for further analysis using a fixed-effects model, while the random model was used for calculations.

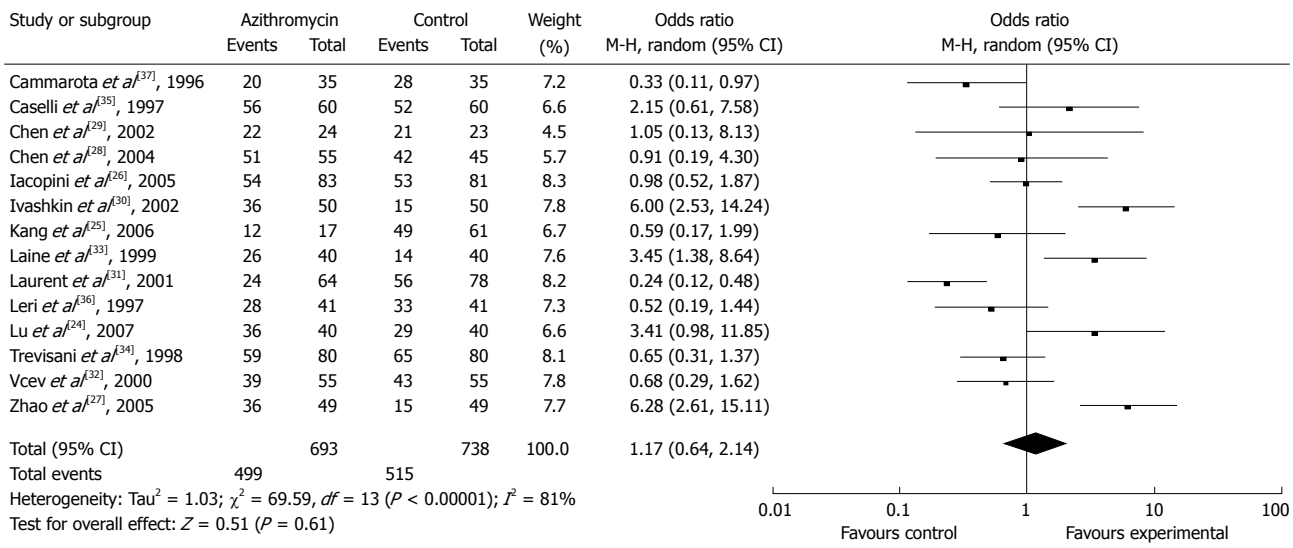


Figure 2 Effect of azithromycin-containing triple therapy *versus* standard triple therapy on eradication rates by intention-to-treat analysis.

Sub-analyses

In the meta-analysis, sub-analyses of *H pylori* eradication efficacy were planned, depending on: (1) the type of drugs co-prescribed with azithromycin (combination with amoxicillin and a PPI was the most widely prescribed); (2) the duration and dose of azithromycin therapy; (3) age of the subjects involved; and (4) quality of the studies (based on quality score proposed by Jadad, see appropriate section). Finally, we used funnel plot asymmetry to detect any publication bias in the meta-analysis, and Egger's regression test to measure funnel plot asymmetry.

RESULTS

Description of the studies

The bibliographical search yielded a total of 69 studies. Of these, 12 articles were excluded owing to publication type, i.e. two reviews, seven letters, two comments, and one editorial. We excluded 18 articles (10 non-clinical trials and eight unrelated articles) after examining the title and abstract, which left 39 potentially relevant articles for more detailed assessment. Of these potential eligible articles, 12 trials without a control group were excluded, and then we excluded another 17 articles, because of no combining with triple eradication regimens[7-11], containing azithromycin in two regimens[12-19], comparison of different treatment course of azithromycin[20,21], no triple first-line therapy in control group[22], and investigating relation between *H pylori* eradication and coronary heart disease[23]. Furthermore, we identified four additional articles from the Chinese Biomedical Database (1981 to May 2009). Finally, 14 RCTs met the inclusion criteria[24-37]. The flowchart of reviews showed the detailed process of selection (Figure 1). The characteristics of 14 trials included in the meta-analysis are summarized in Table 1, including quality score.

Eradication rates

Fourteen studies that described *H pylori* eradication rates were selected for the meta-analysis. Four of these reported significantly improved eradication rates, and the remaining 10 had similar efficacy for *H pylori* eradication. Pooled eradication rates were achieved in 499 of 693 patients with azithromycin supplementation (72.01%, 95% CI: 58.09%-85.93%) and in 515 of 738 patients with azithromycin without regimen (69.78%, 95% CI: 66.47%-73.09%) by intention-to-treat analysis, the OR was 1.17 (95% CI: 0.64-2.14) (Figure 2). Overall, per-protocol eradication rates were 75.81% (95% CI: 72.44%-79.18%) and 72.44% (95% CI: 69.05%-75.83%) for azithromycin supplementation and azithromycin without regimen, respectively (OR 1.22, 95% CI: 0.61-2.43).

Side effects

Total side effects were initially performed for meta-analysis. Data for the occurrence of side effects were obtained from 10 RCTs. Five of these studies reported a significant decrease in the occurrence of gastrointestinal side effects. The total number of side effects with azithromycin supplementation differed significantly from azithromycin without regimen: 15.81% (95% CI: 12.50%-19.12%) and 25.20% (95% CI: 21.44%-28.96%), and the summary OR was 0.58 (95% CI: 0.41-0.82) (Figure 3A). Individual symptoms during eradication therapy, such as nausea, diarrhea, abdominal pain, and taste disturbance were also analyzed. Incidence of diarrhea (2.13% *vs* 6.98%) (Figure 3B), nausea (3.85% *vs* 10.14%) (Figure 3C) and taste disturbance (3.17% *vs* 11.05%) (Figure 3D) were lower in the azithromycin supplementation group (OR: 0.33 *vs* 0.37 *vs* 0.28, 95% CI: 0.12-0.96 *vs* 0.14-0.96 *vs* 0.11-0.70).

Sub-analyses

Sub-analyses for the meta-analysis were planned depending on subject age, symptoms before enrollment, course of azithromycin, and choice of antibiotics. We divided all eligible trials into long- and short-course subgroups, Az+A subgroup, Az+Lev subgroup and Az+M/T subgroup. There was no significant difference between the long-course and short-course subgroups; the summary ORs were 0.89 (95% CI: 0.43-1.85) and 1.56 (95% CI:

Table 1 Characteristics of included studies comparing *Helicobacter pylori* (*H pylori*) eradication efficacy of azithromycin-containing triple therapy versus standard triple therapy

Authors	Country	Form	Trial design	Case No. (Az/con)	Patients	Diagnostic methods	Azithromycin regimen	% Eradication (*n*)	% Adverse effects (*n*)	Triple therapy	Days of antibiotics	% Eradication (*n*)	% Adverse effects (*n*)	Q
Lu *et al*[24], 2007	China	JA	Single centre RCT	85 (43/42)	*H pylori* positive	RUT/	O (20 mg *bid*)	ITT 84 (36/43)	26 (11/43)	O (20 mg *bid*)	7	ITT 69 (29/42)	19 (8/42)	3
					Chronic active gastritis	RUT (30 d later)	Lev (200 mg *bid*) Az (500 mg *o.d.*)	PP 90 (36/40)		A (1 g *bid*) C (500 mg *bid*)		PP 72.5 (29/40)		
Kang *et al*[25], 2006	Korea	JA	Single centre RCT	78 (17/61)	*H pylori* positive	Histology + RUT or UBT/	O (20 mg *bid*)	ITT 70.6 (12/17)	11.8 (2/17)	O (20 mg *bid*)	7	ITT 80.3 (49/61)	41.0 (25/61)	3
					Adults	Histology + RUT or UBT (8 wk later)	Lev (500 mg *o.d.*) Az (500 mg *o.d.*)	PP 70.6 (12/17)		A (1 g *bid*) C (500 mg *bid*)		PP 80.3 (49/61)		
Iacopini *et al*[26], 2005	Italy	JA	Single centre RCT	164 (83/81)	*H pylori* positive	Histology + UBT/	E (20 mg *o.d.*)	ITT 65 (54/83)	12 (9/77)	E (20 mg *bid*)	7	ITT 65 (53/81)	30 (22/70)	4
					Peptic ulcer and GERD adults	UBT + HpSA (8 wk later)	Lev (500 mg *o.d.*) Az (500 mg *o.d.*)	PP 70 (54/77)		A (1 g *bid*) C (500 mg *bid*)		PP 76 (53/70)		
Zhao *et al*[27], 2005	China	JA	Single centre RCT	98 (49/49)	*H pylori* positive	Histology + RUT/	O (20 mg *bid*)	ITT 73.5 (36/49)	/	O (20 mg *bid*)	7	ITT 30.6 (15/49)	/	3
					Active duodenal ulcer	RUT + Histology (4 wk later)	A (1 g *bid*) Az (1 g *o.d.*) 3 d	PP 76.6 (36/47)		A (1 g *bid*) M (500 mg *bid*)		PP 31.3 (15/48)		
Chen *et al*[28], 2004	China	JA	Single centre RCT	100 (55/45)	*H pylori* positive	Histology + UBT/	O (40 mg *o.d.*)	ITT 92.7 (51/55)	5 (3/55)	O (40 mg *o.d.*)	7	ITT 93.3 (42/45)	17.8 (8/45)	2
					Active duodenal ulcer	UBT (6 wk later)	A (1 g *bid*) Az (500 mg *o.d.*) 3 d	PP 92.7 (51/55)		A (1 g *bid*) C (500 mg *bid*)		PP 93.3 (42/45)		
Chen *et al*[29], 2002	China	JA	Single centre RCT	47 (24/23)	*H pylori* positive	Histology + RUT/	L (30 mg *o.d.*)	ITT 92 (22/24)	4 (1/24)	O (20 mg *bid*)	7	ITT 91 (21/23)	9 (2/23)	3
					Chronic gastritis adults	UBT (4 wk later)	M (400 mg *bid*) 3 d Az (500 mg *o.d.*) 3 d	PP 92 (22/24)		M (400 mg *bid*) C (500 mg *bid*)		PP 91 (21/23)		
Ivashkin *et al*[30], 2002	Russia	JA	Multicenter RCT	100 (50/50)	*H pylori* positive	Histology + RUT/	O (20 mg *bid*)	ITT 72 (36/50)	/	O (20 mg *bid*)	7	ITT 30 (15/50)	/	4
					Active duodenal Ulcer adults	Histology + RUT (8 wk later)	A (1 g *bid*) Az (1 g *o.d.*) 3 d	PP 75 (36/48)		A (1 g *bid*) M (500 mg *bid*)		PP 31 (15/49)		
Laurent *et al*[31], 2001	France	JA	Multicenter RCT	247 (64/78/70)	*H pylori* positive	Histology + RUT/	O (20 mg *bid*)	ITT 37.5 (24/64)	56.9 (33/58)	O (20 mg *bid*)	7	ITT 71.8/61.4 (56/78) (43/70)	57.7/66.1 (41/71) (41/62)	4
					Non-ulcer dyspepsia	UBT (4-6 wk later)	A (1 g *bid*) Az (500 mg *o.d.*) day 1 + (250 mg *o.d.*) days 2-5	PP 41.4 (24/58)		C (500 mg *bid*)/ C (250 mg *bid*) A (1 g *bid*)/ M (500 mg *bid*)		PP 78.9/69.4 (56/71) (43/62)		
Vcev *et al*[32], 2000	Croatia	JA	Single centre RCT	110 (55/55)	*H pylori* positive	Histology + RUT/	P (40 mg *bid*)	ITT 71 (39/55)	14 (7/50)	P (40 mg *bid*)	7	ITT 78 (43/55)	17 (9/53)	3
					Active duodenal ulcer	Histology + RUT (8 wk later)	A (1 g *bid*) Az (500 mg *o.d.*) 6 d	PP 78 (39/50)		A (1 g *bid*) C (500 mg *bid*)		PP 81 (43/53)		
Laine *et al*[33], 1999	USA	JA	Single centre RCT	120 (40/40/40)	*H pylori* positive	Histology or serology + UBT/	O (80 mg *o.d.*)	ITT 65 (26/40)	3 (1/38)	O (80 mg *o.d.*)	10	ITT 35/78 (14/40) (31/40)	8/15 (3/37) (5/33)	3
					Symptomatic and	UBT (6 wk later)	M (750 mg *o.d.*)	PP 66 (25/38)		M (750 mg *o.d.*)		PP 35/79 (13/37) (26/33)		

Trevisani *et al*[34], 1998	Italy	JA	Single centre RCT	160 (80/80)	Asymptomatic adults *H pylori* positive	RUT + Histology/ (4 wk later)	Az (500 mg *o.d.*) 7 d L (30 mg *bid*) days 1-4	ITT 73.3 (59/80)	1.3 (1/73)	A (1.5 g *o.d.*)/C (1 g *o.d.*) O (20 mg *o.d.*)	7	ITT 81.2 (65/80)	2.6 (2/76)	4
					Symptomatic adults	RUT + Histology (4 wk later)	T (2000 mg *o.d.*) day 3 Az (500 mg *o.d.*) days 2-4	PP 80.8 (59/73)		C (250 mg *bid*) T (500 mg *bid*)		PP 85.5 (65/76)		
Caselli *et al*[35], 1997	Italy	JA	Multicenter RCT	120 (60/60)	*H pylori* positive Gastritis with or without peptic ulcer	Histology + RUT/ Histology (7-8 wk later)	L (30 mg *o.d.*) M (250 mg *bid*) 3 d Az (500 mg *o.d.*) 3 d	ITT 93.3 (56/60) PP 93.3 (56/60)	/	O (20 mg *o.d.*) C (250 mg *bid*) T (500 mg *bid*)	7	ITT 86.7 (52/60) PP 91.2 (52/57)	/	3
Leri *et al*[36], 1997	Italy	Ab	Single centre RCT	123 (41/41/41)	*H pylori* positive	Histology + RUT	O (20 mg *bid*) M (500 mg *bid*) 10 d Az (500 mg *o.d.*) 6 d	ITT 68 (28/41)	/	O (20 mg *bid*) M (500 mg *bid*) 10 d A (1 g *bid*) / C (500 mg *t.d.*)	14	ITT 80/97 (33/41) (40/41)	/	2
Cammarota *et al*[37], 1996	Italy	JA	Single centre RCT	70 (35/35)	*H pylori* positive Symptomatic adults	Histology + RUT/ Histology + RUT (8 wk later)	L (30 mg *o.d.*) A (1 g *bid*) Az (500 mg *o.d.*) 3 d	ITT 57 (20/35) PP 61 (20/33)	18 (6/33)	L (30 mg *o.d.*) A (1 g *bid*) C (250 mg *bid*)	7	ITT 80 (28/35) PP 82 (28/34)	26 (9/34)	3

Ab: Abstract; JA: Journal article; C: Clarithromycin; A: Amoxicillin; Az: Azithromycin; M: Metronidazole; T: Tinidazole; Lev: Levofloxacin; E: Esomeprazole; P: Pantorazole; O: Omeprazole; L: Lanzoprazole; UBT: 13C-urea breath test; RUT: Rapid urease test; HpSA: *H pylori* stool antigen; Q: quality score; RCT: Randomized controlled trial; ITT: Intent-to-treat analysis; PP: Per-protocol analysis.

0.60-4.08), respectively (Figure 4A). For antibiotics sub-analysis, Az+A subgroup, Az+Lev subgroup and Az+M/T subgroup all had no significant difference; the summary ORs were 1.11 (95% CI: 0.32-3.89), 1.19 (95% CI: 0.51-2.81) and 1.20 (95% CI: 0.53-2.69), respectively (Figure 4B).

Publication bias

We found that the funnel plot had a slightly asymmetrical distribution, but Egger's regression test[38] suggested no significant asymmetry of the funnel plot ($P = 0.84$), which indicated no evidence of substantial publication bias.

DISCUSSION

For *H pylori* eradication therapy, clinical trails are undertaken to search for simpler but equally or more effective regimens. The modern macrolides are a focus of attention from that point of view. Azithromycin, a new-generation macrolide, has some special attributes that make it a promising compound in regimens for *H pylori* eradication. Following the administration of a single oral dose, azithromycin readily accumulates in the human gastric mucosa, subsequently redistributes from mucosal tissue to the mucus layer, and from the mucus to gastric juice. There, it reaches gastric tissue concentrations that persist above the minimal concentration for 90% inhibition (MIC_{90}) for *H pylori* (0.25 μg/mL) over a 5-d period, thus leading to exposure of the microorganism to consistent amounts of this drug. The high tissue affinity and the absorption of the drug after oral administration are reduced when given during or after a meal. The pharmacological properties of azithromycin make it possible to use shorter courses, therefore, the problem was to define an optimal dose and duration of azithromycin in triple therapy.

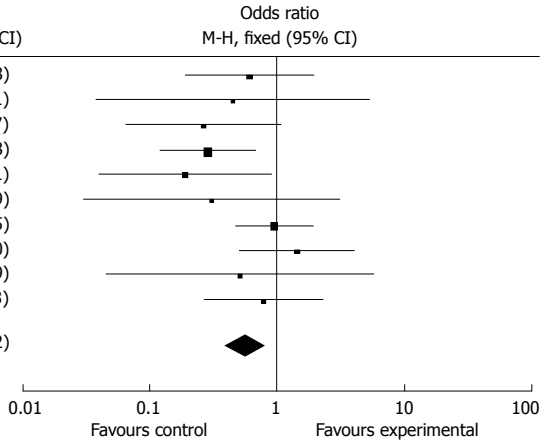
Azithromycin is able to reach high gastric concentrations that persist for several days, and therefore, it can be administered at a dose of 500 mg once daily for only 3 d during a 7-d triple eradication regimen. The published trials that have used this antibiotic have yielded conflicting results, and have reported a wide range of eradication rates. Administration with meals markedly reduces azithromycin absorption, therefore, this might account for the low eradication rates observed in some studies[21]. In treatment regimens in which azithromycin was given to fasting patients, the cure rate was in the range 86%-93%[13,37]. Recently, short-term treatments of only 3 d, using a PPI plus azithromycin 500 mg and tinidazole 1000-2000 mg daily, have been found to promote eradication in 81%-88% of cases[23,39]. In contrast with the results reported in early studies that have used azithromycin for 2 wk and in repeated daily doses[40], side effects are scarce if the drug is administered once daily for a few days. In subanalyses, we also found that *H pylori* eradication rate had no significant difference between the long-course and short course subgroups.

H pylori eradication depends on a number of factors, including patient compliance, side effects, bacterial resistance, poor drug distribution or concentration, geographic differences, and socio-economic conditions. Optimization of *H pylori* eradication therapy remains an

A

Study or subgroup	Azithromycin Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, fixed (95% CI)
Cammarota *et al*[37], 1996	6	33	9	34	8.9	0.62 (0.19, 1.98)
Chen *et al*[29], 2002	1	24	2	23	2.4	0.46 (0.04, 5.41)
Chen *et al*[28], 2004	3	55	8	45	10.2	0.27 (0.07, 1.07)
Iacopini *et al*[26], 2005	9	77	22	70	24.9	0.29 (0.12, 0.68)
Kang *et al*[25], 2006	2	17	25	61	11.8	0.19 (0.04, 0.91)
Laine *et al*[33], 1999	1	38	3	37	3.6	0.31 (0.03, 3.09)
Laurent *et al*[31], 2001	33	58	41	71	19.4	0.97 (0.48, 1.95)
Lu *et al*[24], 2007	11	43	8	42	7.4	1.46 (0.52, 4.10)
Trevisani *et al*[34], 1998	1	73	2	76	2.4	0.51 (0.05, 5.79)
Vcev *et al*[32], 2000	7	50	9	53	9.2	0.80 (0.27, 2.33)
Total (95% CI)		468		512	100.0	0.58 (0.41, 0.82)
Total events	74		129			

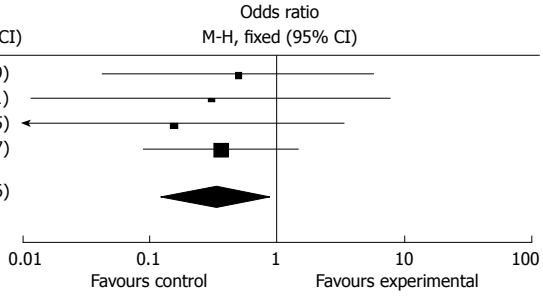
Heterogeneity: χ^2 = 11.46, *df* = 9 (*P* = 0.25); I^2 = 21%
Test for overall effect: *Z* = 3.02 (*P* = 0.002)



B

Study or subgroup	Azithromycin Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, fixed (95% CI)
Cammarota *et al*[37], 1996	1	33	2	34	14.5	0.50 (0.04, 5.79)
Chen *et al*[29], 2002	0	24	1	23	11.4	0.31 (0.01, 7.91)
Chen *et al*[28], 2004	0	55	2	45	20.6	0.16 (0.01, 3.35)
Iacopini *et al*[26], 2005	3	77	7	70	53.5	0.36 (0.09, 1.47)
Total (95% CI)		189		172	100.0	0.33 (0.12, 0.96)
Total events	4		12			

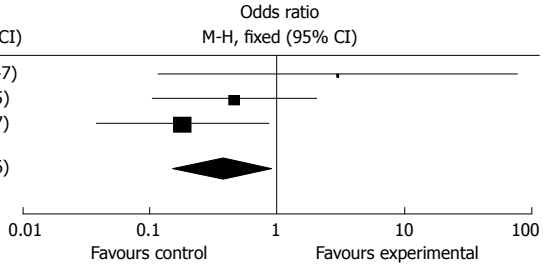
Heterogeneity: χ^2 = 0.36, *df* = 3 (*P* = 0.95); I^2 = 0%
Test for overall effect: *Z* = 2.03 (*P* = 0.04)



C

Study or subgroup	Azithromycin Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, fixed (95% CI)
Chen *et al*[29], 2002	1	24	0	23	3.2	3.00 (0.12, 77.47)
Chen *et al*[28], 2004	3	55	5	45	35.0	0.46 (0.10, 2.05)
Iacopini *et al*[26], 2005	2	77	9	70	61.8	0.18 (0.04, 0.87)
Total (95% CI)		156		138	100.0	0.37 (0.14, 0.96)
Total events	6		14			

Heterogeneity: χ^2 = 2.48, *df* = 2 (*P* = 0.29); I^2 = 19%
Test for overall effect: *Z* = 2.05 (*P* = 0.04)



D

Study or subgroup	Azithromycin Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, fixed (95% CI)
Cammarota *et al*[37], 1996	1	33	5	34	24.8	0.18 (0.02, 1.64)
Chen *et al*[29], 2002	1	24	2	23	10.1	0.46 (0.04, 5.41)
Chen *et al*[28], 2004	0	55	1	45	8.5	0.27 (0.01, 6.72)
Iacopini *et al*[26], 2005	4	77	11	70	56.6	0.29 (0.09, 0.97)
Total (95% CI)		189		172	100.0	0.28 (0.11, 0.70)
Total events	6		19			

Heterogeneity: χ^2 = 0.31, *df* = 3 (*P* = 0.96); I^2 = 0%
Test for overall effect: *Z* = 2.71 (*P* = 0.007)

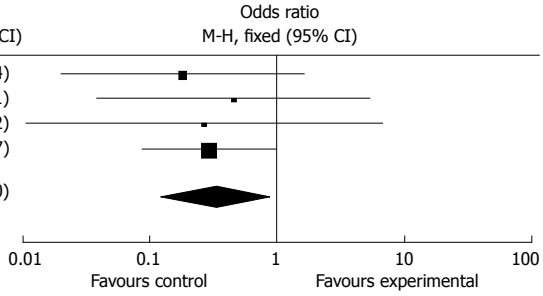
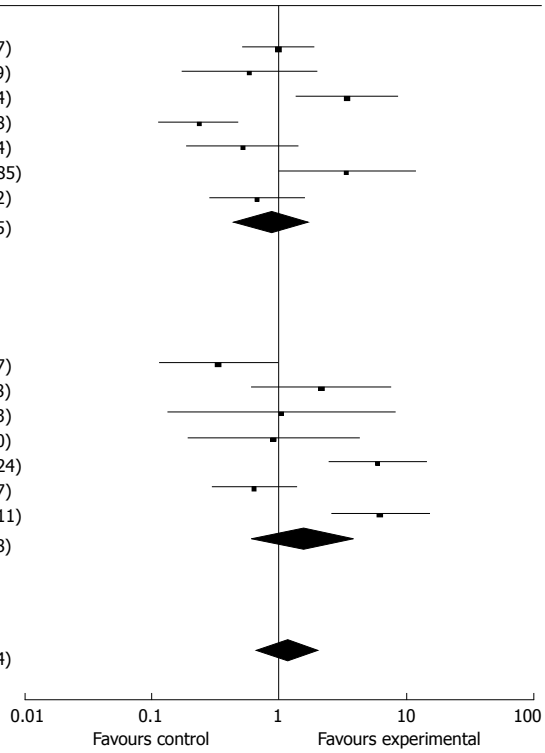


Figure 3 Effect of azithromycin-containing triple therapy *versus* standard triple therapy on the incidence of total side effects (A), diarrhea (B), nausea (C), and taste disturbance (D).

ongoing challenge worldwide. Although a great deal of research has focused on treatment of *H pylori* since the discovery of its crucial role in gastrointestinal disease, currently up to 25% of patients enrolled in clinical trials are treatment failures, even using the widely accepted and efficacious regimens that have gained inclusion in consensus guidelines[41]. A disappointing cure rate of < 80% after 7-d triple therapy was confirmed in the present study. Guidelines often suggest that an acceptable success rate for a particular therapy against *H pylori* infection should be > 80% on an intention-to-treat basis. However, clinical trials with azithromycin have displayed considerable variation with respect to the regimens used and the results obtained. Eradication rates varying between 93% and 22% have been reported[20,30,42]. The results of our meta-analysis demonstrated pooled

A

Study or subgroup	Azithromycin Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, random (95% CI)
3.1.1 Eradication rate-long						
Iacopini *et al*[26], 2005	54	83	53	81	8.3	0.98 (0.52, 1.87)
Kang *et al*[25], 2006	12	17	49	61	6.7	0.59 (0.17, 1.99)
Laine *et al*[33], 1999	26	40	14	40	7.6	3.45 (1.38, 8.64)
Laurent *et al*[31], 2001	24	64	56	78	8.2	0.24 (0.12, 0.48)
Leri *et al*[36], 1997	28	41	33	41	7.3	0.52 (0.19, 1.44)
Lu *et al*[24], 2007	36	40	29	40	6.6	3.41 (0.98, 11.85)
Vcev *et al*[32], 2000	39	55	43	55	7.8	0.68 (0.29, 1.62)
Subtotal (95% CI)		340		396	52.5	0.89 (0.43, 1.85)
Total events	219		277			
Heterogeneity: Tau^2 = 0.75; χ^2 = 27.89, *df* = 6 (*P* < 0.0001); I^2 = 78%						
Test for overall effect: *Z* = 0.31 (*P* = 0.75)						
3.1.2 Eradication rate-short						
Cammarota *et al*[37], 1996	20	35	28	35	7.2	0.33 (0.11, 0.97)
Caselli *et al*[35], 1997	56	60	52	60	6.6	2.15 (0.61, 7.58)
Chen *et al*[29], 2002	22	24	21	23	4.5	1.05 (0.13, 8.13)
Chen *et al*[28], 2004	51	55	42	45	5.7	0.91 (0.19, 4.30)
Ivashkin *et al*[30], 2002	36	50	15	50	7.8	6.00 (2.53, 14.24)
Trevisani *et al*[34], 1998	59	80	65	80	8.1	0.65 (0.31, 1.37)
Zhao *et al*[27], 2005	36	49	15	49	7.7	6.28 (2.61, 15.11)
Subtotal (95% CI)		353		342	47.5	1.56 (0.60, 4.08)
Total events	280		238			
Heterogeneity: Tau^2 = 1.31; χ^2 = 32.98, *df* = 6 (*P* < 0.0001); I^2 = 82%						
Test for overall effect: *Z* = 0.90 (*P* = 0.37)						
Total (95% CI)		693		738	100.0	1.17 (0.64, 2.14)
Total events	499		515			
Heterogeneity: Tau^2 = 1.03; χ^2 = 69.59, *df* = 13 (*P* < 0.00001); I^2 = 81%						
Test for overall effect: *Z* = 0.51 (*P* = 0.61)						
Test for subgroup differences: Not applicable						



B

Study or subgroup	Azithromycin Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, random (95% CI)
5.1.1 Eradication rate-A						
Cammarota *et al*[37], 1996	20	35	28	35	7.2	0.33 (0.11, 0.97)
Chen *et al*[28], 2004	51	55	42	45	5.7	0.91 (0.19, 4.30)
Ivashkin *et al*[30], 2002	36	50	15	50	7.8	6.00 (2.53, 14.24)
Laurent *et al*[31], 2001	24	64	56	78	8.2	0.24 (0.12, 0.48)
Vcev *et al*[32], 2000	39	55	43	55	7.8	0.68 (0.29, 1.62)
Zhao *et al*[27], 2005	36	49	15	49	7.7	6.28 (2.61, 15.11)
Subtotal (95% CI)		308		312	44.3	1.11 (0.32, 3.89)
Total events	206		199			
Heterogeneity: Tau^2 = 2.18; χ^2 = 54.20, *df* = 5 (*P* < 0.00001); I^2 = 91%						
Test for overall effect: *Z* = 0.17 (*P* = 0.87)						
5.1.2 Eradication rate-Lev						
Iacopini *et al*[26], 2005	54	83	53	81	8.3	0.98 (0.52, 1.87)
Kang *et al*[25], 2006	12	17	49	61	6.7	0.59 (0.17, 1.99)
Lu *et al*[24], 2007	36	40	29	40	6.6	3.41 (0.98, 11.85)
Subtotal (95% CI)		140		182	21.7	1.19 (0.51, 2.81)
Total events	102		131			
Heterogeneity: Tau^2 = 0.31; χ^2 = 4.32, *df* = 2 (*P* = 0.12); I^2 = 54%						
Test for overall effect: *Z* = 0.40 (*P* = 0.69)						
5.1.3 Eradication rate-M or T						
Caselli *et al*[35], 1997	56	60	52	60	6.6	2.15 (0.61, 7.58)
Chen *et al*[29], 2002	22	24	21	23	4.5	1.05 (0.13, 8.13)
Laine *et al*[33], 1999	26	40	14	40	7.6	3.45 (1.38, 8.64)
Leri *et al*[36], 1997	28	41	33	41	7.3	0.52 (0.19, 1.44)
Trevisani *et al*[34], 1998	59	80	65	80	8.1	0.65 (0.31, 1.37)
Subtotal (95% CI)		245		244	34.0	1.20 (0.53, 2.69)
Total events	191		185			
Heterogeneity: Tau^2 = 0.51; χ^2 = 11.01, *df* = 4 (*P* = 0.03); I^2 = 64%						
Test for overall effect: *Z* = 0.44 (*P* = 0.66)						
Total (95% CI)		693		738	100.0	1.17 (0.64, 2.14)
Total events	499		515			
Heterogeneity: Tau^2 = 1.03; χ^2 = 69.59, *df* = 13 (*P* < 0.00001); I^2 = 81%						
Test for overall effect: *Z* = 0.51 (*P* = 0.61)						
Test for subgroup differences: Not applicable						

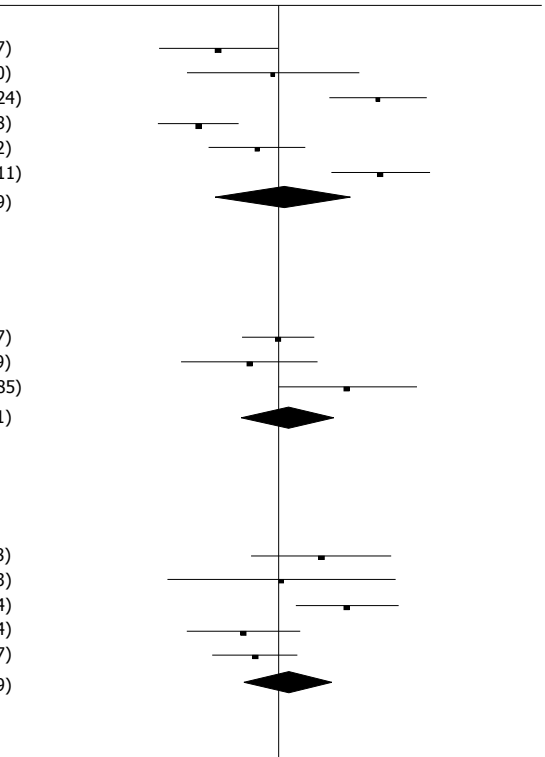


Figure 4 Meta-analysis of eradication rates by treatment course (A) and different antibiotics (B).

H pylori eradication rates were 72.01% and 69.78% for patients with or without azithromycin by intention-to-treat analysis, respectively, and no significant difference was observed between the two regimens.

H pylori has cross resistance to macrolides; e.g. a strain that is resistant to clarithromycin is resistant to every other macrolide. The level of clarithromycin resistance is unfortunately showing a tendency to increase. The effect of drug synergism is of great value in combination treatment to heal *H pylori* infection. Lepper *et al*[43] have demonstrated an *in vitro* synergistic effect of azithromycin and the PPI lansoprazole. They have speculated that this effect might enhance eradication rates even with macrolide-resistant *H pylori* strains, because of the unique pharmacological properties of the combination. Azithromycin could provide a potent anti-*H pylori* effect and could simplify the bulky triple therapy.

Antibiotic-associated gastrointestinal side effects such as diarrhea, nausea, vomiting, bloating and abdominal pain represent a serious drawback of anti-*H pylori* therapy, although they are mild in most cases, but usually result in non-compliance. The quadruple regimen is associated with a relatively high incidence of side effects. In contrast, azithromycin is generally well tolerated, and most side effects associated with its use are mild to moderate in severity and transient. In our systematic review, we found that the total number of side effects with azithromycin supplementation was significantly lower than with azithromycin without regimen: 15.81% *vs* 25.20%; the summary OR was 0.58 (95% CI: 0.41-0.82). Moreover, the incidence of diarrhea (2.13% *vs* 6.98%), nausea (3.85% *vs* 10.14%) and taste disturbance (3.17% *vs* 11.05%) were lower in the azithromycin supplementation group. Our results showed that azithromycin had a positive impact on some *H pylori* therapy-related side effects. Several methodological weaknesses may limit the validity and generalizability of our meta-analysis. For example, there were no studies involving patients from Africa and South America.

In summary, the conclusion of this systematic review and meta-analysis is that, for first-time treatment, azithromycin-containing triple therapy has equal efficacy to that of standard triple eradication therapy. A combination of azithromycin, amoxicillin and a PPI constitutes an encouraging empirical first-line strategy. Furthermore, azithromycin-containing triple therapy showed a lower occurrence of drug-related side effects.

COMMENTS

Background

Colonization with *Helicobacter pylori* (*H pylori*) causes a wide range of upper gastrointestinal disorders in humans. Unfortunately, eradication therapy is not always successful, and can even induce several side effects. Azithromycin has some special attributes that make it a promising compound in the regimens for *H pylori* eradication.

Research frontiers

In first-line therapy, *H pylori* eradication rates using proton-pump inhibitor (PPI)-based triple therapy are about 80%. This signifies that up to 20% of patients are expected to be treatment failures and it could be even higher in areas with a high prevalence of resistant *H pylori* strains. In this study, the authors demonstrated that, for first-time treatment, azithromycin-containing triple therapy has equal efficacy to standard triple eradication therapy.

Innovations and breakthroughs

Recent studies have shown that azithromycin is a promising compound in regimens for *H pylori* eradication. Our meta-analysis demonstrated that azithromycin-containing triple therapy has equal efficacy to standard triple eradication therapy, and has a lower occurrence of side effects. A combination of azithromycin, amoxycillin and a PPI constitutes an encouraging empirical first-line strategy.

Applications

By understanding the effect of azithromycin in *H pylori* eradication, this study represents a new encouraging strategy for first-time treatment, and it could decrease the physiological and pharmacoeconomic burden of second courses of therapy.

Terminology

Azithromycin is a new-generation macrolide and has some special attributes. It is able to reach high gastric concentrations that persist for several days, and therefore may be administered at a dose of 500 mg once daily for only 3 d during 7-d triple eradication therapy.

Peer review

The authors performed a meta-analysis and demonstrated that azithromycin-containing triple therapy has equal efficacy to standard triple *H pylori* eradication therapy. This was an original and good study.

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BRIEF ARTICLE

Performance value of high risk factors in colorectal cancer screening in China

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Abstract

AIM: To analyze the performance value of high risk factors in population-based colorectal cancer (CRC) screening in China.

METHODS: We compared the performance value of the immunochemical fecal occult blood test (iFOBT) and other high risk factors questionnaire in a population sample of 13214 community residents who completed both the iFOBT and questionnaire investigation. Patients with either a positive iFOBT and/or questionnaire were regarded as a high risk population and those eligible were asked to undergo colonoscopy.

RESULTS: The iFOBT had the highest positive predictive value and negative predictive value in screening for advanced neoplasia. The iFOBT had the highest sensitivity, lowest number of extra false positive results associated with the detection of one extra abnormality for screening advanced neoplasias and adenomas. A history of chronic cholecystitis or cholecystectomy, chronic appendicitis or appendectomy, and chronic diarrhea also had a higher sensitivity than a history of adenomatous polyps in screening for advanced neoplasias and adenomas. The sensitivity of a history of chronic cholecystitis or cholecystectomy was highest among the 10 high risk factors in screening for non-adenomatous polyps. A history of chronic appendicitis or appendectomy, chronic constipation, chronic diarrhea, mucous and bloody stool, CRC in first degree relatives, malignant tumor and a positive iFOBT also had higher sensitivities than a history of adenomas polyps in screening for non-adenomatous polyps. Except for a history of malignant tumor in screening for non-adenomatous polyps, the gain in sensitivity was associated with an increase in extra false positive results associated with the detection of one extra abnormality.

CONCLUSION: The iFOBT may be the best marker for screening for advanced neoplasias and adenomas. Some unique high risk factors may play an important role in CRC screening in China.

Key words: Colorectal cancer; Cancer screening; Feces; Occult blood; Risk factors; Predictive value of tests

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INTRODUCTION

The incidence of colorectal cancer (CRC) is increasing rapidly, and there is a similar incidence in some Asian populations to that in Western countries because of a more "Westernized" lifestyle and dietary habits[1]. A relatively long time for malignant transformation, together with improved survival associated with early detection of cancer, makes CRC an ideal target for screening. In source-limited Asian countries, the fecal occult blood test (FOBT) is the first choice for CRC screening because of its better population impact[1]. However, bleeding from cancers and precancerous polyps may be intermittent and

most small colorectal neoplasias do not tend to bleed[2]. Therefore, the immunochemical FOBT (iFOBT) alone inevitably misses some important lesions that do not bleed, or bleed intermittently. The iFOBT and a high risk factors questionnaire approach as primary screening followed by full colonoscopy examination as follow-up screening, has been recommended by the Department of Disease Control, the Ministry of Health of China as the protocol for population-based CRC screening in China[3]. However, the performance value of the iFOBT and a high risk factors questionnaire is still unknown in CRC screening practice in China. According to the definition of high risk factors by American Cancer Society[4], individuals at higher risk for CRC include "individuals with a history of adenomatous polyps (HAP)". Therefore we used the data available in CRC screening practice in China to examine the performance value of each high risk factor using an acknowledged high risk factor - HAP - as a reference in CRC screening practice in China.

MATERIALS AND METHODS

CRC screening protocol in China

The CRC screening protocol of China has been published in a recent study[5]. Subjects (age should be defined as $\geqslant$ 40 years and $\leqslant$ 74 years) who have one or more of the following items are considered to be at high risk of CRC and should undergo colonoscopy: (1) Positive results from the iFOBT; (2) First-degree relatives with CRC; (3) A personal history of cancers or intestinal polyps; (4) 2 or more of the following items: (a) chronic diarrhea; (b) chronic constipation; (c) mucous and bloody stool; (d) history of appendicitis or appendectomy; (e) history of chronic cholecystitis or cholecystectomy; (f) history of psychiatric trauma (e.g. divorce, death of relatives).

Study population

From July 2006 to December 2008, a screening program was implemented following the CRC screening protocol recommended by the Ministry of Health of China, for individuals aged 40-74 years in Xiacheng District, Hangzhou City, China. Among 33778 targeted residents, 16918 declined, 3646 participated only in the questionnaire investigation, and 13214 (39.1%) undertook both the iFOBT and questionnaire investigation.

Study design

The 33778 subjects, aged 40-74 years, who lived in Xiacheng District were enrolled as the target population for our CRC screening practice. Therefore the targeted population can be classified into average, intermediate or high risk individuals. The targeted population was contacted by Chronic Disease Control (CDC) staff to explain the aim of the study, with an invitation to undergo both tests.

The aim of primary screening was to determine the high risk population among the targeted population by the iFOBT and questionnaire approach. Therefore the primary screening test kits included an iFOBT kit (Acon Biotech Co. Ltd., Hangzhou, China), a detailed instruction sheet, a consent form, and a questionnaire containing high risk items. The iFOBT kit used is a qualitative method, with a hemoglobin detection threshold of 200 ng/mL. Participants were asked to prepare a fecal sample from 3 areas of a stool specimen. No specific dietary restriction was stipulated.

The study was approved by the local ethics committee and all participants gave written informed consent.

Identification of high risk subjects

All participants learned how to use the iFOBT kit and how to fill in the questionnaire sheet under guidance of CDC staff. Feces samples were processed and results were obtained at the central laboratory of the local CDC. Processing and evaluation were not automated but were performed by trained staff and under strict quality control (double reading, control of frequency of positive tests, reproducibility). Scrutineers of the iFOBT were blinded to the subject's medical records. The screening procedure was considered positive when at least one of the tests was positive. All positive cases resulting from the primary screening were regarded as high risk subjects and those eligible were invited to the follow-up colonoscopy examinations. The CDC staff and primary care managers were responsible for inviting eligible high risk subjects for further colonoscopy examination.

Colonoscopy examination

Colonoscopy examination was performed by gastroenterologists in endoscopy units of local hospitals and all participants gave written informed consent. The gastroenterologists recorded data using a standard form, including the quality of bowel preparation, the completeness of the colonoscopy, the number, size, and localization of any detected lesions, and the occurrence of complications. All polyps detected during the colonoscopy were immediately removed and/or biopsied for histologic diagnosis by pathologists. Those who were suspected of having CRC or had polyps that could not be removed endoscopically were referred for surgery. If a colonoscopy examination failed because of inadequate bowel preparation, inaccessibility of the cecum, or lack of satisfactory colonoscopy results, a subsequent colonoscopy would be performed within 1 mo.

Pathologic examination

In subjects with more than one polyp, the most advanced pathological lesions or the largest lesion was included in the analysis. An advanced neoplasia was comprised of advanced adenomas (an adenoma measuring 10 mm or more in size, adenomas with high grade dysplasia, or an adenomas with villous component $\geqslant$ 25%) and invasive cancer[6,7]. Non-adenomatous polyps included juvenile polyps, inflammatory polyps and hyperplastic polyps. Invasive cancer was defined as invasion by malignant cells through the muscularis mucosae. Intramucosal carcinoma and carcinoma *in situ* were categorized as high grade dysplasia. Pathologic slides of positive lesions were re-examined and diagnosed by consensus by pathologists.

Statistical analysis

The population of participants in the primary screening

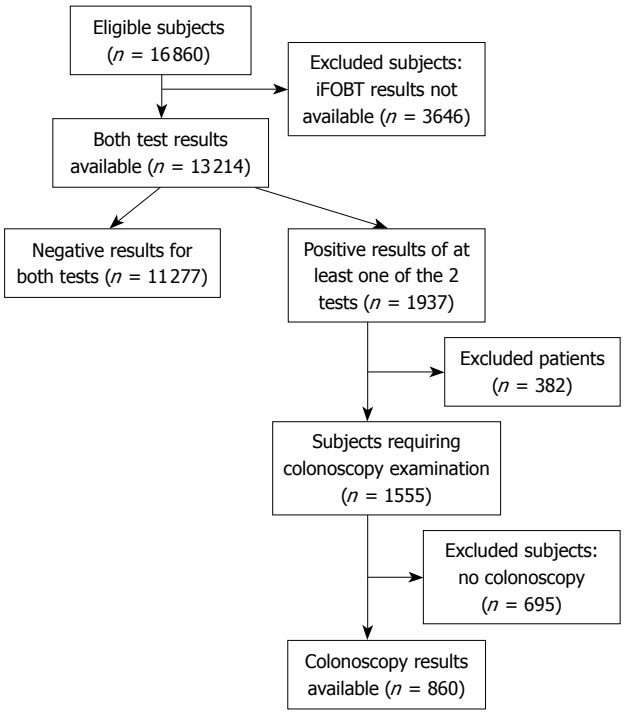

Figure 1 Flow diagram of the study. iFOBT: Immunochemical fecal occult blood test.

comprised all patients who had given written consent (*n* = 16860). Subjects who accepted only questionnaire investigation (*n* = 3646) in the primary screening were excluded from the study. Subjects with at least one test positive (*n* = 1937 were regarded as positive. A colonoscopy examination was not conducted in 382 subjects because of death, health problems, moving or other reasons. A further 695 subjects rejected a colonoscopy examination. Figure 1 provides a flow diagram of the study.

As the confirmatory procedure (colonoscopy examination) was restricted to subjects classified as positive in at least one of 2 tests (iFOBT and questionnaire examination) positive, the sensitivity of each high risk item could not be directly estimated. According to the theory originally suggested by Schatzkin *et al*[8], we therefore compared the relative sensitivity (RSN) by calculating the ratio using HAP as reference. For example, if the number of true positive subjects for one high risk factor is denoted by m and the number of true positive subjects for HAP by n, RSN is calculated as m/n. Confidence intervals (95%) were calculated according to the formulae suggested by Cheng *et al*[9]. Using the theory recommended by Chock, the number of extra false positives associated with the detection of one extra true positive was denoted FP:TP, which was calculated as the ratio between the difference in the number of false positive subjects with one high risk factor versus HAP and the difference in the number of true positive subjects with one high risk factor versus HAP[10].

RESULTS

Colonoscopic results of the iFOBT and questionnaire

A total of 21 CRC (2.4%) cases, 48 (5.6%) subjects with advanced adenomas, 147 (17%) subjects with adenomas, and 54 (6.3%) subjects with non-adenomatous polyps were detected in 860 colonoscopies. Table 1 shows colonoscopic results of the iFOBT and questionnaire. The iFOBT alone diagnosed 13 cases of cancer, 22 cases of advanced adenomas, 44 cases of adenomas, and 9 cases of non-adenomatous polyps while the questionnaire alone found 4 cases of CRC, 21 cases of advanced adenomas, 91 cases of adenomas, and 43 cases with non-adenomatous polyps. Four cases of CRC, 5 of advanced adenomas, 12 of adenomas, and 2 of non-adenomatous polyps were found in both positives. Table 2 shows the results of colonoscopy according to each high risk item. One perforation was recorded after colonoscopy (0.1%).

Table 1 Colonoscopy results of iFOBT and questionnaire *n* (%)

	Colorectal cancer	Advanced adenomas	Adenoma	Non-adenomatous polyps
iFOBT positive only	13 (61.9)	22 (45.8)	44 (29.9)	9 (16.7)
Both positive[1]	4 (19)	5 (10.4)	12 (8.2)	2 (3.6)
Only questionnaire positive	4 (19)	21 (43.8)	91 (61.9)	43 (79.7)
Total	21 (100)	48 (100)	147 (100)	54 (100)

[1]Both iFOBT and questionnaire positive. iFOBT: Immunochemical fecal occult blood test.

The characteristics of the study population

Table 3 shows the characteristics of the study population. Of 13214 subjects who completed both the iFOBT and questionnaire investigation, 1937 had at least one positive test. The positive rate of the questionnaire investigation was markedly higher than that of the iFOBT (11.7% *vs* 3.6%). A colonoscopy examination was not conducted in 382 subjects (19.7%) because of death, health problems, or other reasons. A total of 860 (55.3%) subjects underwent colonoscopy. In subjects undergoing endoscopic examination, 60.3% were iFOBT positive only, 53.2% were questionnaire positive only, 64.9% were positive for both the iFOBT and questionnaire.

Performance of high risk factors in screening for advanced neoplasia

Using HAP as the reference, the sensitivity of iFOBT was highest among all high risk factors. The sensitivities of history of chronic cholecystitis or cholecystectomy, chronic appendicitis or appendectomy, history of chronic diarrhea were also higher than that of HAP. The positive predictive value (PPV) and negative predictive value (NPV) of iFOBT were highest among all high risk factors for advanced neoplasias, but the gain in sensitivity was accompanied by an increase in FP:TP. The iFOBT had the lowest FP:TP ratio (Table 4).

Performance comparison among high risk factors in screening for adenomas

Using HAP as standard, the sensitivity of iFOBT was also highest among 10 high risk factors. Higher sensitivities were also found in history of chronic appendicitis or appendectomy, chronic diarrhea, CRC in first degree relatives, and chronic cholecystitis or cholecystectomy. The

Table 2 Colonoscopy results of high risk questionnaire items

	Cancer	Advanced adenomas	Adenomas	Non-adenomatous polyps	Normal results
iFOBT	17	27	56	11	128
History of malignant tumor	1	3	9	7	41
Colorectal cancer (CRC) in first degree relatives	2	7	29	11	81
History of adenomatous polyps (HAP)	0	9	25	6	48
History of mucous and bloody stool	5	4	15	10	101
History of chronic diarrhea	2	10	29	10	120
History of chronic constipation	0	8	24	13	102
History of chronic appendicitis or appendectomy	3	9	33	14	122
History of chronic cholecystitis or cholecystectomy	3	14	50	19	169
History of psychiatric trauma	4	5	18	2	73

Table 3 Characteristics of the study population *n* (%)

	Subjects with 2 analyzable tests (*n* = 13214)
Sex	
Male	5391 (40.8)
Female	7823 (59.2)
Age (yr)	
40-49	2711 (20.5)
50-59	4704 (35.6)
60-69	3683 (27.9)
70-74	2116 (16.0)
Positive items	
iFOBT	481 (3.6)
History of malignant tumor	172 (1.3)
CRC in first degree relatives	367 (2.8)
HAP	158 (1.2)
History of mucous and bloody stool	430 (3.3)
History of chronic diarrhea	709 (5.4)
History of chronic constipation	902 (6.8)
History of chronic appendicitis or appendectomy	1126 (8.5)
History of chronic cholecystitis or cholecystectomy	1538 (11.6)
History of psychiatric trauma	655 (5)

PPV of the iFOBT was 22.5%, just behind that of HAP (26%). The NPV of iFOBT was highest among all high risk factors. The gain in sensitivity was also accompanied by an increase in FP:TP ratio (Table 4).

Performance comparison of high risk factors in screening for non-adenomatous polyps

Using HAP as standard, a history of chronic cholecystitis or cholecystectomy was the most sensitive marker in screening for non-adenomatous polyps. The sensitivities of other high risk factors except history of psychiatric trauma were also higher than that of HAP. The PPV of history of malignant tumor (10.6%) was highest among all high risk factors in screening for non-adenomatous polyps. Except for history of malignant tumor, the gain in sensitivity was accompanied by increase in the FP:TP ratio (Table 4).

DISCUSSION

Colonoscopy is often regarded as the "gold standard" for detection of CRC[11,12]. Direct colonoscopy screening is the most accurate test for CRC. However because of its potential harm, acceptability[13], availability, and expense[14], the use of colonoscopy as a one-step screening method for the whole targeted population is unpractical in China. The use of noninvasive screening tests in primary screening, such as iFOBT and questionnaire investigation, have been adopted as the large scale population screening program in China[15]. The iFOBT and questionnaire investigation focused on different aspects. The iFOBT can detect bleeding lesions and the questionnaire can find lesions which do not bleed or bleed intermittently. Thus they may have different performance in screening colorectal abnormalities. To the best of our knowledge, the current study is the first analysis comparing the performance value of high risk factors in mass CRC screening in China. Because confirmatory examination was limited to subjects who had at least one positive test, studies calculated the RSN and relative false-positive rate in comparing the 2 screening methods[16,17].

Colorectal adenomatous polyps are recognized as precancerous lesions and are responsible for most cases of CRC[18]. Thus far, the important indicator for transition from adenomas to cancer has been the pathologic characteristics of the advanced adenomas. Thus it is important to find advanced adenomas and block the adenoma-carcinoma sequence in CRC screening. The iFOBT had the highest PPV, NPV and RSN, and the lowest FP:TP ratio in screening for advanced neoplasias, indicating that the iFOBT may be superior to other factors in screening for advanced neoplasias. Though some studies in Asian countries have shown that iFOBT is effective in CRC screening[19,20], iFOBT alone may not be enough in CRC screening, because iFOBT inevitably misses some important lesions which do not bleed or bleed intermittently. A history of chronic cholecystitis or cholecystectomy, chronic appendicitis or appendectomy, and chronic diarrhea also had higher sensitivity than HAP, indicating that these unique Chinese high risk factors can detect a larger number of advanced neoplasias. Some studies have found an increase in the risk of CRC following cholecystectomy for gallstones[21-27]. Cholecystectomy also influences the adenoma to cancer transition, ultimately predisposing to the development of CRC[28]. A study from France supported the hypothesis that the appendix, as a lymphoid organ, plays a protective role in colon carcinogenesis[29]. These unique Chinese high risk factors for CRC may play an important role in screening for advanced neoplasias because of their higher sensitivity, which contributed to detection of a greater number of advanced neoplasias.

Table 4 Comparison of the performance of high risk factors in screening advanced neoplasias, adenomas, non-adenomatous polyps using HAP as reference

	Advanced neoplasias				Adenomas				Non-adenomatous polyps			
	PPV (%)	NPV (%)	RSN	FP:TP	PPV (%)	NPV (%)	RSN	FP:TP	PPV (%)	NPV (%)	RSN	FP:TP
iFOBT	17.70	95.70	4.9 (2.42-9.87)	2.29 (1.36-3.83)	22.50	85.10	2.24 (1.4-3.52)	2.58 (1.37-4.87)	4.40	93	1.8 (0.71-4.58)	16 (3.39-75.58)
HMT	6.10	91.70	0.4 (0.17-1.19)		13.60	82.60	0.36 (0.18-0.7)		10.60	94	1.17 (0.39-3.48)	
FDR	5.70	91.30	1 (0.4-2.519)		18.50	83.20	1.16 (0.69-1.96)	8.25 (0.44-154.47)	7	93.90	1.8 (0.67-4.88)	6.6 (1.16-37.6)
HAP	9.40	92	1		26	84	1		6.30	93.70	1	
MBS	5.70	91.30	1 (0.42-2.39)		9.50	81.20	0.6 (0.33-1.08)		6.30	93.70	1.7 (0.66-4.38)	13.25 (2.02-86.77)
HCD	5.40	91	1.2 (0.54-2.63)	24 (1.87-307.35)	13.90	82	1.16 (0.72-1.88)	18 (1.1-293.1)	4.90	93.30	1.7 (0.71-4.08)	18 (3.2-101.33)
HCC	4.80	91	0.9 (0.35-2.33)		14.30	82.20	0.96 (0.56-1.65)		7.70	94.10	2.2 (0.88-5.49)	7.7 (2.26-26.21)
CAA	5.50	91	1.3 (0.59-2.83)	24.6 (1.63-370.18)	15.10	82.20	1.32 (0.8-2.16)	9.25 (1.52-55.7)	6.40	93.80	2.3 (0.88-5.99)	9.25 (2.96-28.79)
CCC	5.80	87.40	1.9 (0.87-4.12)	15.13 (4.47-51.23)	16.90	82.80	2 (1.26-3.16)	4.84 (2.43-9.65)	6.40	93.80	3.2 (1.38-7.42)	9.3 (4.5-19.22)
HPT	7.60	91.80	1 (0.42-2.39)		15.30	82.60	0.72 (0.43-1.2)		1.70	93	0.33 (0.07-1.63)	

HMT: History of malignant tumor; FDR: History of CRC in first degree relatives; MBS: History of mucous and bloody stool; HCD: History of chronic diarrhea; HCC: History of chronic constipation; CAA: History of chronic appendicitis or appendectomy; CCC: History of chronic cholecystitis or cholecystectomy; HPT: History of psychiatric trauma; PPV: Positive predictive value; NPV: Negative predictive value; RSN: Relative sensitivity; FP:TP ratio: The ratio between the difference in the number of false positive subjects with one high risk factor *vs* HAP and the difference in the number of true positive subjects with one high risk factor *vs* HAP. Values for RSN and FP:TP are mean (95% CI). RSN > 1: Sensitivity of the high risk factor is greater than that of HAP.

It would be unsafe to ignore adenomas < 10 mm because 30% of cancer is derived from 6-9 mm adenomas[30]. The questionnaire detected a greater number of adenomas than the iFOBT in our screening study. We also found that the iFOBT still had the highest sensitivity among all high risk factors, followed by history of chronic cholecystitis or cholecystectomy, chronic appendicitis or appendectomy, and chronic diarrhea. The iFOBT may also be superior to other factors in screening for adenomas because of high PPV and NPV, high RSN and low FP:TP ratio. Higher sensitivities indicated the important performance value of these unique Chinese high risk factors.

A history of chronic cholecystitis or cholecystectomy was the most sensitive marker in screening for non-adenomatous polyps, followed by history of chronic appendicitis or appendectomy, and chronic constipation. Though subjects with non-adenomatous polyps were not regarded as having increased risk of CRC, these polyps do not require surveillance colonoscopy, they may serve as a precursor to CRC in subjects with specific genetic and other molecular characteristics[31-34]. Thus it would be unsafe to ignore these polyps.

The study had several drawbacks. Firstly, to evaluate screening test performances among the general population, the ideal is to obtain sensitivity and specificity for all individuals. Because only eligible high risk subjects were invited and only about 55% of population accepted the colonoscopy examination, these results may not be completely representative of the general population. Secondly, although all the study population accepted both the iFOBT and questionnaire investigation, the colonoscopy uptake rate of the iFOBT positive only was higher than that of the questionnaire positive only. This would slightly overestimate the RSN of the iFOBT.

HAP, an acknowledged high risk factor was used as the reference to calculate the relative ratio in this study. Therefore the other variables being compared may be underestimated. Even so, the iFOBT and some unique Chinese high risk factors - history of chronic cholecystitis or cholecystectomy, chronic appendicitis or appendectomy, and history of chronic diarrhea - still play an important role because of the higher sensitivities than that of HAP. The iFOBT may be superior to other factors in screening for advanced neoplasias and adenomas.

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COMMENTS

Background

The immunochemical fecal occult blood test (iFOBT) and high risk factors questionnaire approach as primary screening followed by full colonoscopy examination as follow-up screening, has been recommended as the colorectal cancer (CRC) screening guideline for population-based CRC screening in China. The performance value of the iFOBT and the high risk factors questionnaire is still unknown in CRC screening practice in China.

Research frontiers

The limitation of the iFOBT is its low sensitivity for CRC. High risk factors for CRC among a Chinese natural population have been identified through a meta-analysis. The major advantage of the high risk factors questionnaire investigation is that it can detect lesions that do not bleed or bleed intermittently.

Innovations and breakthroughs

This is believed to be the first study comparing the performance value of high risk factors in mass CRC screening in China. In this study, because participants with at least one positive factor were asked to undergo colonoscopy, the sensitivity of each high risk item could not be directly estimated. The authors therefore compared sensitivities by calculating the relative sensitivity using HAP (history of adenomatous polyps, an acknowledged high risk factor) as a reference.

Applications

The study suggests that the iFOBT may be the best marker for screening advanced neoplasias and adenomas. Some unique Chinese high risk factors (history of chronic cholecystitis or cholecystectomy, chronic appendicitis or appendectomy, and history of chronic diarrhea) may play an important role in CRC screening in China because of higher sensitivities than that of HAP.

Peer review

The authors have done much work in this study. The study is worthwhile and well performed.

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BRIEF ARTICLE

High expression of osteoglycin decreases gelatinase activity of murine hepatocarcinoma Hca-F cells

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Abstract

AIM: To investigate the possible correlation between osteoglycin expression and gelatinase activity of mouse hepatocarcinoma Hca-F cells.

METHODS: A eukaryotic expression plasmid pIRESpuro3 osteoglycin(+) was constructed and transfected into Hca-F cells to investigate the possible correlation between osteoglycin expression and gelatinase activity of Hca-F cells cultured with extract of lymph node, liver, spleen or in DMEM medium. The activity of gelatinases was examined through zymographic analysis.

RESULTS: High expression of osteoglycin attenuated the gelatinase activity of Hca-F cells cultured with extract of lymph node, and at the same time, decreased the metastatic potential of Hca-F cells to peripheral lymph nodes *in vivo*.

CONCLUSION: High expression of osteoglycin decreases the gelatinase activity of Hca-F cells cultured with extract of lymph node; regulation of gelatinase activity might be one of mechanisms that osteoglycin contributes to lymphatic metastasis suppression.

Key words: Osteoglycin; Transfection; Hepatocellular carcinoma; Neoplasm metastasis; Genes; Gelatinases

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INTRODUCTION

Most cancer lesions metastasize through the lymphatic system and the status of regional lymph nodes is the most important indicator of a patient's prognosis[1]. But the molecular mechanism of lymphatic metastasis remains unclear. Hca-P and Hca-F are synogenetic mouse hepatocarcinoma cell lines, when inoculated subcutaneously in 615-mice, they metastasized only to the lymph nodes but not to other organs, Hca-P cells illustrated a low metastatic potential (lymphatic metastasis rate < 30%), while Hca-F cells showed a high metastatic potential (lymphatic metastasis rate > 80%)[2,3]. In our previous study, we found that osteoglycin was highly expressed in Hca-P cells and lowly expressed in Hca-F cells with suppressively subtracted hybridization (SSH) technique. Osteoglycin (OGN) is a member of proteoglycans (PGs) called small leucine-rich proteoglycans (SLRPs) residing in the extracellular matrix of connective tissues which are involved in matrix assembly, cellular growth and migration[4]. There are few reports about the relationship between osteoglycin and tumor metastasis. We subsequently transfected osteoglycin into Hca-F cells and found that high expression of osteoglycin inhibited the metastatic behavior of Hca-F cells[5]. However, the mechanism of osteoglycin regulating metastasis is elusive.

Gelatinases/type Ⅳ collagenases belong to matrix metalloproteinase (MMP) family, including gelatinase A (also known as MMP2, 72 kDa) and gelatinase B (also known as MMP9, 92 kDa), they are secreted in a proenzyme form and activated extracellularly[6]. Gelatin-

ases mainly degrade collagen Ⅳ and a number of other ECM proteins, such as Col Ⅰ, Ⅴ, Ⅶ, Ⅸ, fibronectin, laminin, elastin and vitronectin[7]. As the most frequently studied MMPs in tumor research, gelatinases are suggested to play critical roles in tumor invasion and metastasis[8].

In this study, we resorted to gene transfection technique to explore the possible correlation between osteoglycin expression and gelatinase activity of murine hepatocarcinoma Hca-F cells with a high metastatic potential. We found that high expression of osteoglycin decreased the gelatinase activity of Hca-F cells cultured with extract of lymph node, and at the same time, decreased the metastatic potential of Hca-F cells to peripheral lymph nodes *in vivo*; regulation of gelatinase activity might be one of mechanisms that osteoglycin contributes to lymphatic metastasis suppression.

MATERIALS AND METHODS

Cell culture and animals

Mouse hepatocarcinoma Hca-P cells and Hca-F cells (established by Department of Pathology, Dalian Medical University) were cultured in DMEM (Invitrogen) supplemented with antibiotics (1 × penicillin/streptomycin 100 U/mL, Invitrogen), 10% FBS (Invitrogen) and cultured in a humidified incubator at 37℃ with 50 mL/L CO_2; inbred 615-mice (male, 8 wk old) were provided by Animal Facility of Dalian Medical University.

Construction of targeting vector

The osteoglycin coding sequence was amplified by polymerase chain reaction (PCR). Briefly, total RNA from 1×10^7 Hca-F cells was isolated with Trizol (Invitrogen). A High Fidelity PrimeScript RT-PCR kit (TaKaRa) was used to synthesize the cDNA according to the manufacturer's protocol. PCR was carried out with primer sets P1, 5'-GAATTCATGGAGACTGTGCACTCTA-3' (forward), and P2, 5'-GCGGCCGCTTAGAAGTATGACCCTA-3' (reverse), containing *Eco*R Ⅰ and *Not* Ⅰ sites, respectively (underlined). Using obtained cDNA as a template, PCR was carried out under the following conditions: 30 cycles of denaturation for 10 s at 98℃, annealing for 15 s at 55℃, and extension for 60 s at 72℃. After digestion by *Eco*R Ⅰ and *Not* Ⅰ enzymes, the PCR product was cloned into pIRESpuro3 vector digested by the same enzymes and designated as pIRESpuro osteoglycin(+). Sequence and orientation were confirmed by DNA sequencing using a BigDye Terminator V3.1 cycle sequencing kit (Applied Biosystems).

Cell transfection and screening

Hca-F cells incubated in antibiotic-free medium with 10% FBS (Invitrogen) were transferred to a 6-well culture plate and incubated at 37℃, CO_2 incubator to obtain 60%-80% confluence, and then were stably transfected with pIRESpuro3 and pIRESpuro3 osteoglycin(+) using *Trans*IT-LT1 Transfection Reagent (TaKaRa) according to the protocol provided by the manufacturer. Two μg plasmid DNA was added to each transfection. The transfected Hca-F cells were selected by puromycin (Clontech) for 2 wk and maintained in medium containing 0.5 mg/L puromycin.

RT-PCR analysis

For RT-PCR analysis of osteoglycin mRNA levels, total RNA was isolated from cells using Trizol (Invitrogen) and cDNA was synthesized with High Fidelity PrimeScript™ RT-PCR Kit (TaKaRa) according to the manufacturer's instruction. The sequences of the primers were as follows: F1: 5'-TTCTCCTGCTACTCTTCGTG-3' and R1: 5'-AAGCAGACACACAACAGGCA-3' for osteoglycin; and F1: 5'-CGGGACCTGACAGACTACCT-3' and R1: 5'-AGCACTGTGTTGGCATAGAG-3' for β-actin, respectively. PCR analysis was performed under the following conditions: 30 cycles of denaturation for 10 s at 98℃, annealing for 15 s at 55℃, and extension for 30 s at 72℃. The amplified products were analyzed by agarose gel electrophoresis using 1.6% gel, followed by ethidium bromide staining. The bands were analyzed with LabWorks (UVP GDS-800 Version 4.0).

Western blotting analysis

Western blotting analysis was carried out to evaluate osteoglycin protein levels. Cellular protein was extracted with lysis buffer [20 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 1 mmol/L $MgCl_2$, 2 mmol/L EGTA, 10% glycerol, 0.15% sodium dodecylsulfate, 1% deoxycholate, 1% Triton X-100, and 1% anti-protease cocktail (Sigma)]. The extracted proteins were subjected to 10% sodiumdodecylsulfate-polyacrylamide gel electrophoresis, blotted onto polyvinylidene difluoride membranes (Invitrogen), then probed with goat anti-mouse osteoglycin polyclonal antibody and β-actin monoclonal antibody (Santa Cruz) followed by secondary antibody conjugated to horseradish peroxidase (Santa Cruz) and detected by enhanced chemiluminescence (Amersham Biosciences). The bands were analyzed with LabWorks (UVP GDS-800 Version 4.0).

In vivo tumor metastasis assay

Ninety inbred 615-mice were randomly divided into 3 groups. Hca-F cells (F), Hca-F cells transfected with pIRESpuro3 (F0), or Hca-F cells transfected with pIRESpuro3 osteoglycin(+) [F(+)] were inoculated subcutaneously at 2×10^6 tumor cells of approximately 0.05 mL cell suspension into the left foot of each mouse in each group. They were terminated on the 28th day after inoculation, the implanted tumor and their axillary lymph nodes, inguinal lymph nodes, and popliteal lymph nodes were hematoxylin eosin (HE) stained and examined under microscope. The mouse which had at least one metastatic axillary lymph node or one metastatic inguinal lymph node or one metastatic popliteal lymph node was considered as a metastatic mouse. The lymph node metastatic rate of tumor-burden mice = metastatic mice/total mice.

The lymph node metastatic rates of F, F0 and F(+) cells burden mice were calculated. The number of positive lymph nodes per mouse was also evaluated.

Zymographic analysis

The F, Hca-P (P), F0 and F(+) cells were put into different wells at 5×10^5, and then added 50 mg extract of lymph node, liver or spleen respectively. The Dulbecco's Modified Eagle Media (DMEM) was placed into each well up to 1 mL. DMEM medium containing only F, P, F0 or F(+) cells, and DMEM medium added only extracts of lymph node, liver or spleen served as controls. These cells were cultured at 37℃ for 24 h. The supernatant of cultured cells was collected by centrifugation at 3000 × *g*. Gelatinases contained in supernatants of each cell with or without extracts of lymph node, liver or spleen were detected through zymographic analysis according to the method described by Fridman[9]. The bands were analyzed with LabWorks (UVP GDS-800 Version 4.0).

Statistical analysis

Data were presented as means ± SD and analyzed by the Student's *t* test, analysis of variance and χ^2 test using SPSS 11.5. $P < 0.05$ was considered statistically significant.

RESULTS

Osteoglycin expression at mRNA and protein level

The relative mRNA and protein levels of osteoglycin were determined by RT-PCR and Western blotting analysis, respectively. Compared with F and F0 cells, F(+) cells showed significantly higher expression of osteoglycin at both mRNA and protein levels; however, no significant difference of osteoglycin expression was found between F0 and F cells. Transfection of osteoglycin into Hca-F cells resulted in high expression of osteoglycin at both mRNA and protein levels. Osteoglycin was highly expression at both mRNA and protein levels in P cells (Figure 1).

In vivo tumor metastasis assay

F, F0 and F(+) cells were injected subcutaneously into the left foot of 615-mice. The implanted tumors were palpable on the 7th day after inoculation. On the 28th day after inoculation, 53.3% (16/30) F(+) cells burden mice developed lymphatic metastasis, while 80% (24/30, $P < 0.05$) F cells burden mice and 83.3% (25/30, $P < 0.05$) F0 cells burden mice developed lymphatic metastasis. Hca-F cells with transfected osteoglycin showed significant decrease in metastasis potential to lymph node (Figure 2). The result supported the fact that osteoglycin acted as a tumor lymphatic metastasis suppressed gene.

No significant difference was found in the number of positive lymph nodes per mouse in F(+), F and F0 cells burden mice.

Zymographic analysis

When cultured in DMEM, no cell produced any gelatinase (no gelatinase was detected in the supernatant of each cell). However, when cultured with extract of lymph node, all cells produced gelatinases (Pro-MMP-9, MMP-9 active, Pro-MMP-2 and MMP-2 active were detected in the supernatant of each cell). The quantity of gelatinases produced by tumor cells were closely associated with the metastatic potential of each tumor cell (quantity of

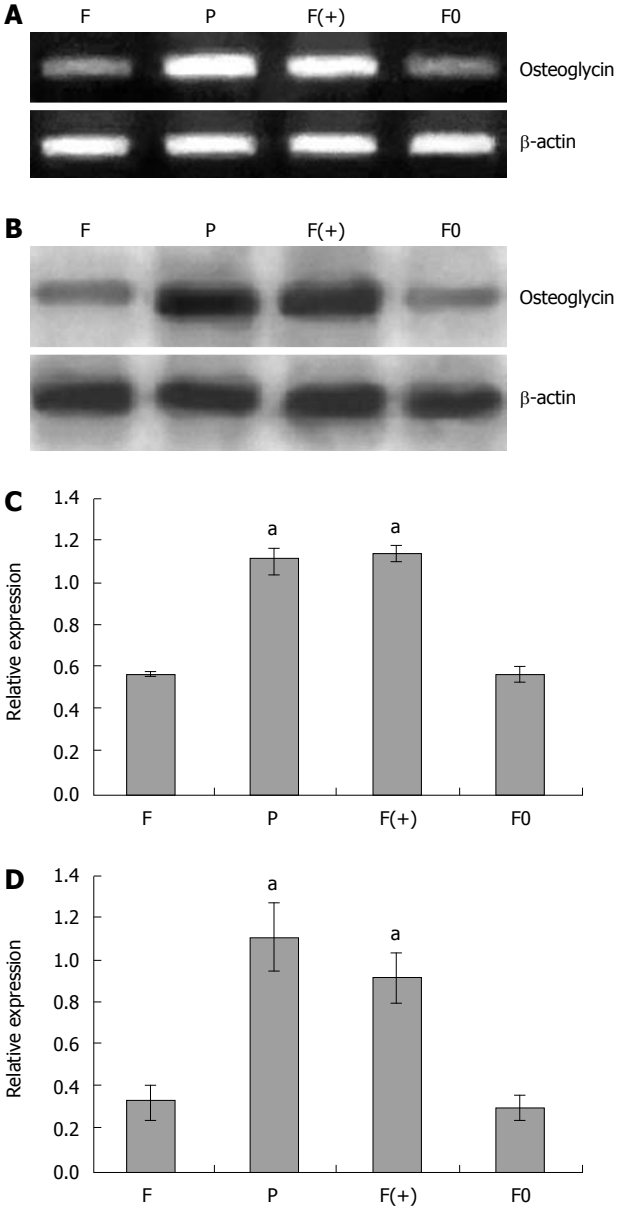

Figure 1 Analysis of osteoglycin expression. RT-PCR analysis (A) and Western blot analysis (B) of osteoglycin expression in mouse hepatocarcinoma cells; relative signal intensities of osteoglycin mRNA (C) and protein (D) levels were normal as against those of β-actin by LabWorks (UVP GDS-800 Version 4.0) analysis (compared with F cells, $^aP < 0.05$). F: Hca-F cells; P: Hca-P cells; F(+): Hca-F cells transfected with pIRESpuro3 osteoglycin(+); F0: Hca-F cells transfected with pIRESpuro3. β-actin was used as an internal control.

MMP2 and MMP9 detected in the supernatant of F and F0 cells were much higher than those detected in F(+) and P cells ($P < 0.05$). High expression of osteoglycin *via* transfecion of osteoglycin attenuated the secretion of gelatinases in Hca-F cells cultured with extract of lymph node (quantities of MMP2 and MMP9 detected in the supernatant of F(+) cells were much lower than those detected in F and F0 cells ($P < 0.05$). The extract of lymph node did not contain any gelatinase (Figure 3). Gelatin lysis bands were found in the zymograms of the supernatant of all cells cultured with extract of liver, and the same gelatin lysis bands were found in the zymograms of the extract of liver, and their intensities were almost the same (Figure 4); gelatin lysis bands were also found in the

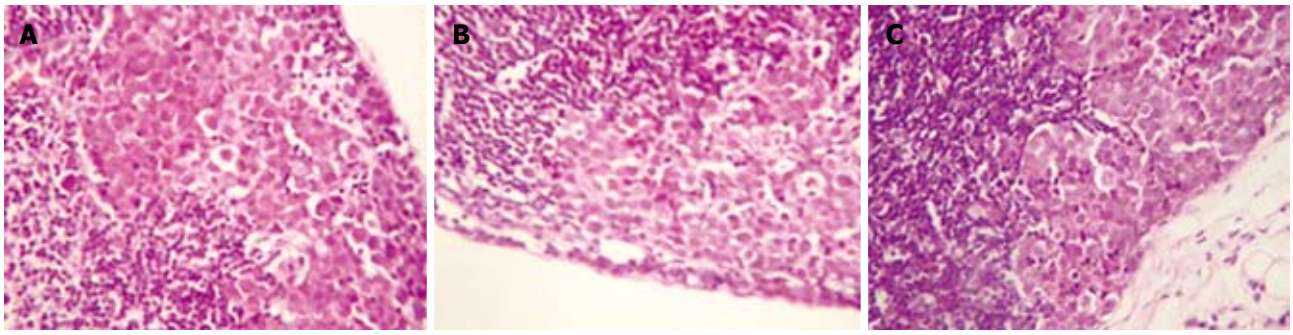

Figure 2 Metastatic lymph nodes of tumor-burden mice inoculated with Hca-F cells (A), Hca-F cells transfected with pIRESpuro3 (B), or Hca-F cells transfected with pIRESpuro3 osteoglycin(+) (C). Lymph nodes of tumor-burden mice were HE stained and examined under microscope.

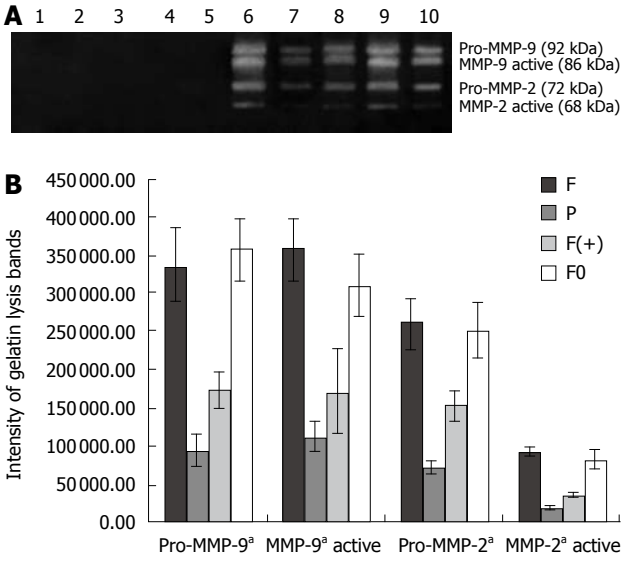

Figure 3 Zymographic analysis of MMPs activity of tumor cells in DMEM with or without lymph node extract (A); the intensity of gelatin lysis bands obtained by scanning densitometry (LabWorks UVP GDS-800 Version 4.0, multiple comparisons, $^aP < 0.05$) (B). 1. L; 2. F; 3. P; 4. F(+); 5. F0; 6. F; 7. P; 8. F(+); 9. F0. 10. Type Ⅳ collagenases. 1. L: lymph node extract; 2-5: cells in DMEM. 6-10: cells cultured with lymph node extract. F: Hca-F cells; P: Hca-P cells; F(+): Hca-F cells transfected with pIRESpuro3 osteoglycin(+); F0: Hca-F cells transfected with pIRESpuro3.

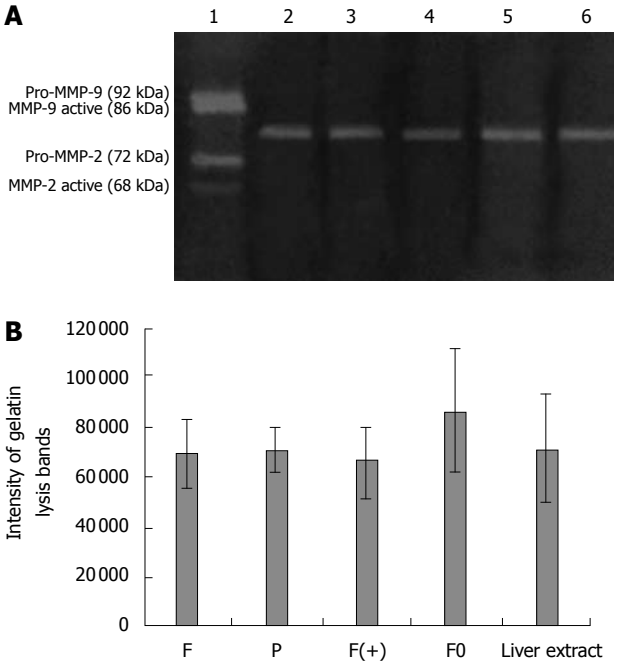

Figure 4 Zymographic analysis of MMPs activity of tumor cells in liver extract (A); the intensity of gelatin lysis bands obtained by scanning densitometry (LabWorks UVP GDS-800 Version 4.0) (B). 1. Type Ⅳ collagenases; 2. F; 3. P; 4. F(+); 5. F0; 6. Liver extract. F: Hca-F cells; P: Hca-P cells; F(+): Hca-F cells transfected with pIRESpuro3 osteoglycin(+); F0: Hca-F cells transfected with pIRESpuro3.

zymograms of the supernatant of all cells cultured with extract of spleen, and in the zymograms of the extract of spleen, with similar intensities (Figure 5). Therefore, we think that all cells in the liver and spleen did not produce any gelatinases.

DISCUSSION

The metastatic potential of tumor cells is believed to be regulated by interactions between the tumor cells and their extracellular environment (extracellular matrix)[10,11]. Being a matrix molecule, osteoglycin participates in the organization and regulation of the extracellular matrix and might influence the tumor metastasis, as exemplified by studies *in vivo* that osteoglycin played a role in collagen fibrillogenesis[12,13], a process essential in metastasis[11,12]. In addition to its extracellular matrix functions, osteoglycin, like other members of SLRPs, also plays a role in regulation of cell biological behavior[4]. As illustrated in the literature, the expression of mimecan was high at mRNA level in corneal keratocytes cultured in low-serum or serum-free media, but was attenuated if these cells were cultured in media containing serum[14]. Osteoglycin mRNA was absent or at a low level in the majority of cancer cell lines and tumors[15]. Bioactive such as p53, basic fibroblast growth factor, interferon-γ and bone morphogenetic protein-1/tolloid-related metalloproteinases interacted with osteoglycin[16-20]. In the earlier studies, we found that osteoglycin was highly expressed in Hca-P cells and lowly expressed in Hca-F cells, and that osteoglycin acted as a tumor lymphatic metastasis suppressed gene[5]. However, no data identified intrinsic mechanism for osteoglycin regulation of tumor lymphatic metastasis.

Hca-P and Hca-F are syngenetic mouse hepato-

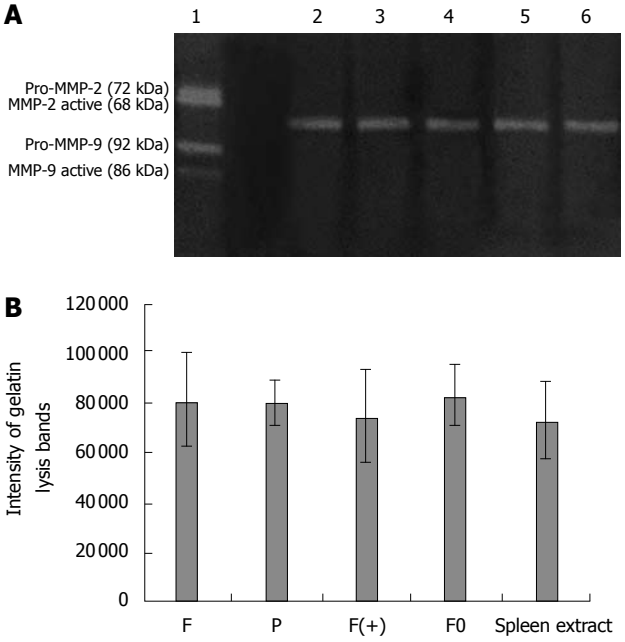

Figure 5 Zymographic analysis of MMPs activity of tumor cells in spleen extract (A); the intensity of gelatin lysis bands obtained by scanning densitometry (LabWorks UVP GDS-800 Version 4.0) (B). 1. Type IV collagenases; 2. F; 3. P; 4. F(+); 5. F0; 6. spleen extract. F: Hca-F cells; P: Hca-P cells; F(+): Hca-F cells transfected with pIRESpuro3 osteoglycin(+); F0: Hca-F cells transfected with pIRESpuro3.

carcinoma cell lines presenting a specific potential of lymphatic metastasis with a significant difference in their potential of metastasis[2,3], which provide good experimental models for lymph node metastasis.

Cell adhesion to extracellular matrices is a determinant for cell migration and invasion[21,22]. Osteoglycin, being a matrix molecule, as we once assumed, would probably affect adhesive capacity of tumor cells, whereby influencing tumor migration and invasion. However, our previous work showed that adhesion was not responsible for the contribution of osteoglycin to lymphatic metastasis inhibition[5]. As the main mediators of extracellular matrix degradation, gelatinases play an important role in tumor metastasis as demonstrated in gastrointestinal cancer[23,24], breast cancer[25], hepatocarcinoma[26], *etc*. Inhibition of the gelatinase activity can reduce the metastatic potential of cancer cells[27]. In the present study, high expression of osteoglycin *via* osteoglycin transfection attenuated the secretion of gelatinases (Pro-MMP-9, MMP-9 active, Pro-MMP-2 and MMP-2 active) in Hca-F cells cultured with extract of lymph node, and at the same time, decreased the metastatic potential of Hca-F cells to peripheral lymph nodes *in vivo*, which suggested that regulation of gelatinase activity might be one of mechanisms that osteoglycin contributes to lymphatic metastasis suppression. Moreover, osteoglycin expression only influenced gelatinase activity of Hca-F cells cultured with extract of lymph node, but failed to influence gelatinase activity of Hca-F cell cultured with extracts of liver and spleen or in DMEM medium, demonstrating a lymph node environment-selective metastasis suppression, which further supported the fact that osteoglycin acted as lymphatic metastasis suppression gene. The mechanism of osteoglycin impact on gelatinases is unclear. Some of the SLRPs members bind and modulate TGF-β and cytokines such as TNF-α[28,29] and play roles in EGFR activation pathway and the NF-κB signal transduction system as well[30,31]. And these bioactives (TGF-β, TNF-α, EGF and NF-κB) are also the regulators of gelatinase activity[6,32], which implicates that SLRPs might involve in the regulation of gelatinase activity. Further studies are needed to clarify the interaction between gelatinases and osteoglycin.

COMMENTS

Background

Lymphatic metastasis is responsible for the early stage of tumor metastasis and acts as the most important indicator of a patient's prognosis. But the molecular mechanism of lymphatic metastasis remains poorly understood.

Research frontiers

The metastatic potential of tumor cells is believed to be regulated by interactions between the tumor cells and their extracellular environment (extracellular matrix). Matrix molecules play important roles in tumor metastasis. Osteoglycin, as one of matrix molecules, is suggested to play a part in matrix assembly, cell growth and migration. However, there has been no report on osteoglycin and tumor metastasis.

Innovations and breakthroughs

The authors first report that osteoglycin acted as a tumor lymphatic metastasis suppression gene, and regulation of gelatinase activity might be one of mechanisms that osteoglycin contributes to lymphatic metastasis suppression.

Applications

This study may help for therapeutic intervention in tumor metastasis.

Terminology

Osteoglycin belongs to a small leucine-rich proteoglycan (SLRP) gene family, as one of the matrix molecules, it is reported to participate in the organization and regulation of the extracellular matrix. In addition to its extracellular matrix functions, osteoglycin, like other members of SLRPs, also plays a role in regulation of cell biological behavior, cell growth and migration, *etc*.

Peer review

In this study, it was observed that osteoglycin upregulation, induced by its transfection into mouse hepatocarcinoma Hca-F cells, results in a decrease in gelatinase activity and metastatic potential of Hca-F cells. The effect of osteoglycin transfection on gelatinase activity has been convincingly demonstrated.

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CASE REPORT

Meckel's diverticulum manifested by a subcutaneous abscess

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Abstract

This case report describes an extremely rare complication of a Meckel's diverticulum: enterocutaneous fistula of the diverticulum. The presence of Meckel's diverticulum is a well known entity, but subcutaneous perforation of the diverticulum is very rare. Here we report the case of a patient with the complaint of a right lower quadrant abscess, preoperatively diagnosed as enterocutaneous fistula, which was determined intraoperatively to be a fistula resulting from Meckel's diverticulum.

Key words: Meckel's diverticulum; Enterocutaneous fistula; Abscess

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Karatepe O, Adas G, Altıok M, Ozcan D, Kamali S, Karahan S. Meckel's diverticulum manifested by a subcutaneous abscess. *World J Gastroenterol* 2009; 15(48): 6123-6125 Available from: URL: http://www.wjgnet.com/1007-9327/15/6123.asp DOI: http://dx.doi.org/10.3748/wjg.15.6123

INTRODUCTION

Meckel's diverticulum is the most common congenital anomaly of the gastrointestinal tract[1-4]. It is an outpouching of the distal ileum located at the antimesenteric border usually within 45-60 cm of the ileocecal valve[4]. The majority of Meckel's diverticulum cases are asymptomatic although they can, occasionally, cause complications such as bleeding, intestinal obstruction and/or inflammatory process[1-4]. The presentation as an abscess in the abdominal wall is a rare clinical entity. Because of insidious onset and subtle clinical signs of the resulting abscess, the diagnosis of such cases is often delayed. Meckel's diverticula such as those forming extensive abscesses may sometimes become complicated and require a prolonged treatment period. These complications should be kept in mind in order to avoid further sequelae. The diagnosis is difficult and is usually performed at surgery[2]. We here report a patient who developed a fistula between an inflamed Meckel's diverticulum and subcutaneous tissue, which is the first case in the literature.

CASE REPORT

A 32-year-old woman with no previous abdominal surgery presented with a 48-h history of right abdominal pain, fever, nausea, vomiting and a palpable mass. There was no significant medical history. Examination of the abdomen showed a marked distension without peritonitis and a mass 8 cm × 5 cm in size. There were significant inflammatory signs such as local heat, swelling, edema and tenderness involving the entire right abdominal wall. Her body temperature was 38.5℃. Laboratory findings showed a white blood cell count of 28000, hemoglobin value of 115 gm/L, and a platelet count of 250000. The other laboratory investigations, including electrolytes and urinalysis, were within normal limits. Chest and abdominal X-ray revealed no abnormality. An abdominal ultrasound scan suggested a fluid collection in the right lower abdominal quadrant, which was evaluated to be an abscess. The magnatic resonance imaging (MRI) finding indicated a mass with a suspicious intraabdominal connection (Figure 1). The clinical, radiological and laboratory findings revealed the presence of a right lower abdominal quadrant abscess as the source of sepsis. The abdomen was opened immediately and the exploration revealed a Meckel's diverticulum with a connection to the right lower quadrant mass (Figure 2A-C). There was not any fluid contamination in the abdomen. A resection of the necrotic segment, Meckel's diverticulum and a functional end-to-

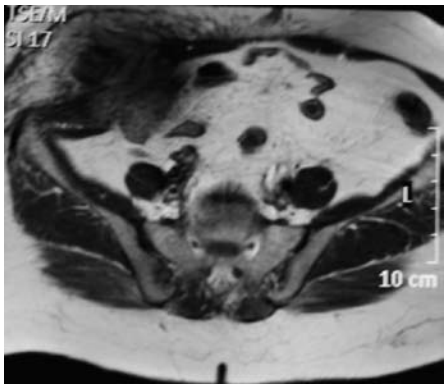

Figure 1 Pre-operative magnatic resonance imaging view of our case.

end anastomosis of the bowel were completed. Afterwards, debridement of the non-viable abdominal wall muscle groups and drainage of the abscess cavity were performed (Figure 2D). The bacterial culture revealed *Escherichia coli* and *Klebsiella pneumoniae* infection. The diverticulum was confirmed as Meckel's diverticulum by histological examination (Figure 3). The patient recovered without incident and was discharged after five days in hospital.

DISCUSSION

Meckel's diverticulum is the most common end result of the spectrum of omphalomesenteric duct anomalies, which also include umbilical-ileal fistula, omphalomesenteric duct sinus, omphalomesenteric duct cyst, fibrous connection of the ileum to the umbilicus, and Meckel's diverticulum[1,2,5]. The lifetime risk of complications is estimated to be about 4%; 40% of which occur in children[1]. The presentation in children is most commonly with gastrointestinal bleeding from ectopic gastric or pancreatic mucosa, whereas adults more commonly develop obstruction, intussusception, ulceration, vesicodiverticular fistula, or tumor[1-6]. Regarding our case, we confronted a rare presentation of Meckel's diverticulum, which first emerged as enterocutaneous fistula. The etiopathogenesis of the disease can be explained by the direct contamination of the right anterior abdominal wall by an inflamed Meckel's diverticulum. The spread of resultant sepsis along the abdominal wall muscles, preperitoneal space and downward behind the inguinal ligament into the thigh, thus presented clinically as an abscess. A review of the literature suggests certain intra-abdominal inflammatory pathologies in the etiology of enterocutaneous fistula, such as diverticulitis, acute appendicitis, Crohn's disease, colorectal carcinoma, rectal trauma and primary staphylococcal abscess[7,8]. Therefore, we should define our case as a new reason for enterocutaneous fistula (Table 1). In the literature another interesting complication of Meckel's diverticulum has been described by Graziotti *et al*[9] They presented a case where an ingested foreign body (chicken bone) entrapped in a Meckel's diverticulum eventually caused a vesicoenteric fistula.

Much rarer complications of Meckel's diverticula include neoplasms, with the most common being benign tumors reported as leiomyomas, angiomas and lipomas. Malignant neoplasms reported include adenocarcinomas, which commonly originate from the gastric mucosa, sarcoma, and carcinoid tumor[10].

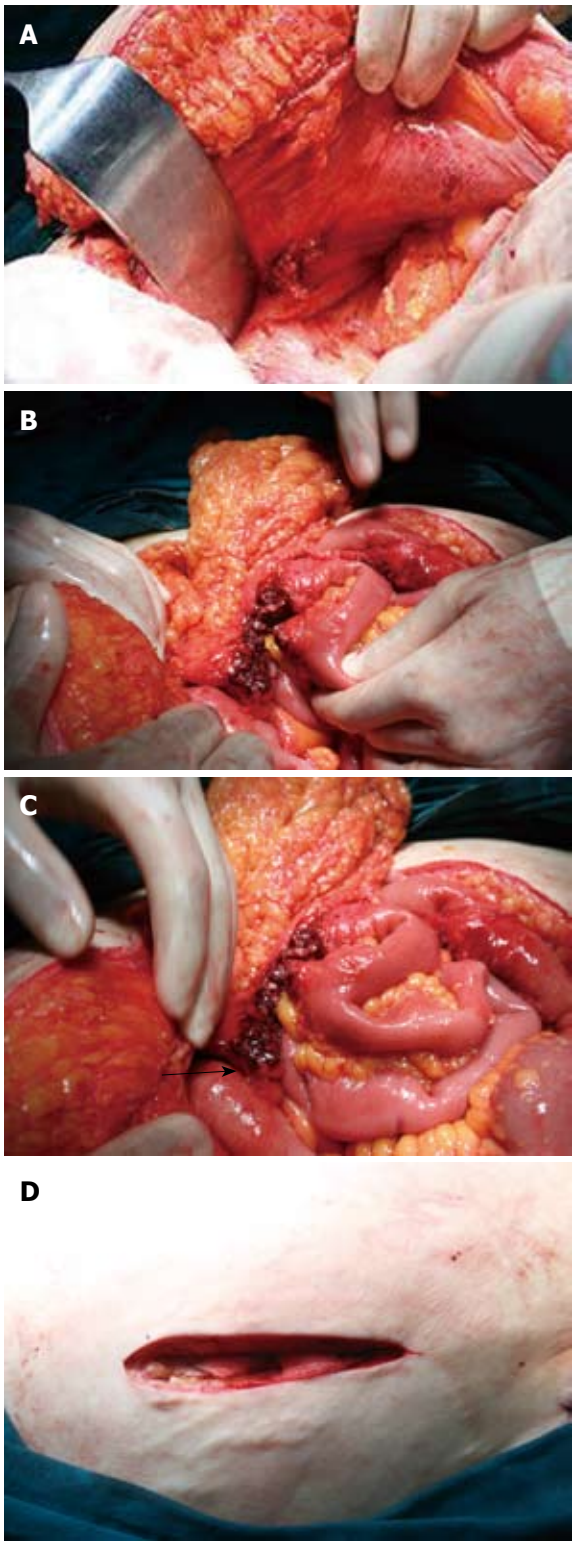

Figure 2 Intraoperative view of the meckel's diverticulum manifested by cutaneous abscess. A: The fistulization area of the diverticulum in the right lower abdominal wall; B: After retraction of the Meckel's diverticulum: intraabdominal view of the diverticulum and necrotic omentum; C: The intraoperative view of the perforated diverticulum (black arrow shows Meckel's diverticulum); D: The view of the abscess cavity.

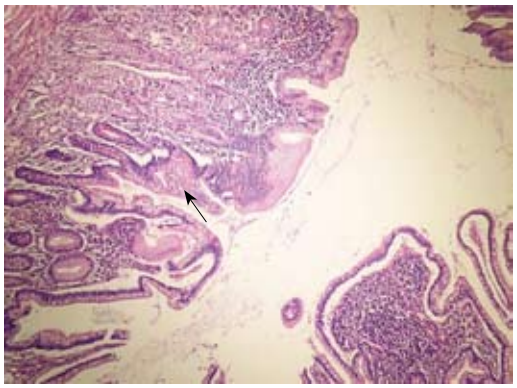

Figure 3 Meckel's diverticulum (HE stain, × 100). Photomicrograph shows the diverticulum composed of all layers of intestinal wall. Normal small intestinal mucosa and a focus of (arrow) gastric mucosa line the diverticulum.

Table 1 Meckel's diverticulitis and abscess[11-13]

Author	Localization
Kundra *et al*[12], 2001	Subphrenic
Hussien *et al*[11], 2001	Liver
Wasike *et al*[13], 2006	Mesenteric
Karatepe *et al*, 2009	Subcutaneous

Clinical diagnosis of Meckel's diverticulum is rarely possible; less than 10% are diagnosed preoperatively[1,2]. It is therefore critical for surgeons to rule out Meckel' s diverticulum in patients undergoing surgical evaluation for chronic abdominopelvic pain[5]. The correct diagnosis of Meckel's diverticulum before surgery is often difficult because a complicated form of this condition is similar to many other abdominal pathologies[1-5]. Arteriography and technetium pertechnetate scanning may be especially useful when there is significant bleeding or ectopic gastric mucosa[5]. In children, the single most accurate diagnostic test for Meckel's diverticula is scintigraphy with sodium ^{99m}Tc-pertechnetate. The diagnostic sensitivity of this scan has been reported to be as high as 85%, with a specificity of 95% and an accuracy of 90% in the pediatric age group. In adults, however, ^{99m}Tc-pertechnetate scanning is less accurate because of reduced prevalence of ectopic gastric mucosa within the diverticulum[10]. However, these scans are not readily amenable in an emergency situation. Contrast-enhanced CT scan may be helpful in patients with enigmatic clinical symptoms of enterocutaneous fistula caused by Meckel's diverticulum. In vesicoenteric fistulas, cystoscopy has a key role in visualization of the fistula[6].

Briefly, the lesson from this case is that Meckel's diverticulum should be kept in mind as one of the differential diagnoses of enterocutaneous fistula.

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CASE REPORT

Carcinoma of the papilla of Vater following treatment of pancreaticobiliary maljunction

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Abstract

Pancreaticobiliary maljunction (PBM) is frequently associated with biliary cancer due to reflux of pancreatic enzymes into the choledochus, and even after surgery to correct the PBM such patients still have a risk of residual bile duct cancer. Here, we report the case of a 59-year-old female with carcinoma of the papilla of Vater which developed 2.5 years after choledochoduodenostomy for PBM. During the postoperative follow-up period, computed tomography obtained 2 years after the first operation demonstrated a tumor in the distal end of the choledochus, although she did not have jaundice and laboratory tests showed no abnormalities caused by the previous operation. As a result, carcinoma of the papilla of Vater was diagnosed at an early stage, followed by surgical cure. For early detection of periampullary cancer in patients undergoing surgery for PBM, careful long-term follow-up is needed.

Key words: Pancreaticobiliary maljunction; Biliary cancer; Carcinoma of the papilla of Vater

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INTRODUCTION

Pancreaticobiliary maljunction (PBM) is a congenital anomaly defined as a union of the pancreatic and biliary ducts outside the duodenal wall[1]. Due to the loss of control by the sphincter of Oddi, pancreatic enzymes reflux into the choledochus, especially in the C-P type of maljunction, in which the common bile duct joins the main pancreatic duct[1]. Consequently, PBM is frequently associated with choledochal cyst and sometimes biliary cancer; a large survey showed that the rate of developing biliary cancer was 10.6% and 37.9% in PBM with and without choledochal dilation, respectively[2].

Once PBM is diagnosed, the development of cancer should be prevented by removal of the entire extrahepatic bile duct, even in patients without biliary dilation[3]. However, it has been reported that residual bile duct cancer could be found during the long-term follow-up period, even after termination of pancreatic enzyme reflux[4-6], and Watanabe *et al*[6] reported that 23 (0.7%) of 1291 patients developed residual bile duct cancer after cyst excision. Besides residual bile duct cancer, other periampullary carcinomas have been reported, but they are very rare[7,8].

In this paper, we report a case of carcinoma of the papilla of Vater that developed following treatment of PBM with choledochal dilation by choledochoduodenostomy. This is the first report of such a case, and particular attention is paid to the incidence, differential diagnosis, and treatment of periampullary neoplasms after treatment for PBM.

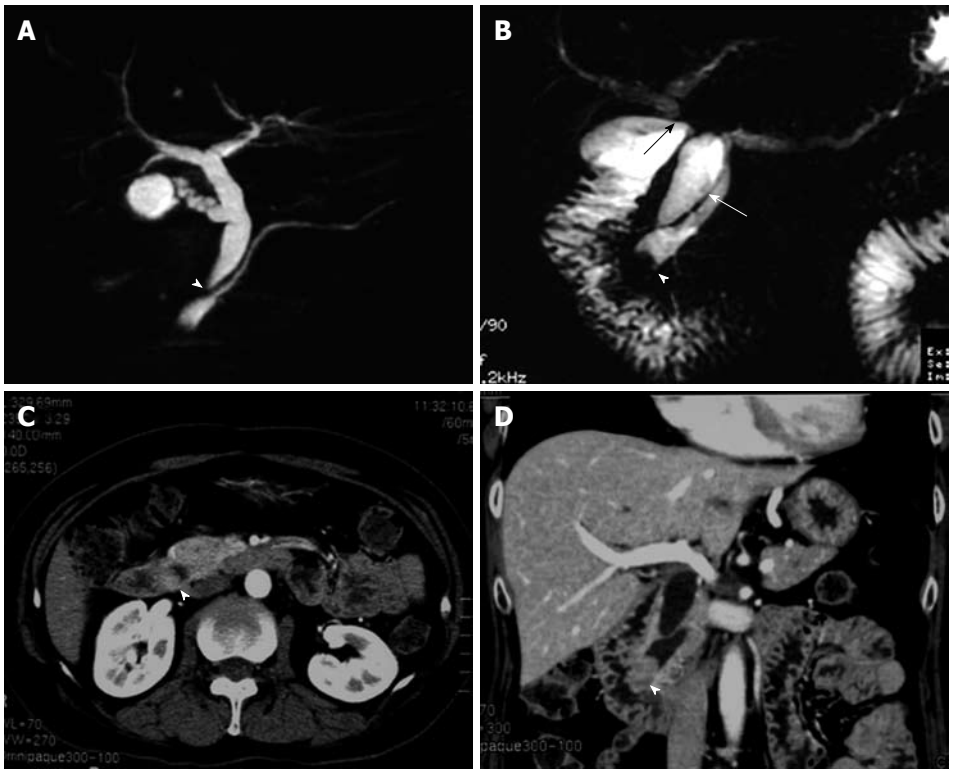

Figure 1 Imaging modalities for pancreaticobiliary maljunction and carcinoma of the papilla of Vater. A: Magnetic resonance choledochopancreatography before first operation shows both maljunction of the main pancreatic duct and the choledochus (arrowhead) and the choledochal dilation, however there is no finding of neoplasm at the papilla of Vater; B: Magnetic resonance choledochopancreatography at follow-up after first operation shows the tumor in the main pancreatic duct (arrowhead). In addition, the choledochoduodenostomy (black arrow) and the residual choledochus (white arrow) can be seen; C, D: Computed tomography obtained 2 years after choledochoduodenostomy for pancreaticobiliary maljunction shows the mass in the distal end of the common bile duct, which had not been detected before first operation (arrowheads).

CASE REPORT

A 59-year-old female was referred to our hospital. She had undergone choledochoduodenostomy and cholecystectomy at another hospital 2.5 years earlier after diagnosis of PBM. Although imaging modalities obtained before surgery showed choledochal dilation, the minimally invasive operation was performed without excision of a choledochal cyst because the patient rejected blood transfusion preoperatively (Figure 1A). During the postoperative follow-up period, magnetic resonance choledochopancreatography demonstrated a tumor in the main pancreatic duct and in the anastomotic site of the choledochus and duodenum from the first operation (Figure 1B). Computed tomography also showed a tumor in the distal end of the residual choledochus, which had not been observed before the first operation despite bile diversion (Figure 1C and D). Endoscopic retrograde pancreatography was then performed, and a 2-cm-diameter filling defect was demonstrated at the papilla of Vater, which was shown to be adenocarcinoma on cytology. Regardless of these findings, the patient did not complain of any symptoms such as jaundice or abdominal pain, and laboratory tests, including hepatobiliary enzymes and tumor markers, showed no abnormalities. With a diagnosis of carcinoma of the papilla of Vater after choledochoduodenostomy for PBM, the patient underwent pancreaticoduodenectomy. Despite moderate adhesions due to the previous operation, we were able to find common hepatic duct which was anastomosed to the duodenum (Figure 2). After Kocher maneuver, residual extrapancreatic choledochus was easily identified behind the anastomotic region and all the residual choledochus could be removed. Because the cancerous lesion was not found at the first operation or at preoperative examination, resection of all the residual bile duct was thought to be sufficient for curative surgery. On pathology of the resected specimen, the tumor was found to be well-to-moderately differentiated adenocarcinoma of the papilla of Vater, with metastasis to the lymph nodes behind the head of the pancreas (Figure 3). The patient's postoperative course was uneventful, and there has been no cancer recurrence 1 year after the second surgery.

DISCUSSION

Even after surgery that stops reflux of pancreatic enzymes into the choledochus and prevents the development of biliary cancer, patients with PBM still have a risk of developing residual bile duct carcinoma, both in the proximal duct and the distal end[6]. In the present case, carcinoma of the papilla of Vater was diagnosed 2 years after choledochoduodenostomy by computed tomography. In addition to complete excision of a choledochal cyst, careful long-term follow-up is necessary; the interval between excision of a choledochal cyst and cancer detection ranges from 1 to 19 years in several reports[6].

Compared with other periampullary carcinomas, including those of the duodenum, bile duct, and pancreas, survival and resectability rates of carcinoma of the papilla of Vater are relatively high[9,10]. In addition to the fact that the rate of resection is one of the predictive factors for survival[9], early detection is of great importance to provide benefit for patients with carcinoma of the papilla of Vater[11]. One of the most common manifestations at presentation in patients with carcinoma of the papilla of Vater is jaundice, as in the other periampullary carcinomas. However, jaundice is not usually observed in patients undergoing a diversion operation of the bile duct, as in the present case. Therefore, unless careful postoperative examinations of the periampullary region are performed,

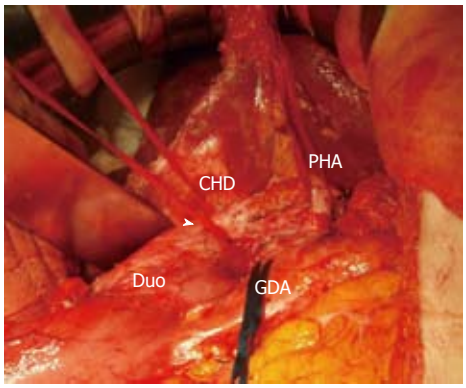

Figure 2 Intraoperative photograph of the second surgery. Common hepatic duct which was anastomosed to the duodenum was found. Behind this anastomotic region, residual choledochus was easily identified after Kocher maneuver. Arrow head indicates the region of choledochoduodenostomy. CHD: Common hepatic duct; Duo: Duodenum; PHA: Proper hepatic artery; GDA: Gastroduodenal artery.

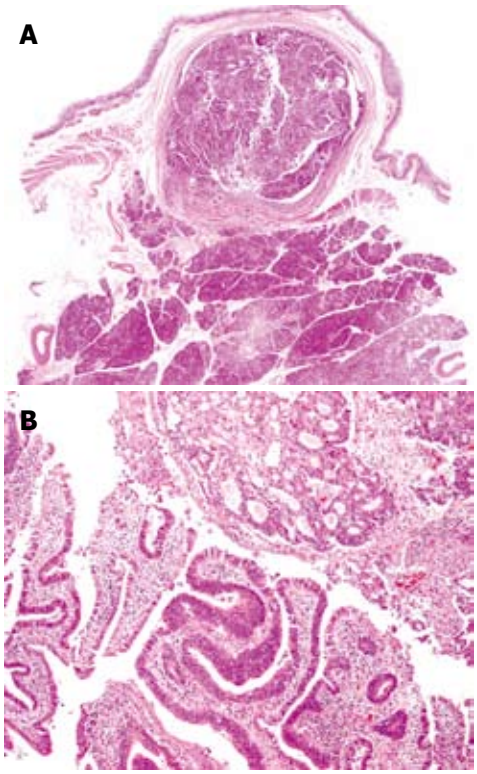

Figure 3 Pathological findings of the tumor. A: Close-up findings of the sliced specimen show a tumor growing mainly in the papilla of Vater. There is minimal invasion, which can hardly be seen at this magnification (HE stain, × 1); B: Histological findings of the tumor show adenocarcinoma composed of two different types of histology: moderately differentiated (upper) and well differentiated (lower) type (HE stain, × 40).

physicians may miss the chance to cure carcinoma of the papilla of Vater.

Compared to bile duct cancer, other residual biliary tree neoplasms are rare but can develop postoperatively. For example, a bile duct Schwannoma that developed 15 years after bypass operation and diversion of bile from a choledochal cyst has been reported[8]. Carcinoma of the papilla of Vater after choledochoduodenostomy for PBM is also very rare and has never been previously reported. Though PBM is not a risk factor for carcinoma of the papilla of Vater[12,13], which was detected only about 2 years after diversion of bile in the present case, inflammation due to the mixture of pancreatic enzymes with bile may have caused the carcinoma of the papilla of Vater in this case.

In summary, a case of carcinoma of the papilla of Vater that developed after choledochoduodenostomy for PBM was reported. In this present case, the lesion was detected at an early stage, and curative resection was performed, suggesting that careful, long-term follow-up of patients following surgery to treat PBM is necessary.

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CASE REPORT

A case of hypereosinophilic syndrome presenting with intractable gastric ulcers

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Abstract

We report a rare case of hypereosinophilic syndrome (HES) presenting with intractable gastric ulcers. A 71-year-old man was admitted with epigastric pain. Initial endoscopic findings revealed multiple, active gastric ulcers in the gastric antrum. He underwent *Helicobacter pylori* (*H pylori*) eradication therapy followed by proton pump inhibitor (PPI) therapy. However, follow-up endoscopy at 4, 6, 10 and 14 mo revealed persistent multiple gastric ulcers without significant improvement. The proportion of his eosinophil count increased to 43% (total count: 7903/mm^3). Abdominal-pelvic and chest computed tomography scans showed multiple small nodules in the liver and both lungs. The endoscopic biopsy specimen taken from the gastric antrum revealed prominent eosinophilic infiltration, and the liver biopsy specimen also showed eosinophilic infiltration in the portal tract and sinusoid. A bone marrow biopsy disclosed eosinophilic hyperplasia as well as increased cellularity of 70%. The patient was finally diagnosed with HES involving the stomach, liver, lung, and bone marrow. When gastric ulcers do not improve despite *H pylori* eradication and prolonged PPI therapy, infiltrative gastric disorders such as HES should be considered.

Key words: Gastric ulcer; Hypereosinophilic syndrome

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INTRODUCTION

Hypereosinophilic syndrome (HES) is a rare disorder characterized by the overproduction of eosinophils in the bone marrow with persistent peripheral eosinophilia, tissue infiltration, and end-organ damage by eosinophil infiltration and the secretion of mediators[1]. The diagnosis of HES is based on marked eosinophilia exceeding 1500/mm^3, a chronic course longer than 6 consecutive months, exclusion of parasitic infestations, allergic diseases and other etiologies for eosinophilia, and signs and symptoms of eosinophil-mediated tissue injury[1,2]. While HES can involve multiple organ systems, including bone marrow, heart, lung, liver, lymph node, muscle, and nerve tissue[1], gastrointestinal tract involvement is rare[1-3]. To date, only a handful of cases of HES presenting with gastritis or enteritis have been reported worldwide[4-9], and HES presenting with intractable gastric ulcers has not been reported. We report our case of a 71-year-old male patient with HES presenting with multiple intractable gastric ulcers with a review of the literature.

CASE REPORT

A 71-year-old man presented with epigastric pain. He underwent cholecystectomy 20 years previously due to acute cholecystitis with gallstones, and has intermittently taken nonsteroidal anti-inflammatory drugs (NSAID) and corticosteroids on account of degenerative arthritis

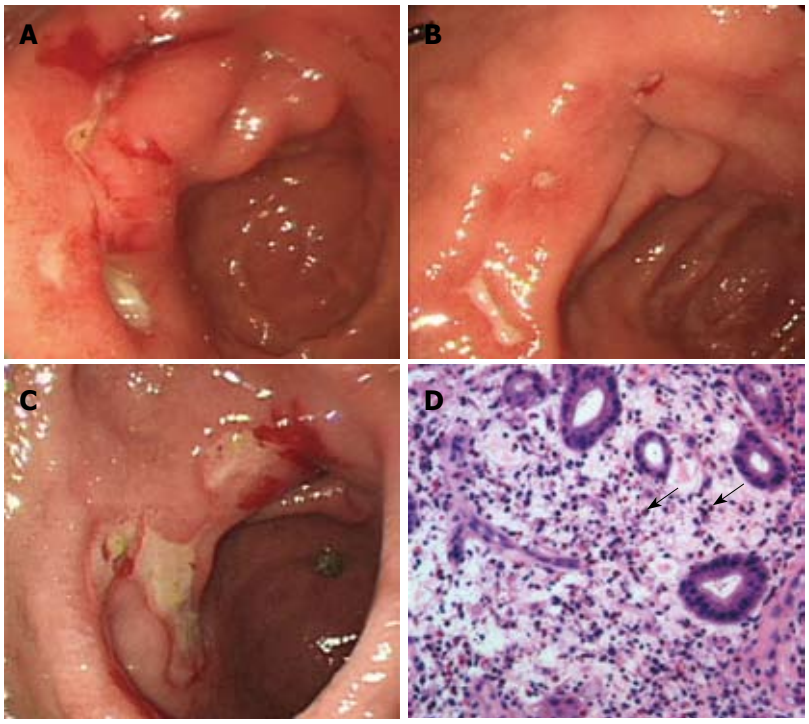

Figure 1 Esophagogastroduodenoscopy (EGD) and biopsy findings. A: Initial EGD findings revealed several active gastric ulcers in the antrum of the stomach; B: In the EGD findings after 2 mo, multiple gastric ulcers were still noticeable with only slight improvement; C: In the EGD findings after 14 mo, multiple gastric ulcers were still found in the antrum; D: Biopsy findings revealed prominent eosinophilic infiltrations > 20 cells/HPF (arrows) (HE stain, × 400).

for 15 years. Other symptoms, as well as his past medical and family history, were otherwise unremarkable. The initial physical examination showed a flat, soft abdomen with normoactive bowel sounds with no sign of direct or rebound tenderness and no hepatosplenomegaly. Thoracic auscultation revealed no remarkable results. Routine complete blood count reported a leukocyte count of 7790/mm^3 with 5.3% eosinophils, hemoglobin level of 12.1 g/dL, and a platelet count of 198000/μL. There were no noteworthy findings on simple chest and abdominal radiography. No specific cardiac abnormalities on standard 12-lead electrocardiogram (ECG) or Doppler echocardiogram were detected. ECG revealed normal sinus rhythm and the echocardiogram showed normal global left ventricular systolic function (estimated ejection fraction 70%).

Esophagogastroduodenoscopy (EGD) findings revealed several active gastric ulcers in the antrum of the stomach (Figure 1A). Biopsy findings showed an ulcer with *Helicobacter pylori* (*H pylori*). He underwent *H pylori* eradication therapy (lansoprazole 30 mg twice a day, clarithromycin 500 mg twice a day and amoxicillin 1000 mg twice a day for 7 d) followed by a proton pump inhibitor (PPI) and gastroprotective agent therapy for 2 mo. Follow-up EGD and biopsy performed after 2 mo showed that *H pylori* was eradicated, whereas multiple gastric ulcers were still noticeable with only slight improvement (Figure 1B). Follow-up endoscopy at 4, 6, and 10 mo showed persistent multiple gastric ulcers in the antrum despite continuous PPI treatment. Therefore, he was readmitted after 14 mo for etiological evaluation of the intractable gastric ulcers.

In the follow-up laboratory data, routine complete blood count showed a leukocyte count of 18380/mm^3 with 43% eosinophils, and an absolute eosinophil count of 7903/mm^3. Serum chemistry showed: Aspartate aminotransferase/alanine aminotransferase (AST/ALT), 39/97 IU/L; total bilirubin/direct bilirubin, 0.3/0.1 mg/dL; alkaline phosphatase, 235 IU/L; total protein/albumin, 7.3/3.2 g/dL; and BUN/Cr, 14/1.1 mg/dL. Serum immunoglobulin E level was elevated to 2147 kU/L. In pulmonary function tests, pre-bronchodilator FEV1 was 2090 mL (95% of predicted value) and the bronchodilator response was negative. The allergen skin test was negative. There were no parasites or ova in stool specimens. ELISA of paragonimiasis westermani, Clonorchis sinensis, cysticercus, and sparganum were negative. Anti-HIV antibody and anti-nuclear antibody were negative.

In the EGD findings, multiple gastric ulcers were still found in the antrum of stomach (Figure 1C). The endoscopic biopsy specimen revealed prominent eosinophilic infiltrations of > 20 cells/HPF (Figure 1D). A retrospective review of the previous endoscopic biopsy specimens disclosed eosinophilic infiltration at the antrum which was overlooked at the initial evaluation.

The chest computed tomography (CT) scan showed very tiny nodules in both lungs and approximately 15-mm-sized nodular lesions in the posterior basal segment of the right lower lobe (Figure 2A and B). In the abdominal-pelvic CT scan, multiple, small, and ill-defined low density lesions were found in both lobes of the liver (Figure 3A and B). The liver biopsy showed eosinophilic infiltration in the portal tract and sinusoid (Figure 3C and D).

The peripheral blood smear report showed that there were no immature or dysplastic cells or morphologically abnormal eosinophils. The bone marrow aspiration smear showed an M:E ratio of 3.8:1 and an elevated eosinophil count of 22.2% (Figure 4A). Bone marrow biopsy findings also indicated eosinophilic hyperplasia, with increased cellularity of 70% and normal distribution of erythroid, myeloid, and megakaryocytic cell lineages (Figure 4B). The Fip1-like 1-platelet-derived growth factor receptor A

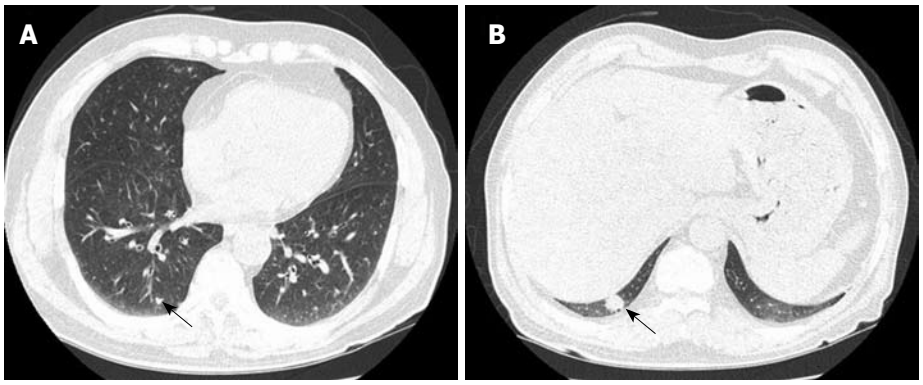

Figure 2 A chest CT scan showed very tiny nodules in both lungs (A) and approximately 15-mm-sized nodular lesions in the posterior basal segment of right lower lobe (B) (arrows).

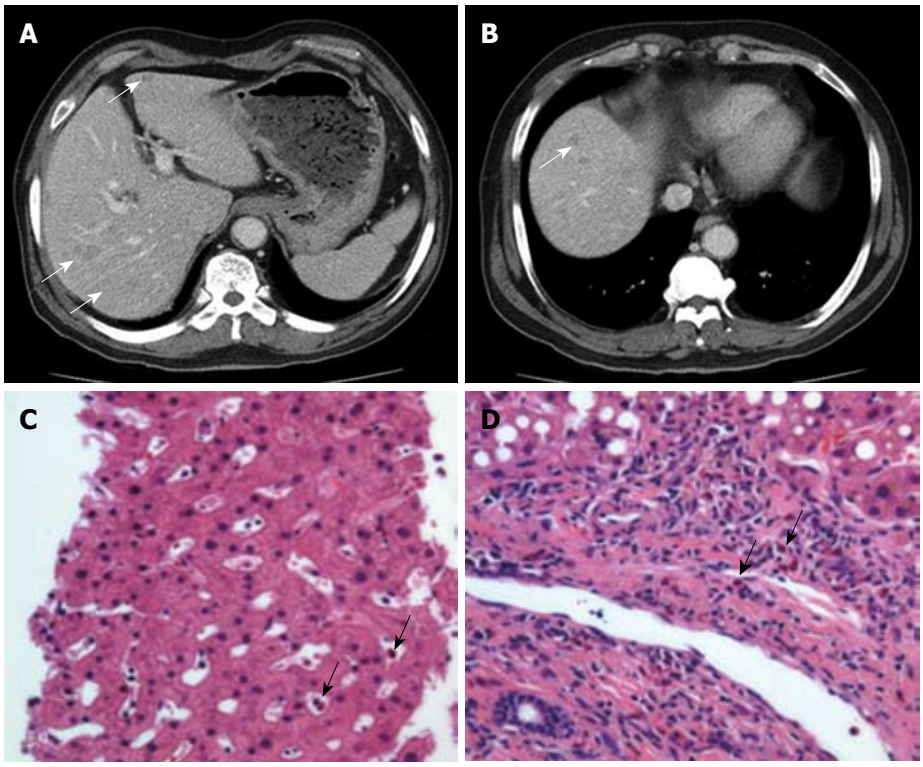

Figure 3 Abdominal-pelvic CT scan and liver biopsy findings. A, B: There were multiple, small, ill-defined low densities found in the liver (white arrows); C, D: Microscopically, eosinophilic infiltration was noted in the sinusoid and the portal tract (black arrows) (HE stain, × 400).

fusion gene (*FIP1L1-PDGFRA*) rearrangement was not detected and there were no cytogenetic abnormalities.

This patient was finally diagnosed with HES involving the stomach, liver, lung, and bone marrow. He was treated with oral prednisolone 60 mg/d and PPI. After two weeks of therapy, clinical manifestations rapidly improved and peripheral blood eosinophilia had subsided.

DISCUSSION

HES is a rare disease characterized by unexplained persistent eosinophilia associated with multiple organ dysfunction[1,2]. In 1968, Hardy and Anderson[10] reported three patients with hypereosinophilia, hepatosplenomegaly, and cardiopulmonary symptoms, and first suggested that they had a nonmalignant disorder that belonged within the spectrum of disease termed hypereosinophilic syndrome. In HES, the degree of end-organ damage is heterogeneous, and there is often no correlation between the level or duration of eosinophilia and the severity of organ damage[1,3]. Also, the clinical manifestations are variable from one patient to another, depending on target-organ infiltration by eosinophils[11]. Virtually any tissue or organ can be affected, but cardiac involvement is the major cause of the morbidity and mortality associated with HES[1,9,12]. We did not find cardiac involvement in our patient.

Since Chusid *et al*[13] reported the analysis of fourteen cases of HES in 1975, some cases of HES involving the gastrointestinal (GI) tract have been reported. Ichikawa *et al*[4] reported a case of probable HES with a gastric lesion, López Navidad *et al*[5] reported a case of HES presenting as a form of epithelioid leiomyosarcoma of gastric origin, and Levesque *et al*[6] reported two cases of HES with predominant digestive manifestations. In Korea, Jung *et al*[8] reported a case of HES presenting as colitis and You *et al*[9] reported a case of HES presenting with various GI symptoms. However, HES presenting with intractable gastric ulcers has not been reported. Our patient suffered from HES presenting with multiple intractable gastric ulcers as well as liver, lung, and bone marrow involvement. The exact mechanism of

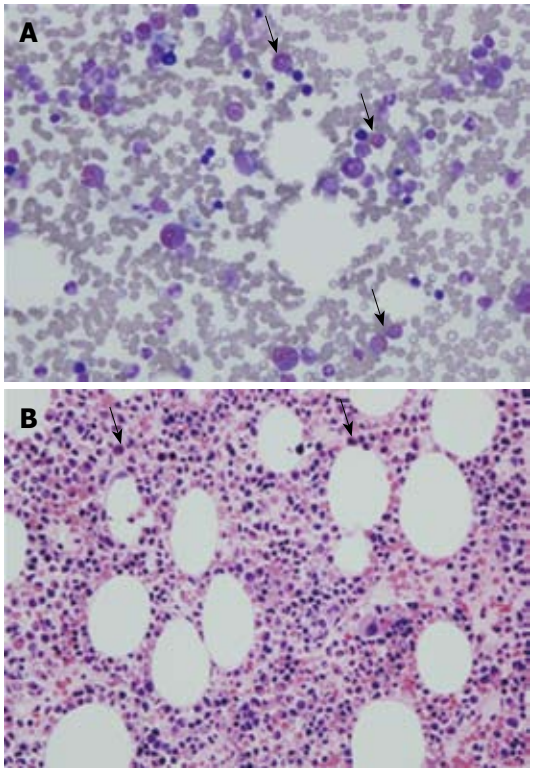

Figure 4 Bone marrow examination. A: In the bone marrow aspiration smear, the M:E ratio was 3.8:1 and the eosinophil count was elevated to 22.2% (arrows); B: Bone marrow biopsy findings showed eosinophilic hyperplasia (arrows) with increased cellularity of 70% and erythroid, myeloid, and megakaryocytic cell lineages with a normal distribution pattern (HE stain, × 400).

eosinophil-related tissue damage, including gastric ulcer, is not known[3], but the accumulation of eosinophils can have direct cytotoxicity through the local release of toxic substances, including cationic proteins, enzymes, reactive oxygen species, pro-inflammatory cytokines, and arachidonic acid-derived factors[14].

The differential diagnosis of HES includes the disparate diseases associated with eosinophilia. Peripheral blood eosinophilia can be associated with allergic disorders, parasite infections, malignancies, and organ diseases, including eosinophilic gastroenteritis (EG) or eosinophilic pneumonitis due to eosinophilic infiltration[15]. In our patients, the bronchodilator response was negative and there was no symptom or sign of allergic disease. Even if allergic disease is present, the severe peripheral eosinophilia noted in our patient is unusual[15]. In addition, *FIP1L1-PDGFRA* gene rearrangement was not detected in bone marrow and there were no cytogenetic abnormalities. Therefore, we could rule out primary clonal eosinophilia such as eosinophilic leukemia.

HES may be confused with EG. The diagnosis of EG is based on the following three criteria: (1) the presence of gastrointestinal symptoms, (2) biopsies showing eosinophilic infiltration of one or more areas of the GI tract, or characteristic radiologic findings with peripheral eosinophilia, and (3) no evidence of parasitic or extraintestinal disease[16]. Because EG is also of unknown etiology, the distinction from HES must be made on clinical and pathologic bases[17,18]. Eosinophilic gastroenteritis characteristically does not extend beyond the target organ[1,18]. Hence, EG lacks the multiplicity of organ involvement often found in HES and does not have the predilection to develop secondary eosinophil-mediated cardiac damage[1,18]. Thus, EG can usually be distinguished from HES, although individual patients may on occasion present with overlapping features that confound classification[1,18]. In our patient, multiple organ involvement was demonstrated, and there was no other possible cause of severe eosinophilia.

In patients with eosinophilia who lack evidence of organ involvement, specific therapy is not needed[1]. Such patients can have prolonged courses without the need for therapeutic intervention[1]. However, patients with vital organ involvement require treatment[1]. The goals in the management of HES are as follows: (1) reduction of peripheral blood and tissue levels of eosinophils; (2) prevention of end-organ damage; and (3) prevention of thromboembolic events[1-3]. Corticosteroids have been used for decades in the treatment of HES and, with the exception of PDGFRA-associated HES, remain the first-line treatment for most patients[17]. Typically high-dose prednisone (1 mg/kg per day or 60 mg/d in adults) can be initiated[1,3]. A good response to corticosteroid therapy is associated with a better prognosis[1]. If patients are refractory or intolerant to corticosteroids, alternative therapies must be considered. Cytotoxic agents, including hydroxyurea, can be considered as second-line therapy[1,3]. Immunomodulatory agents including IFN-α, cyclosporine, and alemtuzumab can also be used[17]. In patients with FIP1L1-PDGFRA-positive HES, imatinib mesylate (Gleevec®), which selectively inhibits a series of protein tyrosine kinases, is considered first-line therapy[17].

In conclusion, we report a case of HES presenting with intractable gastric ulcers. The final diagnosis in this patient was HES involving the stomach, liver, lung, and bone marrow. Clinicians should bear in mind that gastric ulcers can develop in association with infiltrative disorders including HES. When gastric ulcers do not improve despite *H pylori* eradication and prolonged PPI therapy, an infiltrative gastric disorder, such as HES, should be considered.

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CASE REPORT

A special growth manner of intrahepatic biliary cystadenoma

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Abstract

We report a case of a 56-year-old woman with intrahepatic biliary cystadenoma (IBC) accompanying a tumor embolus in the extrahepatic bile duct, who was admitted to our department on October 13, 2008. Imaging showed an asymmetry dilation of the biliary tree, different bile signals in the biliary tree, a multiloculated lesion and an extrahepatic bile duct lesion with internal septation. A regular left hemihepatectomy *en bloc* was performed with resection of the entire tumor, during which a tumor embolus protruding into the extrahepatic bile duct and originating from biliary duct of segment 4 was revealed. Microscopically, the multiloculated tumor was confirmed to be a biliary cystadenoma with an epithelial lining composed of biliary-type cuboidal cells and surrounded by an ovarian-like stroma. An aggressive *en bloc* resection was recommended for the multiloculated lesion. Imaging workup, clinicians and surgeons need to be aware of this different presentation.

Key words: Intrahepatic biliary cystadenoma; Growth manner; Tumor embolus

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Yi B, Cheng QB, Jiang XQ, Liu C, Luo XJ, Dong H, Zhang BH, Wu MC. A special growth manner of intrahepatic biliary cystadenoma. *World J Gastroenterol* 2009; 15(48): 6134-6136 Available from: URL: http://www.wjgnet.com/1007-9327/15/6134.asp DOI: http://dx.doi.org/10.3748/wjg.15.6134

INTRODUCTION

Intrahepatic biliary cystadenoma (IBC) is a rare benign epithelial tumor characterized by unicystic or multicystic growth, accounting for less than 5% of solitary non-parasitic cysts of biliary origin[1,2]. IBC was first described in 1892 by Keen[3], followed by Edmondson as a multilocular cystic lesion lined by mucus-secreting cuboidal or columnar epithelium accompanying a densely cellular "ovarian-like" stroma[4]. The most common symptoms of IBC patients at presentation are abdominal pain and mass in which 35% have obstructive jaundice[1,5]. Knowledge about the pathogenesis of IBC is limited. IBC manifested as a tumor embolus in the extrahepatic bile duct occurs rarely. In addition, no other case report has yet described its clinicopathological features. We report a case of IBC with a tumor embolus in the extrahepatic bile duct with its clinicopathological features described and its diagnostic approaches and surgical procedures discussed.

CASE REPORT

A 56-year-old woman with hypertension, diabetes and chronic B hepatitis was transferred to our hospital on October 13, 2008 due to a 2-wk history of right hypochondrial pain and spontaneously remitted jaundice. She denied nausea, vomiting, fever, shoulder or back pain. She was admitted to a local hospital but not received surgical treatment. Before admission, her peak bilirubin level was 434 μmol/L and magnetic resonance cholangiopancreatography (MRCP) demonstrated a multiloculated cystic lesion in segment 4, measuring 5.5 cm in diameter, and an obviously dilated left intra- and extra-hepatic biliary tree (Figure 1) but no evidence of choledocholithiasis. The signal was different between extrahepatic and marginal bile ducts on T2-weighted magnetic resonance images (MRI)

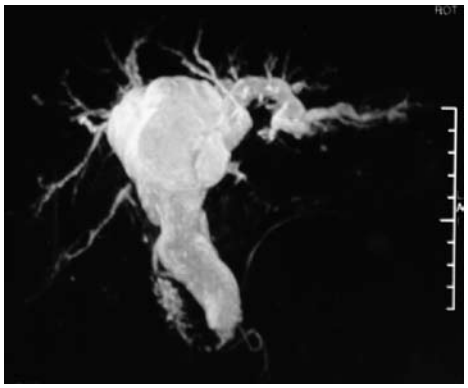

Figure 1 Magnetic resonance cholangiopancreatography (MRCP) showing the dilation of extrahepatic and intrahepatic bile ducts lacking of asymmetry.

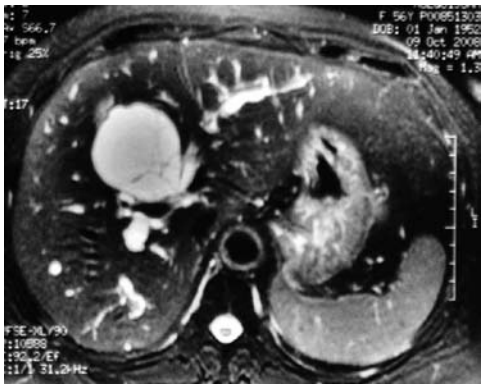

Figure 2 Magnetic resonance imaging (MRI) showing different bile signals between the right and left intrahepatic ducts in a multiloculated lesion with internal septation.

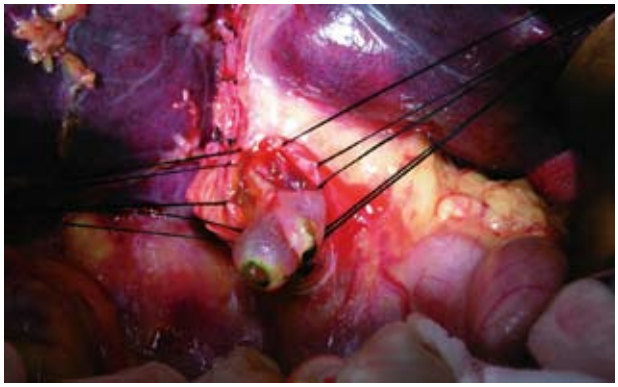

Figure 3 A tumor embolus originating from left intrahepatic duct with smooth surface protrudes the extrahepatic bile duct.

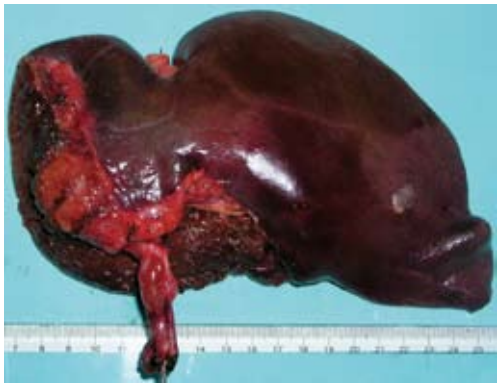

Figure 4 Macroscopy revealing a multilocular cystic lesion containing serous fluid with tumor embolus protruding into the left hepatic duct and the extrahepatic bile duct.

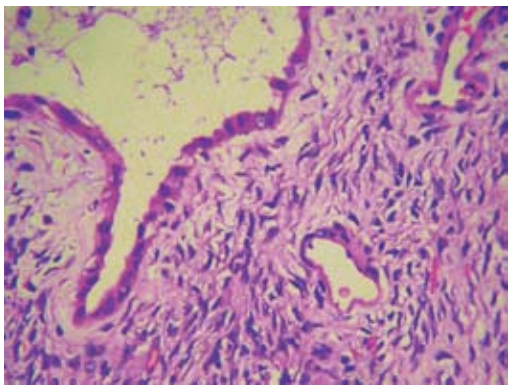

Figure 5 Postoperative pathology showing epithelial lining composed of biliary-type cuboidal cells surrounded by an ovarian-like stroma (400 ×).

showing thin septa in bile duct (Figure 2). The patient had no history of tobacco smoking, alcohol abuse or recent travel or previous surgery.

After admission, she demonstrated obstructive jaundice features, her bilirubin level declined spontaneously to 103.8 μmol/L, carbohydrate antigen 19-9 (CA19-9) was elevated to 92.1 μg/L, and carcinoembryonic antigen (CEA) level was normal. Abdominal ultrasound showed a dilated biliary tree and a multilocular cyst in segment 4. The extrahepatic bile duct (1.8 cm in diameter) was filled with fluid-like matters. Prior to a planned left hepatectomy, percutanous transhepatic biliary drainage (PTBD) of the right lobe was performed to decompress the right biliary system with an 8.5F tube. The TB level was 71 μmol/L and 35.8 μmol/L, respectively, before PTBD and hepatectomy. Volumetric computed tomography (CT) scan revealed that the ratio of remnant liver to total liver volume was 63.2%, with no change in cystic tumor size and no dilation of the right bile ducts, and CT scans showed a small septum in the extrahepatic bile duct, thus the cystic tumor was diagnosed as an intrahepatic tumor infiltrating the extrahepatic bile duct.

The patient underwent choledochotomy under general anesthesia on November 5, 2008, during which no palpable mass was found on the liver surface, but a tumor embolus originating from the intrahepatic duct of segment 4 with smooth surface that protruded into the extrahepatic bile duct was observed without mucus-secretion (Figures 3 and 4). Coexistence of a confluence variation in separate posterior right bile ducts and a variation in the replaced right hepatic artery from superior mesenteric artery was observed. A multiloculated cyst in segment 4 adhering to the bile duct wall of the anterior right lobe was found. So a regular left hemihepatectomy *en bloc* was performed with resection of the entire tumor, segment 5 and the wall of 8 bile ducts. The common hepatic duct was transected to facilitate reconstruction of

the orifice wall of the anterior right bile duct, and Roux-en-Y anastomosis between the hilar bile duct and jejunum was performed. Microscopically, the multiloculated tumor infiltrating the left hepatic duct was confirmed to be a biliary cystadenoma with an epithelial lining composed of biliary-type cuboidal cells and surrounded by an ovarian-like stroma (Figure 5). The bile duct margin was negative. The patient had an uneventful postoperative recovery. Follow-up imaging within twelve months showed no signs of local or distant tumor recurrence.

DISCUSSION

IBC is an uncommon tumor of biliary tract, but IBC with a tumor embolus in the extrahepatic bile duct is rare. The clinical features of IBC, especially with a tumor embolus in the extrahepatic bile duct, are unclear. No risk factors for IBC have been identified, although its predominance in females suggests a hormonal factor for its etiology. The lesion grows slowly but it is believed to be premalignant[6]. Clinically, it can be differentially diagnosed from other cystic hepatic lesions, such as simple cysts, liver abscesses, cystic degeneration of a liver neoplasm, Caroli's disease, *etc.*[6,7].

Imaging studies are of great importance in its diagnosis. Characteristic ultrasound, CT and MRI findings, including a multiloculated lesion with internal septation, a thickened and irregular wall, mural nodules and papillary projections, calcifications, and wall enhancements, have been well described[7,8]. However, IBC with a tumor embolus extending into the extrahepatic bile duct is uncommon and demonstrates some specific clinicopathological features, such as jaundice with spontaneous remission or recurrence[9]. Generally, jaundice often occurs when a big tumor embolus fills the extrahepatic bile duct. When the pressure of intra-biliary tract is high, the bile duct is dilated and the pressure of cystadenoma is counteracted, remitting the obstructive jaundice. The MRCP imaging manifestations of IBC, with a tumor embolus protruding into the extrahepatic bile duct, are different from those of IBC without a tumor embolus. In our case, MRCP showed different bile signals in the peripheral and extrahepatic bile ducts, while MRI and CT showed distinctive thin septa in the extrahepatic bile duct. In IBC with a tumor embolus in the extrahepatic bile duct, the extrahepatic bile duct was dilated much greater than the intrahepatic bile duct, and septa in the extrahepatic bile duct should be the main feature which is different from that of mucin-producing IBC without a tumor embolus, although their manifestations on MRCP are similar. Cystadenoma with an epithelial lining composed of biliary-type cuboidal cells is surrounded by an ovarian-like stroma. Before surgery for our patient, PTBD was performed with no mucin or serous fluid observed in the drainage. Since the tumor did not communicate with the bile duct tree, and was filled with serous fluid, intraductal papillary neoplasm of bile duct (IPNB) and biliary papillomatosis were excluded. This tumor develops intraductally but rarely infiltrates the distal bile duct. The base point of this tumor infiltrating the extrahepatic bile duct is intrahepatic, and the lower end of its embolus is dissociated, thus facilitating complete resection of IBC with a tumor embolus as that without a tumor embolus[10-12]. Complete resection of the tumor provides the chance of cure. An early preoperative diagnosis of IBC is essential to improve the prognosis and survival of such patients.

In conclusion, IBC has a special growth manner[9,10,13-15], but its clinical features have not been fully illustrated. Imaging workup, clinicians and surgeons need to be aware of its different presentations, such as recurrent jaundice, different bile signals on MRI, distinctive thin septa in extrahepatic bile duct, and asymmetry dilation of bile ducts. An aggressive complete resection of the lesion is recommended. Large randomized controlled trials are warranted.

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Comments on the editorial by Riggio & Ageloni on the ascitic fluid analysis

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Abstract

Angeloni *et al* published a landmark study on the use of Coulter counters in spontaneous bacterial peritonitis (SBP) diagnosis. Riggio and Angeloni have recently published an editorial on the ascitic fluid analysis in diagnosis and monitoring of SBP. Herein, some points of interest are discussed.

Key words: Peritonitis; Reagent; Dipstick; Paracentesis; Guidelines; Ascites

Koulaouzidis A. Comments on the editorial by Riggio & Ageloni on the ascitic fluid analysis. *World J Gastroenterol* 2009; 15(48): 6137-6138 Available from: URL: http://www.wjgnet.com/1007-9327/15/6137.asp DOI: http://dx.doi.org/10.3748/wjg.15.6137

TO THE EDITOR

I read with great interest the editorial of Riggio & Angeloni on "The ascitic fluid analysis for diagnosis and monitoring of spontaneous bacterial peritonitis"[1]. In 2003, Angeloni *et al*[2] published the landmark paper, which set a new era in the diagnostic algorithm of spontaneous bacterial peritonitis (SBP), allowing many clinicians and laboratory staff to feel secure in switching from polymorphonuclear (PMN) manual count to the automated one.

I would like to comment on a few points presented in this editorial. First of all, I would appreciate if the authors could clarify the statement on the need for collection of 10 mL ascitic fluid (AF) in ethylenediaminetetraacetic acid (EDTA) containing tube. Universally, most of the EDTA tubes ("purple-top or red-top tubes", used for blood collection) have a maximum capacity of 2.5-3.0 mL. If Riggio & Angeloni meant the universal containers, it is my understanding that these tubes, except for being sterile, they do not contain any anticoagulant. On top of that, only 1 mL of fluid is enough for most laboratories to do the differential diagnosis. I disagree with the statement that "following hospitalization of any cirrhotic patient with newly diagnosed ascites, a diagnostic paracentesis is advised". In fact, all cirrhosis with ascites should have diagnostic paracentesis on hospital admission[3].

There are indeed 4 well-disseminated practical guidelines and expert's consensus reports, but many other national guidelines have been produced as well[4].

Riggio & Angeloni's comprehensive "Table 2" should list 90 AF samples and not 47 in the study by Wisniewski *et al*[5], 2123 samples and not 1041 in the study by Nousbaum *et al*[6], and 78 samples and not 72 in the study by Vanbiervliet *et al*[7], although three studies have not been included[8-11]. In addition, Castellote *et al*[12] in a recently published paper argued that the leucocyte reagent strips may have a role in repeated paracentesis and hence management of SBP.

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Relationship between abdominal trauma or surgery and mesenteric panniculitis

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Abstract

Mesenteric panniculitis is a rare disease characterized by chronic non-specific inflammation of mesenteric fat tissue. Several etiologic and/or associated factors have been reported in the literature so far. Although trauma or surgery is one of the potential etiologic factors for mesenteric panniculitis, to the best of our knowledge, no strong correlation has been shown in the literature until now.

Key words: Mesentery; Pathology; Mesenteric panniculitis; Trauma; Surgery

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TO THE EDITOR

We read with a great interest the article "Mesenteric panniculitis: Various presentations and treatment regimens" by Issa *et al*[1] in the August issue of the *World Journal of Gastroenterology*.

Mesenteric panniculitis is a rare chronic inflammatory disorder of adipose tissue of the intestinal mesentery. This entity has several different names, such as mesenteric Weber-Christian disease, fibrosing mesenteritis, retractile mesenteritis, mesenteric lipodystrophy, and sclerosing mesenteritis. Its etiology still remains unclear, although a variety of possible causative and associated factors, such as infective and autoimmune causes, vascular insufficiency, prior abdominal surgery, and malignancy, have been reported[1-4].

Issa *et al*[1] reported that 84% of patients with mesenteric panniculitis have a history of abdominal trauma or surgery as its etiological factor[2]. However, the actually reported rate of trauma or surgery as an etiologic factor is 4.76% rather than 84%[2]. A same mistaken rate of 84% has also been reported in a case series[3], showing that trauma and surgery are closely correlated with mesenteric panniculitis. Upon reviewing the literature, we were not able to find this strong correlation in any study.

Several studies are available on the etiology of mesenteric panniculitis[1-4]. Daskalogiannaki *et al*[4] reported that mesenteric panniculitis is associated with 69.3% of malignancies, such as lymphoma, breast cancer, colon cancer, lung cancer and melanoma, demonstrating that mesenteric panniculitis is an associated and/or causative factor for malignancies. Although trauma or surgery is one of the potential etiologic factors, to the best of our knowledge, no strong correlation has been shown in the literature until now.

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Meetings

Events Calendar 2009

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Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

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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
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Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
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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

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Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
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April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
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AACR 100th Annual Meeting 2009

April 22-26, 2009
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http://www.easl.ch/

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Digestive Disease Week 2009

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Orange County Convention Center
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45th ASCO Annual Meeting
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Chicago, Illinois
Endpoints Workshop: NASH

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Flims, Switzerland
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ESMO Conference: 11th World Congress on Gastrointestinal Cancer
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World Conference on Interventional Oncology
http://www.chinamed.com.cn/wcio2009/

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World J Gastroenterol 2009 December 28; 15(48): 6142-6144
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4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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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]

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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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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

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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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