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BRIEF ARTICLE

Xba I polymorphisms of apolipoprotein B gene: Another risk factor of gallstone formation after radical gastrectomy

Feng-Lin Liu, Wen-Bin Lu, Wei-Xin Niu

Feng-Lin Liu, Wen-Bin Lu, Wei-Xin Niu, Department of General Surgery, Zhongshan Hospital, Fudan University, Shanghai 200032, China
Author contributions: Liu FL and Lu WB contributed equally to this work; Liu FL and Lu WB performed all of the experiments and wrote the manuscript; Niu WX designed and guided the study in addition to revising and editing the manuscript.
Supported by Zhongshan Hospital, Fudan University, Shanghai, China
Correspondence to: Dr. Wei-Xin Niu, MD, Department of General Surgery, Zhongshan Hospital, Fudan University, #180 Fenglin Road, Shanghai 200032, China. niu.weixin@zs-hospital.sh.cn
Telephone: +86-21-64041990 Fax: +86-21-64037224
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Abstract

AIM: To prospectively investigate the association between the *Xba* I polymorphisms of apolipoprotein B (*APOB*) gene and gallstone formation following gastrectomy.

METHODS: The study was conducted between January 2005 and December 2006. A total of 186 gastric cancer patients who had undergone radical gastrectomy were grouped according to *Xba* I polymorphisms of *APOB* gene (X^+X^- group, n = 24 and X^-X^- group, n = 162) and compared. The *Xba* I polymorphisms of *APOB* gene were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

RESULTS: The incidence of gallstone was significantly higher in the X^+X^- group than in the X^-X^- group [54.2% *vs* 9.3%, RR = 5.85 (2.23-15.32), P < 0.001]. The serum levels of total cholesterol (TC) and low-density lipoprotein (LDL) were higher in the X^+X^- than in the X^-X^- group (4.02 ± 1.12 *vs* 3.48 ± 0.88, P = 0.004 before surgery and 3.88 ± 1.09 *vs* 3.40 ± 0.86, P = 0.008 after surgery). LDL was 2.21 ± 0.96 *vs* 1.89 ± 0.84 (P = 0.042) before surgery and 2.09 ± 0.95 *vs* 1.72 ± 0.85 (P = 0.029) after surgery in the two groups. No relationship was found between *Xba* I polymorphisms and gallbladder motility.

CONCLUSION: In Chinese patients after radical gastrectomy, X^+ allele of *APOB* gene is another risk factor for the development of gallstone besides the gallbladder motility disorder after surgery.

Key words: Gastric cancer; Gastrectomy; Gallstone; Apolipoprotein B gene; Polymorphism

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INTRODUCTION

The incidence of gallstones is higher in patients after radical gastrectomy than in the general population[1]. The current literature suggests that this higher incidence is related to gallbladder motility disorder after surgery resulting from severance of the vagus nerve, non-physiological reconstruction of the gastrointestinal tract and lymph node dissection[2-5]. However, it is also observed in clinical practice that following the same type of surgery on patients with the same stage of gastric cancer, some of them

do and the others do not develop gallstones. This suggests that besides gallbladder dysmotility, there are some possible inherent factors of gallstone formation after gastrectomy. In recent years, several studies have reported that polymorphisms in the apolipoprotein B (*APOB*) gene were associated with gallstone diseases[6,7]. Apolipoprotein B is a key protein in lipid metabolism. It plays an important role in the homeostasis of low-density lipoprotein (LDL) cholesterol in plasma serving as a ligand for receptor-mediated endocytosis of LDL. Several polymorphic forms of *APOB* alleles have been reported to be associated with disorders like coronary heart disease and non-insulin dependent diabetes mellitus[8]. The *APOB-Xba* I polymorphism has been found to be associated with increased serum lipids. Genetic polymorphisms in the *APOB* gene have also been reported to be associated with susceptibility to cholesterol gallstones[9]. However, the effects of *APOB-Xba* I gene polymorphism in patients who had undergone radical gastrectomy for gastric cancer without any previous history of cholelithiasis, abnormal gallbladders motility, diabetes, hyperlipidemia, hyperparathyroidism and other metabolic disorders is unknown. The present study prospectively investigated the association between the *Xba* I polymorphisms of *APOB* gene and gallstone formation after radical gastrectomy.

MATERIALS AND METHODS

Patients

The study enrolled 206 patients who underwent radical gastrectomy for gastric cancer in Zhongshan Hospital between January 2005 and December 2006. The inclusion criteria included age less than 60 years; biopsy proven gastric cancer; normal gallbladder motility; no gallstone or other hepatobiliary system diseases, no metabolic disorders and important organs dysfunction. To avoid biases, all patients were operated upon by the same surgical team with D2 dissection. Complete data were available for only 186 patients as 20 patients were lost to follow-up (3 patients in X^+X^- group and 17 patients in X^-X^- group). These patients were grouped according to *Xba* I polymorphisms of *APOB* gene. For analyzing the effects of *Xba* I polymorphisms, blood was also collected before and 6 mo after gastrectomy. The serum lipids, lipoproteins and apolipoproteins were determined. The levels of total cholesterol (TC), LDL and *APOB* were tested by biochemical autoanalyser (HITACHI7600). The gallbladder motility was examined by detecting gallbladder emptying fraction (GBEF) using nuclide imaging before and 6 mo after gastrectomy. Gallstone formation was determined by periodic ultrasonography after gastrectomy.

Informed consent was obtained from all patients, and the study was approved by the ethics committee of the hospital.

Determination of DNA polymorphism

Leukocyte genomic DNA was extracted from 5 mL of peripheral blood by TianGen Genetic DNA Kit (TianGen Biotech Co., Ltd.). The desired segments were amplified by PCR using the APOB *Xba* I protocols with the primers: 5'(5'GGAGACTATTCAGAAGCTAA3') and 3'(3'GAAGAGCCTGAAGACTGACT5'). The final amplification products were submitted to digestion with the restriction enzymes (*Xba* I) and the variations were visualized after electrophoresis on 1.5% agarose gel with ethidium bromide under ultra-violet light, followed by photographic documentation. PCR cycle for amplification of *APOB* gene was performed at 95℃ for 5 min followed by 95℃ for 45 s, annealing at 58℃ for 45 s and extension at 72℃ for 45 s in 33 cycles. A final extension was conducted for 10 min at 72℃. The products were digested by *Xba* I restriction enzyme at 37℃ for 12 h.

Statistical analysis

Data are expressed as the means ± SD. Means of age, body mass index (BMI), TC, LDL and *APOB* in different groups were compared using Student's t test. Gender, stage, types of gastrectomy and reconstruction were compared using χ^2 test. The differences between each genotype of *Xba* I polymorphisms and the incidence of gallstones were assessed by Fisher's exact test. Interaction between *Xba* I polymorphisms and gallbladder motility in the development of gallstone after gastrectomy was estimated by logistic regression analysis. SPSS statistical software, version 15.0 for Windows was used for all analyses. $P < 0.05$ was considered statistically significant.

RESULTS

Demographic data of X^+X^- group and X^-X^- group

The frequencies of X^+X^- and X^-X^- in 186 patients were 24 (13%) and 162 (87%), respectively. No genotype with X^+X^+ was detected in the study. Demographic data of X^+X^- group and X^-X^- groups are shown in Table 1. The median follow-up time was 28 mo (range: 24-32 mo). The mean age and BMI at the time of gastrectomy was 56.1 ± 8.2 years (range: 38-80 years) and 21.9 ± 1.8 kg/m^2 (range: 19.2-25.1 kg/m^2), respectively. With regard to type of gastrectomy, 7 (4%), 162 (87%), and 17 (9%) patients underwent a proximal, distal and total gastrectomy, respectively. Age and BMI did not differ significantly between the X^+X^- and X^-X^- groups. With respect to TMN stage, gallbladders motility after surgery, types of gastrectomy and reconstruction, there were no significant differences between the two groups (Table 1).

Gallstone formation after radical gastrectomy in X^+X^- and X^-X^- groups

Of the 186 patients, 28 (15.1%; 13 in X^+X^- group, 15 in X^-X^- group) developed gallstones with in a mean follow-up period of 28.4 ± 2.7 mo. The incidence of gallstone formation was significantly higher in the X^+X^- group than in the X^-X^- group (54.2% *vs* 9.3%, relative risk 5.85, 95% CI: 2.23-5.32, $P < 0.001$). The time period from gastrectomy to detection of the gallstones ranged from 6 to 32 mo with a median of 20 mo. The timing of gallstones formation showed no difference between the two groups

Table 1 Demographic data of 186 gastric cancer patients who underwent gastrectomy with D2 dissection in the X^+X^- and X^-X^- groups (mean ± SD) *n* (%)

	X^+X^- group (*n* = 24)	X^-X^- group (*n* = 162)	*P* value
Age (yr)	52.4 ± 5.1	56.8 ± 7.4	0.998
Gender			
Male	16 (66.7)	110 (67.9)	0.904
Female	8 (33.3)	52 (32.1)	
BMI (kg/m^2)	22.0 ± 1.2	21.7 ± 1.6	0.379
TNM stage[1]			
Ⅰ A	1 (4.2)	7 (4.3)	0.996
Ⅰ B	4 (16.7)	31 (19.1)	
Ⅱ	8 (33.3)	52 (32.1)	
Ⅲ A	8 (33.3)	49 (30.3)	
Ⅲ B	3 (12.5)	23 (14.2)	
Type of gastrectomy			
Proximal gastrectomy	1 (4.2)	6 (3.7)	0.820
Distal gastrectomy	20 (83.3)	142 (87.7)	
Total gastrectomy	3 (12.5)	14 (8.6)	
Type of reconstruction			
Without duodenal exclusion	14 (58.3)	96 (59.3)	0.991
With duodenal exclusion	10 (41.7)	66 (40.7)	
Gallbladders motility after surgery			
Normal	10 (41.7)	62 (38.3)	0.750
Abnormal	14 (58.3)	100 (61.7)	

[1]According to the Classification of IUCC. BMI: Body mass index.

Table 2 Gallstone formation after radical gastrectomy

	X^+X^- group (*n* = 13)	X^-X^- group (*n* = 15)	*P* value
Incidence of gallstone	54.2% (13/24)	9.3% (15/162)	< 0.001
Time period from gastrectomy to detection of the gallstones (mo)	16.4 ± 8.4	21.7 ± 7.4	0.085
Gallbladders motility			
GBEF before surgery	50.9% ± 12.5%	51.3% ± 10.3%	0.863
GBEF after surgery	30.3% ± 20.1%	29.6% ± 19.1%	0.868
Follow-up period after gastrectomy (mo)	28.3 ± 1.1	28.6 ± 1.2	0.249

n: Number of patients with gallstone; GBEF: Gallbladder emptying fraction.

(Table 2). For further analysis, whether interaction existed between *Xba* I polymorphisms and gallbladder motility in the development of gallstone after gastrectomy was estimated by logistic regression. As a result, no interaction effect of *Xba* I polymorphisms was found with gallbladder motility (Table 3). To analyze the higher incidence of gallstone in X^+X^- group, the serum concentrations of TC, LDL and APOB were compared between the two groups (Table 4). The serum levels of TC and LDL were significantly higher in X^+X^- group than in X^-X^- group both before and after surgery, but there was no statistical difference in APOB values between the two groups.

DISCUSSION

Formation of gallstones after radical gastrectomy is the result of a very complex interaction of various factors.

Table 3 Association of *Xba* I genotypes with gallstone after gastrectomy, stratified by gallbladder motility status

	Normal gallbladder motility[1]		Abnormal gallbladder motility[1]		Pinteraction
	Gallstone (+)	Gallstone (-)	Gallstone (+)	Gallstone (-)	
X^+X^- group	3	7	10	4	0.701
X^-X^- group	1	61	14	86	

[1]According to the GBEF (gallbladder emptying fraction), estimated by logistic regression analysis.

Table 4 Serum levels of TC, LDL and APOB in X^+X^- and X^-X^- groups (mean ± SD)

	X^+X^- group (*n* = 24)	X^-X^- group (*n* = 162)	*P* value
Total cholesterol (mmol/L)			
Before surgery	4.02 ± 1.12	3.48 ± 0.88	0.004
After surgery	3.88 ± 1.09	3.40 ± 0.86	0.008
Low-density lipoprotein (mmol/L)			
Before surgery	2.21 ± 0.96	1.89 ± 0.84	0.042
After surgery	2.09 ± 0.95	1.72 ± 0.85	0.029
Apolipoprotein B (μg/L)			
Before surgery	0.73 ± 0.15	0.70 ± 0.12	0.142
After surgery	0.72 ± 0.14	0.68 ± 0.12	0.072

TC: Total cholesterol; LDL: Low-density lipoprotein; APOB: Apolipoprotein B.

Destruction of neural structures, such as the vagal nerves, represents one well-known risk factor[2,10,11]. It is speculated that complete amputation of the vagal trunk with dissection of the esophagus (as in total gastrectomy) has a great influence on the contractile ability of the gallbladder. A previous experimental study showed that gastrectomy abolishes phasic contraction of the gallbladder, resulting in an absence of agitation of the gallbladder bile and mixing of gallbladder bile with fresh hepatic bile, increasing the propensity for salt precipitation and gallstone formation[12]. The type of reconstruction is also closely related to gallstone formation because the passage of food through the duodenum stimulates a variety of hormonal secretions such as cholecystokinin[13,14] and this hormone causes contraction of the gallbladder through the hormonal regulation system. It is postulated that exclusion of the duodenum leads to changes in the pattern of cholecystokinin secretion, resulting in decreased gallbladder contraction and an increased risk of gallstone formation[15].

Almost all previous studies have attributed gallstone formation to gallbladder motility disorder after radical gastrectomy. However, it is known from clinical experience that after the same type of surgery is performed on patients with the same stage of gastric cancer, some of them do and the others do not develop gallstones. In the present study, of 114 patients with abnormal gallbladder motility after surgery, 24 patients (21%) developed gallstones and the others did not. It suggests that the theory

of gallbladder motility disorder cannot completely explain gallstone formation after radical gastrectomy. The present study showed that the incidence of gallstone formation was significantly higher in the X^+X^- group than in the X^-X^- group (54.2% *vs* 9.3%), RR 5.85 (2.23-15.32), suggesting that the X^+X^- genotype may be another risk factor in gallstone formation after radical gastrectomy besides gallbladder motility disorder. Furthermore, we found that X^+X^- genotype had no interaction effect with gallbladder motility.

The *Xba* I RFLP-PCR in exon 26 of the *APOB* gene involves the 2488th nucleotide (ACC→ACT). The presence of thymine creates a restriction site for the *Xba* I enzyme characterizing the X^+ allele, whereas its absence determines the X^- allele. These are synonymous variations and so they do not affect the amino acid sequence of *APOB*[16,17]. Hegele *et al*[18] suggested that the allelic frequencies varied between races or within genetic subgroups of a single race. Caucasians have a much higher frequency of X^+ allele than Chinese. In our study, the frequency of X^+ allele in Chinese population was 13% similar to other regional reports and much lower than that reported in Caucasians, South Asians and Brazilians[8,19-21]. Law *et al*[22] and Rajput-Williams *et al*[23] also found a positive association between the X^+ allele and cholesterol concentration, whereas others did not demonstrate such correlation[24,25]. In the present study, we observed that in Chinese population, the serum levels of TC and LDL were significantly higher in X^+X^- group than in X^-X^- group both before and after gastrectomy, but no statistical difference was found in APOB values between the two groups. Since the X^+ and X^- variations are synonymous, the APOB concentration showed no difference between X^+X^- and X^-X^- groups. It is possible that the X^+ allele is in linkage disequilibrium with an unknown variation in the *APOB* gene or with a variation in another gene that influences the levels of TC and LDL, resulting in an increased saturation of bile cholesterol, leading to the gallstone formation[26,27].

In conclusion, the X^+ allele of *APOB* gene in Chinese patients is another important risk factor of gallstone formation after radical gastrectomy. Since this is the first report in a relatively small cohort, the findings should be validated in more independent studies.

COMMENTS

Background

The cause of higher incidence of gallstone after radical gastrectomy is very complex. Besides the gallbladder motility disorder, *Xba* I polymorphisms of apolipoprotein B (*APOB*) gene may be associated with gallstone development.

Research frontiers

APOB is a key protein in lipid metabolism. However, the effects of *APOB-Xba* I gene polymorphism on patients who underwent radical gastrectomy for gastric cancer are unknown. In this study, the authors demonstrate that X^+ allele of *APOB* gene is an important risk factor of gallstone formation after gastrectomy.

Innovations and breakthroughs

Previous studies have attributed higher incidence of gallstone after radical gastrectomy to gallbladder motility disorder. This is the first study to report the relationship between genetic factor (*APOB-Xba* I gene polymorphism) and gallstone formation in gastrectomized patients.

Applications

It is important to screen the high-risk group of gallstone in patients who underwent radical gastrectomy in order to prevent the gallstone formation after surgery.

Peer review

This is a small but interesting study with a reasonable rationale - examining whether a suspected genetic risk factor for gallstones increases the risk of gallstones after gastrectomy (which is a known major risk factor for gallstones). The authors indicated X allele of *APOB* gene is another risk factor for development of gallstone formation after radical gastrectomy. This present data is clear and interesting.

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