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Nonalcoholic fatty liver was associated with asymptomatic gallstones in a Chinese population

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Abstract

The aim of this study was to determine the association between nonalcoholic fatty liver disease (NAFLD) and asymptomatic gallstones in a Chinese population.

The study had a cross-sectional design and enrolled 7583 subjects who visited the physical check-up center at Sir Run Run Shaw Hospital between 2009 and 2011. Colorimetric methods were used to measure the levels of cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), whereas fasting plasma glucose (FPG) level was measured using a dextrose-oxidizing enzyme method. Subjects who completed a questionnaire and underwent a medical and ultrasound examinations were included in the study.

The prevalence of NAFLD was significantly higher in patients with asymptomatic gallstones than in those without asymptomatic gallstones (58.98% vs 46.58%, respectively; $P < .0001$). The age-adjusted odds ratio (OR) for NAFLD being accompanied by asymptomatic gallstones was 1.35 [95% confidence interval (CI), 1.13–1.61; $P = .0009$] in male and 1.92 (95% CI, 1.45–2.54; $P < .0001$) in female subjects. Asymptomatic gallstones were associated with NAFLD in subjects aged < 50 years (OR = 1.74, 95% CI, 1.44–2.12; $P < .0001$), but not in subjects aged > 50 years (OR = 1.17, 95% CI, 0.92–1.48; $P = .2040$). The OR of NAFLD for asymptomatic gallstones was 1.28 after multivariate logistic regression analysis (95% CI, 1.07–1.52; $P = .006$).

Our results indicated that asymptomatic gallstones are strongly associated with NAFLD in the Chinese study population.

Abbreviations: BMI = body mass index, CI = confidence interval, DBP = diastolic blood pressure, FPG = fasting plasma glucose, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OR = odds ratio, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride, WC = waist circumference.

Keywords: asymptomatic gallstones, hyperlipidaemia, hypertension, impaired fasting glucose, metabolic syndrome, nonalcoholic fatty liver disease, obesity

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a general health problem in both Western and Eastern countries. The estimated prevalence of NAFLD among adults in Western countries ranges from 20% to 33%.[1] In China, the prevalence of NAFLD has consistently been increasing in recent years with improved living conditions and adoption of westernized diets and is now estimated to be 26% and 20.82% among adults in the cities of Guangzhou[2] and Shanghai,[3] respectively. NAFLD includes a wide spectrum of liver conditions ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and may eventually develop into cirrhosis, portal hypertension, or hepatocellular carcinoma. The main risk factors for NAFLD include physiological characteristics such as age, sex, and ethnicity, as well as metabolic factors such as obesity, hyperlipidaemia, and type 2 diabetes.[3,4]

Asymptomatic gallstones have become prevalent in the general population and impose a heavy economic burden due to diagnosis, treatment, and indirect health care.[5,6] The prevalence of asymptomatic gallstones is 12.1% in China and shows an increasing trend.[7] Risk factors for asymptomatic gallstones include age, diabetes, obesity, hyperlipidaemia, hepatitis C, high caloric intake, and metabolic syndrome.[7–9] As NAFLD and asymptomatic gallstones have some common risk factors, asymptomatic gallstone patients may theoretically be prone to develop NAFLD as a result of impaired gallbladder motility and increased bile lysogenicity. Associations between NAFLD and gallstones in populations in the United States, Italy, Pakistan, Korea, and Taiwan have been studied.[10–16] No associations were found between NAFLD and gallstones in the studies on the US and Korean populations,[10,13] whereas NAFLD was associated with gallstones in the studies on the Pakistani and Taiwanese populations.[12,15,16] To the best of our knowledge, only 1 study has been conducted to investigate whether asymptomatic gallstones are related to the risk of NAFLD in a Chinese urban population; that longitudinal cohort study revealed that gallstones are risk factors for NAFLD. However, the correlation between NAFLD and asymptomatic gallstones in a Chinese general population has not been studied.

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In this study, cross-sectional analysis of 7583 subjects who underwent health check-ups at Sir Run Run Shaw Hospital from 2009 to 2011 was conducted to clarify the correlation between asymptomatic gallstones and NAFLD. All the subjects were asked to complete a questionnaire. In addition, each subject underwent physical examination to obtain the body mass index (BMI), diastolic blood pressure (DBP), systolic blood pressure (SBP) and chest circumference, and certain biochemical indicators were determined.

2. Materials and methods

2.1. Sample collection

Subjects who visited the physical check-up center at Sir Run Run Shaw Hospital in Hangzhou, Zhejiang, China between 2009 and 2011 were recruited in this cross-sectional study. Subjects diagnosed with asymptomatic gallstones were categorized as the experimental group, and subjects without gallstones were categorized as the control group. All the subjects provided a signed informed consent document and the study protocol was approved by the Ethics Committee of the hospital. Subjects were included in the study if they met the following criteria: absence of history of liver or gallbladder surgery; no hepatitis, gallbladder polyps, or inflammatory bowel disease; and complete abdominal ultrasound (US) results, physical examination data and clinical indicators. Finally, 7583 subjects (4989 men and 2594 women) were included in the study.

2.2. Questionnaire

Each subject was asked to complete a questionnaire to collect demographic data and clinical indicators. The questionnaire was designed by the authors and included the following items: telephone numbers, address, marital status, gravidity, oral contraceptives, hypertension, history of diabetes mellitus, hyperlipidaemia, gastrointestinal surgery, chronic liver disease, systemic diseases, family medical conditions, and other medications.

2.3. Physical examination

The weight and height of each subject were measured with the subject dressed in light clothing and without shoes. BMI was defined as weight (kg) divided by height (m) squared (kg/m^2). According to the midpoint between the lower edge of the thorax and the iliac crest (to the nearest 0.1 cm), the waist circumference (WC) was measured. One blood pressure reading was taken every minute for 3 minutes, and the means of the second and third systolic and diastolic measurements were calculated and used for analysis.

Blood samples used for laboratory tests were collected via venipuncture from each study participant after an overnight fast. The levels of fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using an AEROSET analysis system (Abbott Diagnostics, Holliston, MA). Colorimetric methods[17] were used to measure the concentrations of cholesterol, HDL-C and LDL-C, and a dextrose-oxidising enzyme method[7] was used to measure the FPG level. US examinations were also performed in all subjects.

2.4. Definition of gallstone disease

Abdominal US examination of the subjects was performed using a 3.5 MHz transducer, and the presence of asymptomatic gallstones was determined by the examination findings. Each US was conducted by a radiologist who did not know the objectives of the study. The presence of intraluminal echoes that were gravitationally dependent or attenuated by ultrasound transmission (acoustic shadowing) was considered diagnostic for gallstones.

2.5. Definition of NAFLD

NAFLD was defined according to the revised definition provided by the Chinese Hepatology Association in February 2006.[8] First, other aetiologic factors known to be related to chronic liver disease (alcohol intake, >20 g/d; autoimmune hepatitis; and hepatitis B antigen or hepatitis C virus antibody positivity) were excluded. Then, NAFLD was diagnosed on the basis of the brightness of the liver and the presence of diffuse echogenicity in the liver parenchyma on abdominal ultrasonography.[18]

2.6. Statistical analysis

The SPSS (Version 18.0, SPSS Inc, Chicago, IL) was used for statistical analysis, and the results are shown as the mean ± standard deviation. Binary variables are presented as the number (n) and percentage. The Student t test and χ^2 test were used to compare differences between cases and controls for all continuous variables and categorical variables, respectively. The association between NAFLD and human gallstones was tested using an age- and sex-adjusted logistic regression model. $P < .05$ (2-tailed) was considered statistically significant.

3. Results

3.1. Patient characteristics

Among the 7583 subjects, 919 (654 male and 265 female subjects) had asymptomatic gallstones and 6664 (4335 male and 2329 female subjects) did not exhibit asymptomatic gallstones; in other words, the prevalence of asymptomatic gallstones was 12.12% (919/7583). However, the prevalence of NAFLD was 48.08% (3646/7583) in all subjects and 58.98% (542/919) in subjects with asymptomatic gallstones and 46.58% (3104/6664) in those without gallstones. Of 7583 subjects, 542 (7.16%) exhibited both the conditions. The results of a univariate analysis of various factors and their relationships with asymptomatic gallstones are shown in Table 1. Compared with subjects without asymptomatic gallstones, subjects with asymptomatic gallstones were significantly older ($P < .0001$) and had higher WC ($P < .0001$), BMI ($P < .0001$), SBP ($P < .0001$), DBP ($P < .0001$), FPG ($P < .0001$), and TG ($P < .0001$) values. Moreover, subjects with asymptomatic gallstones had significantly smaller HDL-C ($P = .0005$) and cholesterol levels than did subjects without asymptomatic gallstones, and the incidences of hypertension ($P < .0001$), diabetes ($P < .0001$), hyperlipidaemia ($P = .0011$), and NAFLD were significantly greater in the subjects with than without gallstones ($P < .0001$). However, the levels of TC ($P = .1478$) and LDL-C ($P = .4122$) did not differ significantly between the 2 groups.

Table 1

Characteristics of the study subjects with and without gallstones (mean ± standard deviation) n (%).

	Group with gallstones (n = 919)	Group without gallstones (n = 6664)	*P* value
Male, n, %	654 (71.16)	4335 (65.05)	.0003
Hypertension, n, %	187 (21.06)	850 (12.97)	<.0001
Diabetes, n, %	51 (6.78)	192 (3.25)	<.0001
Hyperlipidaemia, n, %	526 (57.24)	3433 (51.52)	.0011
NAFLD, n, %	542 (58.98)	3104 (46.58)	<.0001
Age, y	48.54 ± 9.07	44.63 ± 8.99	<.0001
BMI, kg/m^2	26.31 ± 2.97	25.22 ± 3.36	<.0001
Waist circumference, cm	91.63 ± 9.40	87.79 ± 10.67	<.0001
SBP, mm Hg	123.65 ± 14.17	119.84 ± 14.49	<.0001
DBP, mm Hg	74.10 ± 9.85	72.14 ± 10.38	<.0001
Log glucose	0.71 ± 0.06	0.70 ± 0.06	<.0001
Log TG	2.19 ± 0.24	2.14 ± 0.26	<.0001
TC, mg/dL	172.30 ± 34.88	170.53 ± 34.42	.1478
Log HDL-c	1.64 ± 0.10	1.65 ± 0.10	.0005
LDL-c, mg/dL	76.75 ± 19.69	76.18 ± 19.76	.4122

The Student *t* test was used to compare group differences for all continuous variables, and Pearson χ^2 test was used to compare categorical variables. Hypertension was defined as any one of the following conditions: systolic blood pressure ≥140 mm Hg; diastolic blood pressure ≥90 mm Hg; or patient receiving drug treatment. Diabetes was defined as an FPG level ≥7.0 mmol/L or drug treatment. Hyperlipidaemia was defined as any one of the following conditions: triglycerides ≥200 mg/mL; cholesterol ≥240 mg/mL; or patient receiving drug treatment.

BMI = body mass index, DBP = diastolic blood pressure, HDL-c = high-density lipoprotein cholesterol, LDL-c = low-density lipoprotein cholesterol, NAFLD = nonalcoholic fatty liver disease, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.

3.2. Effect of sex on the relationship of NAFLD and asymptomatic gallstones in the Chinese study population

Sex-adjusted logistic regression analysis showed that NAFLD was significantly associated with the risk of developing asymptomatic gallstones, irrespective of sex (Table 2). The sex-adjusted odds ratio (OR) for NAFLD being accompanied by asymptomatic gallstones was 1.35 [95% confidence interval (CI), 1.13–1.61; $P = .0009$] in male subjects and 1.92 (95% CI, 1.45–2.54; $P < .0001$) in female subjects.

3.3. Effect of age on the relationship of NAFLD and asymptomatic gallstones in the Chinese study population

Multivariate regression analysis showed that among subjects aged < 50 years, the prevalence of asymptomatic gallstones accompanied by NAFLD was higher than the prevalence of NAFLD alone. The age-adjusted OR for NAFLD being accompanied by asymptomatic gallstones was 1.74 (95% CI, 1.44–2.12; $P < .0001$). However, there was no significant difference in the prevalence of asymptomatic gallstones with and without accompanying NAFLD among subjects aged > 50 years (OR = 1.17, 95% CI, 0.92–1.48; $P = .2040$) (Table 3).

3.4. Effects of clinical indicators on the relationship of NAFLD and asymptomatic gallstones

Multivariate logistic regression analysis of model 1 (adjusted by age and sex) showed that NAFLD was significantly associated with an increased incidence of asymptomatic gallstones (OR = 1.440, 95% CI, 1.2–1.68; $P = .000$) (Table 4). To avoid the influence of other relevant factors, further multiple logistic regression analysis was conducted to study the correlation between NAFLD and asymptomatic gallstones. After making adjustments for BMI ≥24, hyperlipidaemia, increased FPG level, and hypertension, multivariate regression analysis showed that NAFLD remained associated with an increased risk of asymptomatic gallstones (OR = 1.28, 95% CI, 1.07–1.52; $P = .006$).

4. Discussion

In this study, we found that Chinese people with gallstones were more likely to suffer from NAFLD, and this correlation was seen mainly in patients aged < 50 years. Among 7583 subjects who underwent abdominal ultrasonography, 3646 (48.08%) showed imaging signs of NAFLD and 919 (12.12%) showed imaging signs of gallstones. Current estimates of NAFLD prevalence in

Table 2

The relationship between nonalcoholic fatty liver disease and asymptomatic gallstones in male and female subjects.

	Male subjects			
	Group with gallstones, n (%)	Group without gallstones, n (%)	OR (95% CI)	*P* value
Fatty liver				
No	220 (33.64)	1774 (40.92)	1.00	—
Yes	434 (66.36)	2561 (59.08)	1.35 (1.13–1.61)	.0009
	Female subjects			
	Group with gallstones, n (%)	Group without gallstones, n (%)	OR (95% CI)	*P* value
Fatty liver				
No	157 (59.25)	1786 (76.69)	1.00	—
Yes	108 (40.75)	543 (23.31%)	1.92 (1.45–2.54)	<.0001

A sex-adjusted logistic regression model was used to test the association between fatty liver and gallstones.

CI = confidence interval, OR = odds ratio.

Table 3

The relationship between fatty liver and gallstones in different age groups.

	Age < 50 y			
	Group with gallstones, n (%)	**Group without gallstones, n (%)**	**OR (95% CI)**	***P* value**
Fatty liver				
No	226 (39.72)	2767 (54.91)	1.00	—
Yes	343 (60.28)	2272 (45.09)	1.74 (1.44–2.12)	<.0001
	Age > 50 y			
	Group with gallstones, n (%)	**Group without gallstones, n (%)**	**OR (95% CI)**	***P* value**
Fatty liver				
No	151 (43.14)	793 (48.80)	1.00	—
Yes	199 (56.86)	832 (51.20)	1.17 (0.92–1.48)	.2040

An age-adjusted logistic regression model was used to test the association between fatty liver and gallstones.
CI = confidence interval, OR = odds ratio.

Western and Asian countries range from 24% to 42% and from 5% to 40%, respectively.[19–21] The prevalence of NAFLD within the general population of China is estimated at 5% to 26%.[2,3] The NAFLD prevalence in our report was higher than those stated in other reports. Possible explanations for this discrepancy are that most subjects in our study were from the middle to upper socioeconomic classes and that their diets were more westernized.[22] Gallstones were present in 919 subjects (12.12%) and the prevalence of NAFLD was higher in the group with than without gallstones (58.98% vs 46.58%, respectively), which was consistent with some previous studies[19,20,23,24] but rejected by others.[21,22,25,26] This may be due to differences in the sample sources, levels of society, and eating habits.

Older age is known to be a significant risk factor for asymptomatic gallstones.[25,26] In our study, age-adjusted logistic regression analysis showed that NAFLD was significantly associated with the risk of developing asymptomatic gallstones both in male and female subjects. However, this association was more pronounced in female than male subjects, which was similar to the previous cohort study.[22] This may be related to glucose metabolism and oestrogen aggravation in the female body. Moreover, multivariate regression analysis showed that the association of NAFLD and asymptomatic gallstones was only seen in patients younger than 50 years of age. Age of more than 50 years can be used as an independent predictor of cholelithiasis.[27] Previous studies have shown that the prevalence of gallstones and NAFLD increases with age,[22,28] particularly in women who are in menopause or postmenopausal.[29] Liu et al[30] have suggested that subjects aged 50 years and older have a risk of gallstones more than 5 times that of subjects younger than 50 years. In addition, the association of NAFLD and asymptomatic gallstones remained significant when adjusted for BMI, hyperlipidaemia, FPG, and hypertension. Thus, regardless of sex, our study indicated that NAFLD was closely related to asymptomatic gallstones. This finding was similar to that of previous studies that indicated that gallstone disease is an independent risk factor for NAFLD, and NAFLD is an independent risk factor for cholelithiasis.[27,31]

Table 4

Multivariable logistic regression analysis for an association between nonalcoholic fatty liver disease and asymptomatic gallstones after adjusting for various factors.

Fatty liver	***P* value**	**OR (95% CI)**
Model 1 (age + sex)	.000	1.44 (1.2–1.68)
Model 2 (model 1 + BMI ≥24)	.002	1.32 (1.11–1.57)
Model 3 (model 2 + hyperlipidaemia)	.003	1.32 (1.1–1.59)
Model 4 (model 3 + raised FPG)	.006	1.28 (1.07–1.53)
Model 5 (model 4 + hypertension)	.006	1.28 (1.07–1.52)

A logistic regression model was used to test the associations between fatty liver and asymptomatic gallstones after adjusting for other factors. Increased FPG means an FPG level ≥5.6 mmol/L or drug treatment.
BMI = body mass index, CI = confidence interval, FPG = fasting plasma glucose, OR = odds ratio.

Several previous reports have suggested an association between asymptomatic gallstones and NAFLD.[31–33] Given that the risk for asymptomatic gallstones and NAFLD is particularly high among patients with obesity, hyperlipidaemia, type 2 diabetes, and hypertension,[7,27,28] this association might be caused by asymptomatic gallstones and NAFLD having certain common pathogenic factors. All of the above-mentioned conditions belong to the metabolic syndrome, and NAFLD as a determinant significantly increases the development of metabolic syndrome.[34–36] In this study, by adjusting for metabolism factors, NAFLD remained closely associated with asymptomatic gallstones.

Insulin resistance may be an important mechanistic factor underlying the positive relationship between NAFLD and asymptomatic gallstones.[37,38] The study conducted by Sekine et al[39] showed that accumulated abdominal visceral fat plays an important role in the development of gallstone disease, and that visceral adiposity can lead to insulin resistance and hyperinsulinaemia. Contraction of the gallbladder is induced by the action of secreted cholecystokinin (CCK) on CCK receptors. NAFLD may be related to gallbladder hypermotility through insulin resistance and a decreased response to CCK as a result of hyperinsulinaemia. Another possible mechanism is that insulin resistance enhances gallstone formation by activating HMG CoA reductase, which leads to not only increased bile secretion but also increased cholesterol in bile produced by the liver.[40] Insulin resistance stimulates lipolysis in adipose tissue and the influx of free fatty acids into the liver, which may aggravate hepatic insulin resistance; thus, these 2 processes influence each other. Besides, hepatic insulin resistance contributes to the secretion of bile cholesterol by stimulating the expression of biliary cholesterol transporter proteins, suppressing the bile acid synthetic enzyme thereby decreasing bile acid synthesis and causing strong gallbladder movement.[41] A community-based study identified the relationship between combined hyperinsulinaemia plus insulin resistance and asymptomatic gallstones among female subjects.[42] When compared with control subjects without asymptomatic gallstone pathology, female diabetics with asymptomatic gallstones

had significantly higher serum insulin levels, higher HOMA-IR values and lower HOMA β-cell function.[42] However, male subjects showed no significant difference from control subjects regarding insulin resistance, suggesting sex differences with regard to lithogenesis.[42]

Our study had some limitations that should be mentioned. The major limitation was that the relationship between NAFLD severity and asymptomatic gallstones was not determined. Another limitation was that this study was conducted at a single institution; a multicenter study would provide more conclusive results regarding the relationship between NAFLD and asymptomatic gallstones. Furthermore, most of the subjects came from Zhejiang, China, and other populations in other cities were not included in our study; thus, more comprehensive Chinese populations should be used to study the relationship between NAFLD and asymptomatic gallstones. Moreover, NAFLD was defined by brightness in the liver and the presence of diffuse echogenicity in the liver parenchyma on abdominal ultrasonography, and semiquantitative indices were not used.

In conclusion, NAFLD was an independent risk factor for asymptomatic gallstones, particularly in female subjects and subjects aged < 50 years in the Chinese study population. Further studies should focus on the relationships between insulin resistance, NAFLD and asymptomatic gallstones, as well as the biological basis for the association of NAFLD and gallstones.

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