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ORIGINAL ARTICLE

High Levels of Serum Triglyceride, Low-density Lipoprotein Cholesterol, Total Bile Acid, and Total Bilirubin are Risk Factors for Gallstones

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SUMMARY

Background: **This study aimed to identify the risk factors for gallstone disease in the Hakka population in the Meizhou area of China.**

Methods: **In total, 816 patients with gallstone disease and 818 control participants were included in the study, and their serum lipid levels were measured. Data on age, gender, and risk factors for gallstone disease (such as smoking and drinking history and the prevalence of hypertension) were recorded.**

Results: **Of the 1,634 enrolled individuals, age 13 - 101 years, 727 were men and 907 were women. Serum triglyceride (TG) ($p < 0.001$), low-density lipoprotein-cholesterol (LDL-C) ($p = 0.043$), total bile acid (TBA) ($p < 0.001$), and total bilirubin (T-BIL) ($p < 0.001$) levels showed significant differences between the patients and controls. However, age, the proportion history of drinking and smoking; the prevalence of hypertension and diabetes mellitus; and serum levels of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), and Apo-A1/Apo-B were similar between the two groups. The frequencies of gallstones in the common bile duct ($\chi^2 = 13.909$, $p < 0.001$) and intrahepatic bile ducts ($\chi^2 = 8.289$, $p = 0.004$) showed significant differences between male and female patients, but the distribution of gallstones of different sizes was similar between the two groups. Serum TBA ($p < 0.001$) and T-BIL ($p < 0.001$) levels were higher in patients with gallstones in the common bile duct than in those with gallstones in the gall bladder and intrahepatic bile ducts. Logistic regression analysis indicated that participants with high serum TG, LDL-C, TBA, and T-BIL levels had a significantly higher risk of gallstone disease.**

Conclusions: **High serum levels of TG, LDL-C, TBA, and T-BIL are found to be the main risk factors for gallstone formation in our study.**

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KEY WORDS

gallstone, serum lipid, serum total bile acid, serum total bilirubin, Hakka

LIST OF ABBREVIATIONS

TG - triglycerides
TC - total cholesterol
HDL-C - high-density lipoprotein-cholesterol
LDL-C - low-density lipoprotein-cholesterol
Apo-A1 - apolipoprotein A1
Apo-B - apolipoprotein B
TBA - total bile acid
T-BIL - total bilirubin

INTRODUCTION

Gallstone disease involves the development of symptoms or complications caused by the formation of gallstone masses in the gallbladder or biliary tract owing to abnormally high cholesterol or bilirubin levels in bile [1]. Gallstones can cause symptoms such as abdominal pain, jaundice, and fever, and can lead to clinical diseases such as cholecystitis, cholangitis, and acute pancreatitis [2]. Moreover, studies have shown that more than 90% of patients with gallbladder cancer have a history of gallstones, making the presence of gallstones an important risk factor for this disease [3,4]. The prevalence of gallstones in adults worldwide is about 10 - 15% [1,5]. In China, it is reported to be between 4.2% and 23% [6-8].

In general, identifying risk factors is essential both for risk prediction and the prevention of gallstone disease. The risk of gallstone disease varies according to various genetic and environmental factors [1,9]. Epidemiological studies have revealed some risk factors for gallstones, and these include metabolic abnormalities [5], obesity [10], hyperinsulinemia [11], and type 2 diabetes mellitus [12,13]. Moreover, genetic factors have also been found to be correlated with gallstone formation. Mutations in genes such as ATP-binding cassette, subfamily G, member 8 (*ABCG8*), UDP glucuronosyltransferase family member A1 (*UGT1A1*), ATP-binding cassette, subfamily B, member 4 (*ABCB4*), ATP-binding cassette, subfamily B, member 11 (*ABCB11*), cystic fibrosis transmembrane conductance regulator (*CFTR*), and cytochrome P450, subfamily VIIa, polypeptide 1 (*CYP7A1*) have been found to be associated with gallstone disease [14-16].

Previous epidemiological studies have shown that the incidence of gallstones in Guangdong province is approximately 8.0% [17]. Meizhou, located in the northeast region of Guangdong Province, at the junction of Fujian, Guangdong, and Jiangxi, has a high population of Hakka people [18], who are a part of the Han ethnic group from northern China. So far, to our knowledge, there has been no research on the risk factors for gallstones in the population in this area. Therefore, the objective of this study was to identify the risk factors for gallstone disease in this population in order to provide a scientific basis for the prevention and treatment of gallstones in this region.

MATERIALS AND METHODS

Population

In total, 1,634 inpatients were recruited from the Meizhou People's Hospital (Huangtang Hospital), Guangdong province, China, between January 2016 and June 2020; the sample consisted of 816 patients with gallstone disease and 818 randomly selected individuals with non-gallstone disease (controls). Diagnoses of gallstone disease were made by gastroenterologists based on clinical symptoms and ultrasound, cholecystography, and computed tomography/magnetic resonance imaging findings. Information on age, gender, and risk factors of gallstone disease (smoking history, drinking history, prevalence of hypertension and diabetes mellitus, etc.) was recorded.

Serum index measurements

On day 2 of admission, about 5 mL of blood was obtained from each subject, and serum was immediately isolated and analyzed. Total cholesterol (TC) analysis was measured using cholesterol esterase/peroxidase (CHOD/PAP) enzymatic method [19], triglyceride (TG) analysis was measured using glycerophosphate oxidase/peroxidase (GPO-PAP) enzymatic method [20], low-density lipoprotein-cholesterol (LDL-C) analysis was measured using direct surfactant removal method [21], high-density lipoprotein-cholesterol (HDL-C) analysis was measured using selective inhibitory direct method [22], apolipoprotein A1 (Apo-A1) and apolipoprotein B (Apo-B) was measured using immunoturbidimetry method [23], total bile acid (TBA) was measured using circulating enzymatic (3α-hydroxysteroid dehydrogenase) method [24], total bilirubin (T-BIL) was measured using chemical oxidation method [25]. Samples were evaluated using the Olympus AU5800 system (Olympus Corporation, Tokyo, Japan).

Statistical analysis

SPSS statistical software version 21.0 (IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA) was used for data analysis. Continuous variables with normal distribution were expressed as mean ± standard deviation (SD), and analyzed using the *t*-test, while data with non-normal distribution were described as medians and range, and analyzed using the Mann-Whitney U test. Analysis of variance (ANOVA) was used to compare the mean values of continuous variables across multiple groups. Further, the chi-squared test was used for analyzing categorical variables, which were presented as percentages. Logistic regression analysis was ap-

plied to assess the interactions between plasma lipid levels and various factors (smoking history, drinking history, prevalence of hypertension and diabetes, etc.) in gallstone disease. $p < 0.05$ was considered statistically significant.

RESULTS

Population characteristics

The characteristics of the 816 patients with gallstones and the 818 controls are shown in Table 1. Of the 1,634 enrolled individuals, age 13 - 101 years, 727 were men (44.5%) (mean age ± SD: 56.4 ± 17.1 years, range 14 - 93 years) and 907 were women (55.5%) (mean age ± SD: 55.6 ± 16.4 years, range 13 - 101 years). The differences in TG ($p < 0.001$), LDL-C ($p = 0.043$), TBA ($p < 0.001$), and T-BIL ($p < 0.001$) levels between patients and controls were statistically significant. However, there were no statistically significant differences in age, the proportion with a history of drinking and smoking, the prevalence of hypertension and diabetes mellitus; and serum levels of TC, HDL-C, Apo-A1, Apo-B, and Apo-A1/Apo-B between the two groups. There were significant differences in TG ($p < 0.001$), Apo-A1/Apo-B ($p < 0.001$), TBA ($p < 0.001$), and T-BIL ($p < 0.001$) levels between male patients and controls, and in TG ($p < 0.001$), TC ($p = 0.001$), LDL-C ($p = 0.013$), TBA ($p < 0.001$), and T-BIL ($p < 0.001$) levels between female patients and controls.

Comparison of gallstone disease according to gender

Among patients with gallstone disease, gallstones were observed in the gall bladder alone, common bile duct alone, intrahepatic bile ducts alone, gall bladder and common bile duct both, common bile duct and intrahepatic bile ducts both, and gall bladder and intrahepatic bile ducts both in 71.7%, 10.2%, 2.8%, 12.9%, 1.6%, and 0.9% of patients, respectively. There were statistically significant differences in the frequencies of gallstones in the common bile duct alone ($\chi^2 = 13.909$, $p < 0.001$), and intrahepatic bile ducts alone ($\chi^2 = 8.289$, $p = 0.004$) between male and female patients (Table 2). The prevalence of gallstones in the common bile duct alone was higher in men than in women, while the proportion of gallstones in the intrahepatic bile ducts alone was lower.

Regarding size, the diameter of the gallbladder stones was less than 0.5 cm in 66 (8.1%) cases, 0.5 - 0.9 cm in 177 (21.7%) cases, 1.0 - 1.9 cm in 313 (38.4%) cases, and more than or equal to 2.0 cm in 260 (31.9%) cases. There were no statistically significant differences in the size distribution of gallbladder stones between male and female patients (Table 2).

Comparison of lipid levels according to gallstone location and size

The relationship of gallstone location and size with serum lipid levels was analyzed. Subjects with gallstones at multiple locations were excluded (gall bladder and common bile duct (n = 105), common bile duct and intrahepatic bile ducts (n = 13), and gall bladder and intrahepatic bile ducts (n = 7)). We found that serum TBA ($p < 0.001$) and T-BIL ($p < 0.001$) levels in patients with gallstones in the common bile duct were higher than those in patients with gallstones in the gall bladder and intrahepatic bile ducts. However, serum lipid levels did not show significant differences according gallstone size (Table 3).

Risk factors associated with gallstone disease

Logistic regression analysis was performed to identify independent predictors of gallstone disease (Table 4). Univariate regression analysis showed that high age, and high TG, TC, LDL-C, TBA, and T-BIL levels (all $p < 0.05$) were associated with significantly higher risks of gallstone disease. Further multiple logistic regression analysis indicated that participants with high TG (adjusted OR 1.136, 95% CI 1.015 - 1.272, $p = 0.026$), LDL-C (adjusted OR 1.483, 95% CI 1.159 - 1.898, $p = 0.002$), TBA (adjusted OR 1.019, 95% CI 1.010 - 1.027, $p < 0.001$), and T-BIL levels (adjusted OR 1.026, 95% CI 1.016 - 1.036, $p < 0.001$) had a significantly higher risk of gallstone disease. Receiver operating characteristics (ROC) curve analysis showed that the positive cut-off values for serum TG, LDL-C, TBA, and T-BIL levels in increasing gallstone risk were 1.095 mmol/L (AUROC = 0.692, $p < 0.001$, 95% CI 0.666 - 0.718), 2.485 mmol/L (AUROC = 0.697, $p < 0.001$, 95% CI 0.671 - 0.722), 11.250 µmol/L (AUROC = 0.593, $p < 0.001$, 95% CI 0.566 - 0.621), and 26.250 µmol/L (AUROC = 0.597, $p < 0.001$, 95% CI 0.570 - 0.625), respectively.

DISCUSSION

Gallstone disease is mainly caused by liver metabolism disorders, biliary motor dysfunction, and abnormal bile cholesterol output, resulting in the dissolution of the solid components in bile, followed by stone formation. The causes of gallstones are complex, and an increasing number of studies have shown that their development is associated with factors such as age, gender, obesity, high-fat diets, and abnormal lipid metabolism [1]. In the present study, we examined the potential risk factors for gallstones in the population in the Meizhou area and identified high serum levels of TG, LDL-C, TBA, and T-BIL as potential predictors of gallstone risk.

Although dyslipidemia is common, the relationship between dyslipidemia and gallstone disease is still unclear. Premkumar et al. reported that obesity, dyslipidemia, high-fat diets, and high caloric intake increased the risk of gallstone disease [26]. A Korean study demonstrated that low HDL-C levels were an independent predictor of gallstone risk, showing a significant association with gallbladder stone formation in premenopausal women [27]. Brasca et al. found that lower HDL-C and higher

Table 1. Clinical features of patients with gallstones and controls.

	Total (n = 1,634)			Male (n = 727)			Female (n = 907)		
	Patient group (n = 816)	Control group (n = 818)	p-values *	Patient group (n = 345)	Control group (n = 382)	p-values *	Patient group (n = 471)	Control group (n = 436)	p-values *
Age, years	57.0 ± 14.3	54.9 ± 18.7	0.011	58.6 ± 14.1	54.3 ± 19.2	0.001	55.8 ± 14.4	55.3 ± 18.3	0.700
Ever drinker (n, %)	21 (2.6%)	14 (1.7%)	0.237 (χ^2 = 1.448)	21 (6.1%)	14 (3.7%)	0.165 (χ^2=2.321)	0 (0)	0 (0)	-
Ever smoker (n, %)	49 (6.0%)	36 (4.4%)	0.149 (χ^2 = 2.131)	49 (14.2%)	36 (9.4%)	0.050 (χ^2 = 4.010)	0 (0)	0 (0)	-
Hyper-tension (n, %)	161 (19.7%)	141 (17.2%)	0.203 (χ^2 = 1.685)	73 (21.2%)	67 (17.5%)	0.222 (χ^2 = 1.528)	88 (18.7%)	74 (17.0%)	0.501 (χ^2 = 0.452)
Diabetes Mellitus (n, %)	105 (12.9%)	90 (11.0%)	0.253 (χ^2 = 1.352)	54 (15.7%)	46 (12.0%)	0.163 (χ^2 = 1.992)	51 (10.8%)	44 (10.1%)	0.718 (χ^2 = 0.131)
TG, mmol/L	1.430 ± 1.164	1.103 ± 1.280	< 0.001	1.409 ± 1.083	1.057 ± 1.208	< 0.001	1.445 ± 1.221	1.143 ± 1.340	< 0.001
TC, mmol/L	6.099 ± 28.336	4.256 ± 3.001	0.064	7.003 ± 43.374	3.863 ± 1.432	0.158	5.437 ± 3.782	4.600 ± 3.855	0.001
HDL-C, mmol/L	2.335 ± 24.617	1.574 ± 3.876	0.383	3.237 ± 37.623	1.373 ± 2.074	0.334	1.674 ± 3.709	1.749 ± 4.938	0.793
LDL-C, mmol/L	3.749 ± 20.866	2.259 ± 2.652	0.043	4.349 ± 31.311	2.005 ± 0.848	0.144	3.310 ± 6.080	2.482 ± 3.531	0.013
Apo-A1, g/L	1.723 ± 9.589	1.202 ± 1.524	0.126	1.668 ± 11.793	1.144 ± 1.576	0.390	1.763 ± 7.593	1.254 ± 1.477	0.169
Apo-B, g/L	1.944 ± 28.065	0.901 ± 3.600	0.292	3.182 ± 43.079	0.896 ± 4.599	0.303	1.038 ± 2.362	0.905 ± 2.412	0.401
Apo-A1/ Apo-B	1.664 ± 5.520	1.801 ± 0.875	0.485	1.320 ± 0.622	1.821 ± 0.910	< 0.001	1.917 ± 7.239	1.783 ± 0.844	0.702
TBA, µmol/L	23.667 ± 57.103	5.795 ± 9.157	< 0.001	32.181 ± 66.397	6.423 ± 9.887	< 0.001	17.430 ± 48.325	5.244 ± 8.440	< 0.001
T-BIL, µmol/L	26.743 ± 37.567	15.440 ± 10.755	< 0.001	34.948 ± 46.030	16.637 ± 11.941	< 0.001	20.732 ± 28.474	14.391 ± 9.487	< 0.001

Continuous variable values with normal distribution expressed as mean ± SD.
TG - triglycerides, TC - total cholesterol, HDL-C - high-density lipoprotein-cholesterol, LDL-C - low-density lipoprotein-cholesterol, Apo-A1 - apolipoprotein A1, Apo-B - apolipoprotein B, TBA - total bile acid, T-BIL - total bilirubin.
*** - Mann-Whitney U or *t*-test for continuous variable values, chi-squared test for categorical variable values (patient group vs. control group).**

Table 2. Comparison of gallstones between males and females.

Parameter	Total (n = 816)	Male (n = 345)	Female (n = 471)	p-value *
Stone location				
Gall bladder	585 (71.7%)	236 (68.4%)	349 (74.1%)	0.084 (χ^2 = 3.179)
Common bile duct	83 (10.2%)	51 (14.8%)	32 (6.8%)	< 0.001 (χ^2 = 13.909)
Intrahepatic bile ducts	23 (2.8%)	3 (0.9%)	20 (4.2%)	0.004 (χ^2 = 8.289)
Gall bladder + Common bile duct	105 (12.9%)	50 (14.5%)	55 (11.7%)	0.246 (χ^2 = 1.408)
Common bile duct + Intrahepatic bile ducts	13 (1.6%)	4 (1.2%)	9 (1.9%)	0.573 (χ^2 = 0.717)
Gall bladder + Intrahepatic bile ducts	7 (0.9%)	1 (0.3%)	6 (1.3%)	0.249 (χ^2 = 2.267)
The diameter of the largest stone				
< 0.5 cm	66 (8.1%)	24 (7.0%)	42 (8.9%)	0.363 (χ^2 = 1.030)
0.5 - 0.9 cm	177 (21.7%)	79 (22.9%)	98 (20.8%)	0.492 (χ^2 = 0.513)
1.0 - 1.9 cm	313 (38.4%)	140 (40.6%)	173 (36.7%)	0.275 (χ^2 = 1.248)
≥ 2.0 cm	260 (31.9%)	102 (29.6%)	158 (33.5%)	0.254 (χ^2 = 1.453)

* - chi-squared test (males vs. females).

TG levels were associated with a higher probability of gallstone disease, especially in women [28]. Finally, Chang et al. reported that hypercholesterolemia may exert an additive effect in increasing the risk of gallstone disease in women in Taiwan [29]. In addition, several studies have reported that high serum TG levels are positively correlated with the development of gallstones. This is not surprising as high TG levels are often part of a multifactorial disorder of VLDL metabolism, and most patients with high TG levels show supersaturated bile and diminished gallbladder motility, both of which contribute to gallstone formation [30]. It was seen that gallstone disease prevalence was associated with high TG in women [28].

Bile is synthesized by the liver and secreted through liver tubules. It contains bile acids, cholesterol, phospholipids, and other components. When lipid levels increase, serum lipids can be secreted into bile through serous membrane transport from liver cells, increasing the concentration of lipids in bile. This causes lipid supersaturation in bile, resulting in lipid crystallization inside the gallbladder, and eventually leading to gallstone formation. Further, dyslipidemia leads to the accumulation of excess lipids in the liver, affecting liver cell function, and reducing the secretion of bile acids, thus resulting in a state conducive to gallstone formation [30]. Moreover, it is also speculated that in patients with gallstones, increased bile acid synthesis and triglyceride concentrations in the liver are caused by increased microsomal triglyceride protein activity [31].

The relationship of serum TBA and T-BIL levels with gallstones remains unclear. One study showed that prolonged orocecal transit time leads to small intestinal bacterial overgrowth, thus increasing serum bile acid levels and leading to the formation of gallstones [32]. However, another study reported no correlation between serum TBA levels and gallstones [33]. Similarly, some studies report that serum T-BIL levels [34] and their changes over time [35] are risk factors and predictors for common bile duct stones and one study reported that high serum TBA levels are a risk factor for gallstone disease in male Taiwanese vegetarians [36]. Some studies have shown no relationship between serum TBA levels and gallstones [37,38].

It is also controversial whether gender is a risk factor for gallstone disease. The results of our study suggest that gender is not associated with gallstone disease, which is consistent with some previous research [39, 40]. However, some studies based on western populations have suggested that women are more likely to develop gallstone disease [41,42]. Further, it is believed that gender hormone levels are associated with cholesterol metabolism, and gender may thus be related to cholesterol stones [43]. The relationship between gender and gallstone disease may be different between Asian and western populations, which warrants further research.

In the present study, age was not found to be associated with gallstone disease. Diehl et al. found that patients with pigment stones were older than those with cholesterol stones [44]. Some studies have found that gallstone prevalence increases with age [45-47], and that old age is a risk factor for gallstone disease [48]. Older people have underlying conditions such as hyperlipidemia, hypertension, and diabetes. Moreover, the function of a few organs, such as the gallbladder, may dęte-

Table 3. Comparison of lipid levels between patients with different gallstones.

	Stone location				Stone size				
	Gall bladder (n = 585)	Common bile duct (n = 83)	Intrahepatic bile ducts (n = 23)	p-values *	< 0.5 cm (n = 66)	0.5 - 1 cm (n = 177)	1 - 2 cm (n = 313)	≥ 2 cm (n = 260)	p-values *
TG, mmol/L	1.515 ± 1.286	1.237 ± 0.880	1.255 ± 0.774	0.109	1.460 ± 1.061	1.471 ± 1.418	1.375 ± 1.122	1.459 ± 1.044	0.771
TC, mmol/L	6.633 ± 33.451	4.682 ± 1.174	5.056 ± 0.940	0.847	4.947 ± 1.320	5.234 ± 5.511	7.617 ± 45.516	5.153 ± 2.303	0.692
HDL-C, mmol/L	2.747 ± 29.069	1.278 ± 0.418	1.420 ± 0.400	0.878	1.370 ± 0.477	1.318 ± 0.363	3.632 ± 39.489	1.710 ± 4.988	0.697
LDL-C, mmol/L	4.189 ± 24.629	2.624 ± 0.845	2.786 ± 0.830	0.815	2.731 ± 0.939	3.220 ± 6.783	4.671 ± 32.863	3.258 ± 5.986	0.796
Apo-A1, g/L	1.973 ± 11.316	1.075 ± 0.381	1.270 ± 0.487	0.737	1.190 ± 0.355	1.327 ± 2.281	2.365 ± 15.003	1.354 ± 3.714	0.514
Apo-B, g/L	2.384 ± 33.143	0.809 ± 0.253	0.883 ± 0.207	0.890	0.878 ± 0.274	0.865 ± 0.284	3.451 ± 45.225	1.136 ± 3.171	0.689
Apo-A1/ Apo-B	1.749 ± 6.502	1.426 ± 0.613	1.535 ± 0.717	0.892	1.478 ± 0.742	1.711 ± 3.296	1.929 ± 8.538	1.362 ± 0.595	0.663
TBA, µmol/L	12.671 ± 37.322	55.354 ± 84.571	17.483 ± 38.810	< 0.001	27.717 ± 59.828	24.444 ± 53.439	21.229 ± 52.955	25.044 ± 63.481	0.781
T-BIL, µmol/L	19.242 ± 22.656	52.131 ± 57.729	23.474 ± 25.426	< 0.001	26.702 ± 34.930	30.812 ± 51.073	24.404 ± 28.157	26.797 ± 37.271	0.349

TG - triglycerides, TC - total cholesterol, HDL-C - high-density lipoprotein-cholesterol, LDL-C - low-density lipoprotein-cholesterol, Apo-A1 - apolipoprotein A1, Apo-B - apolipoprotein B, TBA - total bile acid, T-BIL - total bilirubin.

*** - ANOVA for comparing the mean values of multiple groups of continuous variable data.**

Table 4. Logistic regression analysis of risk factors associated with gallstone disease.

Variables	Unadjusted values			Adjusted values		
	p-value *	β	OR (95% CI)	p-value *	β	Adjusted OR (95% CI)
Age, y	0.011	0.008	1.008 (1.002 - 1.013)	0.243	0.004	1.004 (0.997 - 1.010)
TG	< 0.001	0.265	1.304 (1.176 - 1.445)	0.026	0.128	1.136 (1.015 - 1.272)
TC	< 0.001	0.349	1.418 (1.312 - 1.533)	0.109	0.147	1.158 (0.968 - 1.386)
LDL-C	< 0.001	0.512	1.668 (1.492 - 1.865)	0.002	0.394	1.483 (1.159 - 1.898)
TBA	< 0.001	0.024	1.024 (1.017 - 1.032)	< 0.001	0.019	1.019 (1.010 - 1.027)
T-BIL	< 0.001	0.031	1.032 (1.023 - 1.040)	< 0.001	0.025	1.026 (1.016 - 1.036)

TG - triglycerides, TC - total cholesterol, LDL-C - low-density lipoprotein-cholesterol, TBA - total bile acid, T-BIL - total bilirubin, OR - odds ratio, CI - confidence interval.
*** - Logistic regression analysis.**

riorate, which can lead to gallstone disease.

In general, our study showed that high serum levels of TG, LDL-C, TBA, and T-BIL are the main risk factors for gallstone formation in the population living in the Meizhou area. This study has several strengths. This was the first study examining the risk factors for gallstone disease in the population in the Meizhou area. Further, the clinical characteristics and serum lipid, TBA, and T-BIL levels were included in the analysis to exclude the influence of related confounding factors on the results. Nevertheless, there were some limitations to this study that should be acknowledged. First, gallstone disease is a multifactorial disease caused by genetic and environmental factors, and there was no assessment of potential gene-environment interactions in our study. Second, the sample size was not very large, which may lead to some deviations in the results. Therefore, further studies with a larger sample size are warranted.

CONCLUSION

In general, in our study, high serum levels of TG, LDL-C, TBA, and T-BIL are found to be the main risk factors for gallstone formation.

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Ethics Approval and Consent to Participate:
The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-Sen University.

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Declaration of Interest:
The authors declare that they have no competing interests with the contents of this paper.

Reference:

1. Lammert F, Gurusamy K, Ko CW, et al. Gallstones. Nat Rev Dis Primers 2016;2:16024 (PMID: 27121416).
2. Shabanzadeh DM. Incidence of gallstone disease and complications. Curr Opin Gastroenterol 2018;34:81-9 (PMID: 29256915).
3. Goetze TO. Gallbladder carcinoma: Prognostic factors and therapeutic options. World J Gastroenterol 2015;21:12211-7 (PMID: 26604631).
4. Shrikhande SV, Barreto SG, Singh S, Udwadia TE, Agarwal AK. Cholelithiasis in gallbladder cancer: coincidence, cofactor, or cause! Eur J Surg Oncol 2010;36:514-9 (PMID: 20537839).
5. Portincasa P, Moschetta A, Palasciano G. Cholesterol gallstone disease. Lancet 2006;368:230-9 (PMID: 16844493).

6. Hsing AW, Gao YT, Han TQ, et al. Gallstones and the risk of biliary tract cancer: a population-based study in China. Br J Cancer 2007;97:1577-82 (PMID: 18000509).

7. Zhang YP, Liu C, Ye L, et al. Early prediction of persistent organ failure by serum Angiopoietin-2 in patients with acute pancreatitis. Dig Dis Sci 2016;61:3584-91 (PMID: 27686934).

8. Zhu L, Aili A, Zhang C, Saiding A, Abudureyimu K. Prevalence of and risk factors for gallstones in Uighur and Han Chinese. World J Gastroenterol 2014;20:14942-9 (PMID: 25356055).

9. Stokes CS, Krawczyk M, Lammert F. Gallstones: environment, lifestyle and genes. Dig Dis 2011;29:191-201 (PMID: 21734384).

10. Méndez-Sánchez N, Chavez-Tapia NC, Motola-Kuba D, et al. Metabolic syndrome as a risk factor for gallstone disease. World J Gastroenterol 2005;11:1653-7 (PMID: 15786544).

11. Chait A, Bierman EL, Albers JJ. Low-density lipoprotein receptor activity in cultured human skin fibroblasts. Mechanism of insulin-induced stimulation. J Clin Invest 1979;64:1309-19 (PMID: 227931).

12. Weikert C, Weikert S, Schulze MB, et al. Presence of gallstones or kidney stones and risk of type 2 diabetes. Am J Epidemiol 2010;171:447-54 (PMID: 20089496).

13. Nervi F, Miquel JF, Alvarez M, et al. Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. J Hepatol 2006;45:299-305 (PMID: 16516330).

14. Buch S, Schafmayer C, Völzke H, et al. A genome-wide association scan identifies the hepatic cholesterol transporter ABCG8 as a susceptibility factor for human gallstone disease. Nat Genet 2007;39:995-9 (PMID: 17632509).

15. Buch S, Schafmayer C, Völzke H, et al. Loci from a genome-wide analysis of bilirubin levels are associated with gallstone risk and composition. Gastroenterology 2010;139:1942-51 (PMID: 20837016).

16. Qayyum F, Lauridsen BK, Frikke-Schmidt R, Kofoed KF, Nordestgaard BG, Tybjærg-Hansen A. Genetic variants in CYP7A1 and risk of myocardial infarction and symptomatic gallstone disease. Eur Heart J 2018;39:2106-16 (PMID: 29529257).

17. Su Z, Gong Y, Liang Z. Prevalence of gallstone in Mainland China: A meta-analysis of cross-sectional studies. Clin Res Hepatol Gastroenterol 2020;44:e69-e71 (PMID: 32446673).

18. Li SM. Population migration, regional economic growth and income determination: a comparative study of Dongguan and Meizhou, China. Urban Studies 1997;7:999-1026. https://journals.sagepub.com/doi/10.1080/0042098975709

19. Trinder P, Webster D. Determination of HDL-cholesterol using 2,4,6-tribromo-3-hydroxybenzoic acid with a commercial CHOD-PAP reagent. Ann Clin Biochem 1984;21(Pt 5):430-3 (PMID: 6508215).

20. Siedel J, Schmuck R, Staepels J, Town MH. Long term stable, liquid ready-to-use monoreagent for the enzymatic assay of serum or plasma triglycerides (GPO-PAP-method). Clin Chem 1993;39. https://www.researchgate.net/publication/285077717

21. Yu HH, Markowitz R, Ferranti SDD, et al. Direct measurement of LDL-C in children: performance of two surfactant-based methods in a general pediatric population. Clin Biochem 2000;33:89-95 (PMID: 10751585).

22. Langlois MR, Descamps OS, van der Laarse A, et al. Clinical impact of direct HDLc and LDLc method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group. Atherosclerosis 2014;233:83-90 (PMID: 24529127).

23. Eugui J, Logrono MJ, Ruiz R, Zugaza C, Mirabel J, Martínez C. Immunoturbidimetry of serum apolipoproteins A-I and B on the Cobas Bio centrifugal analyzer: method validation and reference values. Clin Biochem 1994;27:310-5 (PMID: 8001295).

24. Zhang GH, Cong AR, Xu GB, Li CB, Yang RF, Xia TA. An enzymatic cycling method for the determination of serum total bile acids with recombinant 3α-hydroxysteroid dehydrogenase. Biochem Biophys Res Commun 2005;326:87-92 (PMID: 15567156).

25. Kawamoto S, Koyano K, Ozaki M, et al. Effects of bilirubin configurational photoisomers on the measurement of direct bilirubin by the vanadate oxidation method. Ann Clin Biochem 2021; 4563221999068 (PMID: 33588585).

26. Premkumar M, Sable T. Obesity, dyslipidemia and cholesterol gallstone disease during one year of Antarctic residence. Rural Remote Health 2012;12:2186 (PMID: 23157579).

27. Kim SS, Lee JG, Kim DW, et al. Insulin resistance as a risk factor for gallbladder stone formation in Korean postmenopausal women. Korean J Intern Med 2011;26:285-93 (PMID: 22016589).

28. Brasca AP, Pezzotto SM, Berli D, et al. Epidemiology of gallstone disease in Argentina: prevalences in the general population and European descendants. Dig Dis Sci 2000;45:2392-8 (PMID: 11258564).

29. Chang CM, Chiu THT, Chang CC, Lin MN, Lin CL. Plant-based diet, cholesterol, and risk of gallstone disease: a prospective study. Nutrients 2019;11:335 (PMID: 30720747).

30. Smelt AH. Triglycerides and gallstone formation. Clin Chim Acta 2010;411:1625-31 (PMID: 20699090).

31. Castro J, Amigo L, Miquel JF, et al. Increased activity of hepatic microsomal triglyceride transfer protein and bile acid synthesis in gallstone disease. Hepatology 2007;45:1261-6 (PMID: 17464999).

32. Kaur J, Rana SV, Gupta R, Gupta V, Sharma SK, Dhawan DK. Prolonged orocecal transit time enhances serum bile acids through bacterial overgrowth, contributing factor to gallstone disease. J Clin Gastroenterol 2014;48:365-9 (PMID: 24598592).

33. Tang WH. Serum and bile lipid levels in patients with and without gallstones. J Gastroenterol 1996;31:823-7 (PMID: 9027646).

34. Chan T, Yaghoubian A, Rosing D, et al. Total bilirubin is a useful predictor of persisting common bile duct stone in gallstone pancreatitis. Am Surg 2008;74:977-80 (PMID: 18942626).

35. Gillaspie DB, Davis KA, Schuster KM. Total bilirubin trend as a predictor of common bile duct stones in acute cholecystitis and symptomatic cholelithiasis. Am J Surg 2019;217:98-102 (PMID: 29929909).

36. Chen YC, Chiou C, Lin MN, Lin CL. The prevalence and risk factors for gallstone disease in taiwanese vegetarians. PLoS One 2014;9:e115145 (PMID: 25521621).

37. Sang JH, Ki NK, Cho JH, Ahn JO, Sunwoo JG. Correlations between metabolic syndrome, serologic factors, and gallstones. J Phys Ther Sci 2016;28:2337-41 (PMID: 27630427).

38. Dhamnetiya D, Goel MK, Dhiman B, Pathania OP. Gallstone disease and quantitative analysis of independent biochemical parameters: Study in a tertiary care hospital of India. J Lab Physicians 2018;10:448-52 (PMID: 30498320).

39. Liu CM, Tung TH, Chou P, et al. Clinical correlation of gallstone disease in a Chinese population in Taiwan: experience at Cheng Hsin General Hospital. World J Gastroenterol 2006;12:1281-6 (PMID: 16534886).

40. Chen CH, Huang MH, Yang JC, et al. Prevalence and risk factors of gallstone disease in an adult population of Taiwan: an epidemiological survey. J Gastroenterol Hepatol 2006;21:1737-43 (PMID: 16984599).

41. Lirussi F, Nassuato G, Passera D, et al. Gallstone disease in an elderly population: the Silea study. Eur J Gastroenterol Hepatol 1999;11:485-91 (PMID: 10755250).

42. Tazuma S. Gallstone disease: Epidemiology, pathogenesis, and classification of biliary stones (common bile duct and intrahepatic). Best Pract Res Clin Gastroenterol 2006;20:1075-83 (PMID: 17127189).

43. Chen LY, Qiao QH, Zhang SC, Chen YH, Chao GQ, Fang LZ. Metabolic syndrome and gallstone disease. World J Gastroenterol 2012;18:4215-20 (PMID: 22919256).

44. Diehl AK, Schwesinger WH, Holleman DR Jr, Chapman JB, Kurtin WE. Clinical correlates of gallstone composition: distinguishing pigment from cholesterol stones. Am J Gastroenterol 1995;90:967-72 (PMID: 7771432).

45. Acalovschi M, Badea R, Dumitraşcu D, Varga C. Prevalence of gallstones in liver cirrhosis: a sonographic survey. Am J Gastroenterol 1988;83:954-6 (PMID: 3046336).

46. Conte D, Fraquelli M, Fornari F, Lodi L, Bodini P, Buscarini L. Close relation between cirrhosis and gallstones: cross-sectional and longitudinal survey. Arch Intern Med 1999;159:49-52 (PMID: 9892330).

47. Stroffolini T, Sagnelli E, Mele A, Cottone C, Almasio PL. HCV infection is a risk factor for gallstone disease in liver cirrhosis: an Italian epidemiological survey. J Viral Hepat 2007;14:618-23 (PMID: 17697013).

48. Acalovschi M, Blendea D, Feier C, et al. Risk factors for symptomatic gallstones in patients with liver cirrhosis: a case-control study. Am J Gastroenterol 2003;98:1856-60 (PMID: 12907344).