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

Dehui Zeng 1,3,*, Heming Wu 2,3,*, Qingyan Huang 2,3, Anxiang Zeng 1,3, Zhikang Yu 2,3, Zhixiong Zhong 2,3

* Contributed equally to this work

1 Center for Digestive Diseases, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-Sen University, Meizhou, P. R. China
2 Center for Precision Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-Sen University, Meizhou, P. R. China
3 Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translational Research of Hakka Population, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-Sen University, Meizhou, P. R. China

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.


Correspondence:
Prof. Zhixiong Zhong
Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translational Research of Hakka Population Meizhou People's Hospital (Huangtang Hospital) Meizhou Academy of Medical Sciences Meizhou Hospital Affiliated to Sun Yat-Sen University No 63 Huangtang Road Meijiang District, Meizhou 514031 P. R. China Phone: +86 753-2131-591 Email: zhongzhixiong01@126.com zhongzhixiong@mzrmyy.com

Manuscript accepted March 15, 2021
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 immunonutribidimetry 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-
applied 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 \( \pm SD: 56.4 \pm 17.1 \) years, range 14 - 93 years) and 907 were women (55.5%) (mean age \( \pm SD: 55.6 \pm 16.4 \) 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).

### 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.

<table>
<thead>
<tr>
<th></th>
<th>Patient group (n = 471)</th>
<th>Control group (n = 436)</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age, years</td>
<td>55.8 ± 14.4</td>
<td>55.3 ± 18.3</td>
</tr>
<tr>
<td></td>
<td>Female (n = 907)</td>
<td>90 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Male (n = 727)</td>
<td>72 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>p-</td>
<td>*0.001</td>
<td>*0.001</td>
</tr>
<tr>
<td></td>
<td>永远饮酒(n, %)</td>
<td>91 (19.7%)</td>
<td>81 (18.7%)</td>
</tr>
<tr>
<td></td>
<td>永远吸烟(n, %)</td>
<td>93 (20.2%)</td>
<td>86 (20.1%)</td>
</tr>
<tr>
<td></td>
<td>高血压(n, %)</td>
<td>92 (20.5%)</td>
<td>83 (19.4%)</td>
</tr>
<tr>
<td></td>
<td>糖尿病(n, %)</td>
<td>94 (21.1%)</td>
<td>85 (21.4%)</td>
</tr>
<tr>
<td></td>
<td>TG, mmol/L</td>
<td>1.409 ± 1.182</td>
<td>1.409 ± 1.182</td>
</tr>
<tr>
<td></td>
<td>TC, mmol/L</td>
<td>4.256 ± 3.691</td>
<td>4.256 ± 3.691</td>
</tr>
<tr>
<td></td>
<td>HDL-C, mmol/L</td>
<td>3.719 ± 2.059</td>
<td>3.719 ± 2.059</td>
</tr>
<tr>
<td></td>
<td>LDL-C, mmol/L</td>
<td>2.315 ± 1.466</td>
<td>2.315 ± 1.466</td>
</tr>
<tr>
<td></td>
<td>ApoA1, g/L</td>
<td>1.133 ± 0.876</td>
<td>1.133 ± 0.876</td>
</tr>
<tr>
<td></td>
<td>ApoB, g/L</td>
<td>0.903 ± 0.723</td>
<td>0.903 ± 0.723</td>
</tr>
<tr>
<td></td>
<td>TBA, µmol/L</td>
<td>3.498 ± 3.828</td>
<td>3.498 ± 3.828</td>
</tr>
<tr>
<td></td>
<td>TBIL, µmol/L</td>
<td>5.797 ± 6.157</td>
<td>5.797 ± 6.157</td>
</tr>
</tbody>
</table>

Continuous variable values with normal distribution expressed as mean ± SD.
* - Mann-Whitney U or t-test for continuous variable values, chi-squared test for categorical variable values (patient group vs. control group).
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 secretion 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 deteriorate.
Table 3. Comparison of lipid levels between patients with different gallstones.

<table>
<thead>
<tr>
<th>Stone size</th>
<th>p-values *</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 2 cm (n = 260)</td>
<td>0.771</td>
<td>0.692</td>
</tr>
<tr>
<td>1 - 2 cm (n = 313)</td>
<td>0.697</td>
<td>0.663</td>
</tr>
<tr>
<td>0.5 - 1 cm (n = 177)</td>
<td>0.781</td>
<td>0.349</td>
</tr>
<tr>
<td>&lt; 0.5 cm (n = 66)</td>
<td>0.771</td>
<td>0.716</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Stone location</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrahepatic bile ducts (n = 23)</td>
<td>0.109</td>
</tr>
<tr>
<td>Common bile duct (n = 83)</td>
<td>0.847</td>
</tr>
<tr>
<td>Gall bladder (n = 585)</td>
<td>0.878</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid</th>
<th>TG, mmol/L</th>
<th>TC, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>LDL-C, mmol/L</th>
<th>Apo-A1, g/L</th>
<th>Apo-B, g/L</th>
<th>Apo-A1/Apo-B</th>
<th>TBA, µmol/L</th>
<th>T-BIL, µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>1.237 ± 0.800</td>
<td>4.682 ± 1.174</td>
<td>1.278 ± 0.418</td>
<td>2.524 ± 0.845</td>
<td>1.075 ± 1.075</td>
<td>1.075 ± 0.235</td>
<td>1.075 ± 1.235</td>
<td>1.075 ± 1.075</td>
<td></td>
</tr>
</tbody>
</table>


* - 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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Unadjusted values</th>
<th>Adjusted values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>p-value *</td>
<td>β</td>
</tr>
<tr>
<td>TG</td>
<td>&lt; 0.001</td>
<td>0.265</td>
</tr>
<tr>
<td>TC</td>
<td>&lt; 0.001</td>
<td>0.349</td>
</tr>
<tr>
<td>LDL-C</td>
<td>&lt; 0.001</td>
<td>0.512</td>
</tr>
<tr>
<td>TBA</td>
<td>&lt; 0.001</td>
<td>0.024</td>
</tr>
<tr>
<td>T-BIL</td>
<td>&lt; 0.001</td>
<td>0.031</td>
</tr>
</tbody>
</table>

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.

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.

**Acknowledgment:**

The author would like to thank other colleagues who were not listed in the authorship of Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translation Research of Hakka Population, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences, Meizhou Hospital Affiliated to Sun Yat-Sen University for their helpful comments on the manuscript.

**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.

**Source of Funds:**

This study was supported by Key Scientific and Technological Project of Meizhou People’s Hospital, (Grant No.: MPHKSTP-20180101 to Dr. Zhixiong Zhong) and the Guangdong Provincial Key Laboratory of Precision Medicine and Clinical Translation Research of Hakka Population (Grant No.: 2018B030322003), the Science and Technology Program of Meizhou (Grant No.: 2019 B0202001).

**Declaration of Interest:**

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

**Reference:**


Risk Factors for Gallstones


