Analysis of Serum Lipid Profile and Apolipoproteins in Patients with Concurrent Gallbladder Stone Disease and Type 2 Diabetes Mellitus: A Population-Based Study in China

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Abstract

Background: Gallbladder stone disease is a common disorder, and prior studies have identified an association between gallstones and abnormal serum lipids. Since patients with type 2 diabetes have higher rates of significant abnormalities in lipid metabolism, the prevalence of dyslipidemia may be higher in patients with gallstones with coexisting type 2 diabetes mellitus (T2DM) than in the general population. The purpose of this study was to compare the serum lipid and lipoprotein abnormalities between T2DM patients with gallstones and controls in China.

Methods: Serum lipid and apolipoprotein levels were retrospectively analyzed in 407 patients aged 40 years and older after they were divided into four groups: gallstones with coexisting T2DM, T2DM, gallstones, and normal. All patients had normal total blood bilirubin and were not taking lipid-lowering medications. Overall, 135 T2DM patients with gallstones were compared with three control groups comprising 102 patients with T2DM, 119 patients with gallstones, and 51 healthy individuals. Triglyceride, total cholesterol, low density lipoprotein (LDL), high density lipoprotein, very low-density lipoprotein, serum apolipoprotein AI (ApoAI), and apolipoprotein B (ApoB) levels and their ratio (ApoAI/ApoB) were measured in the four groups and compared. One-way analysis of variance and nonparametric tests (Kruskal-Wallis test) were utilized to compare the groups. Values of p<0.05 were considered statistically significant.

Results: Compared to the three control groups, T2DM patients aged 40 years and older with gallstones demonstrated significantly abnormal changes in lipids. Among the lipid parameters, LDL and ApoB levels were found to be significantly elevated in patients with coexisting gallstones and T2DM (p<0.05), while LDL levels were significantly higher in men with gallstones and T2DM (p<0.05); however, no significant differences in the parameters were found in women.

Conclusions: Serum lipid abnormalities appear to be common in patients over 40 years of age with gallstones and T2DM. LDL and ApoB
levels were significantly different in patients over 40 years of age with concurrent gallstone disease and T2DM, with men having significantly higher LDL levels. However, the effect of serum lipid concentration on the high prevalence of gallstones in women over 40 years of age with concurrent gallstones and T2DM could not be determined.

**Key Words:** Serum lipids, Apolipoproteins, Gallstone disease, Type 2 diabetes mellitus.

**Abbreviations**

HDL—high-density lipoprotein; LDL—low-density lipoprotein; TC—total cholesterol; TG—triglyceride; ApoAl—apolipoprotein AI; ApoB—apolipoprotein B; N—normal group; D—diabetic group; G—gallstone group; GD—diabetes plus gallstone group; GSD—gallbladder stone diseases; T2DM—type 2 diabetes mellitus; ANOVA—one-way analysis of variance; SD—standard deviation; IQR—interquartile range.

**Introduction**

Gallstone disease (GSD) is one of the most common gastrointestinal disorders worldwide, which imposes a substantial economic burden on health care systems with a reported incidence rate of 15% [1]. Possible risk factors include sex, age, obesity, diabetes, dyslipidemia, rapid appetite loss, hepatitis C, and cirrhosis, with diabetes being strongly associated with the incidence of GSD [2-8]. Type 2 diabetes mellitus (T2DM) and prediabetes are commonly associated with dyslipidemia and hypertension [9]. The similar risk factors for T2DM and gallstones may be responsible for the increased risk of gallstones in patients with T2DM. A growing number of studies have suggested that lipid disorder is a common risk factor for both GSD and T2DM, which can also increase the risk of coronary heart disease and stroke [10-13]. Although retrospective clinical studies have shown that changes in the lipid profile occur not only in patients with cholelithiasis but also in patients who undergo laparoscopic cholecystectomy, few studies have compared the serum lipid abnormalities in patients with concomitant gallstones and T2DM with healthy controls [14-16]. The aim of this study was to analyze the serum lipid profile and apolipoprotein levels in Chinese patients with concurrent gallstones and T2DM aged 40 years and above.

**Methods**

This retrospective study was conducted in the Department of General Surgery of Lu’An People’s Hospital affiliated to Anhui Medical University from July 2016 to June 2018. The study was approved by the Ethics Committee of the People’s Hospital of Lu’An. Written informed consent was obtained from the enrolled patients or their guardians for the use of test results and personal information in this study. A total of 407 individuals were divided into four groups: T2DM and gallstones, gallstones only, T2DM only, and healthy controls. All participants were 40 years or older and underwent serum lipid profile and apolipoprotein analysis from July 2016 to June 2018. The inclusion criteria were age ≥40 years, residency in China, normal total bilirubin concentration, and no intake of lipid-lowering drugs. Patients with alcoholic cirrhosis, intrahepatic stones, renal failure, nephrotic syndrome, pancreatitis, heart failure, morbid obesity, hypothyroidism, sickle cell disease, hemoglobinopathy, and pregnancy, as well as patients undergoing cholecystectomy or taking antilipemic drugs were excluded from this study.

Patients with T2DM and gallstones were classified into the diabetic stones group, with ages ranging from 40 to 84 years (mean age 58.92±9.59 years); patients with T2DM without gallstones were classified into the diabetic group, with ages ranging from 40 to 76 years (mean age 56.74 ± 9.35 years); patients with gallstones without T2DM were classified into the gallstone group, with ages ranging from 40 to 91 years (mean age 58.03±12.04 years); and in the healthy group, ages ranged from 40 to 84 years with a mean age of 57.21 ± 10.40 years. The diabetic stones group was abbreviated as the D group and used as the case group. The diabetic group, gallstone group, and normal group were abbreviated as the D group, G group, and N group, respectively, and were all designated as the control group.

Patients in the D and G groups visited the hospital to undergo cholecystectomy. Cholesterol gallstones were identified by the visual inspection of a representative section of the gallstones or, if required, by enzymatic cholesterol analysis. Fasting blood samples were obtained from all patients by venipuncture under aseptic conditions before starting the treatment. The fasting serum lipid profile sample was collected along with the samples for other preoperative investigations. Blood samples collected from the cases and controls were used to measure creatinine, uric acid, fasting glycemia, the serum lipid profile, and apolipoproteins. These measurements were performed using the Beckman Coulter AU5800 automated biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA). The serum lipid profile and apolipoprotein parameters measured included total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL) as well as serum apolipoprotein AI (ApoAI), apolipoprotein B (ApoB), and their ratio (ApoAI/ApoB).

**Statistical analysis**

The statistical analyses were performed using SPSS software, version 25.0 for Windows (SPSS, Chicago, IL), and figures were generated using GraphPad Prism software (v 5.01; GraphPad Software, La Jolla, CA, USA). The continuous variables were tested for normality by the Shapiro-Wilk normality test. A one-way analysis of variance (ANOVA) was used to compare the age differences among the groups, while the sex differences between the groups were assessed using chi-square statistics. If numerical variables were normally distributed, they are expressed as means ± standard deviation (SD). The quantitative data were compared using one-way ANOVA; for multiple comparisons, the Bonferroni’s test was used when the variance was homogeneous, while the Tamhane’s T2 test was used when the variance was not homogeneous. If the numerical variables were not normally distributed, they were expressed as medians (interquartile range [IQR]), and the Kruskal-Wallis test with Bonferroni correction was used for multiple comparisons. Values of p<0.05 were considered statistically significant.
Results

The mean ages of the GD group, G group, D group, and N group were 58.92 ± 9.59 years, 58.03 ± 12.04 years, 56.74 ± 9.35 years, and 57.21 ± 10.40 years, respectively. The GD group consisted of 86 women and 49 men, with a female to male ratio of 1.76:1; the N group comprised 28 women and 23 men, with a ratio of 1.22:1; the D group comprised 36 women and 66 men with a ratio of 0.55:1; and the G group comprised 84 women and 35 men, with a ratio of 2.4:1. Data regarding age, body weight, creatinine, uric acid, fasting glycemia, and sex are presented in Table 1. No significant difference was observed in the sex distribution among the four groups (P=0.36). Although the sex ratios differed significantly, the comparisons among the groups were performed separately for each sex; therefore, the parameter of sex did not affect the results. Between the GD and D groups, there were no statistically significant differences in creatinine (67.65 ± 20.42 vs 64.14 ± 15.50 μmol/L, P=0.58) and fasting glycemia (8.54 ± 2.98 vs 10.79 ± 21.92 mmol/L, P=0.89) (Supplementary Table 1), and creatinine and glycemic values were higher in the D and GD groups than those surveyed in the N and G groups (Table 1). In terms of body weight and uric acid values, none of these variables were assessed too differently among the four groups (P > 0.05).

The mean serum lipid and apolipoprotein concentrations, including TC, TG, LDL, HDL, VLDL, APOAI, ApoB, and APOAI/APOB, in patients aged 40 years and above are shown in Table 2. Comparison of the total serum lipid profile showed that the mean serum levels of TC, TG, and VLDL were higher in the GD group than in the other three groups, although the difference was not significant (P > 0.05). However, LDL levels and ApoB levels showed significant differences (P < 0.05) (Tables 4, 5). The mean levels of ApoA1/B were lower in patients with T2DM and gallstones, but the difference was not significant. The differences in the serum levels of HDL and ApoB in patients with gallstones and T2DM were not significant (P > 0.05) compared to other groups (Table 2). The extracted data are represented by violin plots, with quartiles represented by dotted lines and medians represented by full lines in figure 1. This analysis revealed significant differences in the levels of LDL (Figure 1a) and apolipoprotein (Figure 1b), with the GD group demonstrating higher levels than the other three groups.

In patients aged over 40 years, we further analyzed the serum lipid profile and apolipoprotein levels in men and women separately. In men, higher serum LDL levels were observed in the GD group than in the three control groups, and the difference was significant (P < 0.05) (Tables 4, 5). The data were used to plot a bar graph showing the means ± SD in the four separate groups (Figure 2). However, no significant differences in the parameters were found among the women in the GD group.

Discussion

GSD remains one of the most common and costly gastrointestinal disorders which requires hospitalization. A widely accepted view is that gallstone formation is closely related to disturbances in the lipid and lipoprotein metabolism [17,18]. Previous studies have confirmed the close association between diabetes mellitus and gallstones, and diabetes mellitus was found to be a risk factor for GSD [8,19,20]. Lipid metabolism disorders, insulin resistance, leptin, and lipocalin, either singly or in combination with each other, ultimately contribute to the development of gallstones in combination with T2DM. Abnormal lipid metabolism is a widely recognized risk factor in the pathogenesis of gallstones, and disorders of the lipid metabolism in diabetic patients are a common cause of gallstones [21].

In the present study, the types of gallstones analyzed were all cholesterol gallstones. The incidence of cholesterol stones continues to rise every year owing to the recent improvements in living standards and changes in the dietary habits in China. Aging is also associated with significant changes in the lipid profile and contributes to the development of gallstones. In this study, the average age of the patients in the GD and G groups was 58.92 ± 9.59 years and 58.03 ± 12.04 years, respectively. A high prevalence of GSD was observed in the elderly population, as the ages of the study participants were up to 75 years. An increasing trend in the cumulative prevalence has been reported, especially after 40 years of age [22]. Age is also an important

| Physical Parameter         | N            | D            | G            | GD           | P   |
|---------------------------|--------------|--------------|--------------|--------------|-----|
| Age (years)               | 57.21 ± 10.40| 56.74 ± 9.35| 58.03 ± 12.04| 58.92 ± 9.59| 0.36*|
| body weight (kg)          | 64.22 ± 9.56 | 66.13 ± 11.87| 63.8 ± 10.32 | 66.85 ± 10.38| 0.11*|
| Creatinine (μmol/L)       | 57.94 ± 14.13| 64.14 ± 15.50| 58.07 ± 13.21| 67.65 ± 20.42| <0.001*|
| uric acid (μmol/L)        | 285.95 ± 72.00| 294.01 ± 91.58| 287.77 ± 71.25| 298.69 ± 81.98| 0.66*|
| fasting glycemia (mmol/L) | 5.36 ± 0.73  | 10.79 ± 21.92| 5.38 ± 0.90  | 8.54 ± 2.98  | 0.001|

The ages, body weight, creatinine, uric acid, and fasting glycemia are shown as means ± SD. Abbreviations: SD, standard deviation; N, normal group; D, diabetic group; G, gallstone group; GD, diabetes plus gallstone group; *One-way analysis of variance (ANOVA), ** chi-square statistics; Significance was accepted at p values <0.05. Units of creatinine and uric acid are μmol/L; units of fasting glycemia are mmol/L; units of body weight are kg.

Table 1: Distribution of age, body weight, creatinine, uric acid, fasting glycemia, and sex in patients >40 years in the case and three control groups
All the results are expressed as medians and interquartile ranges as the distribution was not normal. The data were analyzed with the Kruskal–Wallis non-parametric test and Shapiro-Wilk test to check for normal distribution. Significance was accepted at \( p < 0.05 \).

### Table 2: Medians and interquartile ranges of lipid profile and apolipoprotein levels in the case and three control groups.

| Physical Parameter | Sample 1-Sample 2 | Test Statistic | Std. Error | Std. Test Statistic | \( P \) | \( P^* \) |
|--------------------|-------------------|----------------|------------|---------------------|-------|--------|
| TC                 | GD-N              | -92.451        | 19.334     | -4.782              | <0.001| <0.01  |
|                   | GD-G              | -75.041        | 14.791     | -5.073              | <0.001| <0.01  |
|                   | GD-D              | -58.657        | 15.432     | -3.801              | <0.001| 0.001  |
| HDL                | GD-N              | -71.026        | 19.332     | -3.674              | <0.001| 0.001  |
|                   | GD-G              | -69.661        | 14.789     | -4.71               | <0.001| <0.01  |
|                   | GD-D              | -41.169        | 15.431     | -2.668              | 0.008 | 0.046  |

LDL concentrations are expressed as means ± SD; concentrations of TC, TG, HDL, VLDL, ApoAI, and ApoB are expressed as medians and interquartile ranges. *One-way analysis of variance (ANOVA); **Kruskal–Wallis non-parametric test; Significance was accepted at \( p < 0.05 \).

### Table 3: Multiple comparisons of LDL and ApoB levels in patients over 40 years of age among the case and three control groups.

| Physical Parameter | Sample 1(I)-Sample 2(J) | Mean Difference (I-J) | Std. Error | 95% Confidence Interval | \( P \) | \( P^* \) |
|--------------------|--------------------------|-----------------------|------------|-------------------------|-------|--------|
| TC                 | GD-N                     | 0.58                  | 0.15       | 0.18                    | 0.99  | 0.001  |
|                   | GD-G                     | 0.38                  | 0.13       | 0.02                    | 0.74  | 0.030  |
|                   | GD-D                     | 0.43                  | 0.13       | 0.07                    | 0.80  | 0.011  |

Concentrations of LDL are expressed as the means ± SD, and between-group differences were assessed by one-way ANOVA followed by the Tamhane’s T2 test for multiple comparisons (homogeneity of variance was not equal). Significance was accepted at \( p < 0.05 \).
risk factor for diabetes. In China, the incidence of diabetes increases with age [23]. In this study, we included patients over 40 years of age with coexisting gallstones and diabetes mellitus as the number of patients younger than 40 years was low.

The mean serum TC and TG were higher in GSD patients with T2DM than in controls, although the difference was not significant. Several studies have reported higher serum cholesterol and triglyceride levels in patients with gallstones compared to healthy controls [16]. However, it was found that the correlation between high levels of cholesterol and triglycerides and gallstones was not significant [24]. Moreover, the relationship between gallstone formation and elevated serum cholesterol and triglyceride levels has been found to be controversial in the literature. We observed higher concentrations of LDL and VLDL in the GD group than in the three control groups. Notably, the differences in the LDL levels between the GD group and control groups were significant, but the differences in VLDL levels were not significant. Additionally, some studies have shown no association between low HDL levels and gallstone disease [25]. Since LDL is the main protein that transports cholesterol, and most cholesterol in the blood is present in LDL, elevated LDL levels lead to increased transport of peripheral cholesterol into the liver, which may lead to cholesterol supersaturation. Furthermore, previous studies have described a positive correlation between serum LDL levels and gallstones [26].

The most prominent application of lipoprotein measurements in clinical biochemistry is as markers of cardiovascular risk [27]. ApoAI and ApoB are the major apolipoproteins for HDL and LDL, respectively. It has been reported that serum apolipoprotein levels are likely to be more sensitive than serum lipid levels in distinguishing patients with gallstones from those without stones, despite the lack of changes in the lipid levels [28]. In the present study, we found increased concentrations of serum ApoB in the GD group, and the difference in the ApoB levels was significant. The main role of ApoAI and ApoB in the pathogenesis of gallbladder disease may be related to the transport of cholesterol and nucleation of bile cholesterol. Our results suggest that the serum concentrations of LDL and HDL and the respective ApoB and ApoAI levels follow a similar pattern of association, and demonstrate same-directional changes in terms of the serum concentration, which is characterized by increased LDL and ApoB levels along with reduced HDL and ApoAI levels. This finding is in agreement with other studies [29]. Meanwhile, we observed higher concentrations of ApoB in the GD group than in the control group, and the difference in the ApoB concentration was significant. This finding further illustrates the significant role of serum LDL and ApoB concentrations in gallstone formation and the occurrence of diabetes among patients with gallstone disease.

The results of the present study also revealed that in men aged 40 years and above, the median concentrations of LDL and ApoB were higher in gallstone patients with T2DM than in patients in the other three groups, and the difference in the LDL concentration was significant. The prevalence
of dyslipidemia depends on the sex, with men having a higher incidence. Lifestyle may be considered a possible cause for such an observation, as there may be more risk factors for dyslipidemia among men due to lifestyle factors such as smoking, drinking alcohol, and so on. However, no association between serum lipids and high incidence of gallstones in women was found in the present study. There could be more complex factors that would influence the changes in the serum lipid profile and apolipoprotein levels in women. Studies carried out by Gul et al. and Halgaonkar et al. have shown that reproductive hormones play a role in the pathogenesis of gallstones in women, in addition to the race and eating habits. Perhaps, further studies are needed to verify our conjectures [30,31].

**Limitations of the Study**

The present study has several limitations. First, the study was retrospective and was conducted in a single center. Second, the records of height values in the study were incomplete, preventing further analysis of body mass index, which is also a limitation of the study.

**Conclusion**

Serum lipid abnormalities appear to be common in patients over 40 years of age with gallstones and T2DM. This study showed that LDL and ApoB levels are significantly higher in patients aged >40 years with gallstones and concurrent T2DM, with men having significantly higher LDL levels than women in each of the three control groups. However, the effect of serum lipid concentration on the high prevalence of gallstones in women over 40 years of age with gallstones and T2DM could not be determined. From the study, the disorder of lipid metabolism may be a contributing factor among the many causes of T2DM with gallbladder stones. Serum LDL and ApoB levels are sensitive indicators and thus can be helpful in the prevention of T2DM combined with gallstones.

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**Availability of Data and Materials**

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors’ Contribution**

Pin Jiang and Rong-rong Zhai collected and analyzed all the included data. Song Liang designed this study and drafted the manuscript. All of the authors have read and approved the final version of the manuscript.

**Ethics Approval and Consent to Participate**

The present study was approved by the Ethics Committee of Lu’an People’s Hospital. Informed consent was obtained from all the participants.

**Consent for Publication**

Not applicable.

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**Competing Interests**

The authors declare that they have no competing interests.

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