

Increased serum total bile acid is independently associated with non-alcoholic steatohepatitis in non-diabetes population

Hu Li

Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

Jin Ma

Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital North

Leilei Gu

Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital North

Li Jin

Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital North

Peizhan Chen

Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital North

Xinxin Zhang

Shanghai Jiao Tong University Medical School Affiliated Ruijin Hospital

li chen (✉ drchenli@163.com)

Shanghai Jiao Tong University School of Medicine

Research

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Abstract

Background and aims Bile acids, which modulate the glucose and lipid metabolism, play an important role in the development of non-alcoholic fatty liver disease (NAFLD). This study aims to explore the association between serum total bile acid (TBA) and non-alcoholic steatohepatitis (NASH) in the general population from a large clinical dataset.

Methods NAFLD individuals confirmed by ultrasonography were enrolled in the study. The NASH was defined as the NAFLD individual with abnormal liver function, and non-alcoholic fatty liver (NAFL) was defined as the NAFLD individual with normal liver function. The demographic characters, biochemical results including serum TBA were compared between NASH and NAFL patients. The independently associated factors with NASH were investigated by multivariate logistic regression analysis.

Results A total of 6862 individuals were enrolled in the study, of which 23.7% were NASH. The median age was 45 (39-55) years old, and the BMI was 26.9(25.1-28.9) kg/m². The NASH patients showed higher levels of serum TBA compared to the NAFL patients [3.30(2.30-5.0) μ mol/L vs. 2.70(1.90-4.20) μ mol/L, $P < 0.001$]. The prevalence of NASH tended to increase with the increasing of serum TBA level ($P < 0.001$). Multivariate logistic regression analysis showed the age, male, serum TBA levels, diabetes mellitus (DM), serum uric acid, total cholesterol and triglyceride were independent risk factors for NASH ($P < 0.05$). Stratification analysis according to DM status showed that serum TBA was independently associated with NASH in individuals without DM (OR: 1.55, 95% CI: 1.11-2.13, $P = 0.008$), while not in individuals with DM (OR: 1.32, 95% CI: 0.53-2.96, $P = 0.515$).

Conclusion Serum TBA level was significantly higher in NASH patients than in NAFL patients, and was independently associated with NASH in non-diabetes population.

Introduction

Non-alcoholic fatty liver disease (NAFLD), a rapidly growing metabolic disease associated with type 2 diabetes mellitus and obesity, has been the most common chronic liver disease worldwide currently [1]. A systemic study showed that the prevalence of NAFLD was about 25 percent in general population [2], and reached up to above 50 percent in the diabetes population globally [3]. NAFLD is a heterogeneous disease comprising of non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis and even hepatocellular carcinoma [4]. NASH, an active subtype accounting for 10–20 percent of NAFLD [5], is characterized by a varied degree of hepatic ballooning, necroinflammation and fibrosis.

Over the past decade, a growing number of studies have been focused on bile acids (BAs) and the regulation of metabolism. Serum total BAs (TBA) comprise substantial amounts of primary BAs and secondary BAs. Primary BAs, including conjugated and non-conjugated BAs, are synthesized from cholesterol in hepatocytes via a series of enzyme-mediated oxidation reaction, and then released into the intestine. In the intestine, primary BAs are deconjugated by gut microbiota to form secondary BAs. A large

part of intestinal BAs is reabsorbed actively and recirculated to the liver through the portal vein. This process is known as enterohepatic circulation of BAs. BAs not only facilitate absorption of lipid and lipid-soluble vitamin but also regulate and maintain lipid and glucose homeostasis as signal molecular via farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) [6]. Therefore, BAs related pathway has been a potential therapeutic target for NASH [7].

A few studies, which enrolled liver biopsy-confirmed NASH, demonstrated that the level of serum total BAs in NASH patients was higher than NAFLD patients or health population [8–10]. And the total BAs level increased with the development of liver fibrosis. In term of the BAs profiles, the primary conjugated BAs proportion increased, whereas unconjugated BAs significantly decreased with the progression of liver fibrosis[8]. Moreover, the specific changes of serum BAs were correlated with histological features of NASH, *i.e.* higher grades of steatosis, lobular and portal inflammation, and hepatocyte ballooning[11]. These studies suggested that serum BAs profile may severe as a biomarker for identifying NASH and evaluating severity or even pathological phenotype of NASH, however, these findings need to be verified in a large cohort. Thus, this study aimed to evaluate the relationship between serum TBAs and NAFLD, and determine whether serum TBA could be an indicator for identifying NASH patients in NAFLD population.

Methods

Study population

In the present study, the study population were screened for enrolment from the Medical Examination Center of Ruijin Hospital North, Shanghai, China, from January 2018 to December 2018. These participants were scheduled to receive a medical evaluation, including medical history, physical examination, serum biochemical and immune tests and some other auxiliary examinations such as abdominal ultrasonography, electrocardiograph and imaging examinations. The adult patients who showed fatty liver on abdominal ultrasonography scan were enrolled in the study. The following subjects were excluded from study 1) less than 18 years old. 2) history of excessive alcohol consumption (> 21 standard drinks on average per week for men and > 14 standard drinks on average per week for women) [12]. 3) had a history of viral hepatitis, autoimmune hepatitis, or other known causes of chronic liver disease. 4) individuals with hypothyroidism and polycystic ovarian syndrome. This study was approved by the Institutional Ethics Committee of Shanghai Ruijin Hospital North and complied with the Declaration of Helsinki.

Definition and Data collection

Medical history, demographic characteristics (including age, gender, height, body weight, body mass index (BMI), blood pressure, etc), lab tests (including blood routine examination, biochemical tests, thyroid function tests, etc) and abdominal ultrasonography, electrocardiograph were collected for all participants meeting above criteria at the time of enrolment. Blood samples were drawn after overnight fasting. Blood samples were processed and measured at the clinical laboratory in the Shanghai Ruijin

Hospital North. Serum biochemical tests were measured using automatic biochemistry analyzer in the hospital according to related protocol.

NAFLD was diagnosed according to the AASLD guideline based on the evidence of fatty liver on abdominal type B ultrasonic examination and excluding the diseases that could induce liver steatosis[12]. Individuals with serum alanine aminotransaminase (ALT) ≥ 50 IU/L or aspartate aminotransferase (AST) ≥ 50 IU/L or γ -glutamyltransferase (γ -GT) ≥ 55 IU/L according to lab tests normal reference value in our hospital were considered as NASH, while individuals with ALT < 50 IU/L and AST < 50 IU/L and γ -GT < 55 IU/L considered as NAFL. Hypertension was diagnosed when blood pressure was above 140/90 mmHg or the participants had a definite history of hypertension and treatment with anti-hypertension drugs[13]. Diabetes mellitus was diagnosed based on the fasting serum glucose levels above 7.0 mmol/L, or 2 hours post-prandial serum glucose above 11.1 mmol/L, according to the 2019 diagnosis and management guideline[14]. Patients who showed normal serum glucose but had a history of diabetes mellitus and treatment with hypoglycemic agents were also regarded as diabetes mellitus. Lean NAFLD was defined as the NAFLD patients with BMI less than 23 kg/m² [15]. Metabolic syndrome referred to the participants with at least 3 of the following criteria: 1) abdominal obesity with waistline above 90cm in male or 85cm in female; 2) blood pressure above 130/85 mmHg or treatment with anti-hypertension drugs; 3) hyperlipemia with fasting serum triglyceride above 1.7mmol/L or treatment with lipid-lowering medicines; 4) fasting serum HDL-C below 1.0 mmol/L in male or 1.3 mmol/L in female; 5) fasting serum glucose above 5.6 mmol/L or having a history of type 2 diabetes mellitus[16]. Hyperbileacidemia was defined as the serum TBA level exceed the normal range (>10 umol/L). Extra-hepatic complications included gallstone or gallbladder polyp, coronary heart disease, and positive urine protein.

Statistical analysis

Categorical variables were expressed as frequency and percentages, and differences between groups were compared using Pearson's χ^2 test. Continuous variables were expressed as the median and interquartile range (IQR), and differences were compared by Wilcoxon rank sum test. Spearman rank correlation analysis was applied to analyze the association between serum TBA levels and the levels of ALT, AST, γ -GT, respectively. Multivariate logistic regression analysis was performed to explore the associated factors with NASH. All statistical analyses were conducted with R software (version 3.6.1). A P value < 0.05 were considered as statistically significant.

Results

1. General characteristics of the study population

A total of 6862 patients who were diagnosed as NAFLD with intact data were enrolled in the study. Of these patients, 1629 (23.7%) were NASH and 5380 (78.4%) were male. The median age and BMI of these individuals were 45 (39-55) years old and 26.9 (25.1-28.9) kg/m², respectively. 684(10.0%) patients had

diabetes mellitus (DM), 1968(28.6%) patients had metabolic syndrome (MetS), 2842 (41.4%) patients had hypertension and 391 (5.7%) patients were lean NAFLD. The median level of serum total BA was 2.90 (1.93-4.40) $\mu\text{mol/L}$. Hyperbileacidemia was identified in 226 (3.3%) patients.

2. Comparison of characteristics between NAFL and NASH patients

The age of NASH patients was older than that of NAFL patients [47(37-56) years vs. 40(37-51) years, $P < 0.001$]. The prevalence of DM and MetS in NASH patients were higher than those in NAFL patients (14.8% vs. 8.4% and 33.7% vs. 27.1%, respectively, $P < 0.001$), but the prevalence of hypertension and lean NAFLD were similar between these two groups ($P > 0.05$). It is noteworthy that the level of serum TBA in NASH patients was significantly higher than that in NAFL patients [3.30(2.30-5.0) $\mu\text{mol/L}$ vs. 2.70(1.90-4.20) $\mu\text{mol/L}$, $P < 0.001$]. In addition, serum glycocholic acid, serum uric acid (UA), and serum triglyceride (TG), serum total cholesterol (TC), serum apolipoprotein, serum ferritin, serum insulin in NASH patients were higher than those in NAFL patients ($P < 0.001$). Although NASH patients had higher serum creatinine, when serum creatinine above 106 $\mu\text{mol/L}$ considered as renal insufficiency, the percentage of renal insufficiency in patients with NASH was similar to those with NAFL ($P = 0.619$). Patients with NASH had higher extrahepatic complications than those with NAFL ($P < 0.001$) (Table 1).

3. Association of serum TBA with the prevalence of NASH in NAFLD patients

As shown in Figure 1, the distribution of serum TBA level in our study was left-skewed. All patients were classified into quartiles by the serum TBA levels. Quartile 1 (Q1) was TBA < 1.93 $\mu\text{mol/L}$, quartile 2 (Q2) was TBA 1.93-2.90 $\mu\text{mol/L}$, quartile 3 (Q3) was TBA 2.90-4.40 $\mu\text{mol/L}$, and Quartile 4 (Q4) was TBA \geq 4.40 $\mu\text{mol/L}$. As shown in Figure 2, prevalence of NASH tended to increase as the level of serum BA increased ($P < 0.001$). Moreover, prevalence of metabolic syndrome, diabetes and hypertriglyceridemia showed a similar pattern ($P < 0.001$). Spearman correlation analysis showed that serum TBA was positively correlated with serum ALT, AST and r-GT (Spearman correlation coefficient Rho = 0.165, 0.167 and 0.162, respectively, all $P < 0.001$) (Figure 3).

Then the association between serum TBAs level and NASH was stratified analysis according to the DM status. The levels of serum TBA in NASH patients were still significantly higher than those in NAFL patients either in DM patients [3.9 (2.6-5.6) $\mu\text{mol/L}$ vs. 3.3 (2.1-4.78) $\mu\text{mol/L}$, $P = 0.001$] or in patients without DM [3.2 (2.2-4.9) $\mu\text{mol/L}$ vs. 2.7 (1.8-4.1) $\mu\text{mol/L}$, $P < 0.001$] (Figure 4).

4. Serum TBA is an independent factor associated with NASH

Multivariate logistic regression analysis was performed to explore factors associated with NASH. Nine variables including age, gender, TBA, hypertension, BMI, DM, UA, TC and TG were entered into the regression equation. As shown in Table 2, all variables except hypertension and BMI were closely associated with the risk of NASH. Particularly, hyperbileacidemia was independently associated with NASH (OR: 1.53, 95% CI: 1.12 - 2.06; $P < 0.001$). Since NAFLD is closely related to DM, then multivariate logistic regressions were performed based on DM status. Interestingly, we found that the increased serum

TBA was still the independent factor associated with NASH in patients without DM (OR: 1.55, 95% CI: 1.11-2.13, $P = 0.008$). However, the aged (>50 years) and elevated serum UA were the only independent factors associated with NASH in patients with DM, while the increased bile acid level was not independently associated with NASH in patients with DM (OR: 1.32, 95% CI: 0.53-2.96, $P = 0.515$) (Table 3, 4).

Discussion

In this study, we found that serum TBA was significantly associated with NASH in NAFLD population. Firstly, NASH patients exhibited higher serum TBA level compared to NAFL patients. Secondly, the prevalence of NASH, DM, MetS and hypertriglyceridemia increased with the increasing of serum TBA level in a dose-dependent manner. Most importantly, multivariate logistic regression analysis showed that elevated serum TBA level was independent factor for NASH. Moreover, further stratification analysis showed that serum TBA was independently associated with NASH in non-diabetes patients, while not in diabetes patients.

In a recent large-scale study, which involved in 152,336 participants, 27.5% of the study population had NAFLD[17]. The level of serum TBA in NAFLD patients was significantly higher than health population (3.4 vs.3.0 $\mu\text{mol/L}$, $p < 0.001$), however serum TBA level was not independently associated with NAFLD in multivariate regression analysis. In our study, the potential association between serum TBA level and NASH was investigated in the NAFLD population. The level of serum TBA was higher in NASH patients than in NAFL patients, which was consistent with the previous study from small sample[9, 18], and our data further confirmed that the hyperbileacidemia was independently associated with NASH.

The protective or adverse role of bile acid on the development of NAFLD remained controversial. The increased serum bile acids level in the high-fat diet (HFD)-induced obese C57BL/6 mice was correlated with weight loss after vertical sleeve gastrectomy. The bile acids and FXR signalling, which regulate the glucose and lipid metabolism, was served as an important molecular underpinning for the beneficial effects of this weight-loss surgery[19]. It has recently been shown that bile acids modulate insulin signalling by activation of FXR and can improve insulin resistance in cell-based and animal studies[20]. However, the FXR signalling pathway was suppressed in NASH patients compared to NAFL or health control[21]. The primary bile acids were increased while second bile acids were decreased in NASH. Moreover, the ratio of total cholate to total chenodeoxycholate was decreased in NASH[11]. Another study observed the increased FXR antagonistic deoxycholic acid and decreased FXR agonistic chenodeoxycholate in percentage, by which inhibited the FXR signalling in the liver of NASH patients[21]. Several studies demonstrated that the level of bile acid was positively correlated with the fibrosis of NASH. In the present study, although the liver fibrosis was not detected in NASH patients, the prevalence of NASH, MetS, DM and hypertriglyceridemia were increased with the increase of serum BAs level, thus the results from our large population supported that the FXR signalling pathway was suppressed in NASH patients.

An interesting finding from our study was that elevated serum TBA was an independent factor for NASH in non-diabetes subjects, while not in diabetes population. Although the bile acid signalling can modulate glucose metabolism, the studies on the relationship between bile acid and diabetes were not consistent. Some studies showed no changes in fasting bile acid level[22, 23], while others studies reported increased fasting bile acid in diabetes patients compared to health control[24, 25]. The recent large-scale population study showed no association between serum total bile acid and NAFLD[17]. Differences in control populations and the degree of insulin resistance or T2DM severity are potential confounding factors between these studies. In the present study, the diagnosis criteria of NASH were based on abnormal ALT, AST and r-GT level. Hyperglycemia can induce elevation of ALT and AST in some diabetes patients, but without obvious alteration of liver histology[26]. These patients might be falsely classified into NASH group, which might introduce bias in logistic regression analysis. Nevertheless, several noninvasive models for predicting NASH or advanced liver fibrosis of NAFLD showed lower performance in diabetes patients than in non-diabetes patients[27, 28]. Thus, the contribution of bile acid to the NASH development in diabetes patients need to further clarify.

There are some limitations to the present study. Firstly, in the present study, NASH was diagnosed based on the levels of serum ALT, AST and r-GT, which might exclude some NASH patients with normal liver function. Liver biopsy is “gold standard” for diagnosis and assessment of NASH, but it is not feasible in the general population for medical examination. Secondly, the composition of serum BAs was not detected in the present study. The alteration of bile acid composition may be more relevant to metabolic homeostasis but the serum TBA was more widely used in the clinic, some of which or its combination might provide a more valuable noninvasive diagnosis approach.

In conclusion, the serum TBA level was higher in NASH than in NAFL patients, and the elevated serum TBA was independently associated with NASH in non-diabetes population. These results need further validation in biopsy-proven NAFLD subjects. Furthermore, measuring levels of specific serum BAs might provide more information for identifying NASH patients, and deserve further research.

Conclusions

Serum TBA level is independently associated with NASH in non-diabetes population, and further research in biopsy-proven NAFLD patients is needed to validate these results.

Abbreviations

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

NAFL: non-alcoholic fatty liver

FXR: farnesoid X receptor

BMI: body mass index

TGR5: Takeda G protein-coupled receptor 5

TBA: total bile acid

DM: diabetes mellitus

ALT: alanine aminotransaminase

AST: aspartate aminotransferase

γ -GT: γ -glutamyltransferase

MetS: metabolic syndrome

UA: uric acid

BUN: blood urea nitrogen

Cr: creatinine

FBG: fasting blood glucose

TG: triglyceride

TC: total cholesterol

LDL-C: LDL cholesterol

HDL-C: HDL cholesterol

FFA: free fatty acid

APOA: apolipoprotein A

APOB: apolipoprotein B

HCY: homocysteine

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of Shanghai Ruijin Hospital North, and informed consents were obtained from all patients.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author Xinxin Zhang or Li Chen on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

Hu Li and Jin Ma performed the statistical analysis and wrote the manuscript

Leilei Gu and Li Jin were responsible for clinical data collection

Peizhan Chen assisted data analysis

Xinxin Zhang and Li Chen designed study and revised the manuscript

Acknowledgements

Not applicable

Conflict of interest

All authors declare there is no conflict of interest

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Figures

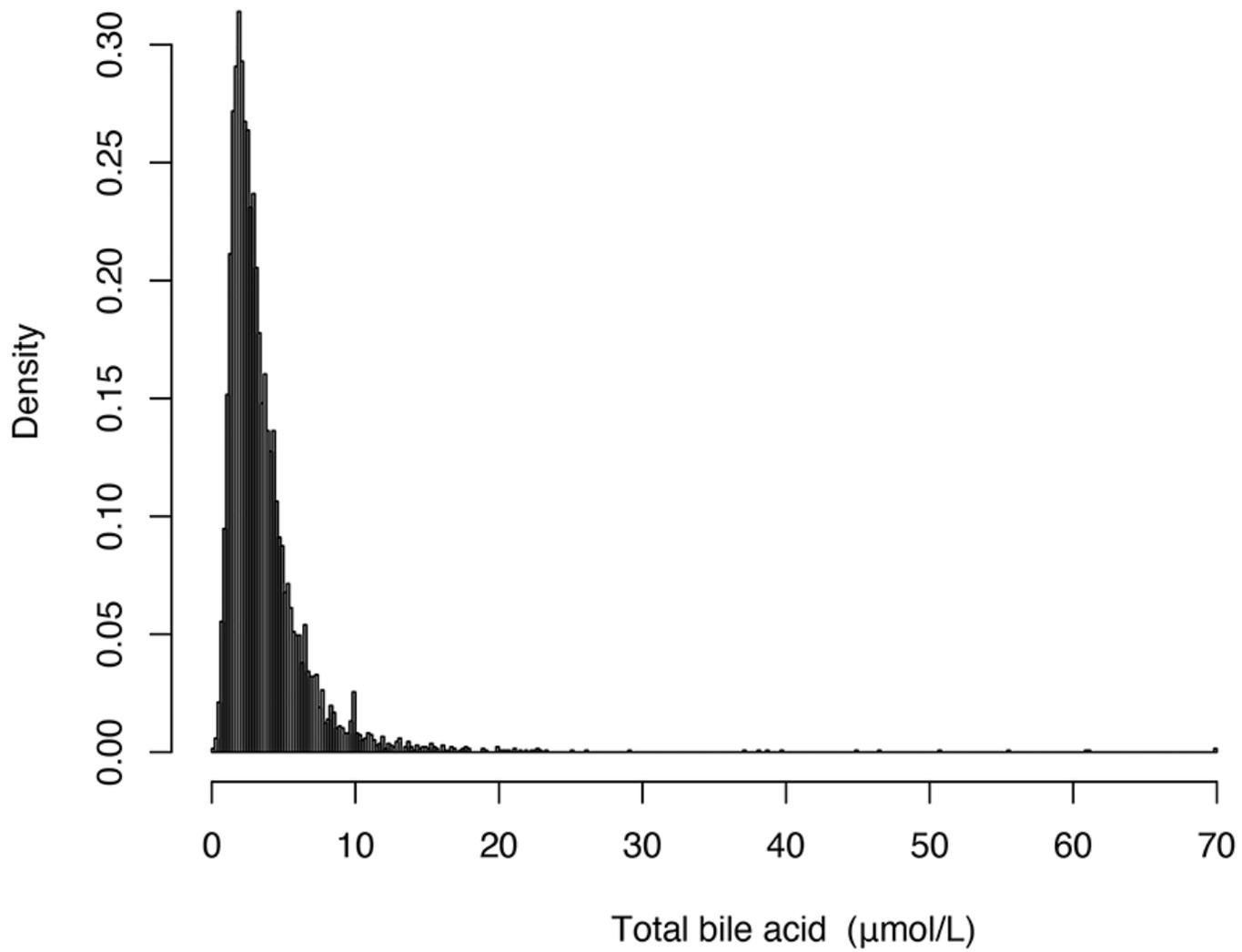


Figure 1

Data of serum TBA presented left skewed distribution

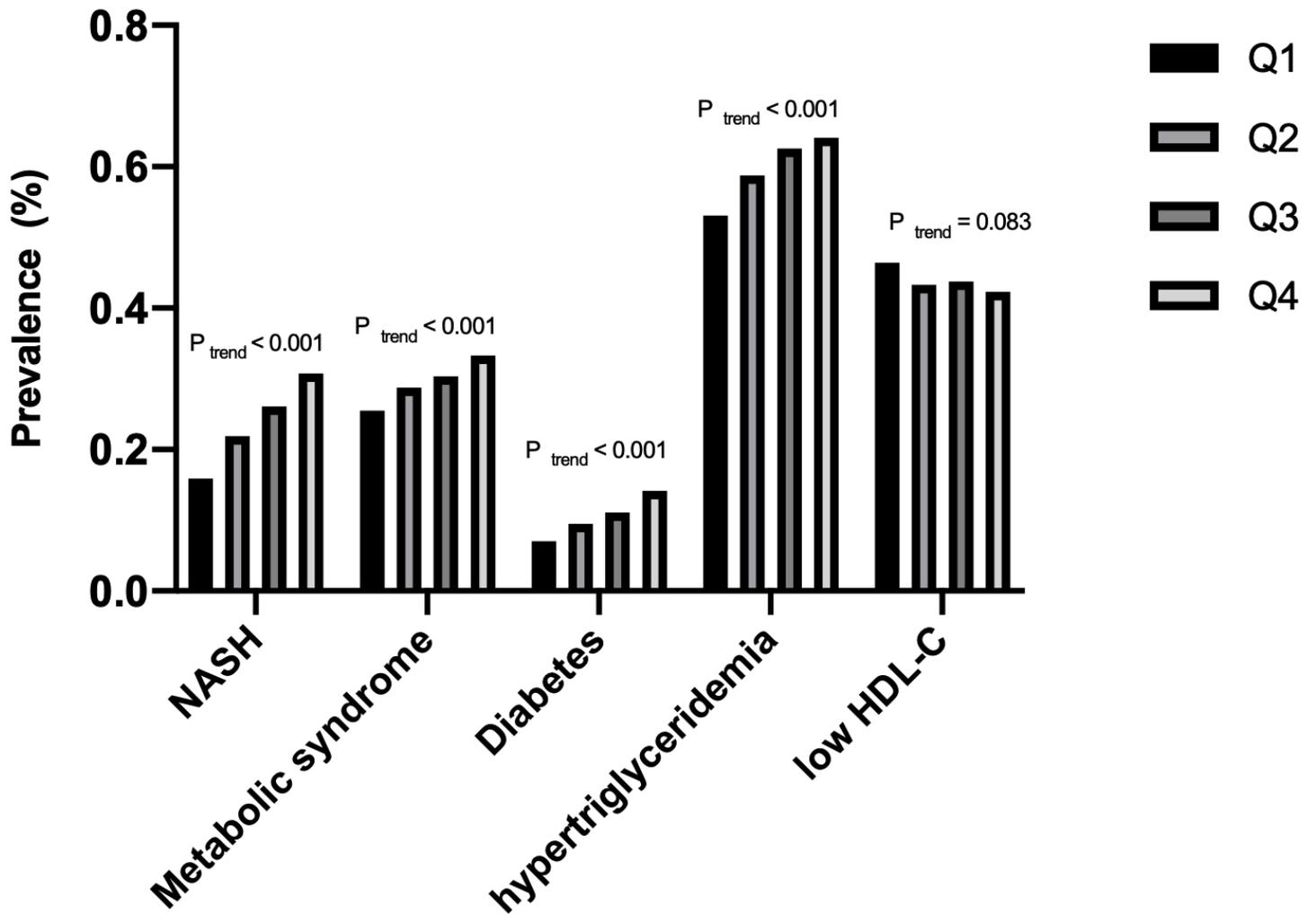


Figure 2

Prevalence of NASH and metabolic syndrome and parts of its components in NAFLD patients with different quartile of serum TBA level

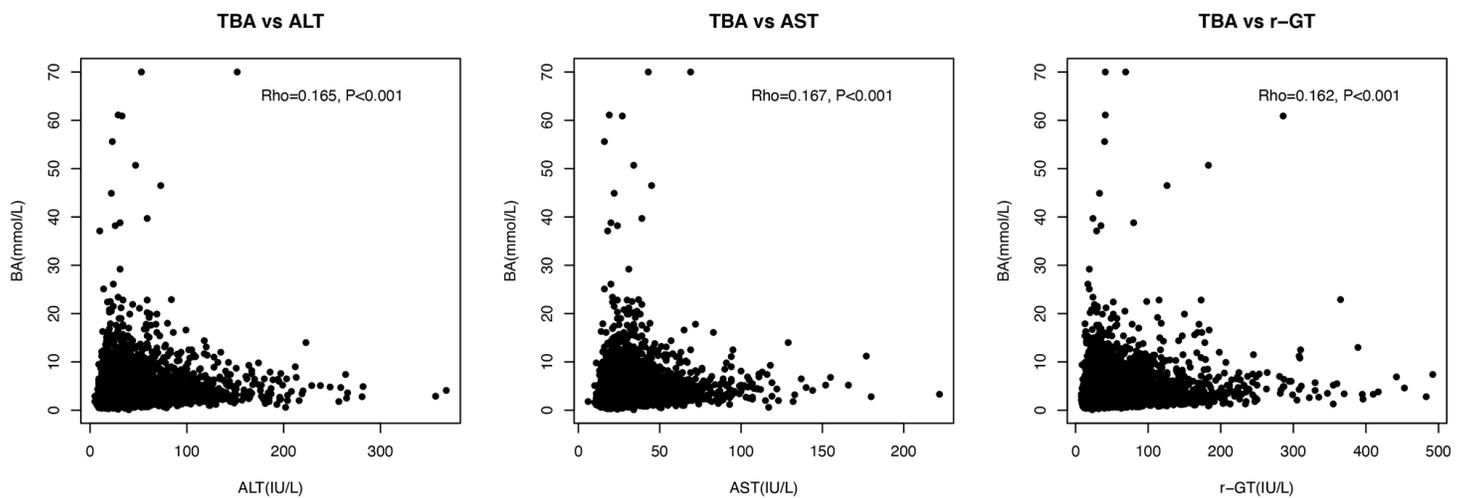


Figure 3

Serum TBA was positively correlated with ALT, AST and r-GT

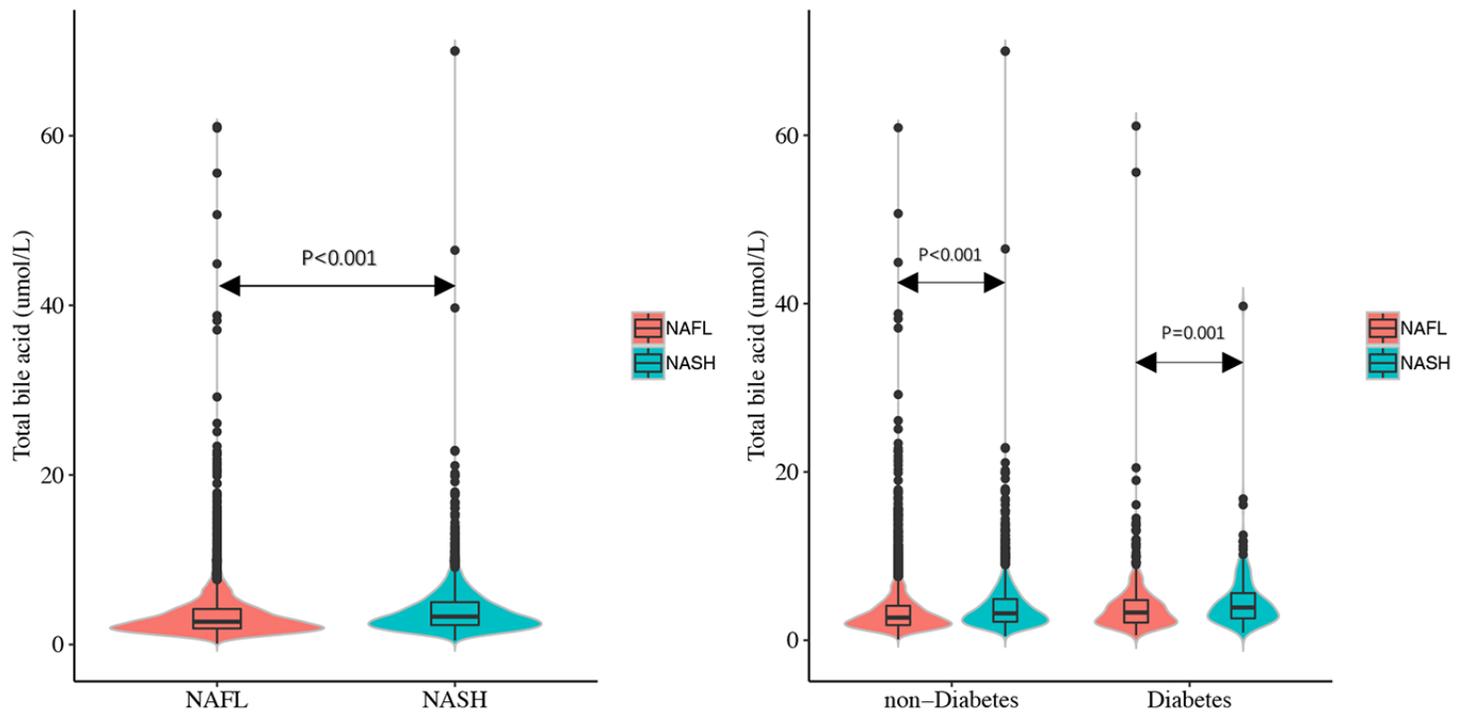


Figure 4

Levels of TBA were higher in individuals with NASH than those with NAFL