Original Article

Correlation between serum total bile acid and nonalcoholic fatty liver disease: A cross-sectional study

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Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) is a common component of chronic liver disease. Total bile acid (TBA) may influence the NAFLD progression through its signaling pathways. We attempted to find out if there is a correlation between TBA and NAFLD.

Methods: 427,507 subjects were enrolled in health examinations conducted by The First Affiliated Hospital of Wenzhou Medical University. Among them, only 67616 met the inclusion criteria. Demographic, clinical, and laboratory data were gathered from all subjects. We used multivariate logistic regression model to find the correlation between serum TBA and NAFLD after adjusting for acknowledged risk factors for NAFLD.

Results: A negative correlation was found between the TBA and NAFLD after adjusting for confounders in the multivariate logistic regression model (OR: 0.80; 95% CI: 0.72, 0.88, $P < 0.001$). After subgroup analysis, we found the interaction between NAFLD and diabetes was significant ($P = 0.043$). In patients with NAFLD without diabetes, TBA showed a protective effect in NAFLD (OR: 0.75; 95% CI: 0.67, 0.85).

Conclusion: TBA is protective for NAFLD, but not in patients with NAFLD and diabetes. Further studies are urgently required to completely explore the underlying mechanisms of TBA in the pathogenesis of NAFLD.

Keywords: Diabetes mellitus, farnesoid X receptor, nonalcoholic fatty liver disease, total bile acid.

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Submitted: 28-Sep-2021 **Accepted:** 13-Nov-2021 **Published:** 14-Feb-2022

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a major component of chronic liver diseases around the world and its prevalence is approximately 20%–30% globally.[1] With the rise of obesity, the incidence of this disorder will continue to rise, which will greatly increase the medical and economic burden on this population. NAFLD refers to the accumulation of lipids in more than 5% of hepatocytes in those without excess alcohol consumption, as well as the exclusion of chronic liver diseases caused by other well-known diseases (autoimmune, viral, etc.).[2,3] Nonalcoholic steatohepatitis (NASH), a more severe spectrum of NAFLD, which is characterized by liver inflammation and hepatocyte damage, shows a potential risk of progressing to cirrhosis and even hepatocellular carcinoma (HCC).[4,5] When NAFLD progresses to its

Access this article online	
Quick Response Code:	Website: www.saudijgastro.com
	DOI: 10.4103/sjg.sjg_512_21

How to cite this article: Chen Y, Su H, Xue H, Wang T, Qian T, Liao C. *et al*. Correlation between serum total bile acid and nonalcoholic fatty liver disease: A cross-sectional study. Saudi J Gastroenterol 2022;28:304-11.

terminal stage, it will greatly affect patients' survival and clinical outcomes. Since there is still lack of a targeted pharmacotherapy, liver transplantation will be the only method of treatment. NAFLD has multiple contributing factors such as insulin resistance, diabetes, hypertension, dyslipidemia, and obesity. It has been recognized as not only a result of abnormal fat metabolism but also a hepatic component of the metabolic syndrome.[6,7] Lately, experts from international panel renamed NAFLD to metabolic associated fatty liver disease (MAFLD), showing the consensus that NAFLD is regarded as a metabolic disorder, which means that certain metabolic factors can influence its development.[8,9]

Bile acids (BA) are cholesterol-derived molecules that are generated exclusively in hepatocytes through multiple enzymatic steps. Their synthesis occurs via two pathways. Most bile acids are produced by the classical pathway with the rate-limiting enzyme, cholesterol-7α-hydroxylase (CYP7A1). The alternative pathway also has two key enzymes called sterol 27-hydroxylase (CYP27A1) and 25-hydroxych olesterol-7α-hydroxylase (CYP7B1). Through these pathways cholesterol mainly produces cholic acid (CA) and chenodeoxycholic acid (CDCA). When primary BAs enter the gut, the intestine microbiota will help to dissociate them into secondary BAs: mainly deoxycholic acid (DCA) and lithocholic acid (LCA).[10,11]

Except for the significant role in lipid absorption, BAs signaling in the intestine and liver also regulate glucose and energy homeostasis through acting on nuclear and membrane receptors, as farnesoid X receptors (FXR) and Takeda G protein-coupled receptor 5 (TGR5).[12,13] FXR improves insulin resistance and glucose homeostasis by stimulating pancreatic β-cells to secrete insulin.[14] TGR5, the other receptor of BA, can ameliorate insulin sensitivity by activating GLP-1 secretion from intestinal L cells.[15] Many studies have indicated that BA-FXR/TGR5 pathway can exert a beneficial impact on lipid homeostasis and inhibit the development of NAFLD.[16,17]

In fact, numerous studies have demonstrated the correlation between serum BA and NAFLD; however, these studies have not reached a consistent conclusion and have not been conducted on large population-based samples. In this study, we aim to evaluate the correlation between serum total bile acid (TBA) and NAFLD in large hospital-based samples.

METHODS

Participants

In this large population-based study, in total 427,507 subjects underwent health examinations conducted by The First Affiliated Hospital of Wenzhou Medical University, from December 2011 to June 2020. The following were the exclusion criteria: (1) under the age of 18; (2) without the examination of abdominal ultrasound, computerized tomography, or magnetic resonance imaging of the liver; (3) with excessive alcohol consumption (>70 g/week for women and >140 g/week for men); (4) a history of viral hepatitis and autoimmune hepatitis; (5) lack of the data of serum TBA. For those who underwent multiple health examinations, we took their first record as our data. After screening this population, 67,616 subjects were ultimately included in our study. Informed written consent was not required since the data were anonymous. This population-based study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (ethical code: KY2020-200) and followed the ethical guidelines of the Helsinki Declaration (1964).

Blood Sampling and biochemical data

Demographic characteristics of all subjects were collected such as age, body mass index [BMI = Weight (kg)/ Height squared (m^2)], sex, blood pressure, and smoking habits (subjects that had smoked more than one cigarette per day in the past year were considered as smokers). Blood samples for laboratory tests were taken by venipuncture in the morning after overnight fasting. Laboratory tests were measured by an automated chemistry analyzer (Beckman Coulter AU5800, Japan), which included total bile acid (TBA), total protein, albumin, total bilirubin (TB), direct bilirubin (DBIL), indirect bilirubin (IBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (γ-GT), creatinine (Cr), uric acid (UA), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycosylated hemoglobin A1c (HbA1c), fasting blood glucose, white blood cells (WBC), neutrophil absolute value (Neu), lymphocyte absolute value (Lym), platelet (PLT), hypersensitive C-reactive protein (hs-CRP), thyroid-stimulating hormone (TSH), thyroid hormone (TH), triiodothyronine (T3), and alpha-fetoprotein (AFP). Platelet to lymphocyte ratio and neutrophil to lymphocyte ratio were calculated. According to the instructions, the levels of serum TBA were measured by the enzyme circulation method (Bile Acid Kit, Tokyo, Japan).

NAFLD was diagnosed based on abdominal ultrasound, computerized tomography, and/or magnetic resonance imaging. Diabetes mellitus (DM) was diagnosed based

on random blood glucose levels ≥11.1 mmol/L, fasting plasma glucose (FPG) values ≥7.0 mmol/L, and/or taking antidiabetic drugs.[18] Those who took antihypertensive medication or had systolic pressure ≥140 mmHg and/or diastolic pressure ≥90 mmHg were defined as having hypertension.[19]

Statistical analysis

In order to avoid the decrease and deviation of statistical test performance caused by the direct elimination of missing values in multivariate analysis, the chain equation was used for multivariate reduction of missing data. Numerical variables were represented as mean (standard deviation) or as median (1st quartile-3rd quartile), while categorical variables were represented as percentage (number). First, differences in numerical variables between the control group and NAFLD group were distinguished by the t-test (normal distribution) and Kruskal–Wallis H test (skewed distribution). The Chi-square test was utilized to compare differences among the groups of categorical variables. Due to the low levels of TBA, we reduced the TBA concentration by a factor of 20 and recorded every 20 changes (TBA/20) to amplify the effects of variables precisely. Second, to assess the correlation between variables and NAFLD, univariate linear regression analysis was indispensable. Third, the correlation between serum TBA and NAFLD was assessed by multivariate regression analysis. Fourth, stratified regression models were used for the subgroup analysis, and forest plot was made to describe the results more concretely. Statistical software packages R (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) were used to perform statistical analysis. For all analyses, P values < 0.05 (two-tailed) were considered statistically significant.

RESULTS

Participants' characteristics

Ultimately 67,616 subjects met the criteria and were divided into NAFLD group (n = 15,937) and control group (n = 51,679). Table 1 presents the demographic and clinical characteristics of subjects. 23.6% of subjects were diagnosed with NAFLD, among which 11,706 (73.45%) were males. The median of BMI in NAFLD group [26.27 (24.53–28.19) kg/m^2 vs 22.77 (20.94–24.70) kg/m^2, P < 0.001] was higher than in the control group. Moreover, subjects with NAFLD had a higher prevalence of hypertension and DM compared to the control group (41.04% vs 22.76%; 15.13% vs 4.96%, P < 0.001). The outcomes of univariate analysis are presented in Table 2. It showed that age, BMI, hypertension, DM, smoking, and serum level of TBA, TB, ALT, AST, ALP, γ-GT, Cr, UA, TC, TG, LDL-C, HbA1c, and FBG were positively correlated with NAFLD, which were consistent with previous studies done by other authors. Sex and serum level of HDL-C were negatively correlated with NAFLD.

Serum TBA and NAFLD

In this study, data were divided into quartile based on the levels of TBA [Table 3]. However, because the levels of TBA were not presented as a normal distribution and mainly distributed on the small side, we could not divide them into four equal groups, and instead we divided TBA into Q2 (2-2), Q3 (3-4), and Q4 (5-311). Then, we found that when TBA levels increased, the prevalence of NAFLD also increased. The Q4 group had the highest prevalence of NAFLD (27%) compared to Q2 (19.64%) and Q3 (26%).

As shown in Table 4, we attempted to find the independent correlation between TBA and NAFLD with multivariate regression analysis. We built nonadjusted models, Model I and Model II, for analysis. In nonadjusted model, TBA/20 was positively correlated with NAFLD (OR: 1.19; 95% CI: 1.12, 1.26, P < 0.001). In Model I (adjusting for age, sex, BMI, hypertension, diabetes, and smoking), the result lost statistical significance (OR: 1.07; 95%CI: 1.00, 1.16, P = 0.057). However, Model II showed that TBA/20 was negatively correlated with NAFLD after adjusting for variables (OR: 0.80; 95%CI: 0.72, 0.88; P < 0.001), which included age, smoking, hypertension, diabetes, sex, BMI, total protein, albumin, DBIL, IBIL, ALT, AST, ALP, Cr, UA, TG, HDL-C, LDL-C, HbA1c, FBG, WBC, PLT, NLR, hs-CRP, TH, and T3. For sensitivity analysis, we also used every 20 serum TBA (TBA/20) as a categorical variable (Q2-Q4), yet the conclusion was different from the results we mentioned above. We set Q2 as a reference group, Q4 versus Q2; the correlation between TBA and NAFLD in nonadjusted model (OR: 1.51, 95%CI: 1.45, 1.58, P < 0.001) and Model I (OR: 1.23, 95%CI: 1.17, 1.30, P < 0.001) was statistically significant. But in Model II, there was no correlation between them (OR: 1.01; 95%CI: 0.95, 1.07, P = 0.8155). Linear trend was not observed after adjusting for Model II (P = 0.531)

In order to explore potential interaction in variables, we further investigated the correlation between TBA and NAFLD in subgroups [Table 5]. As listed in Table 5, the P for interaction for NAFLD was significant to DM (P = 0.043) but not to age, smoking, hypertension, sex, and BMI (P = 0.5886, 0.4341, 0.2331, 0.9089, and 0.8851, respectively). In patients of NAFLD without DM, TBA was negatively correlated with NAFLD (OR: 0.75; CI: 0.67, 0.85), whereas in NAFLD with DM, the result

Table 1: Demographic and clinical characteristics of subjects

	Control group (*n*=51679)	NAFLD group (*n*=15937)	*P*
Clinical characteristics			
Age	45 (38-54)	47 (40-55)	<0.001
BMI, kg/m^2	22.77 (20.94-24.70)	26.27 (24.53-28.19)	<0.001
Male % (*n*)	47.01% (24,293)	73.45% (11,706)	<0.001
Female % (*n*)	52.99% (27,386)	26.55% (4,231)	<0.001
Hypertension, % (*n*)	22.76% (11,764)	41.04% (6,540)	<0.001
DM, % (*n*)	4.96% (2,565)	15.13% (2,412)	<0.001
Smoking	2.20% (1,136)	3.09% (493)	<0.001
Biochemical indicators			
TBA, μmol/L	3.0 (2.0-4.0)	3.0 (2.0-5.0)	<0.001
TBA, μmol/L/20	0.15 (0.10-0.20)	0.15 (0.10-0.25)	<0.001
Total protein, g/L	75.2 (72.3-78.2)	76.4 (73.6-79.3)	<0.001
Albumin, g/L	45.4 (43.4-47.5)	46.3 (44.4-48.3)	<0.001
TB, μmol/L	12 (9-15)	12 (9-15)	<0.001
DBIL, μmol/L	3 (2-4)	3 (2-4)	0.059
IBIL, μmol/L	8 (6-11)	8 (7-11)	<0.001
ALT, U/L	18 (13-26)	33 (23-50)	<0.001
AST, U/L	21 (17-25)	25 (21-32)	<0.001
ALP, U/L	70 (58-86)	78 (66-94)	<0.001
γ-GT, U/L	20 (14-32)	41 (27-68)	<0.001
Cr, μmol/L	63 (53-75)	70 (59-80)	<0.001
UA, μmol/L	310 (258-372)	384 (324-444)	<0.001
TC, mmol/L	5.03 (4.41-5.70)	5.36 (4.72-6.08)	<0.001
TG, mmol/L	1.18 (0.85-1.69)	2.11 (1.50-3.03)	<0.001
HDL-C, mmol/L	1.32 (1.12-1.55)	1.11 (0.97-1.27)	<0.001
LDL-C, mmol/L	2.85 (2.37-3.39)	3.07 (2.51-3.64)	<0.001
HbA1c %	5.4 (5.2-5.7)	5.7 (5.4-6.2)	<0.001
FBG, mmol/L	5.1 (4.8-5.5)	5.5 (5.1-6.2)	<0.001
WBC, ×10^9/L	5.76 (4.87-6.83)	6.53 (5.57-7.67)	<0.001
Neu, ×10^9/L	3.21 (2.57-4.00)	3.59 (2.93-4.44)	<0.001
Lym, ×10^9/L	1.91 (1.59-2.31)	2.21 (1.85-2.66)	<0.001
PLT, ×10^9/L	231 (197-269)	235 (201-272)	<0.001
PLR	119.57 (96.35-149.34)	105.16 (85.20-130.05)	<0.001
NLR	1.66 (1.29-2.15)	1.62 (1.28-2.06)	<0.001
hs-CRP, mg/L	0.53 (0.26-1.17)	1.15 (0.61-2.39)	<0.001
TSH, mIU/L	1.64 (1.15-2.34)	1.65 (1.18-2.33)	0.296
TH, nmol/L	103.64 (92.58-116.05)	103.45 (92.26-115.75)	0.001
T3, nmol/L	1.60 (1.42-1.79)	1.65 (1.47-1.85)	<0.001
AFP, ng/ml	2.63 (1.95-3.60)	2.80 (2.10-3.70)	0.405

Data are shown as mean (standard deviation) or as median (1st quartile-3rd quartile) or percentage (number). Two-tailed $P<0.05$ was considered statistically significant. NAFLD: nonalcoholic fatty liver disease, BMI: body mass index, DM: diabetes mellitus, TBA: total bile acid, TB: total bilirubin, DBIL: direct bilirubin, IBIL: indirect bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, γ-GT: gamma-glutamyl transferase, Cr: creatinine, UA: uric acid, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, HbA1c: glycosylated hemoglobin A1c, FBG: fasting blood glucose, WBC: white blood cell, Neu: neutrophil absolute value, Lym: lymphocyte absolute value, PLT: platelet, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio, hs-CRP: hypersensitive C-reactive protein, TSH: thyroid-stimulating hormone, TH: thyroid hormone, T3: triiodothyronine, and AFP: alpha-fetoprotein

showed no correlation between TBA and NAFLD (OR: 1.00; 95%CI: 0.79, 1.27). Forest plot of the effect values of TBA on NAFLD in subgroups is shown in Figure 1, which revealed the size of effect value in each subgroup of variables more intuitively and concretely.

DISCUSSION

NAFLD, an emerging health problem worldwide, is a complex disease that metabolic, genetic, and nutritional factors can assist in its morbidity. Abnormal lipid metabolism and insulin resistance exert a crucial role in the occurrence and development of NAFLD.[20,21] The pathologic mechanisms between TBA and NAFLD are complex and affected by multiple factors. TBA affects glucose homeostasis and lipid metabolism via specific receptors FXR and TGR5, which give us a hint TBA may have an impact on NAFLD development.[22] Consistent with our hypothesis, several studies in mice have demonstrated that stimulation of FXR pathway could protect hepatocytes from lipotoxicity by decreasing hepatic lipogenesis and lowering plasma triglyceride (TG).[23,24]

This study used large hospital-based samples, (*n*=67,616) in order to evaluate the correlation between TBA and NAFLD. After multivariate logistic regression analysis, the results showed TBA was negatively correlated with NAFLD. We found that TBA became a protective factor for NAFLD without DM, but showed no correlation in NAFLD with DM patients after stratified analysis.

Table 2: Univariate analysis of NAFLD

	Statistics	OR	95% CI	*P*
Clinical characteristics				
Age	46.42±11.44	1.01	1.01, 1.01	<0.001
BMI, kg/m^2	23.74±3.22	1.54	1.53, 1.55	<0.001
Sex				
Male	35,999 (53.24%)	Reference		
Female	31,617 (46.76%)	0.32	0.31, 0.33	<0.001
Hypertension, *n* (%)				
No	49,312 (72.93%)	Reference		
Yes	18,304 (27.07%)	2.36	2.27, 2.45	<0.001
DM, *n* (%)				
No	62,639 (92.64%)	Reference		
Yes	4,977 (7.36%)	3.41	3.22, 3.62	<0.001
Smoking				
No	65,987 (97.59%)	Reference		
Yes	1,629 (2.41%)	1.42	1.28, 1.58	<0.001
Biochemical indicators				
TBA, μmol/L	4.20±5.38	1.01	1.01, 1.01	<0.001
TBA, μmol/L/20	0.21±0.27	1.19	1.12, 1.26	<0.001
Total protein, g/L	75.57±4.43	1.06	1.06, 1.07	<0.001
Albumin, g/L	45.63±3.13	1.1	1.09, 1.11	<0.001
TB, μmol/L	12.66±5.28	1.01	1.01, 1.01	<0.001
DBIL, μmol/L	3.69±1.95	0.99	0.98, 1.00	0.059
IBIL, μmol/L	8.97±3.93	1.02	1.02, 1.03	<0.001
ALT, U/L	26.92±27.36	1.04	1.04, 1.05	<0.001
AST, U/L	24.35±17.73	1.04	1.04, 1.04	<0.001
ALP, U/L	75.51±25.03	1.01	1.01, 1.01	<0.001
γ-GT, U/L	38.67±63.18	1.01	1.01, 1.01	<0.001
Cr, μmol/L	66.25±18.76	1.02	1.02, 1.02	<0.001
UA, μmol/L	335.83±90.38	1.01	1.01, 1.01	<0.001
TC, mmol/L	5.19±1.04	1.36	1.34, 1.38	<0.001
TG, mmol/L	1.70±1.39	2.14	2.10, 2.18	<0.001
HDL-C, mmol/L	1.30±0.32	0.07	0.07, 0.08	<0.001
LDL-C, mmol/L	2.95±0.81	1.31	1.28, 1.34	<0.001
HbA1c%	5.66±0.87	1.8	1.75, 1.84	<0.001
FBG, mmol/L	5.46±1.35	1.42	1.40, 1.44	<0.001
WBC, ×10^9/L	6.17±1.78	1.3	1.28, 1.31	<0.001
Neu, ×10^9/L	3.51±1.29	1.25	1.23, 1.27	<0.001
Lym, ×10^9/L	2.06±0.60	2.23	2.17, 2.30	<0.001
PLT, ×10^9/L	236.87±58.05	1.00	1.00, 1.00	<0.001
PLR	123.51±44.47	0.99	0.99, 0.99	<0.001
NLR	1.82±0.88	0.92	0.90, 0.94	<0.001
hs-CRP, mg/L	1.77±5.43	1.03	1.03, 1.04	<0.001
TSH, mIU/L	2.03±3.13	1.00	0.99, 1.00	0.297
TH, nmol/L	105.50±20.75	1.00	1.00, 1.00	0.001
T3, nmol/L	1.64±0.39	1.30	1.24, 1.35	<0.001
AFP, ng/ml	4.43±213.52	1.00	1.00, 1.00	0.276

Data are shown as mean (standard deviation) or as median (1st quartile-3rd quartile) or percentage (number). Two-tailed $P<0.05$ was considered statistically significant. NAFLD: nonalcoholic fatty liver disease, BMI: body mass index, DM: diabetes mellitus, TBA: total bile acid, TB: total bilirubin, DBIL: direct bilirubin, IBIL: indirect bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, γ-GT: gamma-glutamyl transferase, Cr: creatinine, UA: uric acid, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, HbA1c: glycosylated hemoglobin A1c, FBG: fasting blood glucose, WBC: white blood cell, Neu: neutrophil absolute value, Lym: lymphocyte absolute value, PLT: platelet, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio, hs-CRP: hypersensitive C-reactive protein, TSH: thyroid-stimulating hormone, TH: thyroid hormone, T3: triiodothyronine, and AFP: alpha-fetoprotein

Recently, studies have shown that serum TBA are strongly correlated with NAFLD. Jiao *et al*.[25] reported serum total bile acid levels in biopsy-proven NASH group ($n = 16$) were elevated compared to healthy controls ($n = 11$). However, this study did not do multiple regression analysis to further elaborate the independent correlation between TBA and NAFLD. CDCA is the most effective natural FXR agonist that can reduce inflammation and improve insulin sensitivity through activating FXR, while DCA, antagonists of FXR, do not activate FXR.[25,26] Notably, the study mentioned above found in the NASH group, the concentration of DCA was higher than in the healthy group, meanwhile the concentration of CDCA was decreased when compared with the healthy group. These results suggest that rather than the change in TBA concentration, alterations in TBA component concentration may be more relevant to metabolism, which have a significant impact on the downstream pathways and cause different physiological effects.

Table 3: General characteristics of subjects categorized by TBA quartiles

	Serum TBA quartiles			*P*
	Q2 (*n*=28574)	Q3 (*n*=21804)	Q4 (*n*=17238)	
Clinical characteristics				
Age	45 (38-53)	46 (38-54)	47 (39-56)	<0.001
BMI, kg/m^2	23.30 (21.26-25.46)	23.75 (21.62-25.90)	23.81 (21.68-26.00)	<0.001
NALFD, % (*n*)	19.64% (56,12)	26.00% (5,670)	27.00% (4,655)	<0.001
Male % (*n*)	45.11% (12,891)	57.92% (12,628)	60.80% (10,480)	<0.001
Female % (*n*)	54.89% (15,683)	42.08% (9,176)	39.20% (6,758)	<0.001
Hypertension, % (*n*)	24.82% (7,093)	27.71% (6,042)	29.99% (5,169)	<0.001
DM, % (*n*)	5.78% (1,652)	7.98% (1,740)	9.19% (1,585)	<0.001
Smoking	2.23% (637)	2.02% (440)	3.20% (552)	<0.001
Biochemical indicators				
Total protein, g/L	75.5 (72.6-78.4)	75.6 (72.8-78.5)	75.4 (72.5-78.5)	0.003
Albumin, g/L	45.4 (43.4-47.4)	45.8 (43.8-47.9)	45.7 (43.6-47.8)	<0.001
TB, μmol/L	12 (9-15)	12 (9-15)	12 (9-15)	<0.001
DBIL, μmol/L	3 (3-4)	3 (2-4)	3 (2-5)	<0.001
IBIL, μmol/L	8 (6-11)	8 (6-11)	8 (6-11)	0.439
ALT, U/L	19 (14-28)	21 (15-32)	23 (16-36)	<0.001
AST, U/L	21 (18-25)	22 (18-27)	23 (19-29)	<0.001
ALP, U/L	70 (57-85)	73 (60-88)	75 (62-91)	<0.001
γ-GT, U/L	21 (14-35)	24 (16-43)	27 (17-50)	<0.001
Cr, μmol/L	62 (53-75)	66 (55-77)	67 (56-78)	<0.001
UA, μmol/L	315 (262-381)	333 (274-400)	337 (276-402)	<0.001
TC, mmol/L	5.09 (4.47-5.78)	5.12 (4.49-5.80)	5.12 (4.48-5.82)	0.040
TG, mmol/L	1.26 (0.88-1.86)	1.39 (0.96-2.10)	1.43 (0.98-2.23)	<0.001
HDL-C, mmol/L	1.28 (1.09-1.51)	1.25 (1.06-1.48)	1.24 (1.06-1.47)	<0.001
LDL-C, mmol/L	2.91 (2.40-3.46)	2.91 (2.40-3.45)	2.90 (2.37-3.45)	0.005
HbA1c%	5.5 (5.2-5.7)	5.5 (5.3-5.8)	5.5 (5.3-5.9)	<0.001
FBG, mmol/L	5.1 (4.8-5.6)	5.3 (4.9-5.7)	5.3 (4.9-5.8)	<0.001
WBC, x10^9/L	5.83 (4.92-6.93)	5.98 (5.04-7.09)	6.07 (5.12-7.19)	<0.001
Neu, x10^9/L	3.28 (2.61-4.10)	3.31 (2.67-4.13)	3.33 (2.68-4.14)	<0.001
Lym, x10^9/L	1.93 (1.60-2.32)	2.00 (1.66-2.43)	2.05 (1.68-2.49)	<0.001
PLT, x10^9/L	236 (201-273)	231 (198-269)	227 (193-265)	<0.001
PLR	121.11 (98.07-150.26)	113.88 (92.22-142.92)	110.18 (87.78-138.00)	<0.001
NLR	1.68 (1.31-2.16)	1.63 (1.29-2.10)	1.62 (1.27-2.10)	<0.001
hs-CRP, mg/L	0.59 (0.28-1.32)	0.67 (0.32-1.48)	0.71 (0.33-1.65)	<0.001
TSH, mIU/L	1.60 (1.13-2.28)	1.65 (1.16-2.35)	1.70 (1.19-2.44)	<0.001
TH, nmol/L	103.76 (92.66-116.08)	103.76 (92.66-116.08)	103.34 (92.26-115.91)	0.051
T3, nmol/L	1.60 (1.42-1.79)	1.62 (1.44-1.82)	1.62 (1.43-1.83)	<0.001
AFP, ng/ml	2.60 (1.93-3.51)	2.70 (2.00-3.69)	2.80 (2.09-3.80)	0.022

Data are shown as mean (standard deviation) or as median (1st quartile-3rd quartile) or percentage (number). Two-tailed $P<0.05$ was considered statistically significant. NAFLD: nonalcoholic fatty liver disease, BMI: body mass index, DM: diabetes mellitus, TBA: total bile acid, TB: total bilirubin, DBIL: direct bilirubin, IBIL: indirect bilirubin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, γ-GT: gamma-glutamyl transferase, Cr: creatinine, UA: uric acid, TC: total cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, HbA1c: glycosylated hemoglobin A1c, FBG: fasting blood glucose, WBC: white blood cell, Neu: neutrophil absolute value, Lym: lymphocyte absolute value, PLT: platelet, PLR: platelet to lymphocyte ratio, NLR: neutrophil to lymphocyte ratio, hs-CRP: hypersensitive C-reactive protein, TSH: thyroid-stimulating hormone, TH: thyroid hormone, T3: triiodothyronine, and AFP: alpha-fetoprotein

Table 4: The correlation between NAFLD and TBA levels after adjustments

TBA (μmol/L)	Nonadjusted		Model I		Model II	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
TBA as a continuous variable						
TBA	1.01 (1.01, 1.01)	<0.001	1.00 (1.00, 1.01)	0.057	0.99 (0.98, 0.99)	<0.001
TBA/20	1.19 (1.12, 1.26)	<0.001	1.07 (1.00, 1.16)	0.057	0.80 (0.72, 0.88)	<0.001
TBA/20 as a categorical variable						
Q2 (2)	Reference		Reference		Reference	
Q3 (3-4)	1.44 (1.38, 1.50)	<0.001	1.21 (1.16, 1.28)	<0.001	1.10 (1.05, 1.16)	<0.001
Q4 (5-311)	1.51 (1.45, 1.58)	<0.001	1.23 (1.17, 1.30)	<0.001	1.01 (0.95, 1.07)	0.816
Trend test		<0.001		<0.001		0.531

OR: odds ratio; CI: confidence interval; NAFLD: nonalcoholic fatty liver disease; TBA: total bile acid; Model I adjusting for age, smoking, hypertension, diabetes, sex, body mass index; Model II adjusting for age, smoking, hypertension, diabetes, sex, body mass index, total protein, albumin, direct bilirubin, indirect bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, uric acid, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glycosylated hemoglobin A1c, fasting blood glucose, white blood cell, platelet, neutrophil to lymphocyte ratio, hs-CRP, thyroid hormone, and triiodothyronine

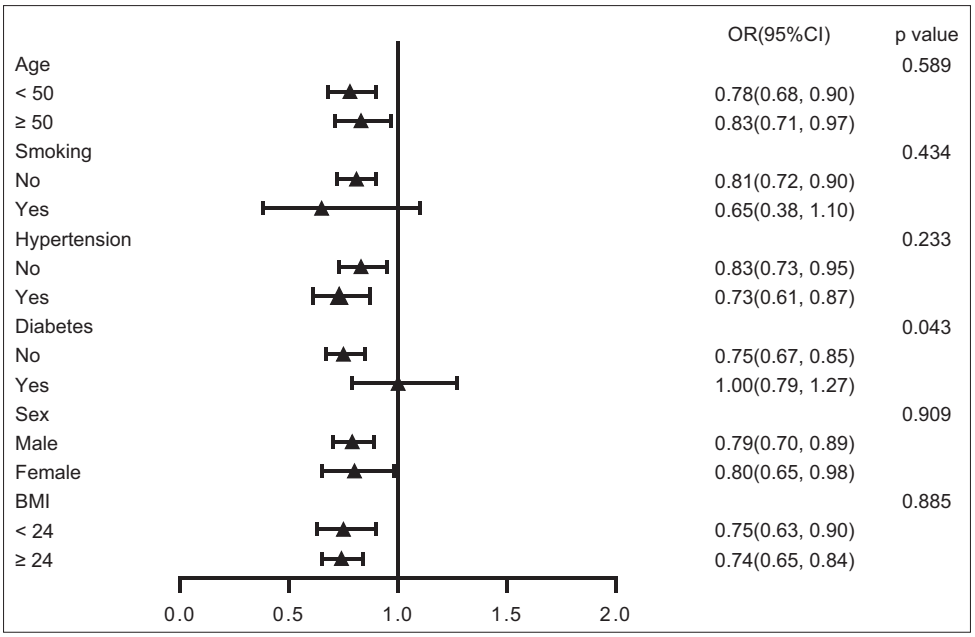

Figure 1: Stratified analysis of TBA and MAFLD in subgroups

In addition, a study by Brufau *et al*.[27] measured the concentration of each component of BA in DM patients ($n = 12$) and healthy subjects ($n = 12$), respectively. They found that DCA concentration increased, whereas CDCA concentration reduced in DM patients. Combined with the study of Jiao *et al*., we hypothesize that changes in DCA and CDCA concentration may also have occurred in our study. Since DCA cannot improve insulin resistance, it may explain why no correlation was observed between TBA and NAFLD in DM patients. Since DCA inhibits the shielding effect of FXR-mediated pathway, its increased levels may explain why in our subgroup analysis the *P* interaction for NAFLD was irrelevant with DM.

Table 5: Stratified analysis of TBA and NAFLD

Characteristic	OR	95% CI	*P* for interaction
Age (years)			0.589
<50	0.78	0.68, 0.90	
≥50	0.83	0.71, 0.97	
Smoking			0.434
No	0.81	0.72, 0.90	
Yes	0.65	0.38, 1.10	
Hypertension			0.233
No	0.83	0.73, 0.95	
Yes	0.73	0.61, 0.87	
Diabetes			0.043
No	0.75	0.67, 0.85	
Yes	1.00	0.79, 1.27	
Sex			0.909
Male	0.79	0.70, 0.89	
Female	0.80	0.65, 0.98	
BMI (kg/m^2)			0.885
<24	0.75	0.63, 0.90	
≥24	0.74	0.65, 0.84	

CI: confidence interval; OR: odds ratio; TBA: total bile acid; NAFLD: nonalcoholic fatty liver disease; BMI: body mass index

A population-based study recruited 152,336 subjects diagnosed by abdominal ultrasonography to demonstrate whether serum TBA was correlated with NAFLD.[28] In the end, they found that there was no correlation between them (OR: 1.00; 95%CI: 1.00, 1.00, $P = 0.797$). Unfortunately, they did not use stratified analysis to further analyze the correlation between TBA and NAFLD in subgroups. Moreover, Adams *et al*.[29] showed that TBA levels increased progressively from controls ($n = 55$), F0-2 NAFLD ($n = 58$) to F3/4 NAFLD ($n = 9$) in biopsy-proven subjects. And TBA turned out to be positively correlated with NAFLD after logistic regression analysis, which was against our finding. Notably, in this study, among the patients in F3/4 NAFLD, 77.8% of them had DM ($n = 7$) and DCA was significantly higher than other groups. Given that the structure of our population was different from theirs and the composition of TBA might be altered in NAFLD or DM patients, it could have a significant disturbance on the conclusion that TBA was a risk factor for NAFLD in their study, and may explain the reason why our conclusion was different from theirs after multivariate regression analysis.

Since we used data from the physical examination center, a possible limitation of our study is the collection of patients' histories may be not very accurate, such as the majority of people reported that they did not smoke, which was doubtful. However, we have objectively documented the indicators that would mainly affect NAFLD, and due to our large sample size, and the bias was normally distributed and did not affect our results. Another limitation of our study is that this was a cross-sectional study, which can

only find the correlation between TBA and NAFLD, but cannot explain the causal relationship. So far there was no convenient technique for evaluating serum TBA composition in clinical laboratories, and we were not able to measure CA and DCA of subjects to assess the influence of BA/FXR pathway. Further studies are needed to focus on the composition of TBA to fully understand the specific role of BA in NAFLD.

In summary, we conclude that serum TBA was a protective factor for NAFLD, but not in those with co-existing DM. The mechanism of the correlation between them remains unclear. Hence, further research needs to be undertaken to figure out the pathophysiological mechanism between TBA and NAFLD, especially the mechanism of BA composition changes in NAFLD patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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