ORIGINAL RESEARCH

Negative Correlation of Serum Total Bile Acid With Albuminuria in Patients With Type 2 Diabetes Mellitus: A Cross-sectional Study

Yan Wu, MM; Zexin Chen, MM; Rendi Deng, BM; Yiqun Wu, MM; Haiyun Xie, BM; Pengfei Shan, MD; Junfen Fu, MD

ABSTRACT

Context • The treatment of diabetic nephropathy (DN) is still quite limited. DN remains poorly understood due to the complexity of and differences in its etiology. Therefore, potential biomarkers for diagnosis and targeted treatments are urgently needed.

Objective • The study aimed to analyze the associations between circulating total bile acid (TBA) levels and the risk of DN in Chinese patients with type 2 diabetes mellitus (T2DM) and to determine the differences in the TBA levels of males and females, including pre- and postmenopausal women, to find clues for the screening of DN.

Setting • The study took place at the Second Affiliated Hospital at the School of Medicine of Zhejiang University in Zhejiang, China.

Participants • Participants were 1785 T2DM patients admitted to the hospital between April 2008 and November 2013.

Groups • The research team separated participants into three groups: (1) the normoalbuminuria or normal group, with a UACR <30 mg/g·Cr (2) the microalbuminuria (MAU) group, with a UACR of 30-299 mg/g·Cr; and (3) the macroalbuminuria (MAC) group, with a UACR of ≥300 mg/g·Cr.

Outcome Measures • Between the three groups, the research team compared: (1) the demographic and clinic characteristics of the normal, MAU, and MAC groups; (2) TBA distribution by age; (3) TBA distribution by gender; and (4) TBA quartiles. The team also examined the associations between TBA and albuminuria, identifying the odds ratios (OR) and relevant 95% confidence intervals (CI) using multiple logistic regression.

Results • The study found that: (1) the MAC group's TBA was significantly lower than those of the normal and MAU groups; (2) the TBA of postmenopausal women was significantly higher than that of premenopausal women; (3) the incidence of MAC was obviously increased with TBA levels; (4) the risks for MAU group didn't change significantly with increasing TBA levels; (5) the MAC group's odds ratios (ORs) were 0.61 between Q2 and Q1, 0.44 between Q3 and Q1, and 0.38 between Q4 and Q1; and (6) for men and postmenopausal women, the TBA levels of those in Q3 and Q4 might decrease the risk of MAC, whereas no such correlation existed for MAU.

Conclusions • An independent negative association exists between TBA levels and MAC in T2DM. The decrease of circulating TBA might be a prospective clinical factor for determining established DN, especially for males and postmenopausal females. (Altern Ther Health Med. [E-pub ahead of print.])

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According to the IDF report, in 2017, the number of people aged 20-79 in China with diabetes was 114 million, ranking first in the world. Additionally, a nationwide investigation in China revealed that a substantial number of diabetics aren’t diagnosed, and newly diagnosed diabetics take up 60% of the overall number of diabetics.

Diabetic nephropathy (DN) is the most commonly seen microvascular complication of diabetic diseases. A meta-analysis including 30 studies found that the overall incidence of DN was 21.8% in China. Among the causes of end-stage renal disease (ESRD), DN accounts for 30-47% in the world. As the leading cause of ESRD, DN has become an important public-health crisis in China.

However, the treatment of DN is still quite limited. Despite the fact that treatments such as intensive control of high blood glucose and high blood pressure can decrease proteinuria and postpone DN development, a substantial number of patients still experience aggravated kidney impairment and develop ESRD. DN remains poorly understood due to the complexity of and differences in its etiology. Therefore, potential biomarkers for diagnosis and targeted treatments are urgently needed.

**Bile Acids (BAs)**

BA is an important substance that cholesterol metabolism produces. Cholesterol 7α-hydroxylase (CYP7A1) and 12-α-Hydroxylase (CYP8B1) are important enzymes in the classical and alternative metabolic pathways of BAs. Although CYP7A1 regulates the size of the BA pool, the activity of CYP8B1 regulates the ratio of cholic acid (CA) to chenodeoxycholic acid (CDCA) within that pool.

In recent years, it has been found that circulating bile acids (BAs) acted as signaling molecules via activation of bile acid receptors, regulated a wide range of metabolic pathways, including glucose and lipid metabolism, energy expenditure and the modulation of immune responses. Ma and Patti also found that BA is a vital signaling molecule and that it was important in regulating glucose and lipometabolism and energy metabolism.

Two studies found that variations in BA metabolism and signaling pathways were associated with obesity and T2DM. Herrema et al’s study discovered that the db/db mice showed increased feces production and a higher fecal bile salt output, representing hepatic bile salt synthesis, compared with lean controls. The db/db mice have an increased pool size and synthesis rate of bile salts compared with lean controls.

Some studies have shown that the total amount of BA doesn’t change, but the proportions of different BAs do change. For example, Brufau et al found that the content of secondary BA deoxycholic acid (DCA) increased while the content of primary BA DCA acid decreased. Another study showed that patients with good glycemic control had lower, plasma CA levels and higher DCA levels.

These results confirmed that BA metabolism is closely related to T2DM. In newly diagnosed T2DM patients, Zhu et al found that the mean total BA was 3.29 μmol/L. Haeusler et al found that serum TBA levels in T2DM patients were almost two times greater than that of normal individuals.

**BAs and DN**

Gai et al’s animal experiments further found that BA and major acceptors, such as the nuclear acceptor—the Farnesoid X receptor (FXR)—and the membrane-bound, G protein-coupled BA receptor 1 (GPBAR1 or TGR5) were associated with DN. Wang et al found that the stimulation of FXR and TGR5 was nephroprotective in animal models by reducing renal inflammatory events, oxidation stress, and fibrosis.

BA is a natural ligand for FXR. Activated by BA, FXR can regulate the expression of various genes in glucose and lipid metabolism, which is conducive to maintaining the homeostasis of glucose and lipid metabolism.

Wang et al treated Western, diet-fed DBA/2J mice with obeticholic acid (INT 747), a FXR agonist, using a daily gavage and observed a significant decrease in urinary protein. Those researchers found that the INT747 could decrease podocyte loss and mesangial dilatation, which they considered to be related to the activation of FXR to regulate fatty acid synthesis and oxidation. The study found that it decreased TG accumulation as well as improved renal structure.

**Albuminuria**

Medical practitioners recognize microalbuminuria (MAU) as an early biomarker of DN that is closely related to the progression and prognosis of DN.

Albuminuria is an important clinical feature of DN and is closely related to the disease’s progression and prognosis. Albuminuria is the result of multiple factors, such as abnormal hemodynamics, podocyte dysfunction, an impaired filtration barrier, and abnormal renal tubule reabsorption.

Hu et al. found that intragastric administration of chenodeoxycholic acid (CDCA) also alleviated renal damage induced by fructose feeding in Wistar rats, such as mesangial membrane dilatation, basal membrane thickening and disappearance of feet process, and urine albumin was significantly reduced to normal.

Jiang et al found that proteinuria, glomerulosclerosis, and tubulointerstitial fibrosis in db/db diabetic mice could improve significantly through treatment with cholic acid for 12 weeks. Another study found that oral enalapril combined with an intraperitoneal injection of taurolursodeoxycholic acid (TUDCA) could significantly reduce UA in 16-week-old db/db mice, and that only TUDCA could improve renal tubule injuries compared to angiotensin-converting enzyme inhibitors (ACEIs). These effects were related to the specific improvement of endoplasmic, reticulum-stress-signal transduction in renal tubular cells.

Therefore, bile acids can activate FXR and play a role in reducing UA in DN by protecting podocytes, tubule cells, and mesangial cells.
TBA and Gender and Age

Gender. Endogenic sex hormones can modulate metabolic homeostasis using nongenetic signal paths or by binding to nuclear acceptors. Jelinsky et al’s animal study found that the signal transmission of estrogen receptors (ERs) can exert a straight influence on genetic expression in the kidney’s extracortical area. The anti-inflammatory effect of estrogen as well as the straight stimulation of ER signal transmission in the kidney, can facilitate renal protection. A female gender and estrogen are vital modulators of BA generation and modulation that use pivotal liver feedback mechanisms.

In mice and humans, the rate of BA biosynthesis and the size of the BA pool are sexually dimorphic. Wild-type female mice display a bigger TBA pool; females secrete less fecal BA and catabolize less cholesterol through BA generation. Consistent with those data, Frommherz et al’s test of BA-pool constituents found that CDCA is greater in males than in females.

Estrogen influences BA biosynthesis by influencing enzyme activities and BA pool constituents. By activating estrogen receptor subtype alpha (ER-α), estrogen is capable of upregulating CYP7A1 and reducing CYP8B1 signal transmissions. ER-β doesn’t seem to exert any influence on the modulation of BA biosynthesis enzymes. In addition, Yamamoto et al found in an animal model that estrogen can reduce the CDCA content in bile through the ERα signaling pathway.

Age. Frommherz et al found that the differences in BA production in women were associated with the differences in age-related hormone levels. Xu et al found that serum TBA levels were positively correlated with age.

Current Study

Exploring the association between albuminuria and serum BAs may provide promising pharmaceutical targets and a therapeutic potential for preventing the progression of DN. However, insufficient research has occurred with regard to the correlation between them.

The current study intended to analyze the associations between circulating TBA levels and the risk of DN for T2DM patient in China and to determine the differences in the TBA levels of males and females, including pre- and postmenopausal women, to find clues for the screening of DN.

METHODS

Participants

The research team performed a retrospective study, which took place at the Second Affiliated Hospital at the School of Medicine of Zhejiang University in Zhejiang, China. The participants were adult patients with type 2 diabetes admitted to the hospital between April 2008 and November 2013.

The study included potential participants if they: (1) had T2DM; (2) had used hypoglycemic drug; and (3) were adult.

The study excluded potential participants if they had: (1) type 1 diabetes; (2) a history of tumors, particularly of the digestive system; (3) serious cardiac vascular or cerebral vascular complications; (4) excessive alcohol intake, with alcohol consumption of >20 g every day for females and of >3 g every day for males; (5) been using hepatotoxicity medicine, which might cast adverse influence on hepatic functions; (6) recently used cholesterol absorption suppressors or BA isolators; (7) persistent cholestasis; (8) persistent viral hepatitis or cirrhosis; (9) experienced gallbladder or bile-duct inflammatory events; (10) cholelithiasis; (11) had surgery related to the gastrointestinal tract, pancreas, liver, or bile ducts; (12) nondiabetic renal illnesses; (13) an estimated glomerular filtration rate (eGFR) of <15 ml/min/1.73 m²; (14) aberrant thyroid functions; (15) recently used steroid hormones; or (16) an autoimmune disease.

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Second Affiliated Hospital of Zhejiang University School of Medicine. The Ethical Board of the hospital approved the study’s protocols (2013LSY083). This study complied with the Helsinki Declaration.

Procedures

Data collection. We obtained various clinical data of patients by searching electronic medical records. All participants underwent a medical-history evaluation and received physical examinations. They wore light clothes during weighing. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Abdominal circumference was measured at the plane of the midpoint of the line between the lower margin of the ribs and the anterior superior iliac crest. Blood pressure was measured twice for each participant using a Kenz BPM SP-1 automatic blood pressure device (Suzuken, Nagoya, Japan). Hypertension was defined as an SBP of ≥140 mmHg, a DBP of ≥90 mmHg, or hospitalization with use of antihypertensive medications.

Venous blood samples were obtained in the morning before 8:00 AM after overnight fasting. FBG, creatinine, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), uric acid were determined with an AU4500 automatic chemistry analyzer (Olympus Corporation, Tokyo, Japan). Glycated hemoglobin (HbA₁c) was measured with a Tosoh HLC-723G8 automatic glycohemoglobin analyzer (Tosoh, Yamaguchi, Japan). Morning spot samples were collected for the urinary albumin/creatinine ratio (UACR). Urine albumin was measured by nephelometry immunoassay with the SIEMENS BN II System (Siemens, Marburg, Germany). The eGFR was calculated according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula

UACR Groups. As per the American Diabetic Association’s categorization of MAU, the research team separated participants into three groups: (1) the normal group, with a UACR of <30 mg/g Cr; (2) the MAU group, with a UACR of 30-299 mg/g Cr; and (3) the MAC group, with a UACR of ≥300 mg/g Cr.
Outcome Measures. Between the three groups, the research team compared: (1) the demographic and clinical characteristics of the normal, MAU, and MAC groups; (2) TBA distribution by age; (3) TBA distribution by gender; and (4) TBA quartiles. The team also examined the associations between TBA and albuminuria, identifying the odds ratios (OR) and relevant 95% confidence intervals (CI) using multiple logistic regression.

Outcome Measures
Demographic and clinical characteristics. Some data was missing in our study, and the missing data was not included in the analysis. The number behind each variable is the actually number of patients for analysis. Besides, in order to show our results clearly, some modification were marked in table 2.

TBA distribution by age. The median and IQR were shown for each age group, and nonparametric trend test was conducted.

TBA distribution by gender. The median and IQR were shown for male, premenopausal and postmenopausal group, and pairwise comparison was conducted between three groups.

Quartiles. The research team classified participants into quartiles according to the concentration of TBA. The ranges of the TBA quartiles were: (1) first quartile (Q1), <2.2 μmol/L; (2) second quartile (Q2), 2.2-3.6 μmol/L; (3) third quartile (Q3), 3.6-6.0 μmol/L; and (4) fourth quartile (Q4), ≥6.0 μmol/L.

Multivariate logistic regressive analyses. The research team modified the analyses for underlying confounders, including age, gender, BMI, diabetic duration, hypertension, smoking, HbA1c, TC, TG, ARB/ACEI use or not, and UA.

Statistical Analysis
The research team analyzed the data using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The team: (1) expressed continuous variables and categorical variables as means ± standard deviations (SDs) and frequencies and proportions (%), respectively; (2) described medians and interquartile ranges for continuous variables that weren't normally distributed; (3) compared the distributions of the continuous variables and categorical variables between the groups, using an analysis of variance (ANOVA) and the Kruskal-Wallis test, respectively; (4) compared the categorical data between the groups using the chi-square test (χ^2) or the Fisher exact test; and (5) conducted a logistic regression analysis to analyze the association between the serum BA and albuminuria. P < .05 indicated a significant difference.

Table 1. Demographic and Clinical Characteristics of T2DM Participants by Albuminuria Type (N = 1785)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal Group (N=1069)</th>
<th>MAU Group (N=452)</th>
<th>MAC (N=264)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n=1785)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>580 (54.3)</td>
<td>212 (46.9)</td>
<td>137 (51.9)</td>
</tr>
<tr>
<td>Female</td>
<td>489 (45.7)</td>
<td>240 (53.1)</td>
<td>127 (48.1)</td>
</tr>
<tr>
<td>Age, y (n=1785)</td>
<td>57.4 ± 12.7</td>
<td>61.8 ± 13.7*</td>
<td>62.0 ± 12.1*</td>
</tr>
<tr>
<td>BMI, kg/m² (n=1694)</td>
<td>24.04 ± 3.66</td>
<td>23.97 ± 3.80</td>
<td>24.25 ± 3.40</td>
</tr>
<tr>
<td>Duration of Diabetes, y (n=1778)</td>
<td>6.0 (1.0, 10.0)</td>
<td>8.0 (3.0, 14.0)</td>
<td>10.0 (6.0, 16.0)</td>
</tr>
<tr>
<td>Hypertension (n=1785)</td>
<td>479 (44.8)</td>
<td>259 (57.3)</td>
<td>202 (76.5)</td>
</tr>
<tr>
<td>ACEI/ARB (n=1772)</td>
<td>217 (20.5)</td>
<td>118 (26.2)</td>
<td>104 (39.5)</td>
</tr>
<tr>
<td>Smokers (n=1777)</td>
<td>710 (66.7)</td>
<td>312 (69.3)</td>
<td>175 (66.8)</td>
</tr>
<tr>
<td>Current smokers</td>
<td>244 (22.9)</td>
<td>74 (16.4)</td>
<td>49 (18.7)</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>111 (10.4)</td>
<td>64 (14.2)</td>
<td>38 (14.5)</td>
</tr>
<tr>
<td>SBP, mmHg (N=1774)</td>
<td>132.8 ± 17.3</td>
<td>141.8 ± 21.1*</td>
<td>151.6 ± 22.4*</td>
</tr>
<tr>
<td>DBP, mmHg (N=1774)</td>
<td>81.1 ± 10.8</td>
<td>81.8 ± 12.3</td>
<td>85.0 ± 11.9*</td>
</tr>
<tr>
<td>HbA1c (N=1785)</td>
<td>9.4 (7.7, 11.3)*</td>
<td>9.6 (7.8, 11.6)</td>
<td>8.8 (7.3, 10.6)*</td>
</tr>
<tr>
<td>Scr, μmol/L (N=1736)</td>
<td>59.2 (49.5, 70.7)*</td>
<td>61.9 (50.0, 76.2)*</td>
<td>87.0 (64.0, 122.0)*</td>
</tr>
<tr>
<td>TC, mmol/L (N=1785)</td>
<td>4.45 (3.80, 5.27)*</td>
<td>4.40 (3.69, 5.14)*</td>
<td>4.66 (3.69, 5.84)*</td>
</tr>
<tr>
<td>TG, mmol/L (N=1785)</td>
<td>1.50 (1.11, 2.19)*</td>
<td>1.69 (1.09, 2.21)*</td>
<td>1.70 (1.27, 2.58)*</td>
</tr>
<tr>
<td>LDL-c, mmol/L (N=1784)</td>
<td>2.78 (2.21, 3.47)*</td>
<td>2.70 (2.18, 3.37)*</td>
<td>3.01 (2.18, 3.90)*</td>
</tr>
<tr>
<td>UA, mmol/L (N=1785)</td>
<td>288.8 (232.3, 345.0)*</td>
<td>288.0 (235.0, 364.7)*</td>
<td>360.6 (294.0, 435.0)*</td>
</tr>
<tr>
<td>TBA, μmol/L (N=1785)</td>
<td>3.7 (2.4, 6.3)*</td>
<td>3.7 (2.1, 5.8)*</td>
<td>2.9 (1.7, 4.8)*</td>
</tr>
</tbody>
</table>

*represents adjusted P < .05 when compared to cases without albuminuria
**represents adjusted P < .05 when compared to cases with microalbuminuria
***represents adjusted P < .05 when compared to cases with macroalbuminuria

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor antagonists; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; LDL-c, low density lipoprotein cholesterol; MAC, macroalbuminuria; MAU, microalbuminuria; sCr, serum creatinine; TC, total cholesterol; TG, triglycerides; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TBA, total bile acid; UA, urinary albumin.

RESULTS
Participants’ Characteristics
The research team enrolled 1918 T2DM patients in the study. After the urine analysis took place, the team excluded those without data for US or TBA, and the team analyzed the data of 1785 participants, 929 men and 856 women, with 205 being premenopausal women and 651 being postmenopausal women.

Of the 1785 participants, 1069 had normoalbuminuria (59.9%), becoming the normal group; 452 had MAU (25.3%), becoming the MAU group; and 264 had MAC (14.8%), becoming the MAC group. Table 1 summarizes participants’ clinical features and metabolic characteristics, based upon the severity of their albuminuria.

The normal group’s mean age was 57.4 ± 12.7 years; the MAU group’s mean age was 61.8 ± 13.7 years; and the MAC group’s mean age was 62.0 ± 12.1 years. The MAU and MAC groups’ mean ages were significantly higher than that of the normal group (both P < .01).
The normal group's mean diabetes duration was 6.0 (1.0, 10.0) years; the MAU group's mean diabetes duration was 8.0 (3.0, 14.0) years; and the MAC group's mean diabetes duration was 10.0 (6.0, 16.0). The MAU and MAC groups' mean diabetes durations were significantly greater than those of the normal group (both \( P < .01 \)). The MAC group's mean diabetes duration was significantly greater than that of the MAU group (\( P < .01 \)).

The MAU group's SBP (\( P < .01 \)), DBP (\( P < .05 \)), and sCr (\( P < .05 \)), were significantly higher than those of the normal group. The MAC group's SBP (\( P < .01 \)), DBP (\( P < .01 \)), HbA\(_1c\) (\( P < .05 \)), sCr (\( P < .05 \)), TG (\( P < .05 \)), LDL-c (\( P < .01 \)), UA (\( P < .01 \)), and TBA (\( P < .01 \)) were significantly higher than those of the normal group.

No significant differences existed among the three groups in terms of gender, BMIs, hypertension, ACE/ARB, or smoking; no significant differences existed between the normal and MAU groups in HbA\(_1c\), TC, TG, LDL-c, UA or TBA levels; and no significant difference existed between the normal and MAC groups in TC levels.

**TBA Distribution by Age**

The median TBA level for all participants was 3.30 \( \mu \text{mol/L} \). Figure 1 shows that the TBA was 3.0 \( \mu \text{mol/L} \) for participants \( \leq 40 \) years old and elevated progressively to 4.5 \( \mu \text{mol/L} \) for patients \( > 70 \) years old (\( P = .001 \)).

**TBA Distribution by Gender**

Figure 2 shows that the TBA of postmenopausal women was significantly higher, at 3.8 \( \mu \text{mol/L} \), than that of premenopausal women, at 2.8 \( \mu \text{mol/L} \) (\( P < .01 \)). The men's TBA was also significantly higher, at 3.6 \( \mu \text{mol/L} \), than that of premenopausal women (\( P < .01 \)). No significant differences existed between the men and the postmenopausal women (\( P > .05 \)).

**TBA Quartiles**

Figure 3 shows that: (1) the normal group's incidence of albuminuria in Q1, Q2, Q3, and Q4 was 20.60%, 15.30%, 11.90%, and 10.80%, respectively; (2) the MAU group's incidence of albuminuria in Q1, Q2, Q3, and Q4 was 26.20%, 22.10%, 28.00%, and 25.10%, respectively; and (3) the MAC group's incidence of albuminuria in Q1, Q2, Q3, and Q4 was 53.20%, 62.60%, 60.10%, and 64.10%, respectively.

**Association Between TBA and Albuminuria Type**

The risk of MAU did not change significantly with increasing TBA levels in the multivariate logistic regression analysis (\( P > .05 \) for Q2, Q3 and Q4 group, when compared with Q1, respectively), adjusting for potential confounding variables (age, gender, BMI, Duration of diabetes, hypertension, Smokers, HbA\(_1c\), TC, TG, ARB/ACEI use or not, UA).

Additionally, a decreased risk of MAC was observed when TBA increased. The OR of multivariate logistic regression was 0.61 (95%CI: 0.41-0.90, \( P = .012 \)) for Q2 vs Q1, was 0.44 (95%CI: 0.29-0.67, \( P < .001 \)) for Q3 vs Q1 and was 0.38 (95%CI: 0.25-0.58, \( P < .001 \)) for Q4 vs Q1, respectively (Table 2).
Table 2. Association Between TBA and Albuminuria Type (N = 716)

<table>
<thead>
<tr>
<th>TBA, μmol/L</th>
<th>MAU Group n = 452 (OR95%CI)*</th>
<th>P value</th>
<th>MAC Group n = 264 (OR95%CI)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (&lt;2.2)</td>
<td>REF</td>
<td></td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Q2 (2.2-3.6)</td>
<td>0.73 (0.52-1.03)</td>
<td>.07</td>
<td>0.61 (0.41-0.90)</td>
<td>.012</td>
</tr>
<tr>
<td>Q3 (3.6-6.0)</td>
<td>0.96 (0.69-1.33)</td>
<td>.002</td>
<td>0.44 (0.29-0.67)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Q4 (≥6.0)</td>
<td>0.72 (0.51-1.01)</td>
<td>.06</td>
<td>0.38 (0.25-0.58)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Adjusted for age, gender, BMI, Duration of diabetes, hypertension, Smokers, HbA1c, TC, TG, using ARB/ACEI use or not, UA

Abbreviations: MAC, macroalbuminuria; MAU, microalbuminuria; REF, reference value; TBA, total bile acid.

Table 3. Association Between TBA and Albuminuria Type By Gender (N=716).

<table>
<thead>
<tr>
<th>Quarter (TBA, μmol/L)</th>
<th>MAU Group N=452 (OR95%CI)*</th>
<th>P value</th>
<th>MAC Group N=264 (OR95%CI)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males (n = 929)</strong></td>
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<tr>
<td>Q1 (&lt;2.2)</td>
<td>REF</td>
<td></td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Q2 (2.2-3.6)</td>
<td>0.72 (0.45-1.17)</td>
<td>.189</td>
<td>0.59 (0.33-1.05)</td>
<td>.071</td>
</tr>
<tr>
<td>Q3 (3.6-6.0)</td>
<td>0.75 (0.46-1.22)</td>
<td>.246</td>
<td>0.44 (0.24-0.81)</td>
<td>.008</td>
</tr>
<tr>
<td>Q4 (≥6.0)</td>
<td>0.75 (0.46-1.22)</td>
<td>.243</td>
<td>0.51 (0.27-0.94)</td>
<td>.030</td>
</tr>
<tr>
<td><strong>Postmenopausal Females (n = 651)</strong></td>
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<tr>
<td>Q1 (&lt;2.2)</td>
<td>REF</td>
<td></td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Q2 (2.2-3.6)</td>
<td>0.65 (0.36-1.17)</td>
<td>.148</td>
<td>0.49 (0.24-1.02)</td>
<td>.058</td>
</tr>
<tr>
<td>Q3 (3.6-6.0)</td>
<td>1.09 (0.63-1.91)</td>
<td>.756</td>
<td>0.34 (0.15-0.74)</td>
<td>.007</td>
</tr>
<tr>
<td>Q4 (≥6.0)</td>
<td>0.77 (0.44-1.35)</td>
<td>.360</td>
<td>0.32 (0.15-0.68)</td>
<td>.003</td>
</tr>
<tr>
<td><strong>Premenopausal Females (n = 205)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 (&lt;2.2)</td>
<td>REF</td>
<td></td>
<td>REF</td>
<td></td>
</tr>
<tr>
<td>Q2 (2.2-3.6)</td>
<td>1.66 (0.61-4.55)</td>
<td>.322</td>
<td>2.64 (0.74-9.45)</td>
<td>.135</td>
</tr>
<tr>
<td>Q3 (3.6-6.0)</td>
<td>2.39 (0.92-6.18)</td>
<td>.074</td>
<td>1.23 (0.29-5.16)</td>
<td>.782</td>
</tr>
<tr>
<td>Q4 (≥6.0)</td>
<td>0.92 (0.30-2.83)</td>
<td>.880</td>
<td>0.19 (0.02-2.01)</td>
<td>.168</td>
</tr>
</tbody>
</table>

*adjusted for potential confounding variables, including age, gender, BMI, Duration of diabetes, hypertension, smoking, HbA1c, TC, TG, ARB/ACEI use or not, and UA

Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor antagonists; BMI, body mass index; HbA1c, glycated hemoglobin; MAC, macroalbuminuria; MAU, microalbuminuria; REF, reference value; TC, total cholesterol; TG, triglycerides; TBA, total bile acid.

Further, a similar multivariate logistic regression analysis was conducted in males, postmenopausal females and premenopausal females three subgroups, respectively (Table 3). Consistent with findings in total subject, no significant association between TBA levels and MAU in three subgroups was observed. In addition, high level of TBA may be associated with decreased risk of MAC (Q3 vs Q1: OR=0.44, 95%CI: 0.24-0.81, P = .008; Q4 vs Q1: OR = 0.51, 95% CI: 0.27-0.94, P = .030). Similar association was found among postmenopausal females (Q3 vs Q1: OR = 0.34, 95%CI: 0.15-0.74, P = .007; Q4 vs Q1: OR = 0.32, 95%CI: 0.15-0.68, P = .003), but not among premenopausal females.

**DISCUSSION**

Similar to past studies, the current study found that the mean TBA level was 3.3 μmol/L and that it increased with age. The current study also showed that the group with higher TBA had a lower number of participants with MAC. Our findings revealed that TBA was negatively correlated to albuminuria. Further, the multivariable regression results indicated that TBA levels was negatively associated with albuminuria, independent of age, duration of diabetes, glycemic control, and use of angiotensin-converting enzyme inhibitors, which were consistent with the aforementioned researches.

Furthermore, the current study also found that the correlation between total BA and albuminuria was different for the different genders and for pre- and post-menopausal women, which might be related to the effects of estrogen on the metabolism of bile acid. The differences that estrogen causes may be responsible for the differences in the correlations between BA and albuminuria in different populations. However, the underlying pathways and receptors related to these differences need further study.

The current study had some limitations. First, the research team collected all the data retrospectively, and the correlation didn’t reveal causality; evaluation of the relationship required prospective studies. Second, all the participants were from Zhejiang, and the outcomes might not be representative for other areas in China. Third, the present research failed to involve normal individuals for comparison, which would comparatively restrict the analysis of diabetic patients. More prospective clinical studies involving normal individuals can assist in revealing the association between BA and DN. Fourth, Shin et al found that statins may be associated with albuminuria. However, the causal relationship between them is still unclear, and the current study didn’t analyze the use of statins, which is also a direction of the current research team’s future research.

In any case, the causality that the current study found between BA and established DN needs more exploration. Future studies in these areas can further enable researchers to find the effects of BA on DN and may facilitate gender-specific therapies for men and women.

**CONCLUSIONS**

An independent negative association exists between TBA levels and MAC in T2DM. The decrease of circulating TBA might be a prospective clinical factor for determining established DN, especially for men and postmenopausal women.
AUTHOR CONTRIBUTIONS
Yan WU and Zexin CHEN contributed equally to this article.

AUTHORS' DISCLOSURE STATEMENT
The Natural Science Foundation of Zhejiang Province (LQ19H100015) supported the study. The authors declare that they have no conflicts of interest related to the study.

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