RESEARCH

Elevated serum trimethylamine oxide levels as potential biomarker for diabetic kidney disease

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Abstract

Background: Diabetic kidney disease (DKD) has become a major cause of chronic kidney disease. However, early diagnosis of DKD is challenging. Trimethylamine oxide (TMAO) is an intestinal microbial metabolite which might be associated with diabetes complications. The aim of this study was to investigate the correlation between TMAO and DKD.

Methods: A cross-sectional study was conducted. A total of 108 T2DM patients and 33 healthy subjects were enrolled in this study. Multiple logistic regression analyses and area under receiver operating characteristic curves (AUROC) were performed to evaluate the correlation between serum TMAO and DKD.

Results: Serum TMAO levels were significantly higher in DKD patients than healthy control group and the NDKD (T2DM without combined DKD) group ($P < 0.05$). TMAO levels were negatively correlated with eGFR and positively correlated with urea nitrogen, ACR and DKD ($P < 0.05$). Logistic regression analysis indicated that serum TMAO was one of the independent risk factors for DKD patients ($P < 0.05$). In the diagnostic model, the AUROC of TMAO for the diagnosis of DKD was 0.691.

Conclusion: Elevated levels of serum TMAO levels were positively associated with the risk of DKD in T2DM patients, which might be a potential biomarker for DKD.

Keywords

- trimethylamine N-oxide
- type 2 diabetes
- diabetic kidney disease

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Introduction

Diabetic kidney disease (DKD) is one of the most common and important microvascular complications of diabetes, with more than 25–40% of diabetic patients developing nephropathy 20–35 years after the onset of the disease (1). DKD has become a major cause of chronic kidney disease (2), which is a serious threat to human health. On this basis, early diagnosis of DKD is particularly important.

In recent years, the relationship between intestinal flora metabolites and chronic metabolic diseases has received increasing attention. Trimethylamine oxide (TMAO) is an intestinal microbial metabolite that is generated from choline and L-carnitine in food by the action of intestinal microorganisms to trimethylamine (TMA), which is catalyzed in the liver by flavin monooxygenase 3 to TMAO (3, 4). Previous studies have confirmed that TMAO was a new predictor of cardiovascular disease and was associated with major adverse cardiovascular events and all-cause mortality (5, 6, 7). TMAO levels were associated with insulin resistance, impaired glucose tolerance, and a positive association with risk of diabetes (8, 9). Recent clinical studies suggested that TMAO levels were not only associated with the development of diabetes but also diabetic complications. Signe *et al* (10) reported that elevated plasma TMAO concentrations were associated with impaired renal function in type 1 diabetes patients; however, the correlation became uncorrelated

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after adjustment for baseline glomerular filtration rate, indicating that TMAO might be a biomarker of renal function or as a risk factor for macro- and microvascular complications, especially impaired renal function. Another cross-sectional survey study showed that elevated plasma TMAO levels in type 2 diabetic patients (T2DM) correlated with the incidence and severity of diabetic retinopathy (11).

In this study, we aimed to investigate the correlation between serum TMAO levels and DKD in T2DM patients and then tried to explore that TMAO might be a promising biomarker for diabetic microangiopathy.

Materials and methods

Study population

A total of 108 patients with T2DM from May 2021 to October 2021 were enrolled. Inclusion criteria: the diagnostic criteria for diabetes mellitus by the World Health Organization in 1999. Exclusion criteria are as follows: (i) patients with combined acute complications of diabetes mellitus; (ii) patients with severe infections, surgery, trauma, and other stressful conditions within the last 1 month; (iii) patients with malignant tumors; (iv) long-term vegetarians; (v) patients with systematic use of antibiotics or probiotics within 3 months; (vi) patients with autoimmune diseases; (vii) patients with severe heart, liver and other organ diseases; and (viii) patients with primary glomerular diseases and other patients with secondary glomerular diseases. Thirty-three healthy individuals who were examined at our hospital during the same period were also included as a control group. All studies in this study were conducted with the informed consent of the study subjects and were approved by the ethics committee of the Second Affiliated Hospital of Fujian Medical University (Ethics No. 2022-81).

Clinical data and biochemical analyses

Clinical data including gender, age, body mass index (BMI, in kg/m^2), duration of diabetes (in years), and history of hypertension were collected. Laboratory parameters included fasting blood glucose (mmol/L), urea nitrogen (BUN in mmol/L), blood creatinine (SCR in μmol/L), blood uric acid (UA in μmol/L), glycated hemoglobin (HbA1c in %), urinary microalbumin (mg/dL), urinary creatinine (μmol/L), and calculate urinary albumin to creatinine ratio (ACR in mg/g) and glomerular filtration rate (eGFR in mL/(min·1.73 m^2)) with the following formulas:

$$\mathrm{ACR} = \left[10^6 \times \text{urinary microalbumin (mg/dL)}\right] / \left[113 \times \text{urinary creatinine (μmol/L)}\right] \quad (1)$$

$$\mathrm{eGFR} = 186 \times (\mathrm{Scr}/88.402)^{-1.154} \times (\mathrm{Age})^{-0.203} \times (\mathrm{Female} \times 0.742) \quad (2)$$

Quantitative detection of serum TMAO concentration

The quantitative detection of serum TMAO concentration was performed by isotope dilution high-performance liquid chromatography-tandem mass spectrometry with ultraperformance liquid chromatography-triple quadrupole tandem mass spectrometry (Shimadzu LC-MS 8050CL).

Statistical analyses

Statistical analysis was conducted using SPSS 26.0 statistical software. Data are presented as frequencies (percentages) or means ± S.D. We assessed differences between groups with Student's *t*-test, Kruskal–Wallis H-test, chi-square test, and Mann–Whitney *U* test. Spearman's correlation analysis and multi-factor logistic regression analysis were used to explore the influencing factors. $P < 0.05$ was considered statistically significant. The diagnostic value of serum TMAO was assessed by the area under the subject's operating characteristic curve (AUROC).

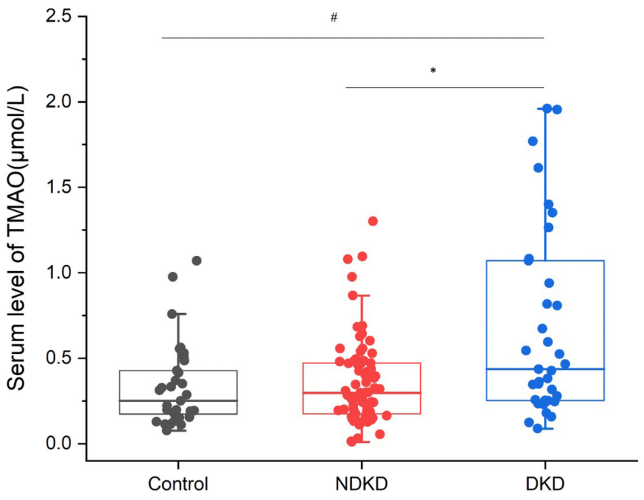

Figure 1
Comparation of serum TMAO concentrations in NDKD and DKD group vs control group, #$P < 0.05$, vs NDKD group,*$P < 0.05$.

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Table 1 Clinical characteristics and serum TMAO levels.

	control group ($n = 33$)	**NDKD group** ($n = 73$)	**DKD group** ($n = 35$)	**F/X²/Z**	***P***
Age (year)	52.00 ± 12.69	51.58 ± 12.52	55.86 ± 14.17	1.356	0.261
Male	60.6% (20/33)	65.8% (48/73)	77.1% (27/35)	2.294	0.318
BMI (kg/m²)	22.18 ± 2.83	25.31 ± 3.42[a]	23.86 ± 3.99	9.651	0.000[d]
Diabetes duration (year)	NA	5.0 (1.0, 10)	10 (4.25,16.0)[b]	−2.752	0.006[d]
Hypertension (%)	NA	32.9% (24/73)	51.4% (18/35)[b]	4.210	0.040[b]
BUN (mmol/L)	4.6 (3.93, 5.55)	5.11 (4.24, 6.07)	5.83 (4.93, 7.30)[a,b]	9.892	0.007[e]
UA (μmol/L)	313 (282, 413.5)	305 (235, 358.5)	370 (309, 407)[b]	8.525	0.014[b]
FPG (mmol/L)	5.39 (5.10, 5.80)	8.0 (6.68, 10.13)[a]	7.85 (6.52, 9.55)[a]	46.249	0.000[d]
HbA1c (%)	NA	9.2 (7.0, 11.05)	9.3 (7.0,10.3)	-0.420	0.674
SCR (μmol/L)	70.0 (56.95, 78.1)	66 (55.1, 76.0)	75.9 (63.0, 96.0)[b]	9.690	0.008[c]
eGFR (mL/min•1.73 m²)	102.32 ± 22.31	113.14 ± 27.38	95.99 ± 41.64[b]	4.10	0.029[b]
TMAO (μmol/L)	0.25 (0.16, 0.46)	0.30 (0.17, 0.48)	0.44 (0.25, 1.07)[a,b]	11.789	0.003[c]

vs control group, [a]$P < 0.05$, vs NDKD group,[b]$P < 0.05$ vs NDKD group, [c]$P < 0.01$, vs NDKD group [d]$P < 0.001$.

Results

Clinical characteristics and serum TMAO levels of study subjects

A total of 108 patients with T2DM and 33 non-diabetic patients (control group) were included in this study. T2DM patients were divided into two groups according to ACR, NDKD group (ACR<30 mg/g, $n = 73$) and DKD group (ACR >30 mg/g, $n = 35$). The differences in age and gender among the three groups were not statistically significant, suggesting that the groups were comparable.

Serum TMAO levels in the control group, NKD group, and DKD group increased sequentially (Fig. 1). Serum TMAO levels in the DKD group were significantly higher than those in the control and NDKD groups ($P < 0.05$). Serum TMAO levels in the NDKD group were higher than those in the control group, without statistically significance ($P > 0.05$) (Table 1).

Clinical characteristics of the high- and low-level groups of TMAO

First, all subjects were divided into two groups according to the median serum TMAO level (0.3126 μmol/L): low TMAO level group ($n = 70$); and high TMAO level group ($n = 71$). High TMAO level group showed higher age, BUN, and lower eGFR ($P < 0.05$) (Supplementary Table 1, see section on supplementary materials given at the end of this article).

Further, we analyzed the clinical characteristics in T2DM patients. 108 T2DM patients were divided into two groups according to the median TMAO level (0.322 μmol/L): low TMAO level group ($n = 54$) and high TMAO level group ($n = 54$). Age, duration of diabetes, BUN, SCR, ACR, and DKD prevalence were significantly higher in the high TMAO level group, while eGFR was significantly lower ($P < 0.05$) (Table 2).

Table 2 Comparison of clinical characteristics between the high-level and low-level TMAO groups in T2DM patients.

	Low TMAO level group ($n = 54$)	**High TMAO level group** ($n = 54$)	**t/X²/Z**	***P***
Age (year)	49.13 ± 12.55	56.80 ± 12.74	−3.150	0.002[b]
Male	72.2% (39/54)	66.7% (36/54)	0.393	0.531
BMI (kg/m²)	25.27 ± 3.99	24.41 ± 3.28	1.220	0.225
Diabetes duration (year)	4 (1, 10)	10 (3, 15)	−2.693	0.007[b]
Hypertension (%)	42.6% (23/54)	35.2% (19/54)	0.623	0.43
BUN (mmol/L)	4.78 (3.91, 5.86)	5.74 (4.95, 7.06)	−3.017	0.003[b]
UA (μmol/L)	324.5 (233.25, 384.25)	331.0 (288.75, 383.75)	−0.839	0.402
FPG (mmol/L)	7.78 (6.47, 9.25)	8.08 (6.72, 10.35)	−1.085	0.278
HbA1c (%)	8.6 (6.9, 10.75)	9.5 (7.15, 11.28)	−0.562	0.574
SCR (μmol/L)	65.5 (54.98, 76.0)	72.4 (60.58, 83.50)	−2.095	0.036[a]
eGFR (mL/min•1.73m²)	116.85 ± 24.68	98.32 ± 38.44	2.981	0.004[b]
ACR (mg/g)	13.35 (7.98, 24.83)	17.22 (11.11, 179.04)	−2.366	0.018[a]
DKD (%)	22.2% (12/54)	42.6% (23/55)	5.115	0.024[a]

[a]$P < 0.05$, [b]$P < 0.01$.

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Table 3 Linear correlation analysis of serum TMAO with clinical characteristics in T2DM patients.

Variables	TMAO	
	r	*P*
Age (year)	0.326	0.001[b]
Male	−0.047	0.626
BMI (kg/m²)	−0.117	0.229
Diabetes duration (year)	0.242	0.012[a]
BUN (mmol/L)	0.423	0.000[c]
UA (μmol/L)	0.181	0.061
FPG (mmol/L)	0.038	0.693
HbA1c (%)	0.065	0.507
SCR (μmol/L)	0.407	0.000[c]
eGFR (mL/min/1.73m²)	−0.445	0.000[c]
ACR (mg/g)	0.231	0.016[a]
Hypertension (%)	0.000	1.000
DKD (%)	0.293	0.002[b]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

Linear correlation analysis between serum TMAO and kidney function

First, we analyzed the correlation between serum TMAO and kidney function in all subjects. Spearman correlation analysis showed that TMAO was negatively correlated with eGFR ($r = -0.370$, $P < 0.001$) and positively correlated with age, BUN, and SCR ($r = 0.304, 0.440, 0.308$, $P < 0.001$) (Supplementary Table 2).

Further, we analyzed the correlation between serum TMAO and kidney function in T2DM patients. Spearman correlation analysis showed that TMAO was negatively correlated with eGFR ($r = -0.445$, $P < 0.001$) and positively correlated with age, disease duration, BUN, SCR, ACR, and DKD prevalence ($r = 0.326, 0.242, 0.423, 0.407, 0.231, 0.293$, $P \leq 0.01$) (Table 3).

Regression analysis of DKD risk factors

Multiple logistic regression analysis showed that serum TMAO (odds ratio (OR) = 7.880,95% CI 1.993–31.152, $P = 0.003$) was independent risk factor for DKD patients after adjusted for eGFR, BUN, and UA (Table 4).

Serum TMAO in DKD diagnosis

As shown in Fig. 2, the AUROC of TMAO was 0.691 (95% CI 0.588–0.795; $P = 0.001$), with a sensitivity of 88.6% and specificity of 58.5% at an optimal cut value of 0.227 mol/L.

Discussion

In the present study, we found a novel relationship between serum TMAO and DKD. TMAO was significantly elevated in DKD patients compared with control group and NDKD patients. Besides, serum TMAO was independent risk factor for DKD patients.

Studies showed gut microbiota-dependent TMAO may be associated with the development of T2DM. The addition of TMAO to the diet has been reported to increase fasting insulin levels and insulin resistance in mice, exacerbating impaired glucose tolerance (9). TMAO levels were significantly higher in patients with T2DM than in controls in a case–control study (12), which confirmed that elevated TMAO levels were associated with an increased risk of diabetes, in line with the results of a meta-analysis (8). However, a recent cohort study (13) showed that plasma TMAO levels were associated with insulin resistance in the elderly and did not significantly correlate with T2DM. In the present study, our results showed that serum TMAO levels were higher in the diabetes group than the control group, but the difference was not statistically significant, which might be related to the small sample size.

Although the difference in serum TMAO levels between the NDKD and control groups was not statistically significant, our study showed that the DKD group had significantly elevated TMAO levels. Besides, the serum TMAO levels in the control, NDKD and DKD groups increased sequentially. The serum TMAO levels in the DKD group were significantly higher than in the other two groups. Previous studies also suggested the relationship between TMAO and renal function.

Table 4 Regression analysis of DKD risk factors

	b	Wald	*P*	OR	OR (95% CI)
TMAO	2.064	8.664	0.003[b]	7.880	(1.993, 31.152)
eGFR	0.008	0.812	0.368	1.008	(0.991, 1.025)
BUN	0.174	1.273	0.259	1.190	(0.880, 1.609)
UA	0.003	1.953	0.162	1.003	(0.999, 1.007)
Hypertension	1.335	8.232	0.004[b]	3.799	(1.526, 9.457)

[b]$P < 0.01$.

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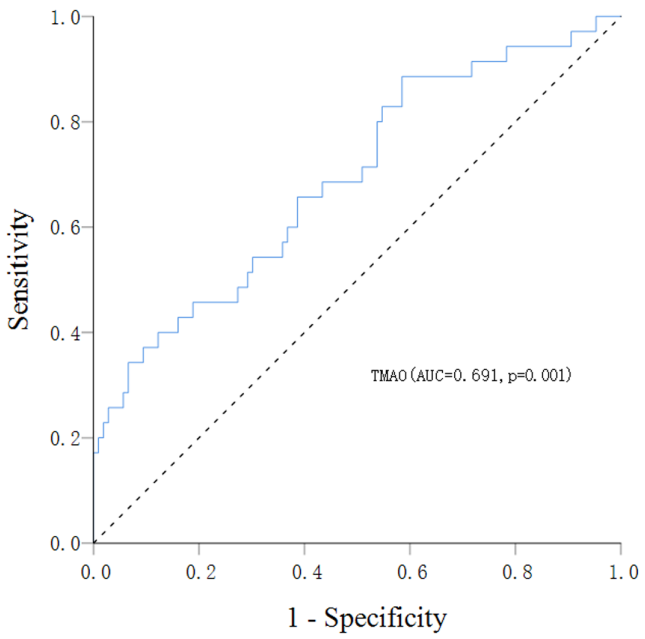

Figure 2
AUROC of serum TMAO level for the diagnosis of DKD.

Another study (14) showed significantly higher TMAO levels in patients with T2DM and advanced CKD than in the control group.

In our study, we found a positive correlation between serum TMAO levels and BUN and SCR levels, and a negative correlation with eGFR, which was consistent with Bell's observations in patients with chronic renal failure (15). We also found that serum TMAO levels were positively correlated with ACR levels. In addition, patients in the high-level group had significantly lower eGFR and higher ACR values, as well as a higher proportion of combined DKD, compared with patients in the low TMAO level group, suggesting that diabetic patients with high circulating TMAO levels have worse renal function. Further, multifactorial logistic regression analysis confirmed that elevated serum TMAO levels were an independent risk factor for the development of DKD. In a cohort study that included patients with end-stage renal disease, patients had significantly higher circulating TMAO levels than normal controls, and TMAO levels were positively correlated with serum BUN and SCR levels (15), which was in consist with our study. As we know, the kidney is the main route of TMAO clearance (16). The cause of the significant increase in circulating TMAO in CKD patients has been suggested to be related to the disruption of gut microbial homeostasis. Mohammed *et al.* showed a significant increase in the number of bacteria that metabolize choline and carnitine and produce TMA in the gut microbiome of T2DM patients with combined chronic kidney disease (14). It has also been suggested that it may be related to increased FMO enzyme-mediated TMAO formation. Alexander *et al.* (17) performed FMO enzyme activity experiments using liver microsomes from experimental CKD rats and control rats and showed for the first time that metabolic activation of FMO enzymes by uremic solutes may contribute to the elevated TMAO levels in CKD. Further basic studies at the *ex vivo* cellular and animal level could help to reveal the causes of elevated TMAO levels in DKD patients.

However, several limitations should be considered. First, our sample size is small. In future studies, we will expand the sample size to provide a better understanding of the changes in TMAO concentrations with DKD. . In addition, our study did not address the underlying mechanisms of TMAO in DKD, which needs to be further explored in the future. It has been shown that TMAO activates the production of NLRP3 (Pyrin structural domain-3) inflammatory vesicles and nuclear factor κB signaling, which is involved in the process of DKD by promoting vascular inflammation and oxidative stress (18, 19). Animal studies have shown that increased dietary TMAO promotes phosphorylation of Smad3, a pro-fibrotic regulator in kidney disease, and increases plasma cystatin C and renal injury marker-1 levels, which are sensitive indicators of impaired kidney function (20, 21).

In conclusion, our study demonstrated that elevated serum TMAO levels are closely associated with the risk of DKD in patients with T2DM and are one of the independent risk factors for the development of DKD.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/EC-23-0048.

Declaration of interest
The authors declare no conflict of interest.

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Availability of data and material
The data presented in this study are available on request from the corresponding author.

Ethics approval and consent to participate
The research proposal was approved by the ethics committee of the Second Affiliated Hospital to Fujian Medical University (ethical approval ID:2022-81). All participants signed an informed consent form.

Consent for publication
All authors consent for publication.

Author contribution statement
YH and ZZ contributed equally to this manuscript. YH and ZZ contributed in data analysis and manuscript writing. JZ conceptualized and designed these studies, performed them, and supervise the programme. ZH contributed in data analysis. All authors contributed to manuscript revision and read and approved the submitted version.

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