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Relationship between Plasma Trimethylamine N-Oxide Levels and Renal Dysfunction in Patients with Hypertension

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Keywords
Chronic kidney disease · Hypertension · Trimethylamine N-oxide

Abstract
Introduction: Trimethylamine N-oxide (TMAO) is a metabolite produced by gut bacteria. Although increased TMAO levels have been linked to hypertension (HTN) and chronic kidney disease (CKD) with poor prognosis, no clinical studies have directly addressed the relationship between them. In this study, we investigated the relationship between TMAO and renal dysfunction in hypertensive patients. ***Methods:*** We included healthy controls ($n = 50$), hypertensive patients ($n = 46$), and hypertensive patients with renal dysfunction ($n = 143$). Their blood pressure values were taken as the highest measured blood pressure. Renal function was evaluated using the estimated glomerular filtration rate. Plasma TMAO levels were measured using high-performance liquid chromatography tandem mass spectrometry. ***Results:*** We found significant differences in plasma TMAO levels among the 3 groups ($p < 0.01$). The plasma TMAO of patients with HTN was significantly higher than that of healthy people, and the plasma TMAO of patients with HTN complicated by renal dysfunction was significantly higher than either of the other groups. Patients in the highest TMAO quartile were at a higher risk of developing CKD stage 5 than those in the lowest quartile. In the receiver operating characteristic curve, the area under the curve of TMAO combined with β 2-macroglobulin for predicting renal dysfunction in patients with HTN was 0.85 (95% confidence interval 0.80–0.90). ***Conclusion:*** An elevated TMAO level reflects higher levels of HTN and more severe renal dysfunction. TMAO, combined with β 2-macroglobulin levels, may assist in diagnosing CKD in hypertensive patients. Plasma TMAO has predictive value for early kidney disease in hypertensive patients.

Introduction

Chronic kidney disease (CKD) is a severe and irreversible global disease; by the end of 2016, approximately 3 million people were known to be undergoing dialysis for CKD [1]. Along with diabetes, hypertension (HTN) is one of the major noninfectious risk factors for renal insufficiency [2]. The possible mechanisms of renal damage in HTN are as follows: (1) secondary elevation of sympathetic nerve activity [3], (2) increased activity of the re-

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nin-angiotensin-aldosterone system [4], (3) increased arterial stiffness [5], (4) genetic susceptibility [6], and (5) impaired water and salt excretion ability of the kidney [7]. HTN not only induces renal injury but also may cause CKD, which could lead to end-stage renal disease [8]. Given the lifestyle changes and aging of the population, the prevalence of HTN is increasing each year. To date, more than a billion people worldwide have HTN [9]. Although HTN and CKD are 2 separate diseases, they affect each other; they also have several common risk factors, including trimethylamine N-oxide (TMAO).

TMAO is a small amine compound that is formed by intestinal microorganisms from foods rich in carnitine. It is then oxidized by flavin monooxygenase 3 in the liver. It has been proven as a causative factor in various diseases and is closely related to poor prognosis [10]. It can be used as an upstream target for therapeutic intervention.

The metabolites of the intestinal flora, mainly TMAO, participate in the regulation of blood pressure [11, 12] and initiate renal interstitial inflammation. This results in a decreased number of perirenal capillaries, hypoxia in the medulla, and kidney injury [13, 14], which aggravates HTN and further damages the target organs affected by HTN, of which the kidney is the most common.

Renal damage is manifested through increased capillary pressure, endothelial damage, barrier rupture, increased protein filtration, glomerular sclerosis, segmental glomerular necrosis [15], interstitial cell proliferation, matrix protein deposition, and impaired renal tubular function. Currently, the reduced glomerular filtration rate (GFR) and/or positive urinary protein is the primary criterion for the diagnosis of renal damage in patients with HTN [16]. The estimation of GFR by assessing the serum creatinine value is inevitably affected by factors such as advanced age and body weight (muscle content). Fever and infection also disturb the evaluation of albuminuria. There are almost no symptoms or signs in the early stages of CKD; it is usually discovered when renal structure and function damage is irreversible or when the patient presents with complications. Moreover, there is no sensitive index for early diagnosis.

The most specific pathological changes seen in hypertensive nephropathy are renal interstitial lesions dominated by renal tubular damage [17]. β2-microglobulin (β2-MG), a specific marker of damaged renal tubules, has a stable rate of synthesis and degradation under physiological conditions [18], with few influencing factors, and is a diagnostic indicator for renal injury in patients with HTN [19]. Therefore, we aimed to investigate the following: (1) whether the plasma TMAO level is related to the grade of HTN, (2) whether TMAO can be combined with β2-MG to predict early damage to renal function in patients with HTN, and (3) whether these are related to the severity of renal function injury.

Materials and Methods

Research Subjects

Our research was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University (No. 202005). The study subjects were patients with HTN admitted to the First Affiliated Hospital of Nanchang University from June 2019 to December 2019. Informed consent was obtained from all the subjects before their inclusion in the study.

This cross-sectional study included 239 subjects, who were then divided into 3 groups: the healthy control group (N_A = 50), HTN group (N_B = 46), and HTN with renal dysfunction group (N_C = 143). The research flowchart is shown in Figure 1. The inclusion criteria were as follows: group A, healthy controls; group B, patients with primary HTN, systolic blood pressure (SBP) ≥140 mm Hg, and/or diastolic blood pressure (DBP) ≥90 mm Hg; and group C, patients having renal insufficiency appearing after HTN for more than a decade and only urine protein positive in the urine test, with other factors being normal. The exclusion criteria were as follows: (1) secondary HTN, such as pheochromocytoma and renal artery stenosis; (2) combination of other diseases known to cause renal dysfunction, for instance, autoimmune-related kidney injury and diabetes; (3) comorbid severe heart failure, liver disease, and gastrointestinal dysfunction; and (4) comorbid autoimmune diseases, various infections, trauma, and malignant tumors.

Blood pressure medications in group B were as follows: 13 (28.26%) patients used angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEI/ARB), 2 (4.35%) used β-antagonists, 36 (78.26%) used calcium blockers, and 7 (15.22%) used diuretics. Medications in group C were as follows: 45 (31%) patients used ACEI/ARB, 8 (5.6%) used beta antagonists, 121 (85%) used calcium blockers, 9 (6%) used diuretics, 76 (53.15%) used a combination of drugs to reduce urine protein and excrete toxins, 58 (40.56%) underwent hemodialysis, and 24 (17.91%) underwent peritoneal dialysis.

According to the Cockcroft-Gault formula [20], the estimated GFR (eGFR) is classified as CKD stage 1, eGFR >90 mL/min/1.73 m^2; CKD stage 2, 60–89 mL/min/1.73 m^2; CKD stage 3, eGFR of 30–59 mL/min/1.73 m^2; CKD stage 4, eGFR of 15–29 mL/min/1.73 m^2; and CKD stage 5, eGFR <15 mL/min/1.73 m^2. In this study, we considered CKD stage 1 as the normal renal functioning group. We categorized CKD stages 2–5 as the renal insufficiency group, which were further divided into 4 subgroups. According to plasma TMAO quartile levels, research object is divided into 4 groups: T1, ≤1.50 mg/L; T2, 1.51–2.26 mg/L; T3, 2.27–3.31 mg/L; and T4, ≥3.32 mg/L.

In the first 3 days after admission, a mercury sphygmomanometer was used to measure blood pressure at the brachial artery of the right upper limb once each day, noting the highest blood pressure. HTN grades were stratified according to the highest recorded blood pressure: HTN grade 1, SBP ≥140 mm Hg and/or DBP ≥90 mm Hg; grade 2, SBP ≥160 mm Hg and/or DBP ≥100 mm Hg; and grade 3, SBP ≥180 mm Hg and/or DBP ≥110 mm Hg.

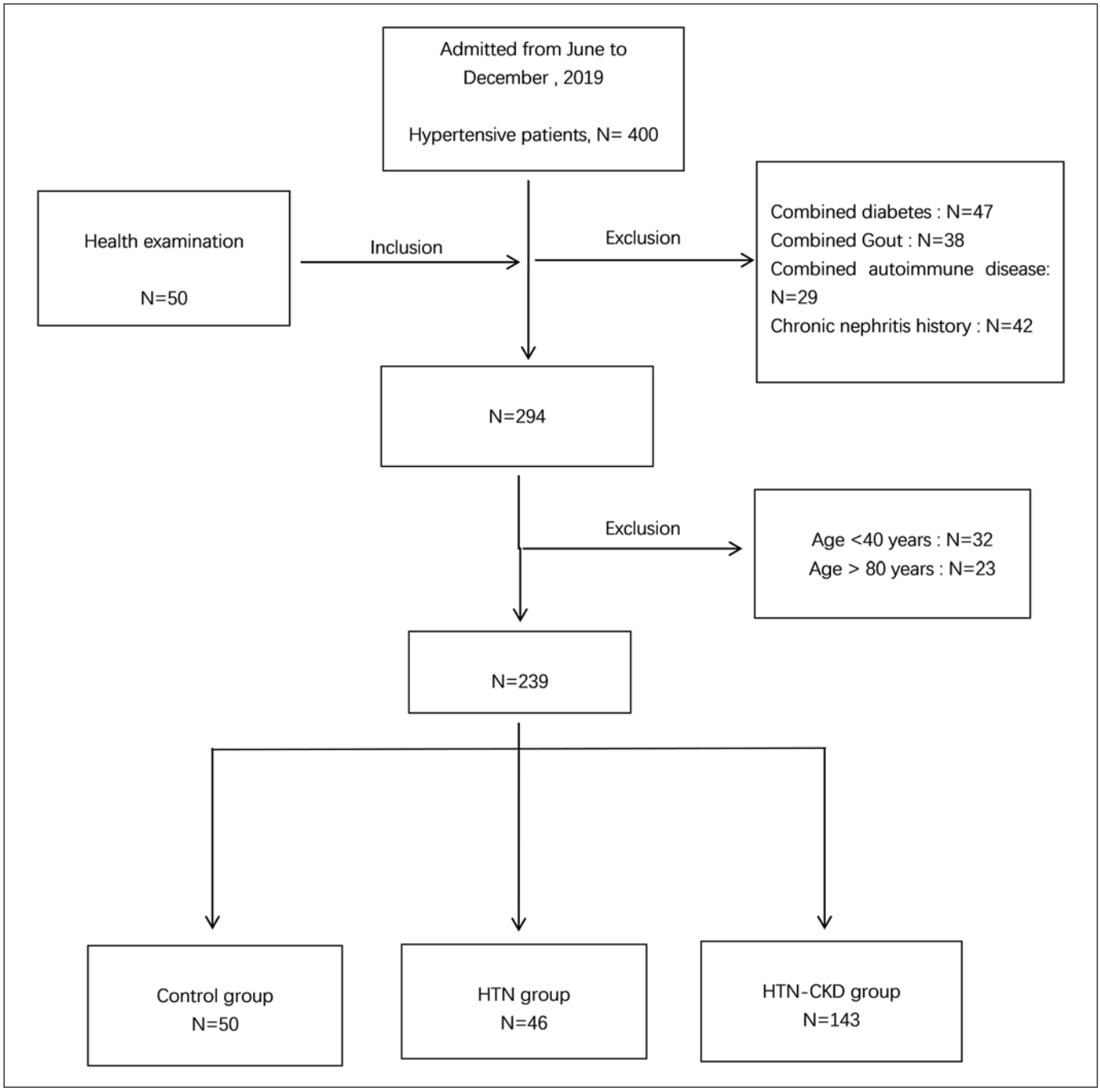

Fig. 1. Research flowchart. CKD, chronic kidney disease; HTN, hypertension.

Demographic characteristics included age, sex, weight, blood pressure treatment, and smoking/drinking history, along with laboratory indicators, including fasting glucose, triglyceride, total cholesterol, low-density lipoprotein cholesterol, lipoprotein a, homocysteine (Hcy), folic acid, creatinine, urea, uric acid, and lactate dehydrogenase. The results are shown in Table 1.

Plasma Analysis

Anticoagulant collection of plasma: blood samples were collected in ethylenediaminetetraacetic acid tubes and centrifuged at 2,000 *g* for 10 min at 4°C. The supernatant was collected and stored at −80°C. TMAO levels were quantified using high-performance liquid chromatography tandem-mass spectrometry on a mass spectrometer (API 4,000; AB Sciex, Co., Ltd.). Next, 40.0 μL of the

Table 1. Demographic and laboratory characteristics between groups

Variable	Normal (N = 50)	HTN (N = 46)	HTN-CKD (N = 143)
Age, years	52.00 (46.00, 59.00)	53.00 (48.75, 57.25)	65.00 (55.00, 73.00)**$^{\#\#}$
BMI, kg/m^2	22.08 (20.68, 25.30)	25.78 (23.50, 28.00)**	21.80 (19.38, 24.00)$^{\#\#}$
Sex, *n* (%)			
Male	19 (38)	18 (39.1)	69 (48.3)
Female	31 (62)	28 (60.9)	74 (51.7)
Smoking, *n* (%)	6 (12)	8 (17.4)	17 (11.9)
Drinking, *n* (%)	2 (4)	5 (10.9)	12 (8.4)
CVD, *n* (%)	–	8 (17.4)	25 (17.5)
Stroke, *n* (%)	–	2 (4.65)	9 (6.29)
HTN grade 3, *n* (%)	–	11 (23.91)	37 (25.87)
HTN course, years	–	12.84±4.3	14.0±4.94
ACEI/ARB, *n* (%)	–	13 (28.3)	43 (30.1)
SBP, mm Hg	116.74±12.51	156.7±15.56	165.23±18.48$^{\#\#}$
DBP, mm Hg	74.18±12.52	94.24±12	93.05±12.66
Hcy, μmol/L	11.00 (8.00, 12.25)	13.00 (11.00, 15.00)	22.00 (15.00, 31.00)**$^{\#\#}$
Urea, mmol/L	4.30 (3.88, 5.30)	4.90 (4.03, 6.23)	11.00 (6.40, 17.60)**$^{\#\#}$
Glucose, mmol/L	4.64 (4.17, 5.23)	5.06 (4.66, 5.53)**	4.82 (4.46, 5.49)
CRE, μmol/L	53.95 (46.68, 63.80)	59.35 (51.03, 68.43)	217.20 (81.30, 545.00)**$^{\#\#}$
UA, μmol/L	283.50 (253.25, 338.00)	340.00 (278.50, 371.5)	356.00 (295.00, 465.00)**$^{\#\#}$
TG, mmol/L	1.21 (0.92, 1.75)	1.66 (1.16, 2.55)**	1.42 (1.03, 2.08)
LDH, U/L	193.50 (174.50, 225.50)	216.00 (197.75, 231.25)	231.25 (209.00, 268.00)**$^{\#\#}$
TC, mmol/L	4.42±0.79	4.52±0.91	4.30±1.41
LDL-C, mmol/L	2.55 (2.33, 2.89)	2.64 (1.98, 3.26)	2.34 (1.84, 2.99)
Lipoprotein a, mg/dL	111.00 (43.50, 238.00)	74.50 (49.75, 194.75)	161.00 (75.00, 435.75)$^{\#\#}$
β2-MG, mg/L	0.60 (0.39, 0.83)	2.11 (1.43, 2.69)**	3.93 (2.68, 10.43)**$^{\#\#}$
TMAO, mg/L	1.00 (0.73, 1.27)	2.01 (1.58, 2.49)**	2.99 (2.08, 3.55)$^{\#\#}$

Continuous data are presented as mean±standard deviation or median (interquartile range), categorical variables are presented as *n* (%). CVD, cardiovascular disease; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; Hcy, homocysteine; TC, total cholesterol; TG, triglyceride; β2-MG, β2-microglobulin; TMAO, trimethylamine-N-oxide; UA, uric acid; CRE, creatinine; LDH, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker. **$p < 0.01$, *$p < 0.05$ versus control group. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus HTN group.

sample or internal standard TMAO-d9 (Toronto Research Chemicals Inc., Toronto, ON, Canada) + 20.0 μL of 10 mmol/L acetonitrile + 300 μL of 1% formic acid solution were mixed, vortexed (MX-S; DalongXingchuang Experimental Instrument [Beijing] Co., Ltd., China), and centrifuged at 4°C to remove impurities. The supernatant (100 μL) was mixed with 100 μL 1% formic acid in water and vortexed. β2-MG was determined using ELISA kits (ELK Biotechnology Co., Ltd, Wuhan, China).

Statistical Analysis

SPSS 25.0 (SPSS Inc., Chicago, IL, USA) and Prism 8.01 (GraphPad Software, San Diego, CA, USA) were used to perform data analyses. Continuous variables are represented as median (interquartile range) or mean ± standard deviation, while percentiles represent categorical variables. Differences in variables among groups were analyzed using the Mann-Whitney U test and Kruskal-Wallis' one-way ANOVA (Dunn's multiple comparison test). Binary and ordered logistic regression analyses were performed to assess whether the TMAO level was an independent risk factor for HTN and HTN-CKD. This was represented using the odds ratio (OR) and 95% confidence interval (95% CI). Correlations between TMAO and other variables were tested using Spearman's correlation. The area under the curve (AUC) of the receiver operating characteristic (ROC) curve was used to analyze the levels of TMAO and β2-MG in the plasma, which enabled us to diagnose the likelihood of renal dysfunction in patients with HTN. When assessing the optimal cutoff values and the sensitivity and specificity of diagnosis, $p < 0.05$ was taken to indicate statistical significance.

Results

Demographic Characteristics of Patients among the Three Groups

Age, sex, blood pressure treatment, smoking history, drinking history, coronary heart disease history, stroke history, HTN course, HTN grade, SBP, and DBP showed no significant differences among the 3 groups ($p >$

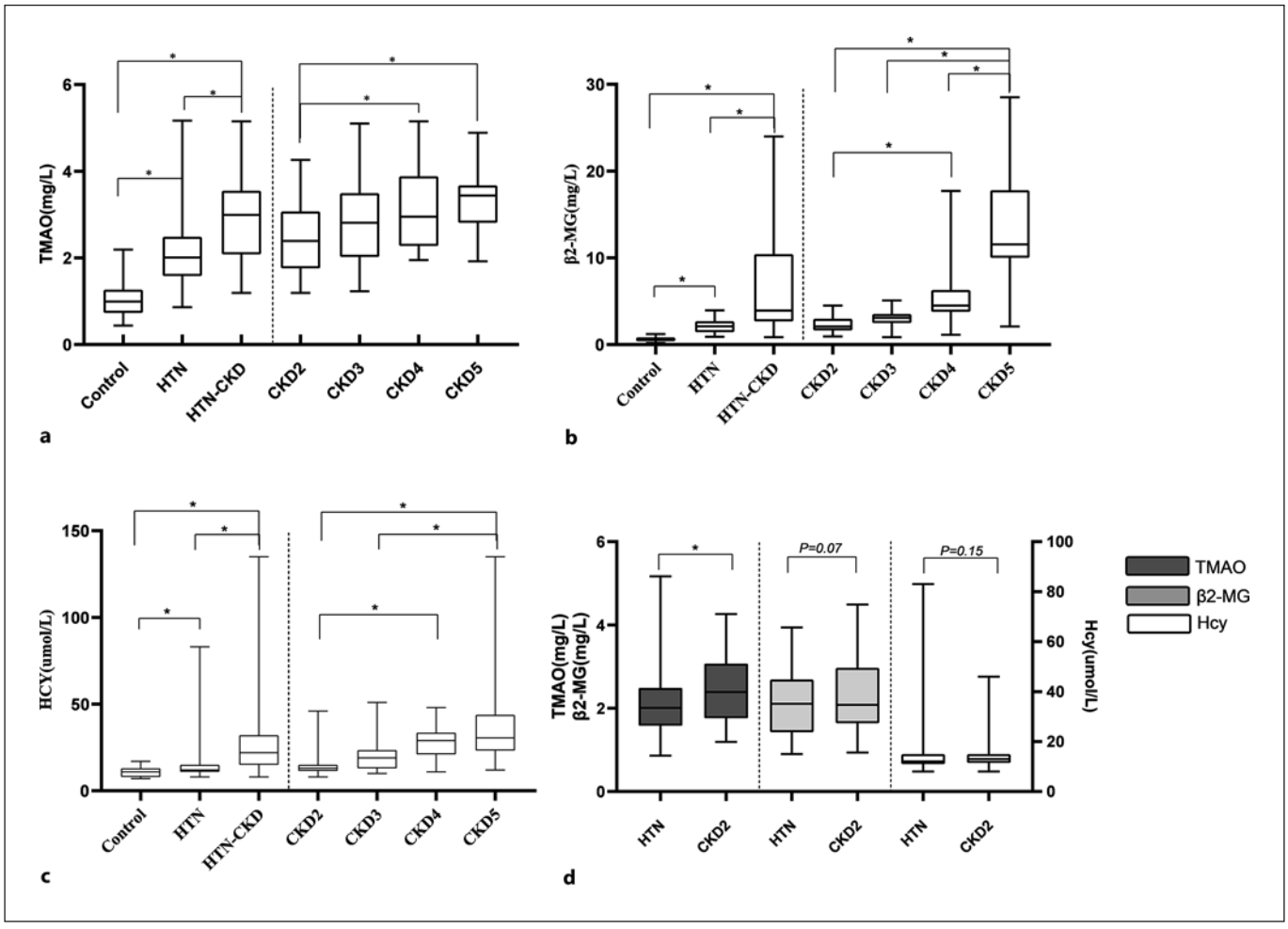

Fig. 2. Association between TMAO, β 2-MG, and Hcy levels and HTN, HTN-CKD, and CKD stage. Increasing plasma levels of TMAO, β 2-MG, and serum Hcy level in HTN-CKD patients (n = 143) compared with HTN (n = 46) and healthy controls (n = 50). **a–c** Increasing plasma levels of TMAO, β2-MG, and serum Hcy level in HTN-CKD stage 5 patients (n = 58) compared with HTN-CKD stage 2 patients (n = 39). **d** Increasing plasma levels of TMAO in HTN-CKD stage 2 patients (n = 39) compared with HTN (n = 46) but not β2-MG and Hcy. Values are expressed as median (min-max). p values analyzed by Kruskal-Wallis' one-way ANOVA, followed by Dunn's multiple comparison test. *$p < 0.01$. CKD, chronic kidney disease; HTN, hypertension; Hcy, homocysteine; β2-MG, β2-microglobulin; TMAO, trimethylamine-N-oxide.

0.05). However, significant differences were found in the levels of creatinine, urea, uric acid, Hcy, lactate dehydrogenase, lipoprotein a, SBP, and BMI ($p < 0.05$) (Table 1).

Elevated TMAO Level Portends Higher Levels of HTN

Patients within the HTN group (n = 46), when compared with the normal control group (n = 50), had increased plasma TMAO levels (2.01 [1.58–2.49] mg/L vs. 1.00 [0.73–1.27] mg/L, $p < 0.01$) (Fig. 2a). Binary logistic regression analysis showed that TMAO was a risk factor for HTN (unadjusted OR 66.81, 95% CI 12.85–347.29, $p < 0.01$). The results based on univariate analysis were included as covariates, and model 1 and model 2 were constructed. On binary logistic regression analysis, TMAO was still an independent risk factor for HTN (adjusted OR 99.88, 95% CI 11.69–853.21, $p < 0.01$) (Table 2). There was a significant positive correlation between the plasma TMAO level and SBP (r = 0.66, $p < 0.01$), DBP (r = 0.504, $p < 0.01$), and HTN grades (r = 0.701, $p < 0.01$) (Fig. 3a, b).

Table 2. Binary/ordered logistic regression analysis: the association between TMAO levels and HTN and HTN-CKD risk

	N	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	
			model 1	model 2
HTN[a]				
TMAO	96	66.81 (12.85,347.29)	81.59 (12.17,546.94)	99.88 (11.69,853.21)
p value		0.001	0.001	0.001
HTN-CKD[b]				
TMAO	189	3.23 (1.99,5.24)	3.87 (2.13,7.04)	4.07 (1.21,13.7)
p value		0.001	0.001	0.02
CKD 5[c]				
T1		Ref	Ref	Ref
T2		2.00 (0.77, 5.18)	1.87 (0.66, 5.35)	4.38 (0.66, 29.17)
p value		0.16	0.24	0.13
T3		3.29 (1.30, 8.32)	4.77 (0.79, 28.93)	3.03 (0.49, 18.89)
p value		0.012	0.09	0.24
T4		5.47 (2.16, 13.87)	14.53 (2.27, 93.03)	12.47 (1.81, 85.86)
p value		0.012	0.005	0.01

Ref, reference; β2-MG, β2-microglobulin; TMAO, trimethylamine-N-oxide; HTN, hypertension; DBP, diastolic blood pressure; SBP, systolic blood pressure. [a]Model 1 was adjusted for BMI, history of coronary heart disease, and stroke. Model 2 was adjusted for all the variables in model 1 plus blood glucose and triglycerides. [b]Model 1 was adjusted for age, BMI, HTN course, SBP, DBP, and HTN grade. Model 2 was adjusted for all variables in model 1 plus homocysteine, uric acid, lactate dehydrogenase, lipoprotein a, creatinine, and urea. [c]Model 1 was adjusted for hemodialysis/peritoneal dialysis, folic acid, homocysteine, and uric acid. Model 2 was adjusted for all variables in model 1 plus creatinine and urea.

Elevated TMAO Level in Patients with HTN Portends Renal Dysfunction

In the HTN cohort (n = 189), the plasma TMAO level in the HTN-CKD group was significantly higher than that in the HTN group (2.99 [2.08–3.55] mg/L) versus (2.01 [1.58–2.49] mg/L, $p < 0.01$) (Fig. 2). Binary logistic regression analysis showed that TMAO was a risk factor for renal dysfunction in patients with HTN (unadjusted OR 3.23, 95% CI 1.99–5.24, $p < 0.01$). After adjusting the mixed interference of 11 covariables such as Hcy and urea, TMAO was considered an independent risk factor for renal dysfunction in patients with HTN (adjusted OR 2.34, 95% CI 1.09–5.00, $p < 0.05$) (Table 2). TMAO levels were positively correlated not only with traditional renal injury markers, such as creatinine (r = 0.54, $p < 0.01$) and urea (r = 0.47, $p < 0.01$) levels but also with β2-MG (r = 0.46, $p < 0.01$) and Hcy (r = 0.48, $p < 0.05$) (Fig. 3c–f).

The ROC analysis of TMAO for the diagnosis of renal insufficiency in patients with HTN showed that the best critical value of TMAO was 2.62 mg/L, sensitivity was 62.2%, specificity was 87%, and the AUC was 0.76 (95% CI 0.68–0.84). The best critical value of β2-MG for predicting renal dysfunction in patients with HTN was 2.93 mg/L, sensitivity was 72%, specificity was 91.3%, and the AUC was 0.83 (95% CI 0.77–0.88). The AUC of TMAO combined with β2-MG for predicting renal dysfunction in patients with HTN was 0.85, 95% CI ranged from 0.80 to 0.90, the best critical value was 0.79 mg/L, sensitivity was 67.8%, and specificity was 93.5% (Fig. 4).

Elevated TMAO Level in HTN-CKD Patients Portends Poor Renal Function

The HTN with the renal dysfunction group (group C) was divided into 4 subgroups, C2–C5, according to CKD stage. The baseline characteristics of all groups are summarized in Table 3.

The levels of Hcy (r = 0.66 and $p < 0.01$), TMAO (r = 0.43 and $p < 0.01$), and β 2-MG (r = 0.80 and $p < 0.01$) increased with the increase in CKD grade (Fig. 2a–c). Although levels of Hcy (15 [13–17] μmol/L) and β 2-MG (2.86 [1.99–3.62] mg/L) in the C2 group were higher than those in the HTN group (13 [11–15] μmol/L, p = 0.15 and 2.27 [1.47–2.80] mg/L, p = 0.07, respectively), the differences were not statistically significant. When compared

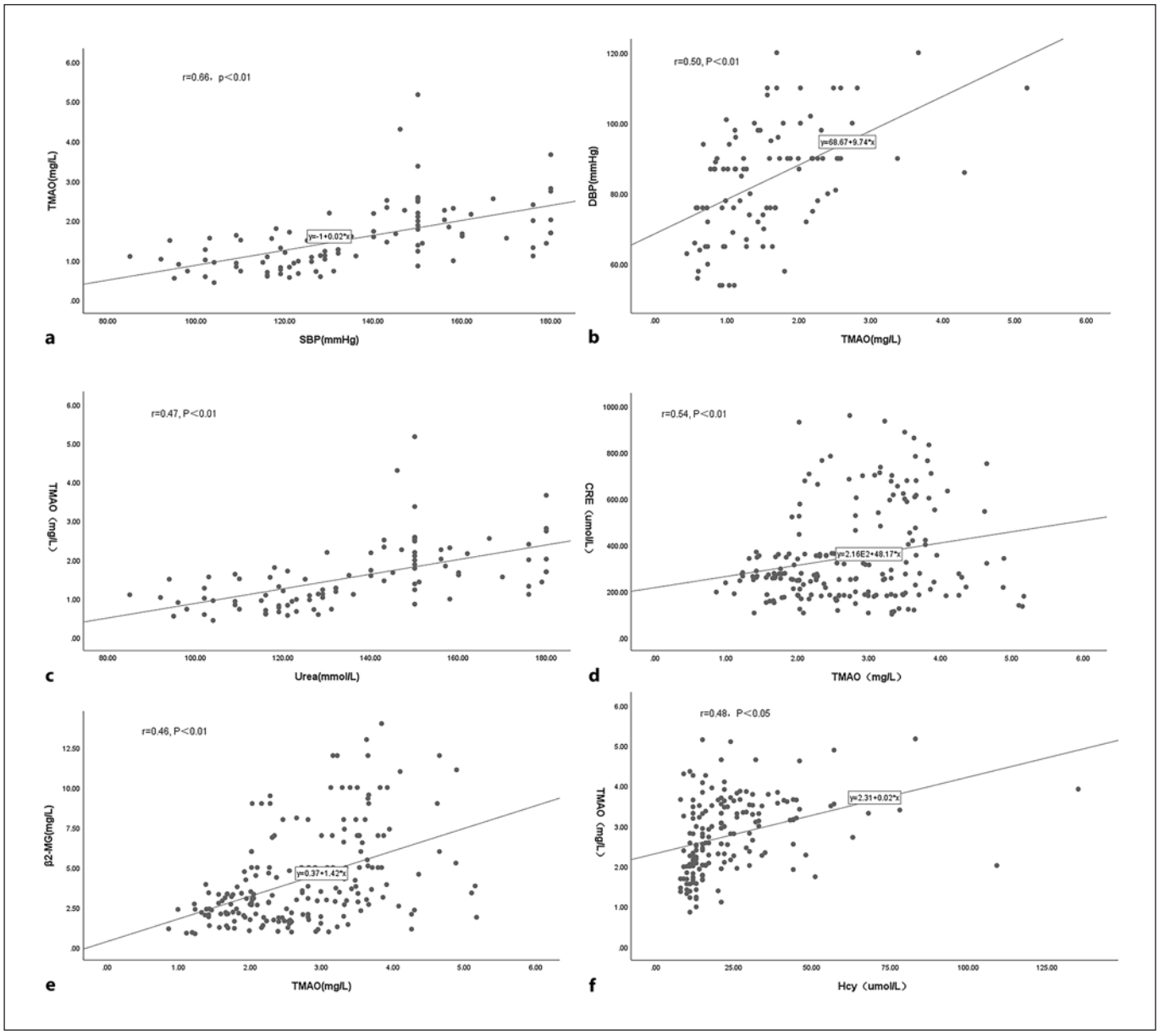

Fig. 3. a, **b** Positive correlation between the elevated levels of TMAO and BP. The positive correlation between the elevated levels of TMAO and traditional renal injury markers: creatinine ($r = 0.54$, $p < 0.01$), urea ($r = 0.47$, $p < 0.01$) (**c**, **d**), and new biomarkers: β2-MG ($r = 0.46$, $p < 0.01$) and Hcy ($r = 0.48$, $p < 0.05$) in HTN-CKD patients (**e**, **f**). BP, blood pressure; CKD, chronic kidney disease; HTN, hypertension; Hcy, homocysteine; β2-MG, β2-microglobulin; TMAO, trimethylamine-N-oxide.

with the HTN group (2.01 [1.66–2.56] mg/L), only TMAO levels in the C2 group (3.28 [2.93–3.39], $p < 0.01$) showed a significant increase (Fig. 2d). This indicates that TMAO was more accurate than β2-MG and Hcy in indicating early renal function damage.

The plasma TMAO levels were divided into 4 subgroups, T1–T4, according to the quartile. Ordered logistic regression analysis showed that, when compared with the lowest TMAO quartile, the highest quartile had a significantly higher risk of being in CKD stage 5 (OR 12.47, 95% CI 1.81–85.86, and $p < 0.01$) (Table 3).

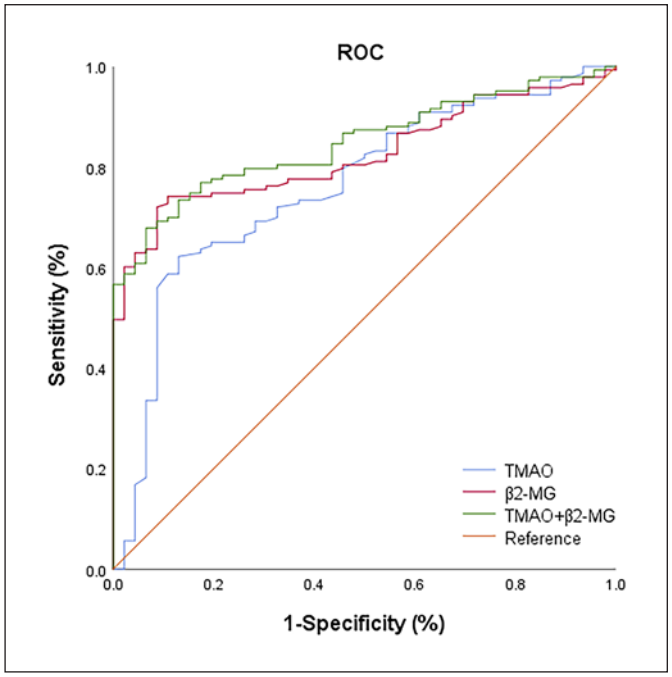

Fig. 4. ROC curve of β2-MG and TMAO in the diagnosis of kidney dysfunction in hypertension. The AUC of TMAO in the diagnosis of kidney dysfunction in HTN was 0.76. The AUC of β2-MG in the diagnosis of kidney dysfunction in HTN was 0.83. The AUC of the combined application of β2-MG and TMAO in the diagnosis of kidney dysfunction in HTN was 0.85. AUC, area under the curve; ROC, receiver-operating characteristics; TMAO, trimethylamine-N-oxide; β2-MG, β2-microglobulin; HTN, hypertension.

Discussion

With population growth, aging populations, and major epidemiological changes, such as the increasing noninfectious risk factors, the incidence of CKD has greatly increased by almost 89% in the past 25 years, thereby aggravating the global burden of the disease [21]. HTN, the most common risk factor for cardiovascular disease [22], has a high incidence and low awareness rate. It is a major risk factor for heart failure, atrial fibrillation, and cognitive decline, as well as being a critical noninfectious risk factor for CKD.

With further investigation into metabolomics, in addition to the traditional risk factors, there are other mechanisms involved in the regulation of blood pressure. One of these is the intestinal flora metabolite TMAO, which can affect the control of blood pressure through the "TMAO-AVP-AQP-2 axis" [11], that is, TMAO can stimulate the release of vasopressin (AVP) by increasing the plasma osmotic pressure , upregulating the expression of aquaporin-2 (AQP-2) in the renal collecting duct, and increasing sodium and water retention. It can also enhance the pressor effect of the renin-angiotensin-aldosterone system , mainly by making the pressor effect mediated by low-dose angiotensin II last longer [12]. Clinical studies have confirmed that the change in plasma TMAO concentration is related to the fluctuation of carotid pressure, as well as the promotion of adverse cardiovascular events by affecting central hemodynamics [23]. As the aortic pulse wave velocity increases, all-cause mortality increases in patients with HTN-CKD stages 2–4 [24].

Circulating TMAO not only causes renal injury by increasing HTN but also directly promotes renal interstitial fibrosis and dysfunction by mediating TGF-β/Smad3 phosphorylation. Trimethylamine inhibitor 3-dimethyl-1-butanol is normally used to reduce TMAO levels, which can mitigate the aforementioned pathological changes [14]. As CKD progresses in severity to uremia, the balance of the intestinal flora is disturbed [25] and, together with impairment of the intestinal barrier function [26], allows potential translocation of uremic toxins and intestinal microbial metabolites. Systemic inflammatory reactions and oxidative stress are amplified, which exacerbates the adverse cardiovascular outcome and mortality [27]. However, there are experimental studies that do not support such a relationship. For example, TMAO reduced mortality in the SHHF mouse model, an effect associated with osmotic diuresis caused by the hyperosmotic state of TMAO. TMAO increased the level of vasopressin, in addition to its diuretic and natriuretic effects [28]. However, the study did not directly measure the blood pressure of mice, and the findings were contradictory to previous animal experiments, showing that TMAO upregulated the effect of AQP-2 and led to sodium and water retention by increasing plasma osmotic pressure, and amplified the boosting effect of Ang-II [11, 12], which may be related to variation in TMAO dosage and methods of intervention. Therefore, the specific mechanism by which TMAO regulates blood pressure requires further investigation. TMAO was initially thought to exist primarily in deep-sea fish and other aquatic products, functioning to maintain cell osmotic pressure and protect protein invariance. Eating these fish is thought to be good for the circulatory system [29–31], but animal experiments have shown that TMAO does not improve the prognosis of SHHF mice by affecting the structure of proteins such as lactate dehydrogenase [28].

Based on the existing studies, damage to renal function has a cause-and-effect relationship with HTN [8] and is associated with the level of circulating TMAO [25, 26, 32].

Table 3. Comparison of baseline and laboratory data by CKD subgroups

Variable	CKD		Stage	
	CKD2 (*N* = 39)	CKD3 (*N* = 29)	CKD4 (*N* = 17	CKD5 (*N* = 58)
Age, yr	61.38±8.83	65.97±11.18	63.18±11.70	64.14±12.75
BMI, kg/m^2	23.49±2.94	21.50±3.30	22.57±3.63	20.76±3.71**
Sex, *n* (%)				
Male	18 (46.2)	16 (44.8)	8 (47.1)	30 (51.7)
Female	21 (53.8)	13 (55.2)	9 (52.9)	28 (48.3)
Smoking, *n* (%)	6 (15.4)	5 (17.2)	0 (0)	6 (10.3)
Drinking, *n* (%)	3 (7.7)	5 (17.2)	1 (5.9)	3 (5.2)
CVD, *n* (%)	8 (20.5)	3 (10.3)	4 (23.5)	10 (17.2)
Stroke, *n* (%)	3 (7.7)	2 (6.9)	0 (0)	4 (6.9)
ACEI/ARB, *n* (%)	17 (43.6)	6 (20.7)	4 (23.5)	18 (31)
HD/PD, *n* (%)	–	7 (8.5)	17 (20.7)$^{\#\#}$	58 (70.7)$^{\#\#\&\&}$
HTN, *n* (%)				
HTN1	15 (38.5)	15 (51.7)	5 (29.4)	22 (37.9)
HTN2	15 (38.5)	7 (24.1)	5 (29.4)	22 (37.9)
HTN3	9 (23.1)	7 (24.1)	7 (41.2)	14 (24.1)
TMAO, mg/L	2.39 (1.76, 3.07)	2.81 (2.02, 3.50)	2.95 (2.28, 3.89)*	3.44 (2.81, 3.67)**
TG, mmol/L	1.51 (1.14, 2.11)	1.56 (1.05, 2.71)	1.34 (0.93, 2.19)	1.21 (1.01, 1.69)
TC, mmol/L	4.47±1.31	4.64±1.05	4.27±0.87	4.04±1.09
LDL-C, mmol/L	2.44 (1.93, 3.45)	2.52 (2.06, 3.40)	2.52 (1.68, 3.00)	2.20 (1.73, 2.78)
Lipoprotein a, mg/dL	81.50 (77.00, 84.00)	73.00 (65.50, 75.00)	183.50 (67.50, 205.00)	238.00 (99.50, 468.00)
Glucose, mmol/L	5.00 (4.40, 5.96)	4.87 (4.55, 5.38)	4.85 (4.42, 5.23)	4.69 (4.39, 5.51)
Hcy, μmol/L	14.00 (12.00, 21.00)	15.00 (12.50, 22.00)	29.00 (21.00, 33.50)**	29.50 (24.00, 42.25)$^{**\#\#}$
FA, ng/mL	7.41 (7.33, 7.74)	4.63 (3.37,5.12)**	5.46 (3.98, 0.03)	6.79 (5.59, 9.98)$^{\#\#}$
UA, μmol/L	332.00 (283.00, 381.00)	417.00 (325.00, 465.50)	505.00 (409.50, 565.00)**	340.00 (281.00, 446.75)$^{\&\&}$
β2-MG, mg/L	2.08 (1.64, 2.97)	3.07 (2.50, 3.53)	4.50 (3.78, 6.30)**	11.55 (10.00, 19.24)$^{**\#\#\&\&}$
Urea, mmol/L	5.90 (4.70, 6.80)	6.90 (6.30, 8.80)	13.60 (12.15, 15.85)$^{**\#\#}$	18.45 (15.45, 23.05)$^{**\#\#}$
Cr, μmol/L	75.00 (57.50, 84.50)	86.10 (79.95, 123.15)	249.20 (213.85, 303.10)**	605.80 (479.60, 744.50)$^{**\#\#}$

Continuous data are presented as mean ± standard deviation or median (interquartile range), and categorical variables are presented as *n* (%). CVD, cardiovascular disease; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; TMAO, trimethylamine-N-oxide; Hcy, homocysteine; TC, total cholesterol; TG, triglyceride; β2-MG, β2-microglobulin; FA, folic acid; UA, uric acid; Cr, creatinine; LDH, lactate dehydrogenase; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HD, hemodialysis; PD, peritoneal dialysis. $^{**}p < 0.01$, $^{*}p < 0.05$ versus CKD2. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus CKD3. $^{\&\&}p < 0.01$, $^{\&}p < 0.05$ versus CKD4.

TMAO can affect renal injury either directly [14] or indirectly through the aggravation of renal insufficiency by increasing HTN [11–13]. Moreover, the clearance rate of TMAO in patients with renal insufficiency is decreased, and the activity of flavin monooxygenase 3 is significantly increased [32], which leads to additional TMAO accumulation and further aggravation of renal injury (Fig. 5). HTN itself may cause an increase in plasma TMAO due to pathological changes in the gut and enhanced passage of TMA, a TMAO precursor, into the circulation [33]. Another study has suggested that TMA is a cardiovascular risk factor and not TMAO [34]. TMAO plays an important role in HTN and HTN-CKD, but no clinical studies have directly demonstrated their relationship. Therefore, this study included healthy controls, patients with HTN, and patients with HTN complicated with renal dysfunction to investigate this relationship thoroughly.

We found that an elevated TMAO level serves as a marker for HTN. This suggests that the increase in the plasma TMAO level can be used not only as a warning of HTN but also as a novel indication for assisting cardiovascular risk stratification in HTN patients.

The levels of TMAO and β2-MG in patients with HTN-CKD were significantly higher than those in patients with HTN alone. After adjusting the confounding interference of all potential risk factors for renal impairment, elevated TMAO levels were found to forecast renal impairment in patients with HTN. On subgroup

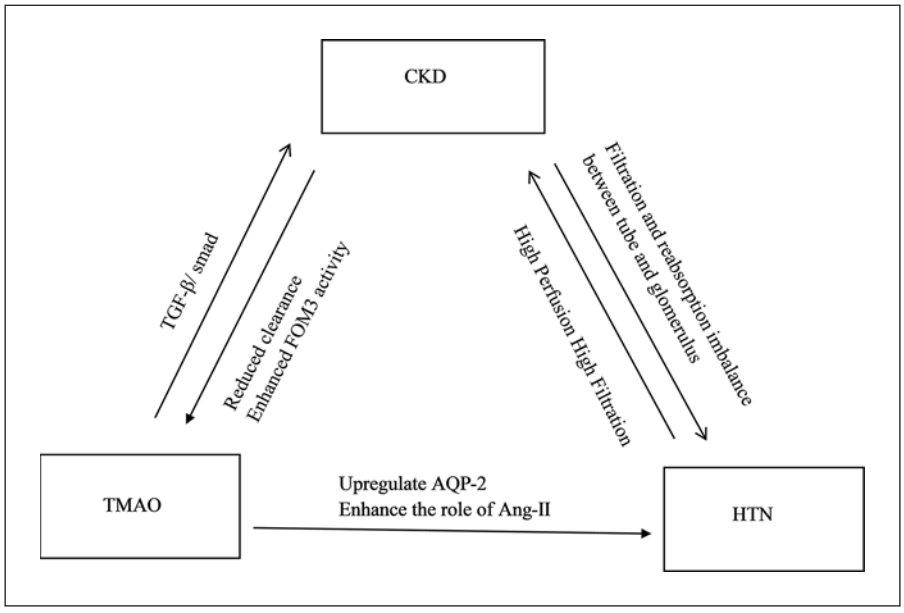

Fig. 5. Relationship between TMAO, HTN, and CKD. CKD, chronic kidney disease; HTN, hypertension; TMAO, trimethylamine-N-oxide. $^{**}p < 0.01$, $^{*}p < 0.05$ versus control group. $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ versus HTN group. Continuous data are presented as mean ± standard deviation or median (interquartile range), and categorical variables are presented as *n* (%).

analysis, we found that even in patients whose eGFR decreased only slightly, as in CKD stage 2, TMAO, but not β2-MG, levels were significantly increased. This suggests that TMAO has an earlier diagnostic value than β2-MG. TMAO is not only a nephrotoxic substance [14] but also a potential novel plasma marker to screen for early renal function damage. The increase in the plasma TMAO level was found to be proportional to the degree of renal function injury, indicating that TMAO levels can also represent the severity of renal function. An observational cohort study found that plasma TMAO levels were independently associated with cardiovascular event mortality in patients with moderate and severe CKD. This suggests that TMAO not only increases HTN and aggravates renal function damage but also increases cardiovascular event mortality in patients with HTN and CKD [27]. TMAO may contribute to the risk stratification of HTN and CKD and thus can potentially be used to identify subclinical patients and improve prognosis.

This study has some limitations. First, the cross-sectional observation study design cannot confirm a causal relationship, amid mutual influence, between TMAO, HTN, and HTN-CKD. Therefore, a prospective intervention study with a large sample size is needed. Second, patients in the HTN-CKD group are older and have a lower body weight than those in the other groups. Therefore, the pairing principle should be adopted in future research. Finally, there is no dynamic monitoring of blood pressure and TMAO during hospitalization, so it is impossible to evaluate the antihypertensive effects of TMAO in HTN patients.

In conclusion, the plasma TMAO level is related to HTN; moreover, the degree of TMAO elevation is related to the HTN grade, suggesting its potential use as a new blood marker to assist in cardiovascular risk stratification of patients with HTN. The increase in plasma TMAO levels in patients with HTN correlated with renal damage, whereas the level of plasma TMAO in patients with HTN complicated with renal dysfunction represented the severity of renal insufficiency. Combined plasma TMAO and β2-MG levels showed favorable efficacy in the diagnosis of renal injury in patients with HTN. Notably, we can use plasma TMAO levels as a biomarker to help identify early renal injury.

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Statement of Ethics

The research complied with the guidelines for human studies and conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given written informed consent, and the study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

B.L. and J.Z. conceived and participated in study design, coordination, and helped draft the manuscript. D.W. and X.L. carried out the mass chromatographic analysis. X.L. and S.L. carried out the immunoassays. N.L. and X.Z. performed the statistical analysis. All the authors read and approved the final manuscript.

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