Serum Trimethylamine N-oxide and the Diversity of the Intestinal Microbial Flora in Type 2 Diabetes Complicated by Diabetic Kidney Disease

Mengxue Yang 1, Rui Zhang 1, Caifang Zhuang 1, Yueyue Wu 1, Qian Yang 1, Zhiyuan Yu 1, Jun Liu 1, Bingbing Zha 1, Qihai Gong 2, Bo Yang 3, Miao Zeng 4, Cuili Yan 1

1 Department of Endocrinology, Shanghai Fifth People’s Hospital, Fudan University, Shanghai, China
2 Key Laboratory of Basic Pharmacology of Ministry of Education and Joint International Research Laboratory of Ethnomedicine of Ministry of Education, Zunyi Medical University, Zunyi, China
3 Department of Endocrinology, Affiliated Hospital of Zunyi Medical University, Zunyi, China
4 Department of Infectious Diseases I, Shanghai Fifth People’s Hospital, Fudan University, Shanghai, China

SUMMARY

Background: Trimethylamine N-oxide (TMAO) serves as a metabolite of intestinal bacteria as well as a urotoxin influencing the prognosis of chronic kidney disease (CKD), which has become a research hotspot in the field of kidney disease. This study preliminarily explored the alternations of the microbial flora and serum TMAO in patients with type 2 diabetes mellitus (T2DM) complicated with diabetic kidney disease (DKD).

Methods: Seventeen T2DM patients at the Affiliated Hospital of Zunyi Medical University between September 2018 and February 2019 were included. Among these patients, 8 patients had T2DM complicated with DKD. Eight healthy volunteers constituted the control group. Fresh stool was collected for Illumina sequencing. Based on the sequencing outcomes, the flora diversity and species differences were analyzed. Serum TMAO, cystatin C, urinary albumin/urine creatinine ratios (ACRs), and routine biochemical outcomes were also compared.

Results: The DKD group exhibited a significantly higher TMAO level than the remaining groups. The high-TMAO group had a significantly increased ACR level compared with the low-TMAO group. TMAO positively correlated with the ACR. Compared with the control group, the DKD group exhibited a decreased flora diversity. At the genus level, both the T2DM group and the DKD group showed decreased numbers of Alloprevotella and Megasphaera compared with the control group. The difference in Megasphaera between the DKD group and the control group was significant.

Conclusions: The alternation of the intestinal microbial flora may participate in the development of DKD, and TMAO and chronic inflammation might be important factors for DKD development.


KEY WORDS
diabetic kidney disease, intestinal microbial flora, metabonomics, trimethylamine N-oxide

INTRODUCTION

Diabetic kidney disease (DKD) is one of the chronic and severe complications of type 2 diabetes (T2DM). Compared with other kidney diseases, DKD is characterized by a short time span to develop into end-stage
renal disease (ESRD) [1]. It is a frequent cause of renal failure, and therefore is associated with high disability and death rates. In terms of the primary etiologies for new cases of hemodialysis, the rank of chronic glomerulonephritis gradually drops while DKD climbs up to the first [2].

Currently, it is accepted that the pathogenesis of DKD is possibly associated with the activation of the renin-angiotensin system (RAS), oxidative stress, the increased activity of protein kinase C, the production of advanced glycation end products, glucose metabolism disorder, inflammation, and genetic factors [3-5]. Besides, numerous studies have shown that intestinal bacterial microbes are closely associated with DKD, DM, and chronic kidney disease [6-9]. Intestines and kidneys have close physiopathological associations, which constitutes the “gut-kidney axis” [10,11]. Diet change and antibiotic utilization can both cause intestinal microbial flora disruption; the increase in lipopolysaccharides (LPS) downregulates the mRNA expression of the tight junction proteins between intestinal cells ZO-1 and occluding via the toll-like receptor (TLR) channel, which damages the intestinal epithelial barrier and increases paracellular permeability [12,13]. Under such a condition, bacterial debris and toxins enter the blood flow through the permeated intestinal tract and cause metabolic endotoxemia; in the meantime, they activate renal TLR4 and then activate the downstream signal molecules via myeloid differentiation factor 88 (MyD88)-dependent and independent pathways to initiate a cascade of inflammatory reactions, aggravate podocyte lesion and the inflammation and fibrosis of the kidneys [14,15] and finally lead to DKD. In addition, urea accumulated due to chronic kidney disease is very likely to diffuse in the intestinal tract. After diffusion, it is degraded by bacterial urease, which increases the pH value of the stool; accordingly, the structure of the intestinal bacterial microbes alters [16], which produce and absorb uremic toxins such as indole sulfate and p-cresol sulfate [17]. The intestinal permeability is further aggravated. Under such condition, endotoxins or even living bacteria migrate into blood from the intestinal tract, which increases the accumulation of metabolic wastes and inflammation level in the blood, thereby further aggravating renal dysfunction [18,19].

According to recent research [20], the structure and diversity of the intestinal microbial flora in diabetic mice were different from that in normal mice, and germ-free mice presented with an abnormal pathological structure of the kidneys after transplantation of the intestinal flora from the diabetic mice, with increased microalbuminuria. Previously, investigations of intestinal bacteria relied on traditional culturing, microscopy, and biochemistry. In recent years, 16S rRNA gene high-throughput sequencing has been developed. This technique has been extensively applied in studies on microbial flora because of its deep sequencing and high accuracy [21]. However, studies on the intestinal microbial flora in patients with T2DM complicated by DKD based on 16S rRNA gene detection are rare.

Based on the aforementioned context, this study compared the structures of the intestinal microbial flora among T2DM patients, DKD patients, and healthy volunteers to explore the possible influence of the intestinal microbial flora on the development of DKD. The results of this study may provide a new idea for the prevention and treatment of DKD.

**MATERIALS AND METHODS**

**Study subjects**

A total of 17 patients who were newly diagnosed with T2DM at the Affiliated Hospital of Zunyi Medical University between September 2018 and February 2019 were included. None of the patients had received T2DM-related treatment. Among the patients, nine suffered from T2DM alone (the T2DM group) and eight had T2DM complicated with DKD (the DKD group). Additionally, eight healthy volunteers who took routine health examinations during the same period constituted the control group.

The inclusion criteria for the T2DM group were as follows: 1) diagnosed according to the Guidelines for the Prevention and Treatment of Type 2 Diabetes in China released by the Chinese Diabetes Society; 2) ACR, < 22 mg/g (male) and < 31 mg/g (female); or 24-hours microalbuminuria (UALB), < 30 mg.

The inclusion criteria for the DKD group included the following: 1) satisfying the diagnostic criteria for T2DM; 2) albuminuria (ACR), > 265 mg/g; 24-hours UALB, > 300 mg; and/or diabetic retinopathy with microalbuminuria (ACR, 22 - 265 mg/g (male) and 31 - 265 mg/g (female); or 24-hours UALB, 30 - 300 mg) and/or eGFR < 60 min/mL.

Those for the control group were as follows: 1) no diabetes or other organic lesions; 2) normal outcomes according to routine laboratory examinations (blood, urine, stool, liver and kidney function, blood lipids, blood glucose, and C-reactive protein) and imaging examinations (e.g., chest radiography, electrocardiographic examination, and abdominal ultrasonography); 3) being matched with the T2DM group and the DKD group in terms of age and gender.

Participants that met one or more of the following criteria were excluded from the current study: 1) administration of microecological preparations, such as probiotics and prebiotics, antibiotics or Chinese herbal medicine that might affect the structure of the intestinal microbial flora within one month prior to this inclusion; 2) infection-related diseases or other diseases within one month prior to this inclusion; or 3) being under stress.

The procedures of this study were in accordance with the Declaration of Helsinki and gained approval from the Ethics Committee of the Affiliated Hospital of Zunyi Medical University (approval no., (2018)1-159).
Written informed consent was obtained from each participant.

**Sample collection**
Approximately 10 g of fresh stool was collected from each participant in the morning. The samples were placed in an aseptic ice box and stored at -80°C.

**DNA extraction**
Stool flora DNA was extracted, and the sequences of microbial ribosomal RNAs that were able to reflect the composition and diversity of the microbial flora were taken as the targets. According to the conservation region of the sequence, primers 338F (5′-ACTCCTACGG GAGGCAGCA-3′) and 806R (5′-GGAC-TACHVGGG TWTCTAAT-3′) were designed. Specific barcode sequences were applied to amplify the variable regions of the 16S RNA gene, i.e., the V3 - V4 region. The PCR amplification products were subjected to 2% agarose gel electrophoresis, and the gels of the target fragments were harvested for fluorescence quantification.

**Illumina MiSeq sequencing**
A TruSeq Nano DNA LT Library Prep kit (Illumina) was used for sequence library preparation [22]. The qualified sequences were sequenced. A sequence similarity of 97% was used as the threshold for operational taxonomic unit (OTU) classification and taxonomic status identification, which corresponded roughly to the sequence difference at the taxonomic species level. The OTUs with an abundance value less than 0.001% of the total sequencing number of all the samples were removed. Based on the sequence number of each OTU in each sample, the abundance matrix of the OTU in each sample was constructed. The taxonomic statuses of the OTU at the phylum, class, order, family, genus, and species levels were identified with the Greengenes database, and statistical analysis was performed.

The diversity of the intestinal microbial flora was calculated according to the Chao1, ACE, Shannon, and Simpson indices, with the former two focusing on community abundance while the latter also giving consideration to community evenness. Differential testing was performed using the Wilcoxon rank sum method, and rarefaction curves were plotted. R software was used for the NMDS analysis of the weighted UniFrac distance matrix, and a two-dimensional ordination was used to describe the structural distribution of the flora samples. According to the OTU classification and taxonomic status identification results, the detailed compositions of each sample at the phylum, class, order, family, genus and species classification levels were obtained.

**Serum TMAO determination**
The serum TMAO concentration was determined using liquid chromatography mass spectrometry (LC-MS) [23]. The chromatographic conditions consisted of an ACQUITY UPLC BEH HILIC chromatographic column (2.1 × 100 mm, 1.7 μm; Waters Corp., Milford, MA, USA), a sampling volume of 5 μL, and a mobile phase of acetonitrile (A) and water containing 0.1% formic acid and 10 mM ammonium formate at a velocity of 0.4 mL/min. The conditions for gradient elution included 80% A for 0 - 1 minutes, 80 - 70% A for 1 - 2 minutes, 70% A for 2 - 2.5 minutes, 70 - 50% A for 2.5 - 3 minutes, 50% A for 3 - 3.5 minutes, 50 - 80% A for 3.5 - 4 minutes, and finally 80% A for 4 - 6 minutes. The mass spectrometry conditions were as follows: electrospray ionization (ESI) source; positive ionization mode; ionization temperature, 500°C; ion source voltage, 5000 V; collisional gas, 6 psi; curtain gas, 30 psi; atomized gas, 50 psi; and auxiliary gas, 50 psi. Multiplex response monitoring (MRM) was adopted for scanning. Based on the detection outcomes, target quantification was performed for all the detected samples, and then, statistical analysis was performed.

**Statistical analysis**
Data were analyzed using SPSS 25.0 software and GraphPad Prism was used for plotting. Measurement data with a normal distribution are presented as x ± s, and one-way analysis of variance (ANOVA) was used to compare among groups. Data with an abnormal distribution are presented as the median (interquartile) and the Mann-Whitney rank sum test was used for pairwise comparisons. p < 0.05 was considered indicative of significance.

**RESULTS**

**Baseline data**
The three groups did not show significant differences in gender, age, body mass index, blood pressure, neutrophil absolute value, glycerin lipid and cholesterol. Compared with the control group, both the T2DM group and the DKD group exhibited a significantly increased platelet/lymphocyte ratio (PLR). The PLR of the DKD group was significantly higher than that of the T2DM group (p < 0.05). Compared with the control group and the T2DM group, the DKD group showed significantly increased urea, uric acid, cystatin C, creatinine, and PLR (p < 0.01). Compared with the control group, both the T2DM group and the DKD group had a significantly increased ACR, and the ACR of the DKD group was significantly higher than that of the T2DM group (p < 0.05). The DKD group showed a significant lower serum eGFR than the control group (p < 0.05), whereas no significant difference was observed between the control group and the T2DM group (p > 0.05). The results are summarized in Table 1.

**Serum TMAO concentration**
The T2DM group had a significantly higher serum TMAO concentration than the control group, and the DKD group had a significantly higher serum TMAO concentration than the T2DM group (p < 0.01, Figure 1).
Comparison based on TMAO stratification
The patients were divided into the low TMAO group (TMAO < 100 μmol/L) and the high TMAO group (TMAO ≥ 100 μmol/L). The two groups showed a significant difference in the urine ACR (65.01 ± 11.27 mg/g vs. 122.56 ± 28.11 mg/g; p < 0.05). No significant difference in the eGFR was observed (p > 0.05).

Correlations of PLR with biochemical outcomes, serum TMAO, and urine ACR
Pearson’s correlation analyses showed that the PLR was positively correlated with cystatin C, urea, and creatinine (r = 0.43, 0.421 and 0.509, respectively; p < 0.05), and serum TMAO was positively correlated with the ACR (r = 0.468, p < 0.05).

Venn graph of the OTUs
According to Illumina sequencing, a total of 1,197,365 high-quality sequences were obtained, which included 400,275 from the control group, 458,103 from the T2DM group and 338,987 from the DKD group, with an average valid sequence number of 47,287 ± 10,615 for each sample. According to the obtained OTU abundance matrix, the OTU numbers of the three groups were calculated with the R software, and a Venn graph was plotted to visually present the numbers of the shared and specific OTUs of each group (Figure 2). The three groups shared 1,847 OTUs, and the numbers of the OTUs specific to the control group, the T2DM group, and the DKD group were 559, 708, and 441, respectively.

Alpha diversity analysis
The three groups were ordered as the DKD group < the T2DM group < the control group in terms of the abundance indexes Chao1 and ACE. In terms of the indexes Simpson and Shannon, the DM (T2DM and DKD) groups showed lower diversity than the control group (Figure 3).

Composition of the intestinal microbial flora at the phylum and genus levels
In the three groups, Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, Fusobacteria, and Tenericutes were detected. Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the dominant phyla, which accounted for 96.7% above of the total number of all bacteria. The Metastats statistical algorithm was used to compare the sequence numbers of each OTU at the phylum level among the groups. The sequence number of Actinobacteria in the DM (T2DM and DKD) was significantly larger than that in the control group (p < 0.05). Although no significant difference in the Bacteroidetes/Firmicutes ratio was observed between the DKD group and the control group, the former group showed a lower ratio than the latter. At the genus level, the sequence numbers of Alloprevotella and Megasphaera in both the

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Table 1. Baseline data of the three groups.

<table>
<thead>
<tr>
<th>Data</th>
<th>Control (n = 8)</th>
<th>T2DM (n = 9)</th>
<th>DKD (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>4/4</td>
<td>4/5</td>
<td>4/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.13 ± 2.80</td>
<td>57.67 ± 4.61</td>
<td>58.75 ± 7.40</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>21.75 ± 1.83</td>
<td>22.33 ± 1.22</td>
<td>23.00 ± 1.60</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>127.38 ± 3.16</td>
<td>129.33 ± 9.68</td>
<td>137.88 ± 15.83</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>75.13 ± 5.14</td>
<td>80.22 ± 8.68</td>
<td>84.88 ± 5.96*</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/L)</td>
<td>4.76 ± 0.58</td>
<td>8.84 ± 3.93*</td>
<td>8.21 ± 3.29*</td>
</tr>
<tr>
<td>Absolute value of neutrophils (10^4/μL)</td>
<td>3.05 ± 1.07</td>
<td>3.42 ± 0.96</td>
<td>3.65 ± 1.03</td>
</tr>
<tr>
<td>Platelet/lymphocyte</td>
<td>110.07 ± 39.35</td>
<td>147.33 ± 24.31*</td>
<td>186.08 ± 28.37 **##</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.19 ± 0.91</td>
<td>4.86 ± 0.82</td>
<td>6.76 ± 1.70 *##</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>63.75 ± 15.00</td>
<td>63.78 ± 13.54</td>
<td>127.88 ± 67.56 ***##</td>
</tr>
<tr>
<td>Uric acid (μmol/L)</td>
<td>308.38 ± 66.27</td>
<td>267.56 ± 64.86</td>
<td>403.63 ± 80.62 ***##</td>
</tr>
<tr>
<td>Cystatin C (mg/L)</td>
<td>0.80 (0.7, 0.94)</td>
<td>0.87 (0.72, 0.90)</td>
<td>1.25 (0.93, 2.38) **##</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.39 (1.21, 1.52)</td>
<td>1.38 (0.99, 1.56)</td>
<td>1.47 (1.24, 1.92)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.06 ± 0.85</td>
<td>4.66 ± 1.66</td>
<td>4.92 ± 1.30</td>
</tr>
<tr>
<td>Urine ACR (mg/g)</td>
<td>21.08 ± 6.26</td>
<td>26.15 ± 9.06</td>
<td>278.60 ± 77.29 ***##</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>90.25 ± 24.80</td>
<td>86.07 ± 18.65</td>
<td>52.60 ± 12.46 #</td>
</tr>
</tbody>
</table>

Notes: * p < 0.05 and ** p < 0.01 vs. the control group, # p < 0.05 and ## p < 0.01 vs. the T2DM group. BMI, body mass index; ACR, albumin/urine creatinine ratio; eGFR, estimated glomerular filtration rate.
Intestinal Flora and Serum TAMO in DKD

Figure 1. Serum TMAO concentrations of the three groups. * p < 0.01 vs. the control group and # p < 0.01 vs. the T2DM group.

Figure 2. Representative Venn graph of the OTUs. OTU, operational taxonomic unit.
Figure 3. Rarefaction curves of the three groups. The curves indicate the control, DKD, and T2DM groups, respectively. In the Shannon figure, the curves of the DKD group and T2DM group are almost completely overlapped.

Figure 4. The composition and abundance distribution at the phylum level in each sample. The x-axis indicates the involved groups and the y-axis indicates the relative abundance of the intestinal microbial flora at the phylum level.
Figure 5. The composition and abundance of Alloprevotella and Megasphaera at the genus level in each group.

T2DM group and the DKD group were smaller than that of the control group. The DKD group also showed a significantly smaller sequence number of Megasphaera than the control group (p < 0.05) (Figures 4 and 5).

DISCUSSION

Intestinal microbial flora is closely associated with DM and ESRD. The alteration of the intestinal microbial flora, intestinal wall damage, increase in the intestinal tract permeability, exhaustion of short chain fatty acids (SCFAs)-producing beneficial bacteria, and increase in harmful bacteria all contribute to the occurrence of chronic inflammation, and both DM and ESRD are characterized with inflammation [24]. However, what the association between DKD and intestinal microbial flora is like remains unclear. In DKD patients, the eGFR decreases, and a large volume of metabolic waste accumulates as it cannot be discharged out of the body through the kidneys. The waste enter the intestinal cavity through the intestinal wall, which alters the intestinal environment and aggravates the alternation of the intestinal microbial flora. In recent years, TMAO, a metabolite as well as aurotoxin affecting the prognosis of chronic kidney disease (CKD) has become a hot spot of research as the understanding of the gut-kidney axis deepens. In the current study, we preliminarily investigated the possible differences in the intestinal microbial flora between patients with T2DM alone and those with T2DM complicated by DKD, as well as the fluctuation of the serum TMAO level in these patients.

According to our study, Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the dominant phyla in all the investigated groups, which is consistent with those reported in literature [25-27]. As the course of DM progressed, the abundance of the intestinal microbial flora decreased. This finding is in consistency with that reported by Larsen et al. [28], which suggests that the development of T2DM and DKD might be associated with the decreased abundance of the intestinal microbial flora. In addition, this study showed that compared with the control group, the DKD group presented with significantly decreased SCFAs-producing Alloprevotella and Megasphaera and decreased beneficial bacteria Blautia and Coprococcus but increased harmful bacteria Escherichia and Enterococcus. Intestinal bacteria play a key role in the generation of TMAO: They metabolize choline, carnitine, and betaine from food into TMA, which is then oxidized into TMAO by FMO3 after entering the liver. Fennema et al. summarized the intestinal bacteria associated with TMA generation [29]. According to them, bacterial species of the phyla Actinobacteria (Campylobacter and Uramericana), Firmicium (Clostridium, Enterococcus, and Streptococcus), Proteobacteria (Vibrio desulfuricus, Edwardsiella, Enterobacter, and Escherichia) can encode the catalysis required for choline degradation and promote the transfor-
flam

mation of choline into TMA. Then the question is, do these discovered beneficial and harmful bacteria activate cytokines and promote systemic inflammatory response by regulating the secretion of the intestinal metabolite TMAO, or does TMAO directly participate in the development of DK or aggravate the pathological condition of DK?

This study also showed that in the patients newly diagnosed with DK, serum TMAO increased. In the meantime, serum TMAO positively correlated with the urine ACR, and the patients with a higher TMAO level tended to have a higher cystatin C level. These findings suggest that TMAO accumulation might occur prior to noticeable renal function damage. Additionally, this study showed that PLR was positively correlated with cystatin C, urea, and creatinine, which suggests that inflammation and an increased TMAO level might be factors for the development of DKD. Our finding is similar to that of the study conducted by Tang et al. [30]. Compared with the non-CKD population, the CKD population with an eGFR level < 60 mL/min/1.73 m² had significantly increased serum TMAO.

Alloprevotella of the Bacteroidetes phylum is a genus of SCFAs-producing beneficial bacteria that exist in the gastrointestinal tract and saliva [31,32]. Supplementation of beneficial bacteria increased the abundance of intestinal Alloprevotella in rats and improved their blood lipid status [33,34]. After a 3-week supplementation of the mixed food of oligosaccharide and dietary fiber, the abundance of Alloprevotella increased in mice, whereas harmful diamine oxidase and/or TMAO decreased [35]. By increasing the abundance of Alloprevotella, interferon significantly decreased the levels of the inflammatory factors cryptidin-5, IL-17, and IFN-γ [36]. As like Alloprevotella, Megasphaera of the phylum Firmicutes is another genus of SCFAs-producing beneficial bacteria in the gastrointestinal tract [37]; it can produce SCFAs such as isovaleric acid, butyric acid, propionic acid, and acetic acid [38]. In this study, the abundance of Megasphaera and Alloprevotella were both decreased in the DKD patients.

In T2DM patients, the permeability of the intestinal tract increases, and the bacteria in the intestinal wall, the primarily lipid component of the outer membrane of most Gram-negative bacteria LPS, and the metabolites of the bacteria enter blood to produce a low-degree inflammation [37] and thus induce the generation of a variety of proinflammatory cytokines. This process is closely associated with inflammation of the kidney tissue, which causes cellular apoptosis, fibrosis, and organ dysfunction, thereby leading to renal failure [38]. Our previous study showed that compared to the normal population, the endotoxin level in patients with uremia and those with T2DM significantly increased [39]. The retention of urea in CKD is one of the mechanisms underlying the rupture of epithelial tight junction. After diffusing from blood into the intestinal tract, urea is metabolized into ammonia by intestinal bacterial urease; then, ammonia is transformed into corrosive ammonium hydroxide, which can damage the proteins that seal the spaces between epithelial cells [38]. Such damage ultimately leads to intestinal leakage, and endotoxins and bacterial fragments enter blood through the paracellular channel, thereby promoting systemic chronic inflammation [39]. PLR is a simple inflammatory indicator, which is independently correlated with DM [40]. In this study, compared with the control group, both the T2DM group and the DKD group showed a significantly increased PLR, and as the disease courses proceeded, the PLR gradually increased. DM patients might have a chronic low-degree inflammation status, and the inflammatory indicators in the DKD patients had higher levels than those in the T2DM patients, which is consistent with the results of our previous studies [41,42]. Therefore, chronic inflammation might participate in the development of DKD.

However, this study was single-centered and the sample size was small. In addition, the intestinal flora in the stool sample might not completely reflect all intestinal bacteria colonizing intestinal mucosa. To overcome these limitations, studies with a larger sample size remain to be conducted, and further research on the role of the intestinal flora in the development of DKD is needed. In addition, experiments involving animal models remain to be conducted to validate the results of this study.

In conclusion, the disproportion of the intestinal microbial flora might be associated with the development of DKD. This association might be that the alteration affects the gut-kidney axis and participates in the development of DKD by increasing the TMAO level and activating inflammatory response. Therefore, adjustment of the structure of the intestinal flora, particularly the supplementation of beneficial bacteria, may provide a new idea for the prevention and treatment of DKD in clinical practice, which, however, needs to be validated in the future.

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Declaration of Interest:
The authors declare no conflicts of interest.
References:


