JOURNAL OF ORAL MICROBIOLOGY
2025, VOL. 17, 2488054
https://doi.org/10.1080/20002297.2025.2488054

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RESEARCH ARTICLE

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Correlation of tongue coating thickness with microinflammatory state and oral microbiome in maintenance hemodialysis patients

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ABSTRACT

Aim: This study investigated the correlation between tongue coating thickness (TCT), microinflammatory state (MIS), and oral microbiome in maintenance hemodialysis (MHD) patients.
Methods: Forty MHD patients (20 thin-tongue coating [BTZ], 20 thick-tongue coating [HTZ]) and 15 healthy controls (DZZ) were enrolled. Blood microinflammatory markers were analyzed in all patients. Saliva samples from 15 HTZ, 15 BTZ, and 15 DZZ underwent 16S rRNA sequencing.
Results: HTZ patients exhibited higher microinflammatory marker levels than BTZ. Oral microbiome species richness in DZZ surpassed that of the MHD groups, with distinct structural differences, particularly between HTZ and DZZ. HTZ showed higher abundances of *Actinobacillus, Peptostreptococcus*, and Lachnospiraceae *NK4A136 group* than BTZ. Correlation analysis revealed a positive correlation between the levels of IL-6 and TNF-α and the abundance of *Fusobacterium*, but a negative correlation with *Streptococcus*. Additionally, the TNF-α level positively correlated with *Campylobacter*.
Conclusion: Thick tongue coating in MHD patients is associated with elevated microinflammation and altered oral microbiome, suggesting a link between inflammation and microbial dysbiosis.

ARTICLE HISTORY
Received 18 August 2024
Revised 31 December 2024
Accepted 12 March 2025

KEYWORDS
Maintenance hemodialysis; tongue coating thickness; microinflammatory status; 16S rRNA sequencing; oral microbiome

Introduction

In recent years, hemodialysis therapy has continuously developed, leading to a significant increase in the long-term survival rate of maintenance hemodialysis (MHD) patients. However, the prognosis for patients with end-stage renal disease (ESRD) remains poor, with a five-year survival rate of only 33.0–54.3% [1]. Research indicates that uremic patients typically exhibit a micro-inflammation state (MIS), which is particularly pronounced in MHD patients, affecting 35–50% of this population [2]. Microinflammation, characterized by elevated levels of inflammatory factors such as high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) [3–7], contributes to the morbidity and mortality of ESRD patients, specifically increasing the incidence of atherosclerosis [8]. Emerging evidence suggests that the microbiome significantly impacts the host's digestive, metabolic, and immune functions, playing a crucial role in human health and disease progression [9]. New research indicates that disturbances in flora microecology may be a key factor in the development of microinflammation [10]. Other studies have also highlighted the role of microbiota dysregulation in promoting chronic systemic inflammation in chronic kidney disease (CKD) [11]. Moreover, early identification and intervention of microinflammatory states are crucial for reducing mortality and enhancing the quality of life for MHD patients [12].

Tongue diagnosis stands as a crucial diagnostic technique in Traditional Chinese Medicine (TCM), enabling practitioners to assess physiological functions and pathological changes in the human body [13]. Tongue coating, the fur-like layer covering the tongue's surface, serves as a highly sensitive indicator of the internal organs' health status and the gravity of illnesses [14,15]. Alterations in the tongue coating's color, thickness, and dryness offer insights into the severity of a disease, thereby aiding in determining its progression and prognosis. Research has revealed that TCT varies in kidney diseases [16,17]. Specifically, studies have demonstrated that CKD patients with declining renal function tend to have a higher prevalence of thick tongue coating compared to those with healthier kidney function [18]. Our prior

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investigation further indicated that CKD (stages 1–3) patients exhibiting a greasy tongue coating possess elevated levels of serum high-density lipoprotein (HDL) compared to those with a thinner coating [19]. Nevertheless, the correlation between MIS and TCT in MHD patients remains underexplored, highlighting a gap in current research.

In this study, we explored the relationship between tongue thickness and MIS in MHD patients and examined the impact of structural and functional alterations of the oral microbiome on the MIS in these patients by high-throughput sequencing, in an attempt to identify correlations between TCT, MIS, and the oral microbiome, and thus to provide new diagnostic and therapeutic perspectives for the early identification and intervention of MIS in MHD patients.

Materials and methods

Study participants

A total of 116 patients with MHD (CKD 5 stage) were recruited from the Hangzhou TCM Hospital of Zhejiang Chinese Medical University. These patients had been receiving regular long-term consultations from December 2022 to June 2023. Additionally, 15 healthy volunteers with moderate tongue thickness (TCT scores of 14–23) were recruited from those attending the hospital medical check-up centre during the same period to serve as the healthy control group (DZZ). This project received approval from the Ethics Committee of Hangzhou Hospital of TCM.

The inclusion criteria for MHD patients in this study were as follows: (1) Regular MHD for ≥6 months and a stable condition during the observation period. (2) Age ≥18 years old. (3) Willingness to voluntarily participate and sign the informed consent form.

The exclusion criteria were as follows: (1) Primary diseases such as vasculitis, systemic lupus erythematosus, and other autoimmune diseases. (2) Organic diseases of the gastrointestinal tract, which include gastrointestinal bleeding, intestinal obstruction, intestinal adhesions, gastrointestinal tumors, and inflammatory bowel disease. (3) The presence of local or systemic infections, acute heart failure, cardiovascular and cerebrovascular accidents, severe oral diseases, hyperthyroidism, tumors, tuberculosis, hepatitis, and other significant systemic diseases. (4) A history of major trauma or surgery within the past three months. (5) Use of acid suppressants, probiotics, antibiotics, immunosuppressants, and glucocorticoids within the past month. (6) Challenges in cooperating with the completion of specimen collection or withdrawal from the test for any reasons during its course.

Acquisition and assessment of tongue coating images [15,20]

All 116 enrolled MHD patients had their tongue images taken using the same Tongue Diagnostic Instrument and the 'TCM Tongue Diagnostic Expert System' in the morning, prior to brushing their teeth or eating. The main features of the Tongue Diagnostic Instrument include that it has a fixed light source and focal length, can automatically focus, and can clearly and stably collect the tongue image, thereby reducing the influence of different light and angles on the tongue coating image acquisition. Each subject sat in front of the Tongue Diagnostic Instrument of the 'TCM Tongue Diagnosis Expert System', placed the jaw on the pedestal of the instrument, extended the tongue naturally, and fully exposed the tongue surface, enabling the researchers to take clear tongue coating images (Figure 1). Two TCM practitioners, each possessing a licensed practitioner qualification, quantitatively scored the thickness of the tongue coating using a modified version of the Japanese Toyo Shimada's TCT quantification standard [21,22]. This standard divides the dorsum of the tongue into three areas (anterior, middle, posterior), and each area is further divided into three parts. The thickness of the tongue coating in each part was scored as follows: no coating scored 0, thin coating scored 1, slightly thick coating scored 2, and thick coating scored 3 (Figure 2). No coating is literally the absence of tongue coating. The difference between thin and thick coating is determined by whether the tongue body of the tongue is visible through the coating (thin coating, through the tongue coating, one can vaguely see the tongue body; thick coating: through the tongue coating, one cannot see the tongue body) [23]. The total TCT was calculated by adding the scores from all areas. Based on the mean values of these scores, the patients were categorized into four groups: less tongue coating group (scores ≤4), thin tongue coating group (scores 5–13), slightly thick tongue coating group (scores 14–23), and thick tongue coating group (scores ≥24). After 6 months of follow-up, the patients' tongue images were collected and scored again using the same method.

Agreement of the assessment of TCT

To evaluate the consistency of scoring within and among two TCM practitioners, they first underwent comprehensive training in the differential criteria for TCT and familiarized themselves with conventional methods of assessing TCT prior to participating in the study. Then, in the same room and light, they independently scored all tongue coating images using the same

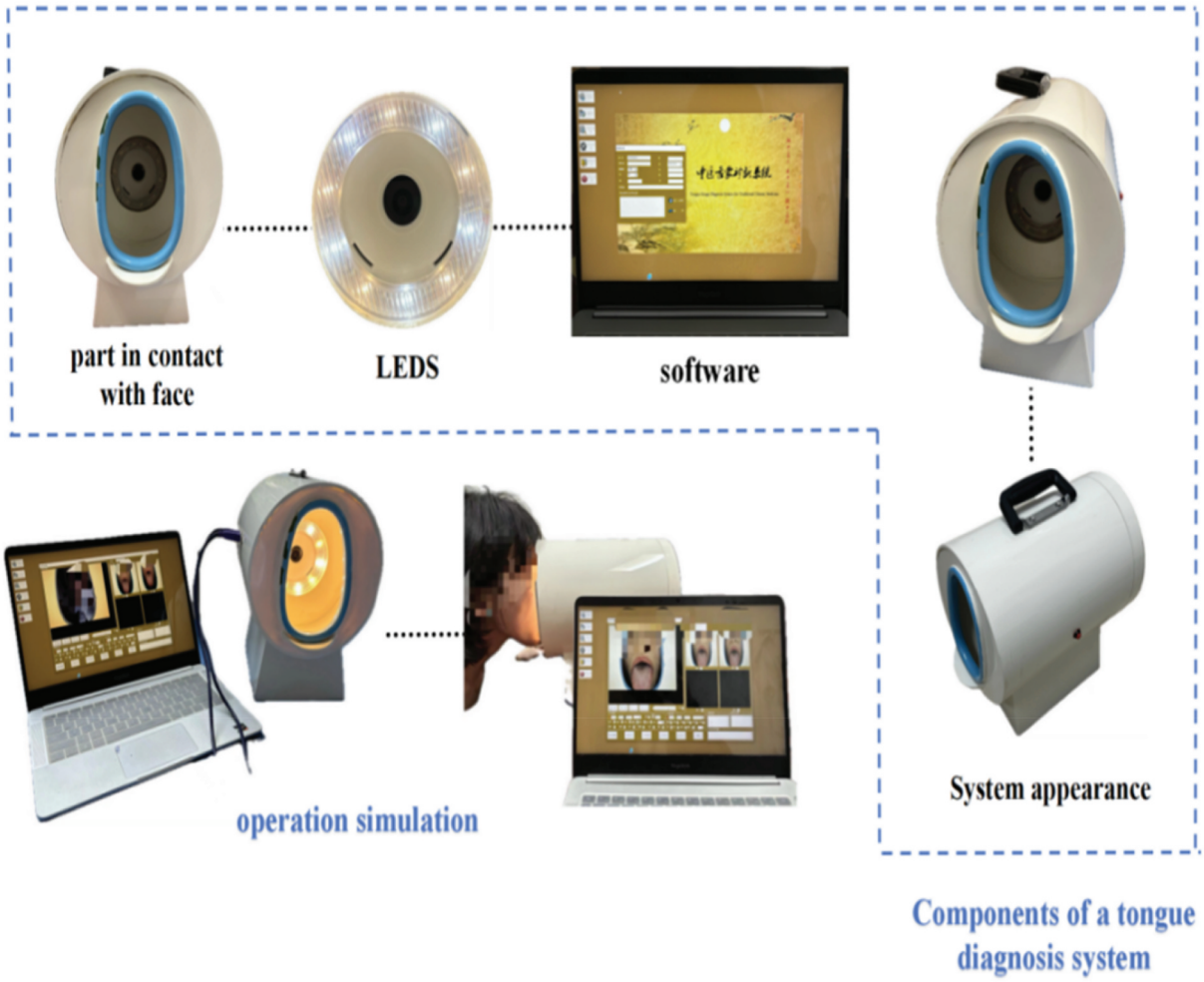


Figure 1. Components and operation simulation of a tongue diagnosis system.

method and criteria. The clinicians were blinded to the assessments of other clinicians and to the clinical data of the patients. Additionally, weighted kappa (k) was calculated to assess the inter-rater reliability.

Clinical data and sample collection

Baseline Data

Clinical data were collected from patients who met the inclusion criteria, including their gender, age, height, primary disease, Kt/V, and duration of dialysis.

Serum Sample Collection and Detection

Before starting dialysis, two tubes of venous blood (3 ml each) were drawn from all enrolled MHD patients. One tube was sent to the clinical laboratory of Hangzhou Hospital of TCM on the same day for tests, including white blood cell count (WBC), neutrophil count (NEU), lymphocyte count (LYM), haemoglobin (HB), platelet count (PLT), Serum creatinine (SCR), urea nitrogen (BUN), albumin (ALB), C-reactive protein (hs-CRP), and parathyroid hormone (PTH), among others. The Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) were calculated based on these results. The other tube was centrifuged at 3000 rpm for 10 minutes at room temperature; the supernatant was then immediately stored in a −80°C freezer. Subsequently, ELISA was used to measure IL-6 and TNF-α levels after collecting all specimens, using kits purchased from Hangzhou Dacheng Biotechnology Co., Ltd (production lot numbers: ELK1156 LOT:27474064, ELK1190 LOT:27473169).

Saliva Samples Collection

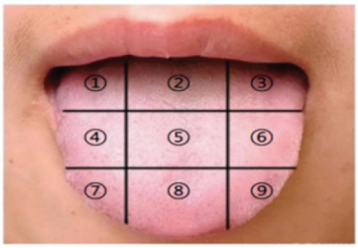
From the study group, 15 cases each from the HTZ and the BTZ were randomly selected, along with an additional 15 healthy volunteers (DZZ) from the medical check-up centre. All of them were collected 1–2 ml of naturally secreted saliva, retained in the mouth for at least 30 seconds, into two sterile tubes on an empty stomach and without brushing their teeth. The saliva was then transferred to a −80°C freezer within 2 hours.

16S rRNA high-throughput sequencing

Total DNA was extracted from the saliva samples using the CTAB method, and the DNA purity and concentration were assessed by agarose gel electrophoresis. The selected 16S rRNA V3-V4 (338F/806 R) variable regions were amplified by PCR using specific primers with

Modified Japanese Yutaka Shimada's Quantitative Criteria

Name_____ Gender_____ Scorer_____

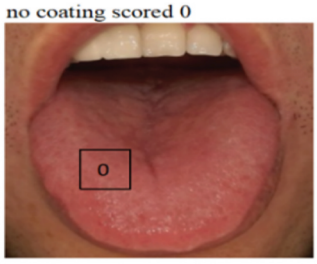


The thickness of the tongue coating in each part was scored as follows: no coating scored 0,
thin coating scored 1,
slightly thick coating scored 2,
thick coating scored 3.

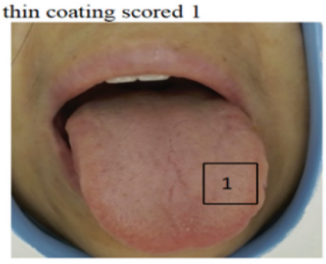
Area	Score
①Anterior right	
②Anterior middle	
③Anterior left	
④Middle right	
⑤Center	
⑥Middle left	
⑦Posterior right	
⑧Posterior middle	
⑨Posterior left	
Total	

Scoring examples

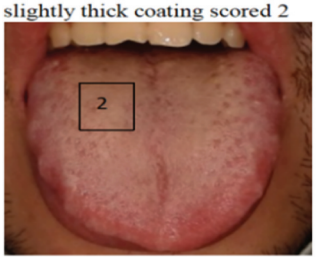
no coating scored 0



thin coating scored 1



slightly thick coating scored 2



thick coating scored 3

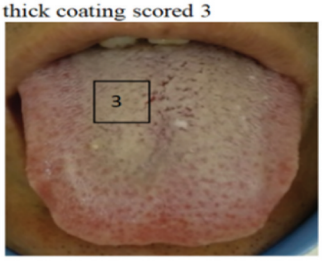


Figure 2. Modified Yutaka Shimada's quantitative criteria for TCT, with examples.

barcodes and high-fidelity DNA polymerase. The PCR products were detected by 2% agarose gel electrophoresis. These PCR products were then purified using AMPure XT beads (Beckman Coulter Genomics, Inc., Danvers, MA, USA), quantified by Qubit (Invitrogen, USA), and recovered using the AMPure XT beads recovery kit. After amplification, the samples were sent to Hangzhou Lianchuan Biotechnology Co., Ltd. under dry ice conditions, where library construction and quality control were completed. Subsequently, on-board sequencing was performed using the NovaSeq 6000 sequencer platform.

Processing of sequencing data

The data obtained from sequencing were split, spliced, filtered, and then further length filtered and denoised by calling DADA2 via qiime dada2 denoise-paired. Subsequently, class OTUs (equivalent to clustering with 100% similarity) were constructed using the concept of ASVs (Amplicon Sequence Variants). This process yielded the final ASV (feature) sequences and ASV (feature) abundance tables.

Microbial community construction analysis

Species Annotation Analysis

Species annotation and classification were conducted using the SILVA and NT-16S databases based on ASV (feature) sequence files. The abundance of each species in each sample was quantified using the ASV (feature) abundance table. Additionally, a species composition analysis was performed for different sample groups across varying levels of species abundance. The results were visualized using column stacking, heat maps, and cluster maps.

Species Diversity

Alpha and Beta diversity analyses were conducted on the obtained ASV (feature) feature sequences and ASV (feature) abundance tables.

Species Differences

An analysis of microbial composition differences between the comparison groups was conducted based on statistical information regarding species abundance. This analysis identified species that showed significant differences in abundance across the groups.

Statistical analysis

The data from this study were analyzed using SPSS version 26.0. Count data were expressed as frequencies or percentages and compared using the chi-square (χ^2) test. Measurement data were described using either the mean ± standard deviation ($x \pm s$) or the median and interquartile range (25% and 75% quartiles). The t-test was utilized for data that followed a normal distribution, while the Wilcoxon rank sum test was applied to data that did not follow a normal distribution. A p-value of less than 0.05 was considered statistically significant.

Results

Assessment of tongue coating

Based on the scoring results, the patients were divided into four groups: 17 patients in the less tongue coating group (scores ≤4), 40 patients in the thin tongue coating group (BTZ, scores 5–13), 23 patients in the moderate tongue coating group (scores 14–23), and 36 patients in the thick tongue coating group (HTZ, scores ≥24). However, due to significant fluctuations in TCT scores and a relatively small number of patients in the group with less tongue coatings (scores ≤4) during the follow-up reassessment, we ultimately randomly selected 20 MHD patients from each of the BTZ and the HTZ who exhibited no or minimal changes in their TCT scores over the follow-up period for further analysis.

Inter-rater reliability of assessors

The level of inter-rater reliability ranged from substantial to almost perfect (range of weighted kappa, 0.631–0.876). in addition, the total reliability of the assessors was Almost perfect and the kappa value was 0.817 as presented (Table 1).

General clinical information

Statistical analysis revealed no significant differences in gender, age, height, and individual weight between the two groups ($p > 0.05$), confirming that the baseline data for both groups were similar and comparable (Table 2). Furthermore, there were no significant differences between the HTZ and BTZ. among MHD patients regarding BUN, PTH, Ca, P, ALB, and HB indexes ($p > 0.05$). However, a significant difference was observed in the Kt/V values ($p < 0.05$) (Table 3), indicating a notable disparity in this parameter.

Table 1. Inter-rater reliability of assessors in evaluating TCT.

Area	Weight kappa	Standard error (95% CI)	Level of agreement
① Anterior right	0.872	0.068(0.739 – 1.00528)	Almost perfect
② Anterior middle	0.876	0.066(0.747 – 1.005)	Almost perfect
③ Anterior left	0.876	0.066(0.747 – 1.005)	Almost perfect
④ Middle right	0.865	0.070(0.728 – 1.002)	Almost perfect
⑤ Center	0.796	0.079(0.641 – 0.951)	Substantial
⑥ Middle left	0.631	0.090(0.455 – 0.807)	Substantial
⑦ Posterior right	0.704	0.089(0.530 – 0.878)	Substantial
⑧ Posterior middle	0.832	0.069(0.697 – 0.967)	Almost perfect
⑨ Posterior left	0.865	0.064(0.740 – 0.990)	Almost perfect
Total	0.817	0.025(0.768 – 0.866)	Almost perfect

Table 2. Comparison of general clinical data of HTZ and BTZ in MHD patients.

		HTZ (n = 20)	BTZ (n = 20)	F	P
Gender (n)	(male/female)	13 (65%)/7 (35%)	8 (40%)/12 (60%)		0.27
Age (years)		64.25 ± 10.36	65.5 ± 14.64	0.12	0.89
Height (cm)		164.00 ± 6.38	162.95 ± 6.68	0.13	0.88
Weight (Kg)		64.05 ± 8.25	59.40 ± 8.52	1.62	0.21
Primary disease (n)	Chronic Glomerular Disease	9	12		
	Diabetic nephropathy	7	5		
	Hypertensive nephropathy	1	2		
	Polycystic kidney	1	1		
	Else	2	0		

Table 3. Comparison of HTZ and BTZ laboratory indices in MHD patients.

Item	HTZ (*n*=20)	BTZ (*n*=20)	T/Z	P
BUN (mmol/l)	20.90 ± 4.79	20.89 ± 5.61	0.01	0.99
P (mmol/l)	1.55 ± 0.39	1.76 ± 0.52	−1.47	0.15
HB (g/l)	109.50(104.00,118.00)	110.00(103.50,118.00)	−0.31	0.76
ALB (g/l)	37.10(35.35,38.65)	36.5(33.93,38.85)	0.50	0.62
Ca (mmol/l)	2.27(2.19,2.34)	2.29(2.17,2.41)	−0.43	0.67
PTH (pg/ml)	149.50(79.75,432.00)	246.35(108.15,392.85)	−0.76	0.45
Kt/V	1.52 ± 0.24	1.73 ± 0.38	−2.09	0.04

Comparison of micro-inflammatory index levels

The statistical analysis demonstrated that hs-CRP, IL-6, and TNF-α levels were significantly higher in HTZ of MHD patients, compared to BTZ, with tshe differences being statistically significant ($p = 0.04$, $p = 0.042$, $p = 0.01$). Conversely, no significant differences were observed in NLR and PLR between the two groups of MHD patients (Table 4).

Characteristics of oral flora in MHD patients with different TCTs

ASV distribution Venn diagram

The analysis used the concept of ASVs to construct class OTUs, resulting in a distribution of 2744 ASVs for HTZ, 2538 ASVs for BTZ, and 3130 ASVs for DZZ. Notably, DZZ's saliva specimens contained significantly more ASVs than those of HTZ and BTZ, with BTZ having the fewest. The Venn Diagram analysis revealed 1167 ASVs common between HTZ and DZZ, 1577 unique to HTZ, and 1963 unique to DZZ. BTZ and DZZ shared 1147 ASVs, while HTZ and BTZ shared 1076, with 1672 and 1466 ASVs unique to each, respectively. The three groups – HTZ, BTZ, and DZZ – shared 851 ASVs. Preliminary evidence suggests notable differences in species composition of the oral flora among HTZ, BTZ, and the healthy DZZ population, indicating fewer species in MHD patients compared to healthy subjects. Greater differences in oral flora composition may exist between HTZ and BTZ compared to DZZ (Figure 3).

Diversity analysis of oral microbiome

Alpha diversity. Alpha diversity, which assesses microbial diversity within a sample, showed that both the Chao1 and Observed Species indices were significantly different between DZZ and the MHD patients (HTZ and BTZ) ($p < 0.05$), but no significant difference was found between HTZ and BTZ. The Shannon index was significantly higher in DZZ compared to BTZ ($p < 0.05$), whereas Simpson's index showed no significant differences among the three groups ($p > 0.05$). Overall, HTZ and BTZ exhibited a lower richness of oral flora compared to healthy controls, yet homogeneity was consistent across all groups. Additionally, the Goods coverage indices were close to 1, and dilution curves flattened with increased sequencing volume, affirming adequate sequencing depth and volume (Figures 4, 5).

Beta diversity. Beta diversity, reflecting differences in colony composition among samples, was assessed using an Unweighted Unifrac distance matrix for Principal Coordinates Analysis (PCoA) and Nonmetric Multidimensional Scaling (NMDS) analysis. The PCoA results showed that samples within each group clustered independently, indicating structural differences in the oral flora among the groups. Notably, HTZ showed significant separation from DZZ, suggesting greater differences in flora composition compared to BTZ. The NMDS analysis supported these findings, indicating closer similarities between HTZ and BTZ, and greater disparities with DZZ (Figure 6).

Species composition

The characteristic sequences of ASVs from three groups of saliva samples were annotated by comparison with the SILVA and NT-16S databases. We obtained species annotation results at six taxonomic levels – phylum, order, family, genus, and species – along with a table detailing the abundance of ASVs in each sample. This study focused on analyzing the colony composition and variability at the phylum and genus levels, which are well-defined and representative taxonomic levels. Results were visually presented using bar charts and stacked plots.

Table 4. Comparison of micro-inflammatory indicators of HTZ and BTZ in MHD patients.

Item	HTZ (*n* = 20)	BTZ (*n* = 20)	T/Z	P
IL-6 (pgmL)	75.86(64.94, 93.28)	56.68(48.19, 69.48)	2.03	0.042
TNF-α (pgmL)	6.82(4.04, 10.12)	4.03(2.43, 6.168)	3.22	0.001
hs-CRP (mg/)	3.69(2.63, 8.98)	1.58(0.79, 5.42)	2.06	0.04
NLR	3.34(2.66, 4.0)	3.31(2.40, 4.66)	0.257	0.797
PLR	137.53 (113.46, 198.34)	145.30 (116.85, 200.52)	−0.352	0.738

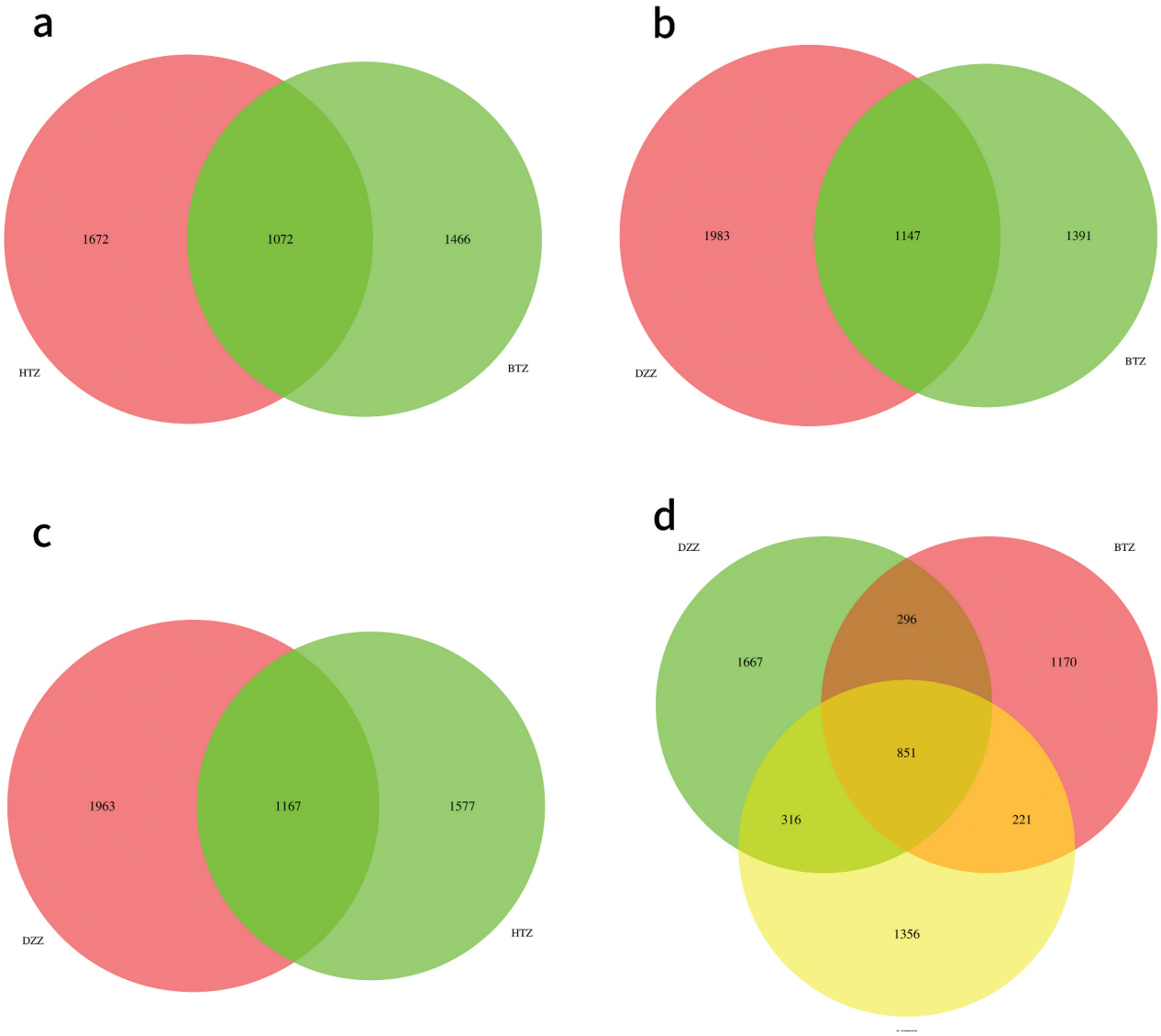


Figure 3. Venn diagram illustrating the distribution of OTUs among the different groups.

At the phylum level, the dominant phyla of oral flora in the HTZ, BTZ, and DZZ groups included Bacillota, Pseudomonadota, Bacteroidota, Actinobacteriota, and Fusobacteriota. Further statistical analysis showed that in MHD patients, BTZ had higher levels of Verrucomicrobiota, Desulfobacterota, Cyanobacteria, and Chloroflexota than HTZ. Additionally, the abundance of Desulfobacterota and Gemmatimonadota was lower in BTZ compared to DZZ ($p < 0.05$). In comparison to DZZ, MHD patients, especially those in the HTZ group, had significantly lower relative abundances of Desulfobacterota and Patescibacteria ($p < 0.05$) (Figures 7, 8).

At the genus level, the top 10 dominant genera of oral microbiome in the HTZ, BTZ, and DZZ groups were *Streptococcus*, *Neisseria*, *Prevotella*, *Haemophilus*, *Veillonella*, *Alloprevotella*, *Rothia*, *Porphyromonas*, *Fusobacterium*, and *Gemella*. Collectively, these genera accounted for more than 70% of the total relative abundance in each group. The HTZ showed a significantly higher abundance of *Prevotella*, *Megasphaera*, and *Escherichia-Shigella* compared to BTZ and DZZ ($p < 0.05$), while the abundance of unclassified *Desulfovibrionaceae* and *Ralstonia* was significantly lower ($p < 0.05$). Furthermore, genera such as *Actinobacillus*, *Peptostreptococcus*, and the *Lachnospiraceae_NK4A136_group* had significantly higher abundances in HTZ compared to BTZ, and genera like *Actinobacillus*, *Lautropia*, and *Corynebacterium* were significantly less abundant compared to DZZ ($p < 0.05$) (Figures 9, 10).

The relative abundance and degree of compositional similarity among samples were further clustered for each group based on the top 30 genera. A heat map using a color gradient reflected the similarities and differences in composition across multiple samples at each taxonomic level. Cluster analysis using dendrites showed that the BTZ and DZZ clusters had shorter branches, indicating more similarity in species abundance between these groups (Figure 11).

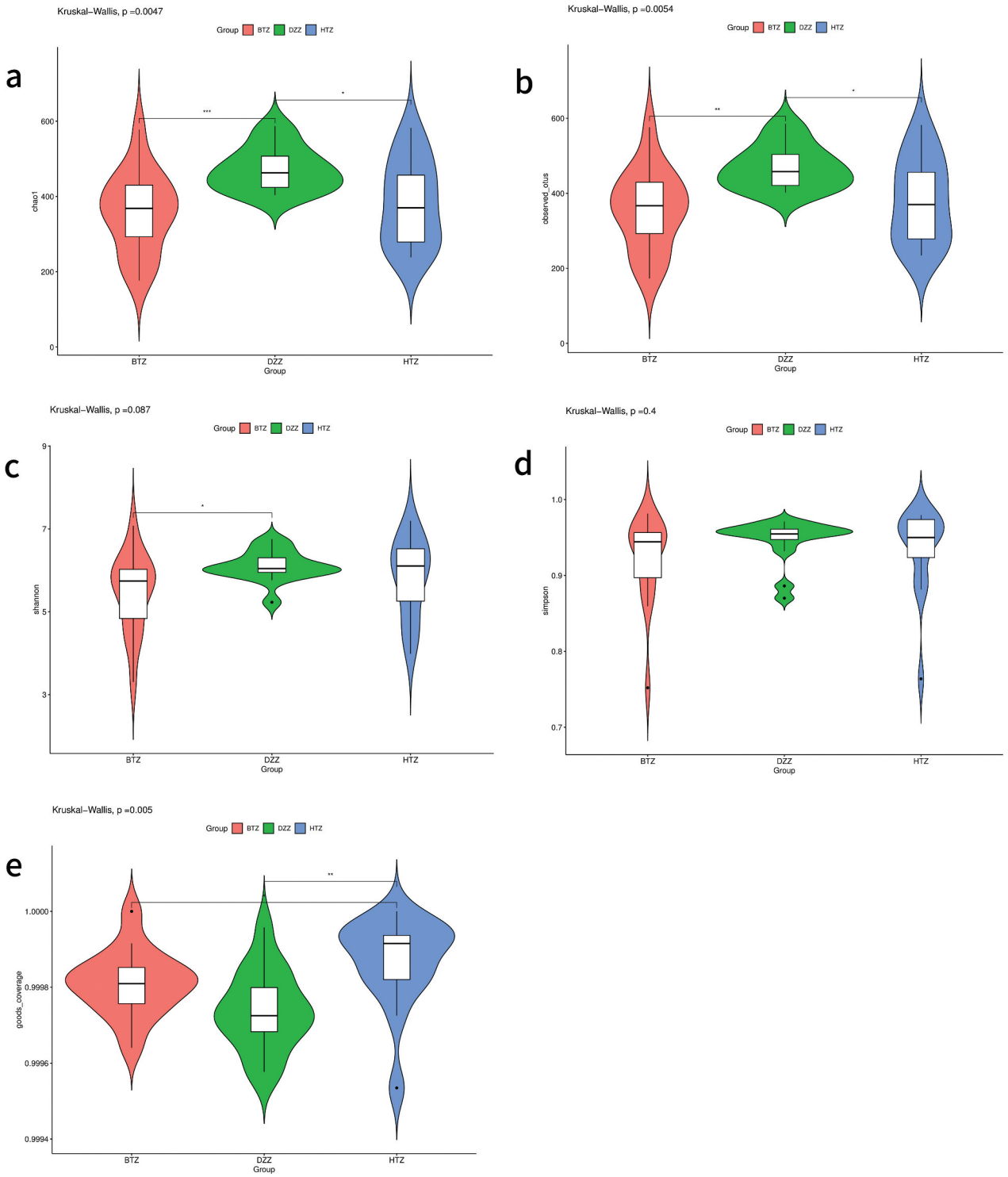


Figure 4. Alpha diversity analysis – violin plot.

Linear discriminant analysis (LDA) effect size (LEfSe)

The magnitude of LDA values in LEfSe analysis was utilized to identify species with significant differences in abundance between the HTZ and BTZ groups in MHD patients. Histograms displaying the distribution of LDA values highlighted a total of 19 species with notable differences across the HTZ, BTZ, and DZZ groups. Specifically, species such as *Prevotella*, *Escherichia Shigella*, *Enterobacteriaceae*, and five others were identified as marker species for HTZ. Meanwhile, 11 taxa including *Burkholderiaceae Lautropia*, *Streptococcus sp.*, and *Actinobacillus* along with 11 other flora were identified as markers for DZZ. Species like *Streptococcus pneumoniae*, *Bifidobacteriaceae*, *Bifidobacteriales*, and three others were marked as characteristic for BTZ (Figure 12).

The LEfSe analysis further revealed nine significant differential flora influencing the HTZ in MHD patients. These included *Prevotellaceae*, *Prevotella*, *Prevotella nanceiensis*, *Actinomycetaceae*, *Actinomycetales*, *Actinomyces*, *unclassified Actinomyces*, *Escherichia*

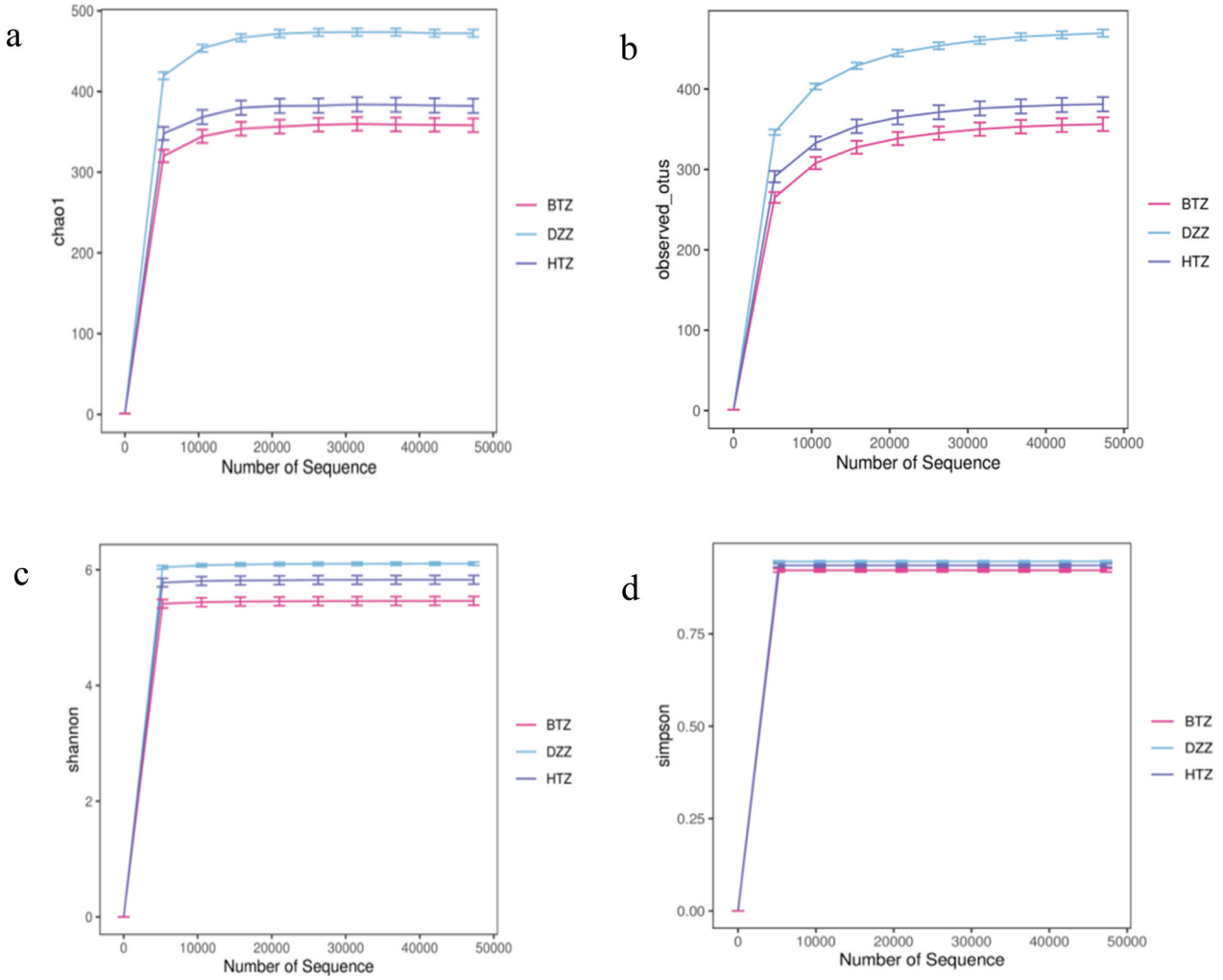


Figure 5. Alpha diversity analysis – rarefaction curve. Different colors in the figure represent different samples. The horizontal axis represents the number of sequences randomly selected, while the vertical axis represents the exponential value. The subplots (a-Chao1, b-observed_species, c-Shannon, d-Simpson) show different diversity indices.

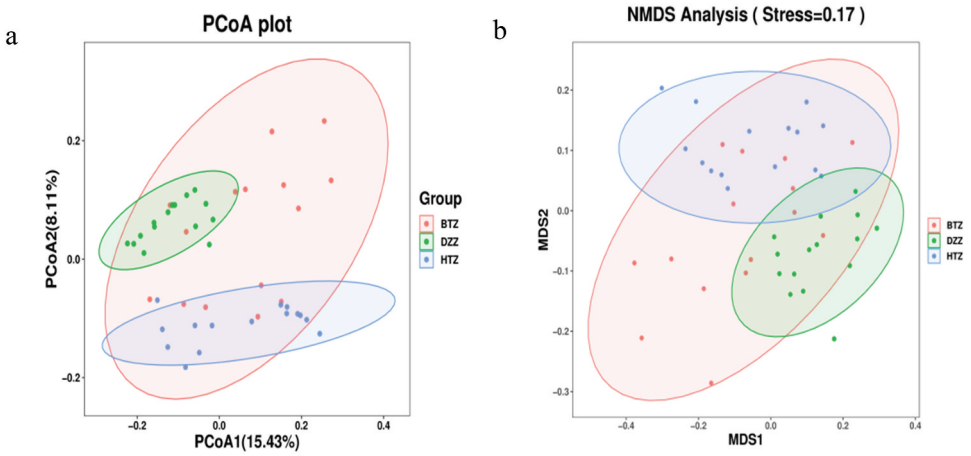


Figure 6. Beta diversity – PCoA and NMDS analysis. (a) presents the PCoA, where the proximity of the samples indicates a higher similarity in microbial composition and structure, suggesting smaller differences among them. The percentages on the horizontal and vertical axes represent the explanatory degree of the first and second axes for sample differences. (b) depicts the NMDS analysis, where the distance between points reflects the degree of difference between samples. Notably, when the stress value is less than 0.2, the two-dimensional point graph of NMDS is considered meaningful.

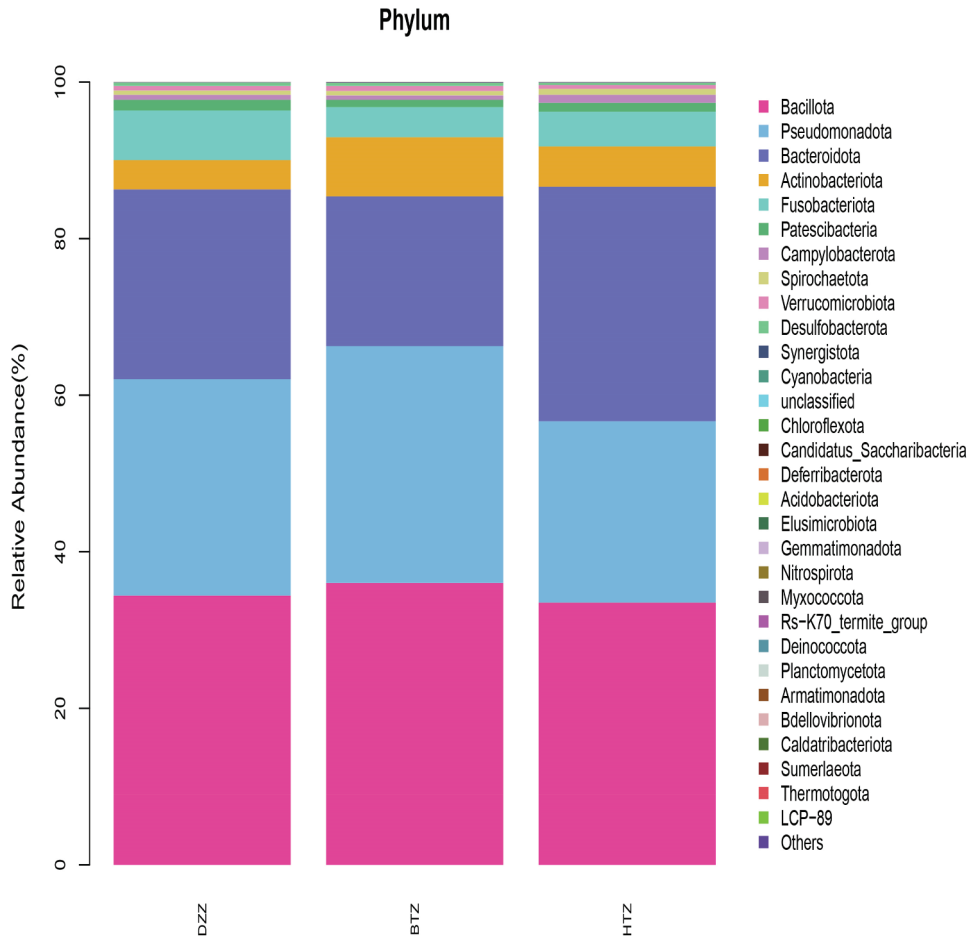


Figure 7. Phylum level species abundance.

Shigella, and *unclassified Escherichia Shigella*. In contrast, *Streptococcus sanguinis* was notably dominant in influencing the BTZ group (Figure 13).

Correlation between oral microbiome and micro-inflammatory indicators

Based on the species abundance table of oral microbiome in MHD patients, the top 30 genera at the genus level were selected. The Spearman tool was utilized to explore the relationships between the dominant oral flora and clinical indicators. The results revealed significant correlations: The levels of microinflammatory indicators IL-6 and TNF-α were positively correlated with the abundance of *Fusobacterium* ($r = 0.68, 0.67$; $p < 0.01$). TNF-α levels also showed a positive correlation with *Campylobacter* ($r = 0.52$; $p < 0.05$). In contrast, the abundance of *Streptococcus* was negatively correlated with both IL-6 and TNF-α levels ($r = -0.61, -0.60$; $p < 0.05$). Additionally, the abundances of *Actinobacillus* and *Megasphaera* were positively correlated with PLR values ($r = 0.58, 0.76$; $p < 0.01, 0.05$) (Figure 14).

Functional prediction

Based on the amplified sequencing data, the functional abundance of oral microbiome between HTZ and BTZ in MHD patients was predicted and analyzed. Using the PICRUSt2 and STAMP tools, the results of the KEGG pathways analysis (Figure 15) revealed significant functional differences: In comparison to BTZ, HTZ showed significantly enhanced roles in various microbial functions, including N-Glycan biosynthesis, Terpenoid backbone biosynthesis, Ribosome, Peptidases, Mismatch repair, DNA replication proteins, Homologous recombination, and Cell cycle – Caulobacter ($p < 0.05$). Conversely, a decreasing trend in functions was observed in Propanoate metabolism, Metabolism of xenobiotics by cytochrome P450, Drug metabolism – cytochrome P450, Glutathione metabolism, and the Secretion system ($p < 0.05$).

Discussion

The micro-inflammatory state (MIS), often triggered by non-pathogenic microbial infections, is essentially an immune inflammatory response that is prevalent among MHD patients [24]. Prompt identification and amelioration of this state can prevent secondary infections, disrupt the vicious cycle of MIA syndrome (consisting of malnutrition, inflammation, and atherosclerosis), enhance patient survival rates, and

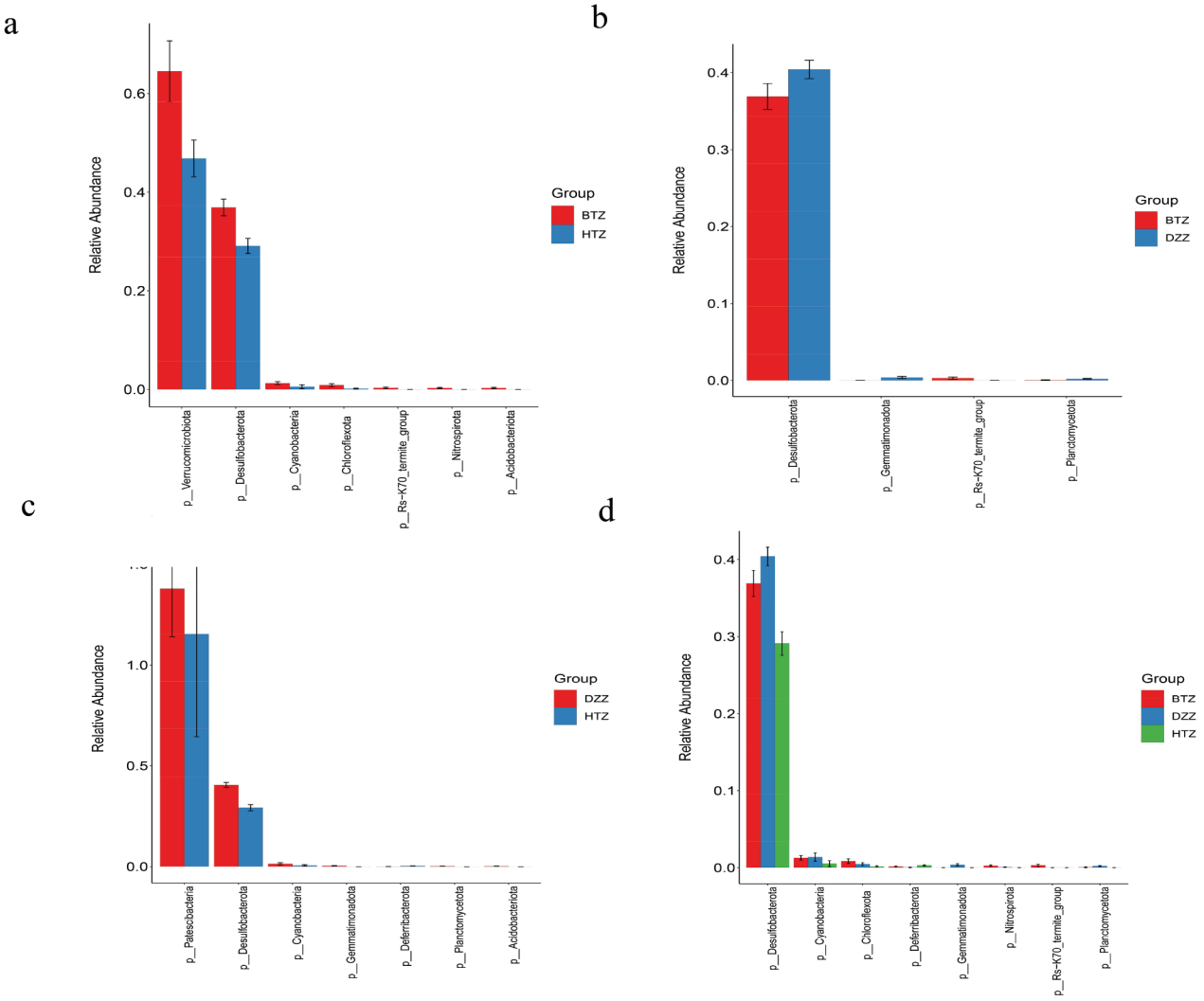


Figure 8. Differences in oral microbiome relative abundance at the phylum level among groups. Significant differences in the relative abundance of oral microbiome at the phylum level between groups are presented. The horizontal axis represents different species (arranged from left to right according to their abundance), and the vertical axis represents relative abundance. (a) BTZ vs HTZ (b) BTZ vs DZZ (c) DZZ vs HTZ (d) BTZ vs DZZ vs HTZ.

minimize related complications [25]. According to TCM principles, tongue manifestations offer valuable insights into bodily changes, serving as a key diagnostic tool for evaluating chronic conditions [26–28]. Tongue features reflect subtle alterations in internal organs, closely correlating with inflammatory reactions. Studies indicate that tongue coatings change with inflammation: a white coating signifies early inflammation, yellow denotes acute inflammation, and varying colors emerge during chronic inflammation. These observations suggest that tongue coatings mirror bodily infection and inflammatory states [29]. Prior research underscores the profound link between the oral microbiome and tongue diagnosis, hinting at the tongue-coating microbiome's potential as a biomarker for patient subtyping [30–32]. Investigations reveal distinct oral microbial community structures, compositions, and functions in MHD patients, closely tied to dialysis duration [33]. Additionally, there may be a connection between specific oral microbiome and CKD, as well as inflammatory kidney biomarkers [34]. Consequently, a potential relationship between microbiota and tongue signs exists. However, tongue features are often overlooked in microbiological and disease-focused studies. Therefore, this study aims to unravel the connections between TCT, MIS, and the oral microbiome in MHD patients, paving the way for advancements in clinical diagnosis and treatment.

In our study, 116 MHD patients were grouped according to the quantified scores of their TCT. We discovered that the BTZ and the HTZ comprised the highest percentages of patients, with BTZ accounting for 34.4% and HTZ for 31%. These were followed by the moderate tongue coating group (19.8%) and the slight tongue coating group (14.6%). We selected 20 patients from each of the BTZ and HTZ, who exhibited minimal TCT changes, to measure

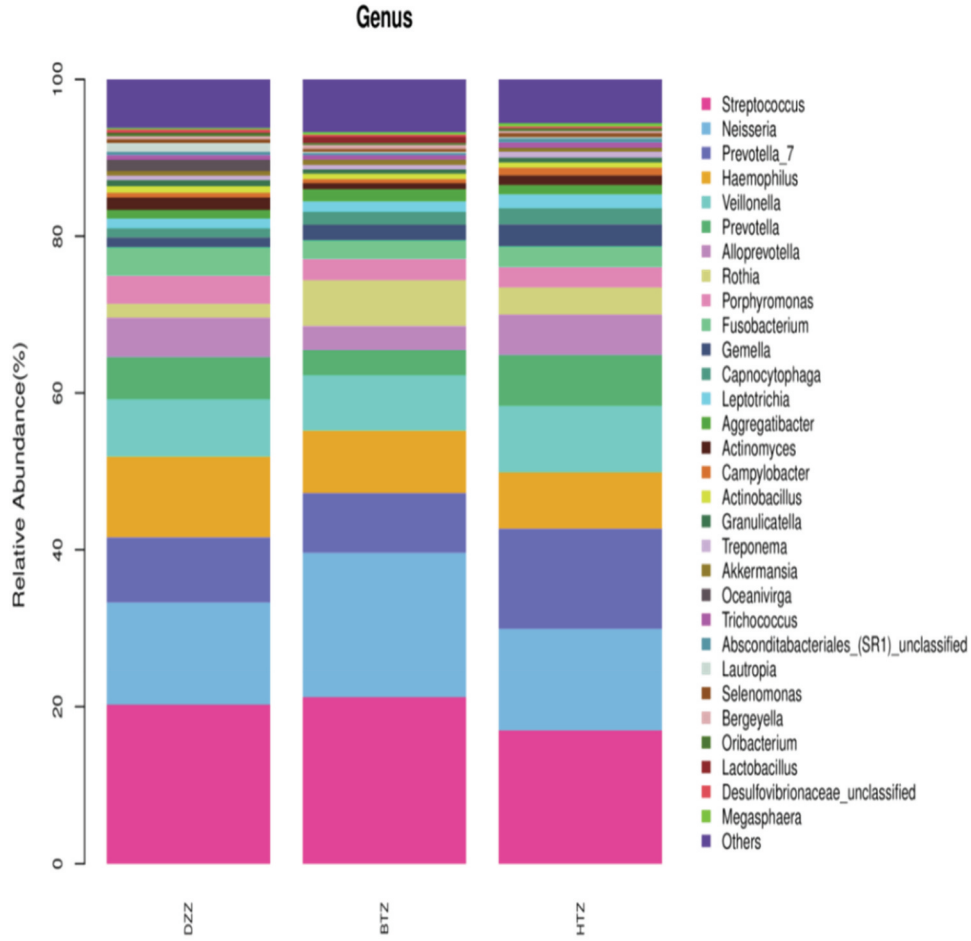


Figure 9. Genus level species. The top 10 dominant genera accounted for more than 70% of the total relative abundance in each group.

microinflammation markers. The results indicated that, compared to the BTZ, the HTZ had significantly elevated levels of hs-CRP, IL-6, and TNF-α ($p < 0.05$), suggesting a more pronounced MIS in the HTZ of MHD patients. This provides initial evidence of a correlation between TCT and MIS. Zhu Yunyun et al. [35] reported a strong correlation between tongue images in TCM and MIS in CKD, noting that a red tongue and thick tongue coating have diagnostic value for CKD MIS, which concurs with our findings. Another study [36] observed the progression of chronic gastritis, finding that gastritis severity gradually escalates from mild to severe as the TCT index increases. This suggests that a thick tongue coating may indicate inflammation progression. Combined with our findings, TCT appears to be an external indicator of inflammation. Hence, we believe that observing TCT could potentially serve as a fast, convenient, and noninvasive method for early MIS identification in MHD patients.

A study found [37] that there are differences in the composition of oral microorganisms between individuals with chronic gastritis who have greasy tongue coating and those who do not. To further investigate, our study conducted genetic sequencing on the saliva of healthy individuals and MHD patients with HTZ and BTZ. The results indicated that MHD patients possess a lower overall diversity of oral microorganisms compared to healthy individuals, along with a notable decrease in microbial species. Furthermore, the oral microbiome structure of MHD patients with HTZ and BTZ displayed a higher degree of similarity. Among the three groups examined, the prevalent phyla were Bacillota, Pseudomonadota, Bacteroidota, Actinobacteriota, and Fusobacteriota. It was observed that the abundance of Desulfobacterota was notably higher in healthy individuals. Moreover, the primary genera across the three groups encompassed *Streptococcus*, *Neisseria*, *Prevotella*, *Haemophilus*, *Veillonella*, *Alloprevotella*, *Rothia*, among others, which align with the known oral microbiome, confirming the findings of other oral ecology studies [38]. When compared to the healthy control group, MHD patients showed a significant decrease in the abundance of *Actinobacillus*, *Lautropia*, and *Peptostreptococcus* in their oral cavity, whereas the presence of *Megasphaera* and *Escherichia-Shigella* increased significantly. Despite the variations in the oral microbiome structure of MHD patients compared to healthy individuals at multiple taxonomic levels, it was evident that the primary dominant microbiome remained largely unchanged,

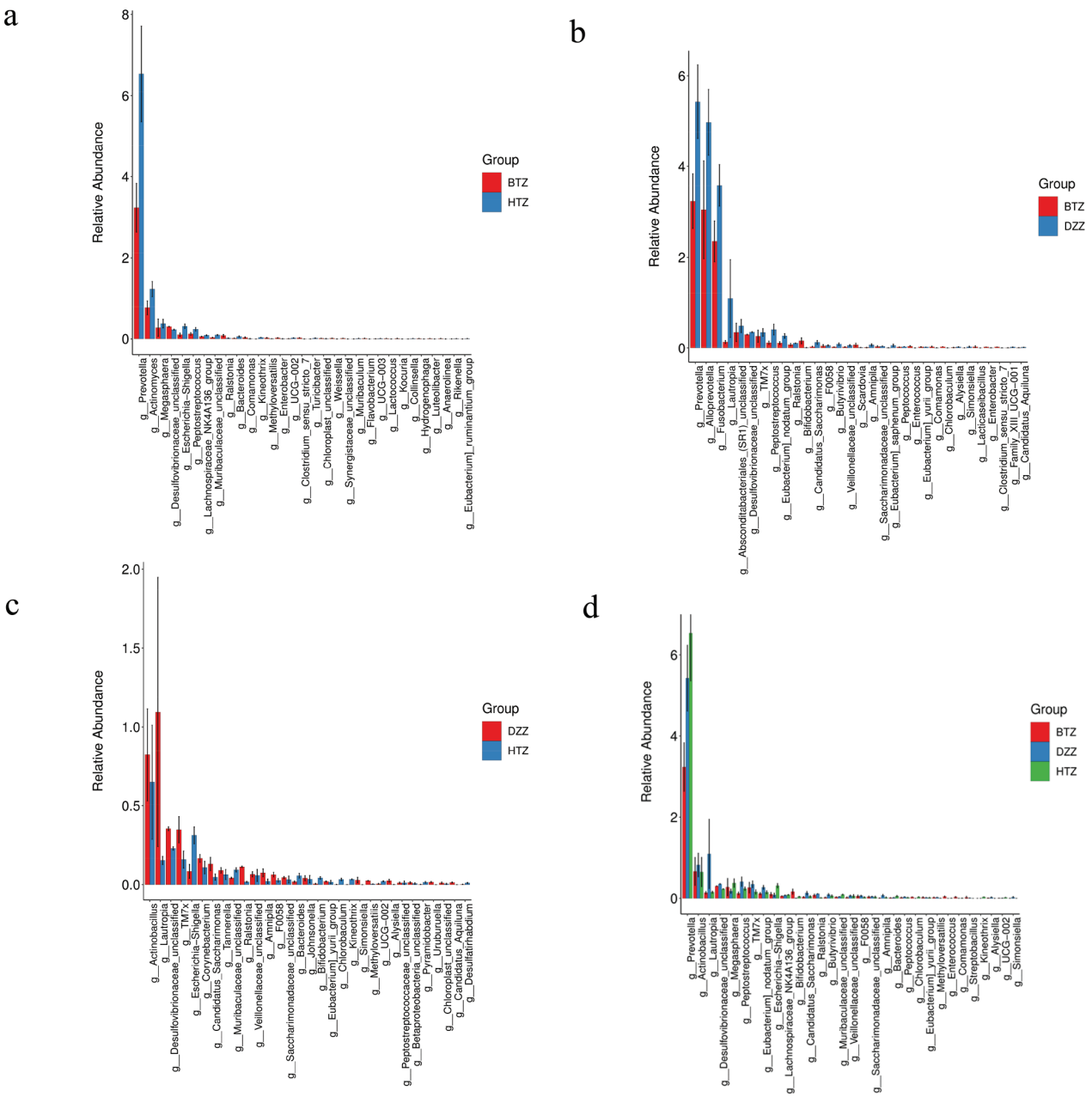


Figure 10. Differences in oral microbiome relative abundance at the genus level among groups. (a) BTZ vs HTZ (b) BTZ vs DZZ (c) DZZ vs HTZ (d) BTZ vs DZZ vs HTZ.

indicating a certain level of stability within the oral microecosystem. Upon further analysis between HTZ and BTZ in MHD patients, it was revealed that HTZ had a greater number of microbial species compared to BTZ. Although no significant difference in biodiversity was observed between the two, there were distinct differences in microbiota abundance at various taxonomic levels. Specifically, HTZ had a significantly higher abundance of *Prevotella*, *Megasphaera*, *Escherichia-Shigella*, *Actinobacillus*, and *Peptostreptococcus* compared to BTZ. Additionally, 19 species with notable differences were identified among the three groups, with five oral species, including *Prevotella*, *Escherichia-Shigella*, and Shigellaceae, emerging as potential marker species for HTZ. On the other hand, *Streptococcus pneumoniae*, *Bifidobacteriaceae*, and *Bifidobacteriales* appeared to be marker species for BTZ. It should be noted that the detection results may exhibit some margin of error due to the inherent limited accuracy of the V3-V4 region at the species level, particularly when dealing with closely related species such as *Pneumococcus* and *Streptococcus mitis*. Consequently, additional verification and confirmation of these results are crucial to further enhance the accuracy of the detection moving forward. Potential methods for such verification include full-length 16S rRNA gene sequencing or metagenomic sequencing.

In this study, we observed Verrucomicrobia, Desulfobacterota, Cyanobacteria, Chloroflexota, and several other atypical oral bacteria, including *Akkermansia*, *Oceanivirga*, *Trichococcus*, *Escherichia/Shigella*, and others. Generally, these bacteria are more prevalent in the natural environment, freshwater, and various other

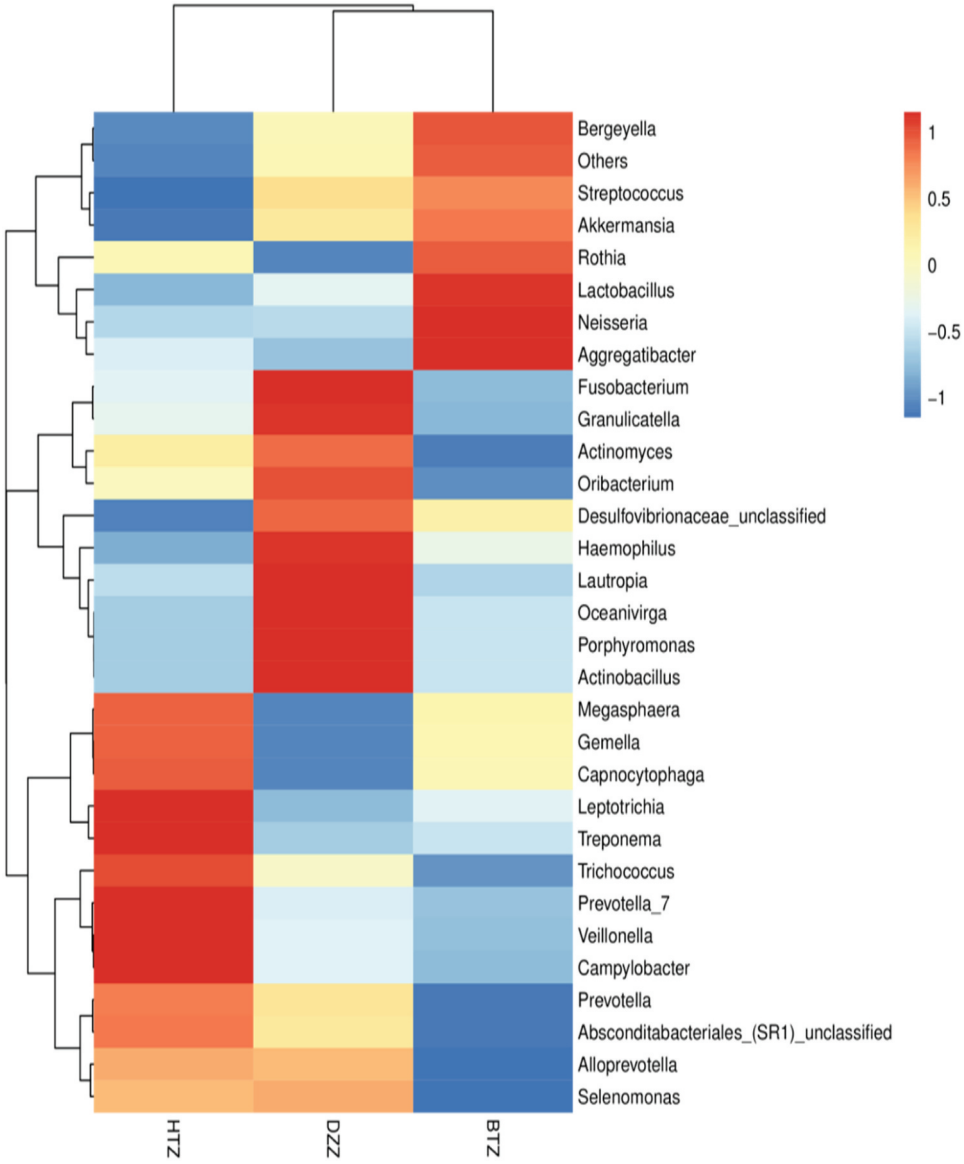


Figure 11. Heat map of species composition. The color gradient reflects the similarities and differences in the composition of multiple samples at each taxonomic level.

media. Considering that the composition of the oral microbiota is influenced by many factors, including diet, lifestyle, geographic location, and host health status, the presence of these atypical bacteria may reflect the complexity and dynamics of the oral microbiota [39,40]. Indeed, previous studies have reported the presence of Verrucomicrobia, Cyanobacteria, and *Trichococcus* in saliva samples [41–43], and one review has clearly listed Chloroflexota as part of the oral microbiota [44]. Desulfovibrio, Desulfomicrobium, Desulfobulbus, and other bacteria in the Desulfobacterota have also been reported in the mouths of healthy people [45]. *Escherichia/Shigella*, in particular, have been shown to be present in saliva, mouth, and faeces [46,47]. Therefore, we cannot rule out the possibility that these environmental bacteria become part of the oral microbiome under certain conditions. They may enter the mouth through external routes such as food or water, or they may represent a 'rare biosphere' in the oral microbiome – a population of bacteria that is usually low in abundance but can proliferate under certain conditions. For example, studies have shown a significant increase in Enterobacteriaceae in the mouths of patients with CKD compared to healthy controls, suggesting that the composition of the oral microbiota may be far more complex and diverse than expected [48].

To explore the relationship between oral microbiome and MIS, we conducted a deeper analysis of the oral microbial species differences between HTZ and BTZ in MHD patients. Our findings revealed an enrichment of the genera *Peptostreptococcus* and *Prevotella* in the oral cavity of HTZ patients. Studies have shown that certain anaerobic species within the genus *Peptostreptococcus* can stimulate cell proliferation and activate NF-κB (Nuclear factor-kappa B) of B cells through its surface protein. In turn, NF-κB triggers a proinflammatory response, linked to the progression of chronic inflammation and

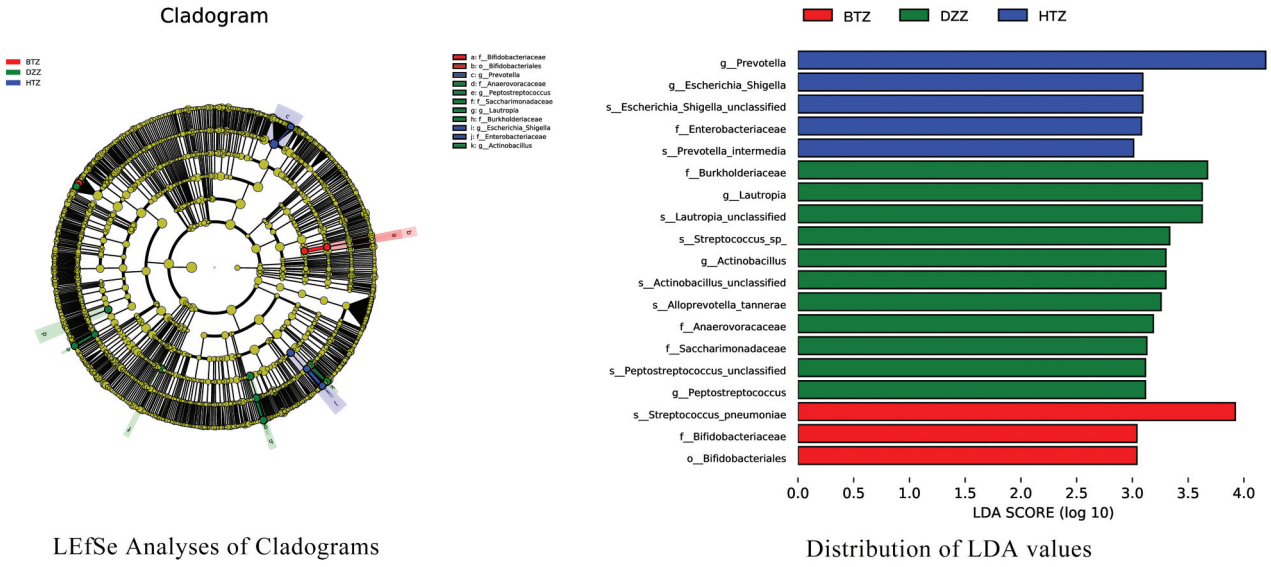


Figure 12. LEfSe differential analysis diagram in three groups. In the left panel (evolutionary branching diagram), different circles radiate from the inside to the outside, representing seven taxonomic levels: kingdom, phylum, class, order, family, genus, and species. Each node represents a taxonomic classification at the corresponding level, with the node circle size proportional to the relative abundance. Yellow nodes indicate no significant difference, while red/green nodes indicate that the species is significantly different in the corresponding comparison group. The right panel (LDA value distribution map) displays species with significantly different LDA values (>3). The color of the histogram represents different groups, and the length of the histogram represents the degree of influence of the significantly different species between different groups.

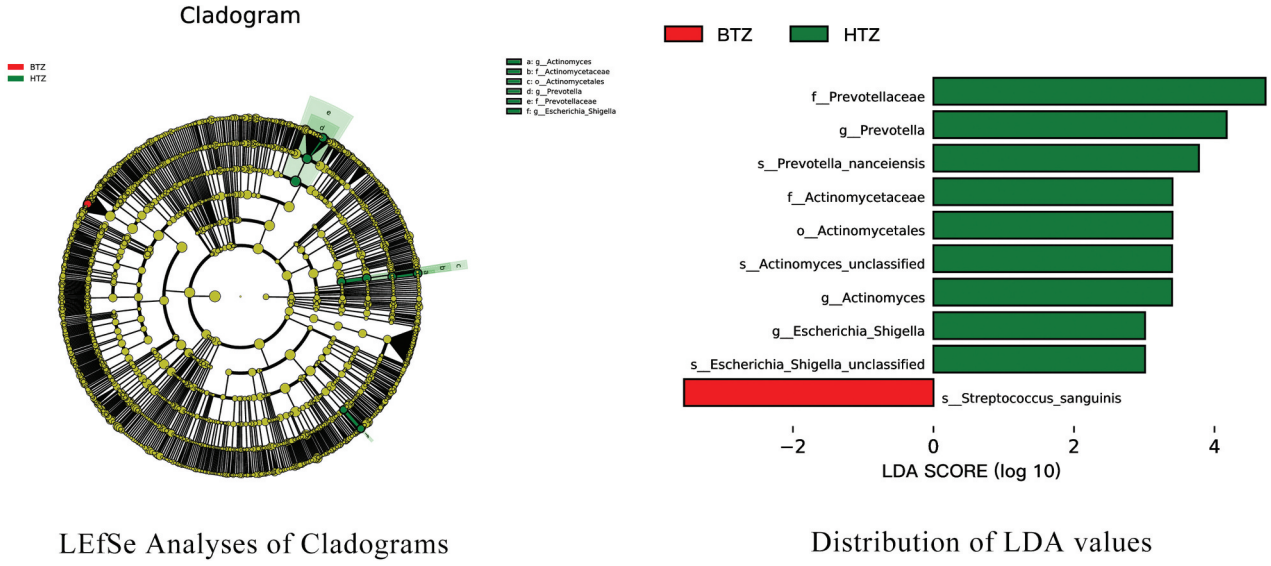


Figure 13. LEfSe differential analysis between HTZ and BTZ.

tumorigenesis [49]. Furthermore, the rise in opportunistic pathogens, such as specific species within the genus *Prevotella* and *Actinobacillus*, in the oral cavity of HTZ patients might explain the elevated inflammatory factors in these MHD patients. For instance, certain *Prevotella* species have been shown to predominate in periodontitis, intensifying inflammatory conditions by inducing inflammatory mediators like CCL20, IL-8, and IL-6 [50]. Additionally, oral flora translocation can impact the microecology's structure and function. Studies have indicated that colonization by specific *Prevotella* species in the intestine alters microbiota metabolism, including a reduction in short-chain fatty acids. This metabolic shift might lead to decreased intestinal IL-18 levels, thereby amplifying intestinal inflammation and potentially triggering a systemic autoimmune response [51]. Scholars postulate that the metabolic mechanism of oral microbiome inducing micro-inflammation might resemble that of intestinal microbiome. These flora can migrate to the respiratory or digestive tract, forming a new equilibrium with local microorganisms and constructing a biological barrier with host epithelial cells. An imbalance in oral microecology

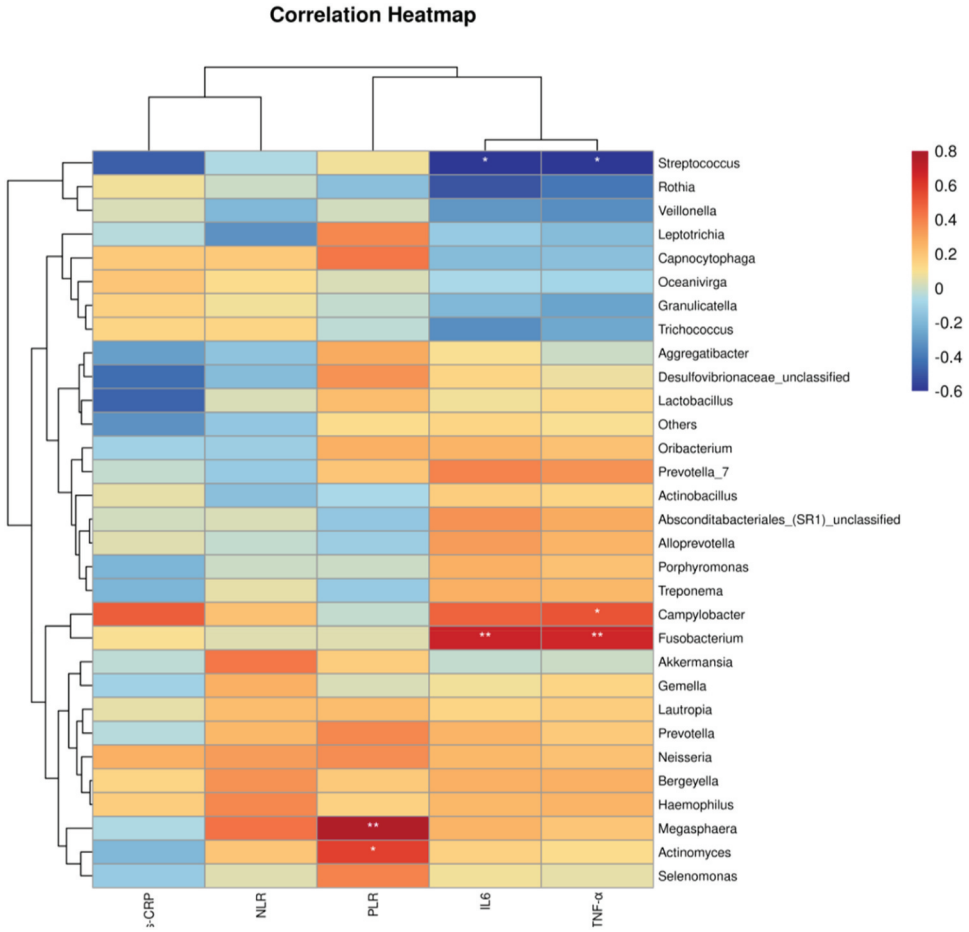


Figure 14. Cluster heatmap of the correlation between dominant oral microbiome and MIS. This heatmap displays the correlation coefficients between the dominant species in the oral microbiome and the MIS index. The color intensity and shade represent the strength and direction of the correlation, respectively.

can result in endotoxin production from cell walls and the disruption of epithelial integrity, ultimately promoting inflammation and tissue damage [52]. Meanwhile, correlation analysis revealed a negative association between the abundance of oral *Streptococcus* in MHD patients and serum IL-6 and TNF-α levels. According to research, *Streptococcus* can alleviate inflammatory reactions by reducing NF-κB in small intestinal epithelial cells, suppressing PPARγ transcription activity, and decreasing the production of the proinflammatory cytokine IL-8 [53]. Interestingly, our study observed a trend of decreasing oral *Streptococcus* abundance in MHD patients with HTZ, albeit non-significant statistically. This trend suggests that the reduction of oral *Streptococcus* might play a role in the micro-inflammatory response in these patients.

Based on the above studies, we found that MHD patients in the HTZ exhibited notably higher micro-inflammatory markers compared to the BTZ. Additionally, there were considerable alterations in the oral microbiome composition between the two groups, indicating potential differences in their functional capabilities. This study continued to conduct a differential analysis between the oral microbiome sequencing results of MHD patients in the HTZ and BTZ utilizing the KEGG bioinformatics analysis database.In comparison to the BTZ, the functional diversity of the oral microbiome in the HTZ showed an overall upward trend, particularly demonstrating significant enhancement in various microbial functions such as N-glycosyl biosynthesis, ribosome, peptidase, DNA replication proteins, mismatch repair (MMR), and homologous recombination (HR). Numerous studies have reported a close correlation between these metabolic pathways and signaling cascades with inflammation.It has been documented that changes in N-glycosylation can significantly affect leukocyte trafficking, promote a shift towards more pro-inflammatory effector functions in leukocytes, and initiate pro-inflammatory transformations in immunoglobulins and acute phase proteins (APP), ultimately leading to the development of various inflammatory

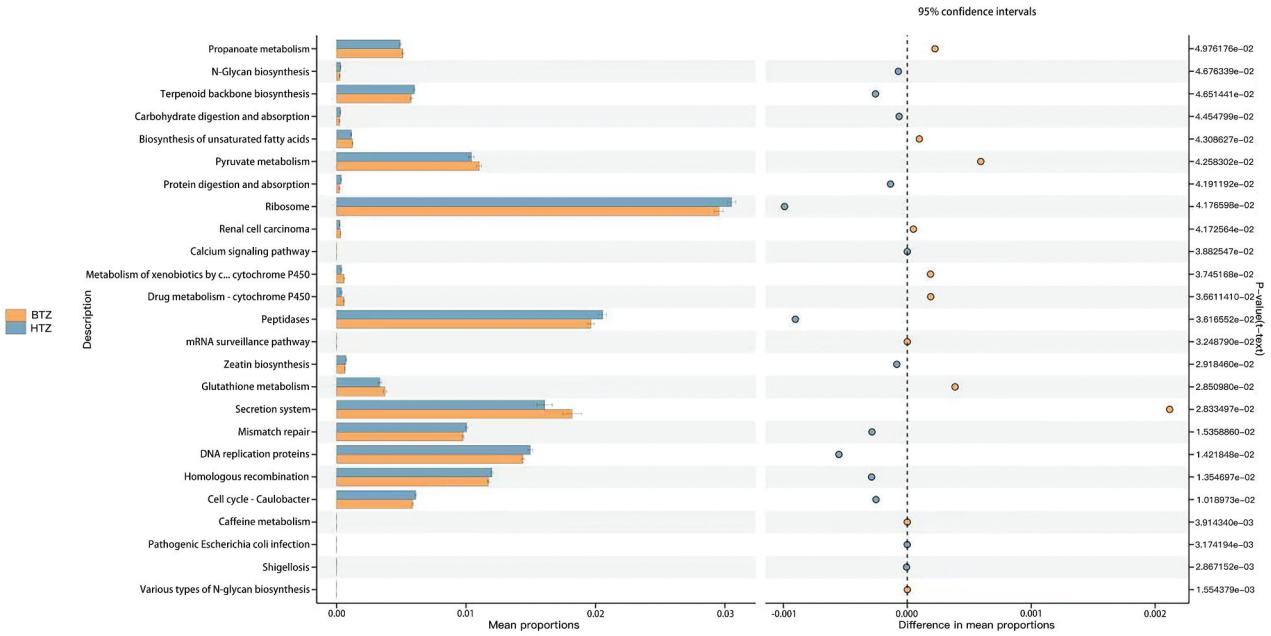


Figure 15. STAMP differential analysis diagram for functional prediction. This diagram displays the results of the STAMP differential analysis, which predicts the functional differences between different groups based on the metagenomic data. The x-axis represents the significance level of the difference, and the y-axis represents the different functional categories.

diseases [54]. For instance, Lu Xinxia's research on the role of N-glycosylation of immunoglobulin G (IgG) in lupus nephritis (LN) revealed that reduced sialylation, galactosylation, core fucosylation, and increased bifurcated N-acetylglucosamine (GlcNAc) may significantly influence LN development by enhancing IgG's pro-inflammatory responses [55]. Furthermore, Zhu Hongming and others found through experimentation that ribosomes have anti-inflammatory effects at the single-cell level. High levels of inflammatory cytokine expression may result from a high inflammation and low ribosome (HI-LR) cell type stagnation, or insufficient basal ribosome activity, and a further reduction in ribosome gene expression could enhance inflammatory cytokine expression [56]. Peptidases in organisms primarily act on hydrolyzing proteins or peptides, engaging in digestion, cellular signaling, apoptosis, and immune responses. Extensive research has demonstrated that various peptidases have pro-inflammatory roles. For example, tryptases produced by mast cells are central in inflammatory and IgE-mediated immediate hypersensitivity reactions. Carvalho and colleagues observed that tryptase levels in the abdominal lavage fluid (ALF) and serum of patients with acute appendicitis (AA) were significantly higher compared to controls, indicating tryptases' involvement in the allergic inflammatory reactions during AA onset [57]. Colotta and colleagues observed that various MMR proteins could regulate chronic inflammation by activating HIF-1α in response to inflammatory cytokines and reactive oxygen species (ROS) [58]. Additionally, alterations in the BER, HR, and NHEJ repair pathways are also closely associated with chronic inflammatory states [59].

Conclusion

In summary, our study initially reveals a correlation between TCT and MIS in MHD patients, with significantly higher micro-inflammation in patients with thick coating compared to those with thin coating. Therefore, TCT could potentially serve as one of the assessment indicators for MIS. Furthermore, we have observed significant disparities in the oral microbiome features between MHD patients and healthy individuals. Notably, MHD patients with different TCT also demonstrate remarkable differences in their flora structure and diversity. This suggests that TCT, to some extent, reflects changes in the composition and diversity of microorganisms in the body. Moreover, changes in the structure and function of the oral microbiome in MHD patients are associated with MIS. In patients with HTZ, we have noticed that the functional abundance of oral microbiome exhibits a significant increasing trend. Hence, we hypothesize that the occurrence of micro-inflammation in MHD patients with HTZ may be closely correlated with alterations in the structure and function of their oral microbiome. This finding lays a foundation for future research utilizing TCT as a predictive factor for microecological disorders in MHD patients.

Future directions

To further enhance the depth and breadth of research, future studies will focus on incorporating more tongue-related features, such as coating color. Additionally, there will be a concerted effort to expand sample sizes and diversify oral sample types. By utilizing more advanced sequencing technologies and integrating various database resources for cross-validation, we aim to comprehensively and deeply analyze the complex relationships between tongue features, micro-inflammation, and the oral microbiome, thereby uncovering their underlying mechanisms and interactions. Ultimately, these efforts will provide more solid data support and theoretical foundations for in-depth research in related fields.

Acknowledgments

The authors would like to express their gratitude to the Zhejiang Province TCM Science and Technology Programme and Hangzhou Hospital of Traditional Chinese Medicine for their financial support of this study. The authors thank Hangzhou Lianchuan Biotechnology Company for technical support of this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by Grants-in-Aid from Zhejiang Provincial Traditional Chinese Medicine Science and Technology Plan [project 2023ZL110 and project 2024ZL110]. Hangzhou TCM Hospital of Zhejiang Chinese Medical University.

Authors' contributions

YQ.Z. and XY.Z. wrote the main manuscript text; AP.Z., MQ.W. and B.L. were responsible for data statistics and analysis; H.L. and FG.Z. revised manuscript; RY. L. reviewed the manuscript and revised the final draft.

Abbreviations

MHD	maintenance haemodialysis
ESRD	end-stage renal disease
MIS	micro-inflammatory state
hs-CRP	high-sensitivity C-reactive protein
IL-6	interleukin-6
TNF-α	tumour necrosis factor-α
CKD	chronic kidney disease
TCM	Traditional Chinese Medicine
TCT	Tongue Coating Thickness
HDL	high-density lipoprotein
BTZ	thin-tongue coating group
HTZ	thick-tongue coating group
DZZ	the healthy control group
WBC	white blood cell count
NEU	neutrophil count
LYM	lymphocyte count
HB	haemoglobin
PLT	platelet count
SCR	Serum Creatinine
BUN	urea nitrogen
ALB	albumin
PTH	parathyroid hormone
NLR	Neutrophil-to-Lymphocyte Ratio
PLR	Platelet-to-Lymphocyte Ratio
ELISA	enzyme-linked immunosorbent assay
CTAB	cetyltrimethylammonium bromide
PCR	polymerase chain reaction
OUT	operational taxonomic unit
ASV	Amplicon Sequence Variant
LDA	Linear Discriminant Analysis
LEfSe	Linear discriminant analysis Effect Size
KEGG	Kyoto Encyclopedia of Genes and Genomes
MIA syndrome	malnutrition, inflammation, atherosclerosis
MMR	mismatch repair
HR	homologous recombination
APP	acute phase proteins
IgG	immunoglobulin G
LN	lupus nephritis
GlcNAc	N-acetylglucosamine
HI-LR	high inflammation and low ribosome
ALF	abdominal lavage fluid
AA	acute appendicitis
ROS	reactive oxygen species

Ethics approval and consent to participate

The study was ethically reviewed by the Research Ethics Committee of Hangzhou Hospital of TCM. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Consent for publication

Written informed consent for publication was obtained from all participants.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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References

[1] Chiu Y-W, Jiwakanon S, Lukowsky L, et al. An update on the comparisons of mortality outcomes of hemodialysis and peritoneal dialysis patients. Semin Nephrol. 2011;31(2):152–158. doi: 10.1016/j.semnephrol.2011.01.004

[2] Schindler R. Causes and therapy of microinflammation in renal failure. Nephrol Dial Transplant. 2004;19 Suppl 5):V34–40. doi: 10.1093/ndt/gfh1054
[3] Madsen T, Schmidt EB, Christensen JH. The effect of n-3 fatty acids on C-reactive protein levels in patients with chronic renal failure. J Ren Nutr. 2007;17 (4):258–263. doi: 10.1053/j.jrn.2007.03.003
[4] Taziki O, Lessan-Pezeshki M, Akha O, et al. The effect of low dose omega-3 on plasma lipids in hemodialysis patients. Saudi J Kidney Dis Transpl. 2007;18(4):571–576.
[5] Micallef MA, Munro IA, Garg ML. An inverse relationship between plasma n-3 fatty acids and C-reactive protein in healthy individuals. Eur J Clin Nutr. 2009;63(9):1154–1156. doi: 10.1038/ejcn.2009.20
[6] Liepa GU, Basu H. C-reactive proteins and chronic disease: what role does nutrition play? Nut In Clin Prac. 2003;18(3):227–233. doi: 10.1177/0115426503018003227
[7] Magee P, Pearson S, Allen J. The omega-3 fatty acid, eicosapentaenoic acid (EPA), prevents the damaging effects of tumour necrosis factor (TNF)-alpha during murine skeletal muscle cell differentiation. Lipids Health Dis. 2008 Jul 18;7(1). doi: 10.1186/1476-511X-7-24
[8] Zimmermann J, Herrlinger S, Pruy A, et al. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int. 1999;55 (2):648–658. doi: 10.1046/j.1523-1755.1999.00273.x
[9] Liu T, Wang P. The impact of yishen bupi prescription on the quality of life and micro-inflammatory state of patients with renal anemia undergoing maintenance hemodialysis. Liaoning J Tradit Chin Med. 2021;48(3):78–81. doi: 10.13192/j.issn.1000-1719.2021.03.023
[10] Kotanko P, Carter M, Levin NW. Intestinal bacterial microflora–a potential source of chronic inflammation in patients with chronic kidney disease. Nephrol Dial Transplant. 2006;21(8):2057–2060. doi: 10.1093/ndt/gfl281
[11] Li F, Wang M, Wang J, et al. Alterations to the gut microbiota and their correlation with inflammatory factors in chronic kidney disease. Front Cell Infect Microbiol. 2019 Jun 12;9(206). doi: 10.3389/fcimb.2019.00206
[12] Ahmadmehrabi S, Wilson Tang WH. Hemodialysis-induced cardiovascular disease. Semin Dial. 2018;31 (3):258–267. doi: 10.1111/sdi.12694
[13] Anastasi JK, Currie LM, Kim GH. Understanding diagnostic reasoning in TCM practice: tongue diagnosis. Altern Ther Health Med. 2009;15(3):18–28.
[14] Shi B, Xu H, Xie J. A brief discussion on the significance of tongue diagnosis in traditional Chinese medicine for the treatment of chronic gastritis. Guoyi Forum. 2008;6:22–24.
[15] Kim J, Han G-J, Choi B-H, et al. Development of differential criteria on tongue coating thickness in tongue diagnosis. Complement Ther Med. 2012;20 (5):316–322. doi: 10.1016/j.ctim.2012.03.004
[16] Xu G. Objective study on tongue manifestations in patients with chronic renal failure at different stages of renal function. Chin J Integr Tradit & West Nephrol. 2006;9:530–531.
[17] Ding J, Wang Y, Xia Y. Observation of tongue manifestations and their clinical significance in 162 patients with chronic renal insufficiency. Gansu J Tradit Chin Med. 1994;2:18–19.
[18] Ma J. Clinical observation on the correlation between tongue manifestations and renal function damage in chronic renal failure. Shaanxi J Tradit Chin Med. 2008;10:1322–1324.
[19] Lin R, HangYing Y, JunYan Q. Characteristic analysis of blood lipids, CRP, and hs-CRP in 50 patients with chronic glomerulonephritis and greasy tongue coating. Chin J Integr Tradit & West Nephrol. 2014;15(6):536–537.
[20] Shimizu T, Ueda T, Sakurai K. New method for evaluation of tongue-coating status. J Oral Rehabil. 2007;34 (6):442–447. doi: 10.1111/j.1365-2842.2007.01733.x
[21] Shimada Y. The relationship between tongue coating and Qi-blood-water imbalance. Jpn J Orient Med. 1994;44 (5):135.
[22] Shimada Y, Furuta K, Shibahara N, et al. The relationship between tongue coating and qi-blood-water as well as spleen-stomach imbalance. Jpn J Orient Med. 1995;45(4):841–847.
[23] Fei Z, Gu Y. Mirror of health: tongue diagnosis in Chinese medicine. Beijing: People's Medical Publishing House; 2007.
[24] Kaysen GA. The microinflammatory state in uremia: causes and potential consequences. J Am Soc Nephrol. 2001;12(7):1549–1557. doi: 10.1681/ASN.V1271549
[25] Yao Q, Axelsson J, Stenvinkel P. Chronic systemic inflammation in dialysis patients: an update on causes and consequences. Asaio J. 2004;50(6):lii–lvii. doi: 10.1097/01.mat.0000147958.87989.eb
[26] Kim J, Son J, Jang S, et al. Availability of tongue diagnosis system for assessing tongue coating thickness in patients with functional dyspepsia. Evid Based Complement Alternat Med. 2013;2013:1–6. doi: 10.1155/2013/348272
[27] Lee T-C, Lo L-C, Wu F-C, et al. Traditional Chinese medicine for metabolic syndrome via TCM pattern differentiation: tongue diagnosis for predictor. Evid Based Complement Alternat Med. 2016;2016 (1):1971295. doi: 10.1155/2016/1971295
[28] Tomooka K, Saito I, Furukawa S, et al. Yellow Tongue coating is associated with diabetes mellitus among Japanese non-smoking men and women: the toon health study. J Epidemiol. 2018;28(6):287–291. doi: 10.2188/jea.JE20160169
[29] Yuanying S, Zhijun L, Xinsheng R, et al. Traditional Chinese medicine diagnosis and treatment of systemic inflammatory response syndrome. China TCM Emergencies. 2005;4:340–341.
[30] Ye J, Cai X, Yang J, et al. Bacillus as a potential diagnostic marker for yellow tongue coating. Sci Rep. 2016 Aug 31;6(1). doi: 10.1038/srep32496
[31] Lu H, Ren Z, Li A, et al. Tongue coating microbiome data distinguish patients with pancreatic head cancer from healthy controls. J Oral Microbiol. 2019 Jan 28;11(1 1563409. doi: 10.1080/20002297.2018.1563409
[32] Zhao Y, Gao X, Guo J, et al. Helicobacter pylori infection alters gastric and tongue coating microbial communities. Helicobacter. 2019;24(2):e12567. doi: 10.1111/hel.12567
[33] Duan X, Chen X, Gupta M, et al. Salivary microbiome in patients undergoing hemodialysis and its associations with the duration of the dialysis. BMC

Nephrology. [2020 Sep 29];21(1):414. doi: 10.1186/s12882-020-02009-y
[34] Hu J, Iragavarapu S, Nadkarni GN, et al. Location-specific oral microbiome possesses features associated with CKD. Kidney Int Rep. 2017 Sep 8;3(1):193–204. doi: 10.1016/j.ekir.2017.08.018
[35] Zhu Y, Zhou R, Chen X, et al. Correlation between tongue manifestations in traditional Chinese medicine and micro-inflammatory state in chronic kidney disease. Zhejiang J Tradit Chin Med. 2019;54(1):15. doi: 10.13633/j.cnki.zjtcm.2019.01.008
[36] Zhang Z, Zhengguang DU, Bei GU, et al. Correlation between objective image recognition of tongue diagnosis and endoscopic classification of chronic gastritis. J Chin Med. 2013;41(2):119–121. doi: 10.19664/j.cnki.1002-2392.2013.02.049
[37] Li F, Zhao J, Qian P, et al. Analysis of oral microbial fingerprint patterns in patients with chronic gastritis and greasy tongue coating. Chin J Integr Tradit & West Med. 2012;32(10):1331–1335.
[38] Yamashita Y, Takeshita T. The oral microbiome and human health. J Oral Sci. 2017;59(2):201–206. doi: 10.2334/josnusd.16-0856
[39] Leão I, de Carvalho TB, Henriques V, et al. Pseudomonadota in the oral cavity: a glimpse into the environment-human nexus. Appl Microbiol Biotechnol. 2023;107(2–3):517–534. doi: 10.1007/s00253-022-12333-y
[40] Wang J, Feng J, Zhu Y, et al. Diversity and biogeography of human oral saliva microbial communities revealed by the earth microbiome project. Front Microbiol. 2022 Jun 13;13(931065). doi: 10.3389/fmicb.2022.931065
[41] Woodall CA, Hammond A, Cleary D, et al. Oral and gut microbial biomarkers of susceptibility to respiratory tract infection in adults: a feasibility study. Heliyon. 2023 Jul 28;98 e18610. doi: 10.1016/j.heliyon.2023.e18610
[42] Yang Z, Cai T, Li Y, et al. Oral microbial communities in 5-year-old children with versus without dental caries. BMC Oral Health. 2023 Jun 16;23(1):400. doi: 10.1186/s12903-023-03055-2
[43] Liu F, Sheng J, Hu L, et al. Salivary microbiome in chronic kidney disease: what is its connection to diabetes, hypertension, and immunity? J Transl Med. 2022 Sep 4;20(1):387. doi: 10.1186/s12967-022-03602-5
[44] Perera M, Al-Hebshi NN, Speicher DJ, et al. Emerging role of bacteria in oral carcinogenesis: a review with special reference to perio-pathogenic bacteria. J Oral Microbiol. 2016 Sep 26;8(1):32762. doi: 10.3402/jom.v8.32762
[45] Deo PN, Deshmukh R. Oral microbiome: unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122–128. doi: 10.4103/jomfp.JOMFP_304_18
[46] Jiao J, Zheng Y, Zhang Q, et al. Saliva microbiome changes in thyroid cancer and thyroid nodules patients. Front Cell Infect Microbiol. 2022 Aug 11;12:989188. doi: 10.3389/fcimb.2022.989188
[47] Troci A, Zimmermann O, Esser D, et al. B-cell-depletion reverses dysbiosis of the microbiome in multiple sclerosis patients. Sci Rep. 2022 Mar 8;12(1):3728. doi: 10.1038/s41598-022-07336-8
[48] Costa CFFA, Merino-Ribas A, Ferreira C, et al. Characterization of oral *Enterobacteriaceae* prevalence and resistance profile in chronic kidney disease patients undergoing peritoneal dialysis. Front Microbiol. 2021 Dec 14;12(736685). doi: 10.3389/fmicb.2021.736685
[49] Long X, Wong CC, Tong L, et al. Peptostreptococcus anaerobius promotes colorectal carcinogenesis and modulates tumour immunity. Nat Microbiol. 2019;4(12):2319–2330. doi: 10.1038/s41564-019-0541-3
[50] Jiang S, Xie S, Lv D, et al. Alteration of the gut microbiota in Chinese population with chronic kidney disease. Sci Rep. 2017 Jun 6;7(1):1 2870. doi: 10.1038/s41598-017-02989-2
[51] Iljazovic A, Roy U, Gálvez EJC, et al. Perturbation of the gut microbiome by Prevotella spp. enhances host susceptibility to mucosal inflammation. Mucosal Immunol. 2021;14(1):113–124. doi: 10.1038/s41385-020-0296-4
[52] Li Y, Cui J, Liu Y, et al. Oral, tongue-coating microbiota, and metabolic disorders: a novel area of interactive research. Front Cardiovasc Med. 2021 Aug 20;8. doi: 10.3389/fcvm.2021.730203
[53] Couvigny B, De Wouters T, Kaci G, et al. Commensal streptococcus salivarius modulates PPARγ transcriptional activity in human intestinal epithelial cells. PLOS ONE. 2015 May 6;10:5 e0125371. doi: 10.1371/journal.pone.0125371
[54] Radovani B, Gudelj I. N-Glycosylation and Inflammation; the not-so-sweet relation. Front Immunol. 2022 Jun 27;13:893365. doi: 10.3389/fimmu.2022.893365
[55] Lu X, Wang L, Wang M, et al. Association between immunoglobulin G N-glycosylation and lupus nephritis in female patients with systemic lupus erythematosus: a case-control study. Front Immunol. 2023 Sep 21;14(1257906). doi: 10.3389/fimmu.2023.1257906
[56] Zhu H, Chen J, Liu K, et al. Human PBMC scRNA-seq-based aging clocks reveal ribosome to inflammation balance as a single-cell aging hallmark and super longevity. Sci Adv. 2023;9(26):eabq7599. doi: 10.1126/sciadv.abq7599
[57] Carvalho N, Carolino E, Ferreira M, et al. Tryptase in acute appendicitis: unveiling allergic connections through compelling evidence. Int J Mol Sci. 2024 Jan 3 1645. 29;25. doi: 10.3390/ijms25031645
[58] Colotta F, Allavena P, Sica A, et al. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis. 2009;30(7):1073–1081. doi: 10.1093/carcin/bgp127
[59] Sahan AZ, Hazra TK, Das S. The pivotal role of DNA repair in infection mediated-inflammation and cancer. Front Microbiol. 2018 Apr 11;9(663). doi: 10.3389/fmicb.2018.00663