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Original Article

Human oral microbiota dysbiosis may be a novel non-invasive indicator of erectile dysfunction

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KEYWORDS
Erectile dysfunction;
Indicator;
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Abstract *Background/purpose:* Dysbiosis of the oral microbiota has been associated with various human diseases, however, the relationship between the oral microbiota and erectile dysfunction (ED) remains unexplored. This study aimed to investigate the connection between the oral microbiota and ED, ultimately identifying a novel non-invasive indicator for ED.
Materials and methods: We recruited 74 male patients diagnosed with erectile dysfunction and 38 men with normal sexual function to serve as the control group. We compared the overall conditions and clinical data between the two groups. Oral samples were collected from the subjects, followed by 16S rRNA sequencing. We assessed the differences in oral microbiota between the two groups. We analyzed the correlation between oral microbiota and clinical indicators, ultimately constructing ten machine learning prediction models based on the oral microbiota data.
Results: There were distinct differences in the oral microbiota at both the phylum and genus levels between the two groups. Furthermore, significant differences in both α-diversity and β-diversity were observed between the two groups. The different oral microbiota between the groups were correlated with certain clinical indicators. We constructed ten machine learning prediction models, among which the Light Gradient Boosting Machine (LGBM) model demonstrated superior performance, achieving a relatively higher area under the curve (0.711), accuracy (0.733), sensitivity (0.784), specificity (0.636) and F1-score (0.795).

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Conclusion: An dysbiosis in oral microbiota may have a potential connection to ED. This dysbiosis could serve as an indicator for assessing the risks associated with the occurrence and progression of ED.

Introduction

Erectile dysfunction (ED) is defined as the inability of a male to consistently achieve and maintain an adequate penile erection necessary for a satisfactory sexual experience.[1] ED is a common sexual dysfunction that significantly affects patients' self-confidence and their relationships with partners. The global prevalence rate of ED is notably high. Risk factors associated with ED include advanced age, obesity, sedentary lifestyle, smoking, diabetes, hypertension, hyperlipidemia, hyperuricemia, hyperhomocysteinemia, cardiovascular diseases, hyperthyroidism, vitamin D deficiency and depression.[1,2] As a psychosomatic condition, the pathogenesis of ED is multifaceted and its clinical diagnosis and treatment face certain limitations, particularly due to the absence of specific biomarkers. Identifying an indicator that reflects the intricate and complex physiological changes in ED patients would significantly enhance the diagnosis, treatment and prevention of this condition in clinical practice. The gut microbiota serves as a valuable indicator in this context. Maintaining the balance of the gut microbiota is crucial for sustaining both physical and mental health. An dysbiosis in the gut microbiota may lead to a range of adverse effects on health and can even result in various diseases. Our previous studies have demonstrated notable differences in the gut microbiota between healthy individuals and ED patients, suggesting a correlation between gut microbiota dysbiosis and ED, gut microbiota composition may be an indicator of erectile dysfunction.[3]

The mouth serves as the primary entry point for microorganisms into the human body and is the second largest habitat for these organisms, following the gut.[4] The oral cavity is home to a diverse array of microorganisms, including bacteria, fungi and viruses, with bacteria being the predominant component. The oral microbiota plays a vital role in maintaining both oral and overall health. The pathogenic mechanisms of oral microbiota primarily occur through three pathways: (1) local colonization, where an increase in oral pathogens can lead to periodontitis; (2) remote transfer, as the oral cavity is interconnected with the digestive and respiratory systems. The oral microbiota can migrate directly to distal sites, causing disease; additionally, periodontitis resulting from dysbiosis of the oral microbiota can compromise the periodontal epithelial barrier. Due to the high vascularization of the oral cavity, bacteria can enter the bloodstream and disseminate throughout the body, leading to bacteremia; (3) the modulation of metabolic pathways and immune functions within the human body.[5–7] Recent studies have demonstrated that dysbiosis in oral microbiota is linked to various diseases, including inflammatory bowel disease, cancer, cardiovascular diseases, Parkinson's disease, Alzheimer's disease, multiple sclerosis, migraines, depression, anxiety, diabetes, rheumatoid arthritis and premature birth.[8–12] Oral microbiota can serve as indicators of the health and disease status of the human body, offering significant potential for disease prevention, diagnosis and treatment.[8] This study aimed to investigate the alterations in the oral microbiota of patients with erectile dysfunction and to explore the potential mechanisms by which these changes may impact male erectile function.

Materials and methods

Recruitment of study subjects

We recruited 74 male patients diagnosed with ED and 38 men with normal erectile function. The study was reviewed and approved by the Ethics Committees of the Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University. All participants signed consent forms. The general conditions and clinical data of the subjects were gathered through electronic medical records and questionnaires. The subjects' levels of depression and anxiety were assessed using the beck depression inventory (BDI) and the beck anxiety inventory (BAI), respectively. Sleep quality was evaluated using the pittsburgh sleep quality index (PSQI), while erectile function was measured with the international index of erectile function-5 (IIEF-5). A score of 21 or less in IIEF-5 was identified as ED patients; conversely, a score greater than 21 was identified as men with normal erectile function. The inclusion criteria for participants in this study were as follows: (1) individuals who had completed middle school or attained a higher level of education; (2) individuals who had engaged in regular sexual activity with a single, stable, heterosexual partner for a minimum duration of six months; (3) individuals exhibiting normal development of the male reproductive system. Participants were excluded from the study based on any of the following criteria: (1) individuals who had received antibiotics, probiotics, hormones or any other treatment or inspection that may affect the oral microbiome within the previous three months; (2) individuals with autoimmune diseases, malignant tumors or infectious diseases; (3) individuals with active oral infections or a history of oral disease treatment within the past three months.

Oral sample collection and storage

The subjects were prohibited from brushing their teeth for 24 h prior to sampling. In the morning, they fasted and rinsed

their mouths with clean water before the procedure. A sterile swab was first inserted into the left side of the mouth, ensuring that the swab head made full contact with the inner mucosa of the left cheek. The swab was moved up and down with appropriate pressure and rotated 20 times. The same method was then applied to the right side of the mouth. After sampling, the sterile cryopreservation tube was opened, and the swab was placed inside. The swab was then broken along the crease, the cap was tightened, and the sample was labeled with the sample number, name and date. The sample was temporarily stored at −20 °C and subsequently transferred to a −80 °C freezer for long-term storage on the same day.

DNA extractions

DNA was extracted from various samples using the CTAB method in accordance with the manufacturer's instructions. This reagent, specifically designed to isolate DNA from trace amounts of samples, has demonstrated effectiveness in preparing DNA from most bacterial species. Nuclear-free water served as a blank control. The total DNA was eluted in 50 μL of elution buffer and stored at −80 °C until measurement by polymerase chain reaction (PCR).

Polymerase chain reaction amplification and 16S rRNA gene sequencing

DNA quantification was performed using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). Total DNA was amplified via PCR employing the universal primers 341F and 805R (341F: 5′-CCTACGGGNGGCWGCAG-3′; 805R: 5′-GACTACHVGGGTATCTAATCC-3′). The PCR protocol was conducted according to the methodology described by Chen et al.[13] The Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA) were used to assess the size and concentration of the amplicon library, respectively. Finally, the libraries were sequenced on an Illumina NovaSeq 6000 platform (PE250).

S rRNA gene sequencing data analysis

The analysis of 16S rRNA gene sequencing data was performed following the methodology outlined by Zhao et al.[14] The QIIME2 was used to calculate the alpha and beta diversities. The alpha diversity of the oral microbiota composition was assessed using the chao1 index, goods coverage index, observed operational taxonomic units (OTUs) index and shannon index. The beta diversity of the oral microbiota was evaluated through principal coordinates analysis (PCoA). Our analysis primarily utilized the jaccard and unweighted unifrac distance matrices. The relative abundance was employed for bacterial taxonomy. Wilcoxon test was applied to identify differentially abundant genera, with significance set at $P < 0.05$. Additionally, we employed linear discriminant analysis (LDA) effect size (LEfSe) with a threshold of 3.0 for the LDA.

Clinical characteristics statistical analysis

We utilized SPSS 23.0 statistical software for data processing and analysis. We performed normality tests for each group of continuous variables. For measurement data that adhered to a normal distribution, results were expressed as $X \pm S$, and comparisons between the two groups were conducted using the t-test. Conversely, for measurement data that did not conform to a normal distribution, values were represented as M (P25, P75), with comparisons between the two groups conducted using the non-parametric Mann–Whitney U test. A p-value of less than 0.05 was deemed statistically significant.

Machine learning prediction model development

In this study, we utilized the Boruta algorithm for variable selection, which is a feature selection method grounded in random forests. The dataset was partitioned into two subsets: a training set comprising 70 % of the data and a test set consisting of the remaining 30 %. We employed ten machine learning classifiers to construct predictive models, including adaptive boosting (AdaBoost), categorical boosting (CatBoost), gradient boosting decision tree (GBDT), k-nearest neighbors (KNN), LGBM, logistic regression (LR), naive bayes (NB), random forest (RF), support vector machine (SVM) and eXtreme gradient boosting (XGBoost). Each model underwent five-fold cross-validation to ensure robustness and reliability. We assessed the predictive performance of each model on the test set by plotting the area under the receiver operating characteristic (ROC) curve (AUC) and the confusion matrix.

Results

The demographic and clinical data of participants

There were no significant differences in demographic characteristics, including age, height, weight and body mass index (BMI), between the two groups. Furthermore, the serum levels of interleukin-1β (IL-1β), Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), trimethylamine N-oxide (TMAO), 5-hydroxytryptamine (5-HT), along with various biochemical indicators were compared between the two groups (Table 1). We found that the serum levels of aspartate transaminase (AST), alanine aminotransferase (ALT), urea (UREA), creatinine (CREA), total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) did not show significant differences between the two groups, whereas the level of uric acid (UA) was higher in the ED group than in the control group. The total scores of the BDI, BAI and PSQI were significantly lower in the control group compared to the ED group.

Oral microbiota composition

The chao1 index, goods coverage index, observed OTUs index and shannon index were used to reflect the α-diversity, which reflected the diversity and uniformity of species between the two groups. We found that the Chao1

Table 1 Clinical characteristics of enrolled patients and healthy controls.

Parameter	Control group (n = 38)	ED group (n = 74)	t/Z	*P*-value
Age (years)	31.00 (29.00,33.00)	31.00 (29.00,36.00)	−0.814	0.416
Height (m)	1.73 (1.71,1.78)	1.72 (1.70,1.77)	−1.215	0.224
Weight (kg)	76.15 ± 14.29	75.04 ± 11.68	0.437	0.663
BMI (kg/m2)	24.85 ± 4.19	24.92 ± 3.83	−0.089	0.929
AST (u/l)	18.50 (15.50,24.50)	19.00 (16.00,23.50)	−0.037	0.970
ALT (u/l)	32.50 (23.00,45.75)	25.00 (17.50,44.00)	−0.227	0.821
UREA (mmol/l)	5.12 ± 1.19	5.15 ± 1.20	−0.112	0.911
CREA (umol/l)	80.77 ± 11.38	78.75 ± 11.12	0.784	0.435
UA (umol/l)	358.46 ± 79.51	398.13 ± 72.96	−2.310	**0.023***
TC (mmol/l)	4.74 ± 0.83	4.82 ± 1.02	−0.367	0.714
TG (mmol/l)	1.17 (0.75,1.70)	1.33 (1.04,1.86)	−0.909	0.364
HDL (mmol/l)	1.24 (1.12,1.43)	1.24 (1.12,1.43)	−0.209	0.834
LDL (mmol/l)	2.54 (2.31,3.18)	2.74 (2.29,3.54)	−0.583	0.560
IL-1β (pg/ml)	44.19 (39.95,47.36)	43.76 (41.71,46.61)	−0.877	0.381
IL-6 (ng/l)	21.77 (21.36,23.37)	22.92 (20.91,24.22)	−1.251	0.211
TNF-α (ng/l)	492.37 (408.81,512.66)	515.85 (445.07,576.95)	−0.645	0.519
TMAO (pg/ml)	165.28 (150.34,178.49)	148.96 (130.37,160.90)	−1.121	0.262
5-HT (ng/l)	399.03 (367.91,425.79)	393.18 (368.30,424.62)	−0.424	0.672
BDI	4.50 (1.75,12.75)	11.00 (4.50,17.00)	−2.740	**0.006****
BAI	30.00 (27.50,39.25)	35.00 (29.00,41.00)	−2.994	**0.003****
PSQI	2.50 (2.00,4.25)	5.00 (3.00,7.00)	−2.829	**0.005****
IIEF-5	22.00 (22.00,24.00)	14.00 (7.50,16.00)	−8.671	**0.000*****

Abbreviations: BMI, body mass index; AST, aspartate transaminase; ALT, alanine aminotransferase; UREA, ureophil; CREA, creatinine; UA, uric acid; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TMAO, trimetlylamine oxide; 5-HT, 5-hydroxytryptamine; BDI, beck depression inventory; BAI, beck anxiety inventory; PSQI, pittsburgh sleep quality index; IIEF-5, the international index of erectile function-5.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

index, goods coverage index, observed OTUs index and shannon index exhibited significant differences between the ED group and the control group, with statistical significance indicated by $P < 0.05$ (Fig. 1A—D). We employed jaccard and unweighted unifrac distance matrices to perform PCoA, allowing us to assess β-diversity. The compositional variation distinguishing the two groups was validated by PERMANOVA analysis based on unifrac data. This analysis revealed the compositional differences in microbial community between the two groups (Fig. 1E and F).

We compared the relative abundance of critical oral microbiota at both the phylum and genus levels between the two groups. At the phylum level, our findings indicate that the relative abundance of Verrucomicrobiota, Desulfobacterota and Deferribacterota were significantly higher in the control group than in the ED group (Fig. 2A and B). At the genus level, Our findings indicate that the relative abundance of Campylobacter was significantly higher in the ED group compared to the control group. Conversely, the control group exhibited a significantly higher relative abundance of Lactobacillus, Escherichia-Shigella, Clostridia_UCG-014_unclassified, Lachnospiraceae_NK4A136_group, Atopobium, Saccharimonadaceae_unclassified, Muribaculaceae_unclassified, Gardnerella, Bacteroides, Cardiobacterium, Johnsonella, Lachnospiraceae_unclassified, Akkermansia, Ligilactobacillus, Firmicutes_unclassified, Bosea, Bacilli_unclassified, UCG005, Megasphaera, Bifidobacterium, Faecalibacterium, Clostridiales_unclassified, Klebsiella, Gracilibacteria_unclassified, HT002, Dubosiella, Achromobacter, Allobaculum and Ruminococcus when compared to the ED group (Fig. 2C and D). To identify differentially abundant bacteria between the two groups, we utilized LEfSe, setting the LDA threshold at 3.0. Our findings were presented in Fig. 2E and F.

Associations of the oral microbiota with clinical indicators

We conducted a Spearman correlation heatmap analysis at the genus level to investigate the relationship between the oral microbiota and clinical indicators (Fig. 3). Our findings indicate that the oral microbiota, which exhibited relatively higher relative abundance in the normal group, had a negative correlation with serum levels of IL-1β, IL-6, TMAO and 5-HT, as well as scores on the BDI and PSQI. Conversely, the relative abundance of Campylobacter, which was elevated in the ED group, showed a positive correlation with these clinical indicators.

Prediction model development and comparison of erectile dysfunction based on the oral microbial markers

We employed the Boruta algorithm for feature selection, identifying the top 20 genera as modeling variables.

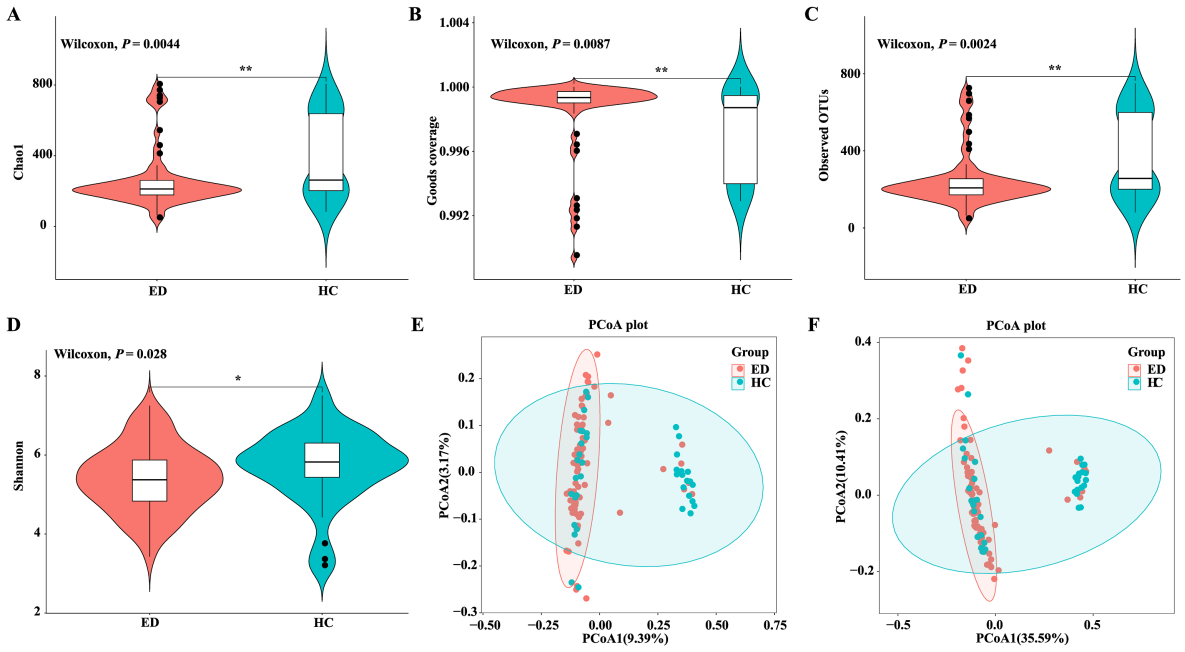

Figure 1 Comparison of α-diversity and β-diversity between the control group and ED group. (A) Chao1 index was compared between the ED group and the control group, $P = 0.0044$. (B) Goods coverage index was compared between the ED group and the control group, $P = 0.0087$. (C) Observed OTUs index was compared between the ED group and the control group, $P = 0.0024$. (D) Shannon index was compared between the ED group and the control group, $P = 0.028$. (E–F) The composition of the microbial community in the ED group was different from that of the control group base on jaccard and unweighted unifrac distance matrices respectively.

Subsequently, we developed ten machine learning (ML) models to predict the risk of ED. The receiver operating characteristic (ROC) curves and confusion matrices for each model are presented in Figs. 4–5. The LR model demonstrated the highest area under the curve (AUC) at 0.726, followed closely by the NB model at 0.720, the RF model at 0.715, the LGBM model at 0.711 and the CatBoost model at 0.707. The KNN model achieved an AUC of 0.690, while the SVM model had an AUC of 0.677. The XGBoost model recorded an AUC of 0.673, the GBDT model had an AUC of 0.646 and the AdaBoost model achieved an AUC of 0.626 (Table 2). Notably, five models exhibited an AUC greater than 0.7: CatBoost, LGBM, LR, NB and RF. Furthermore, we calculated the accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and F1-score for all models. Although the LGBM model did not have the highest AUC, it exhibited the best accuracy, sensitivity, specificity and F1-score. When considering these metrics holistically, the LGBM model demonstrated the best overall predictive performance.

Discussion

The oral microbiome is a crucial component of the human microbiome, interrelated and mutually influential with microbiomes from other body regions, thereby forming a complex microbial ecosystem. Oral dysbiosis is characterized by significant alterations in the composition and function of the microbial community, which tends to favor the dominance of pathogenic bacteria while excluding commensal species. This dysbiosis is associated not only with oral diseases but also with numerous systemic conditions, including cardiovascular diseases, respiratory diseases and endocrine and metabolic disorders. These systemic diseases are recognized as risk factors for ED.[5,6,15] Periodontal disease, resulting from changes in the oral microbiome, is linked to the onset and progression of cardiovascular diseases and metabolic syndrome. Notably, these conditions share common pathophysiological mechanisms with ED, such as endothelial dysfunction and chronic inflammation.[16] Our research indicates significant differences in the oral microbiome between healthy individuals and patients with ED. Alpha diversity, which refers to the diversity within a group and is primarily used to reflect the richness and evenness of species, and beta diversity, which refers to the differences in species composition among groups, were both observed to be significantly different between the two groups.

Oral microbiota dysbiosis can lead to endothelial dysfunction, which plays a critical role in the process of penile erection. Many patients with ED exhibit vascular endothelial damage. Endothelial nitric oxide synthase (eNOS) is responsible for producing nitric oxide (NO), a key factor in initiating and maintaining penile erection. Furthermore, endothelial dysfunction can impair the synthesis and release of NO, reduce its bioavailability, accelerate its degradation, ultimately contributing to the development of ED.[17]

Oral microbiota dysbiosis can promote atherosclerosis. Patients with periodontal disease exhibit elevated levels of serum total cholesterol and low-density lipoprotein.

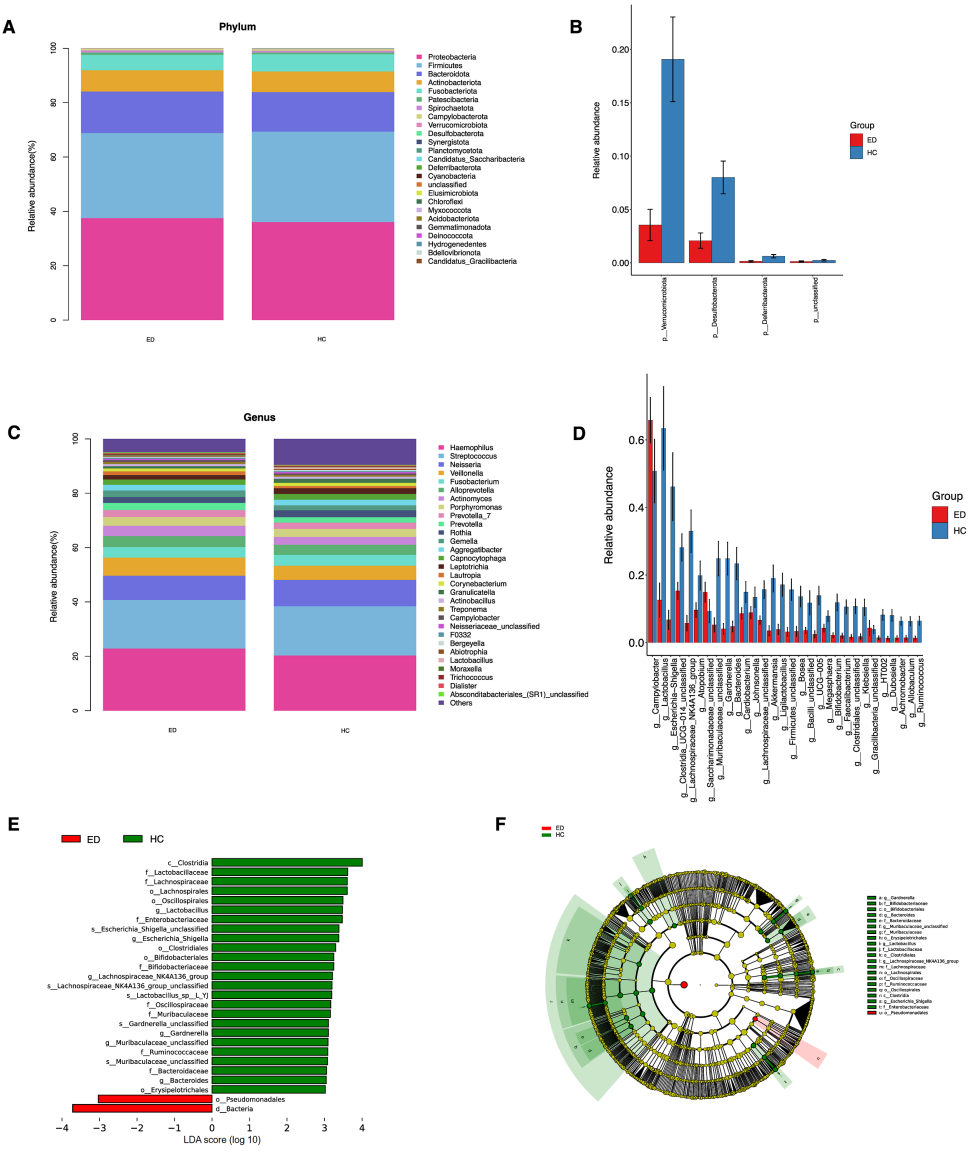

Figure 2 Comparisons of the relative abundances of the major oral microbiota between the two groups. (A) The relative abundance of oral microbiota on phylum level was different between the two groups. (B) The column chart of top 4 species with significant difference on phylum level between the two groups. (C) The relative abundance of oral microbiota on genus level was different between the two groups. (D) The column chart of top 30 species with significant difference on genus level between the two groups. (E) Histogram of the LDA scores for differentially abundant species between the two groups. The LDA scores >3.0 were listed. (F) Cladogram showing the most differentially abundant bacterial taxa and their relationship identified by LEfSe between the two groups. Red bars indicated taxa were enrichment in the ED group, while green bars indicated taxa were enrichment in the control group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Lipopolysaccharides derived from periodontal disease pathogens may facilitate the development of atherosclerosis through multiple mechanisms.[17] Periodontitis is recognized as a significant risk factor for cardiovascular diseases and atherosclerosis.[18] IL-6 has been identified as an independent risk factor for the rupture of atherosclerotic plaques and coronary artery disease, with significantly higher levels of IL-6 observed in patients suffering from chronic periodontitis.[19] Additionally, elevated levels of trimethylamine oxide (TMAO) are recognized as an independent risk factor for atherosclerosis. Increased serum TMAO levels can contribute to vascular inflammation and damage to endothelial and smooth muscle cells within the spongy tissue, ultimately leading to the development of ED.[3] Our findings indicate that the relative abundance of Campylobacter in the oral cavity is positively correlated with serum TMAO levels. Oral Campylobacter is suggested to be associated with cardiovascular diseases.[20]

Oral microbiota dysbiosis can trigger inflammatory responses. The disruption of the periodontal epithelial barrier allows pro-inflammatory mediators to enter the bloodstream. Elevated levels of C-reactive protein (CRP), IL-1β

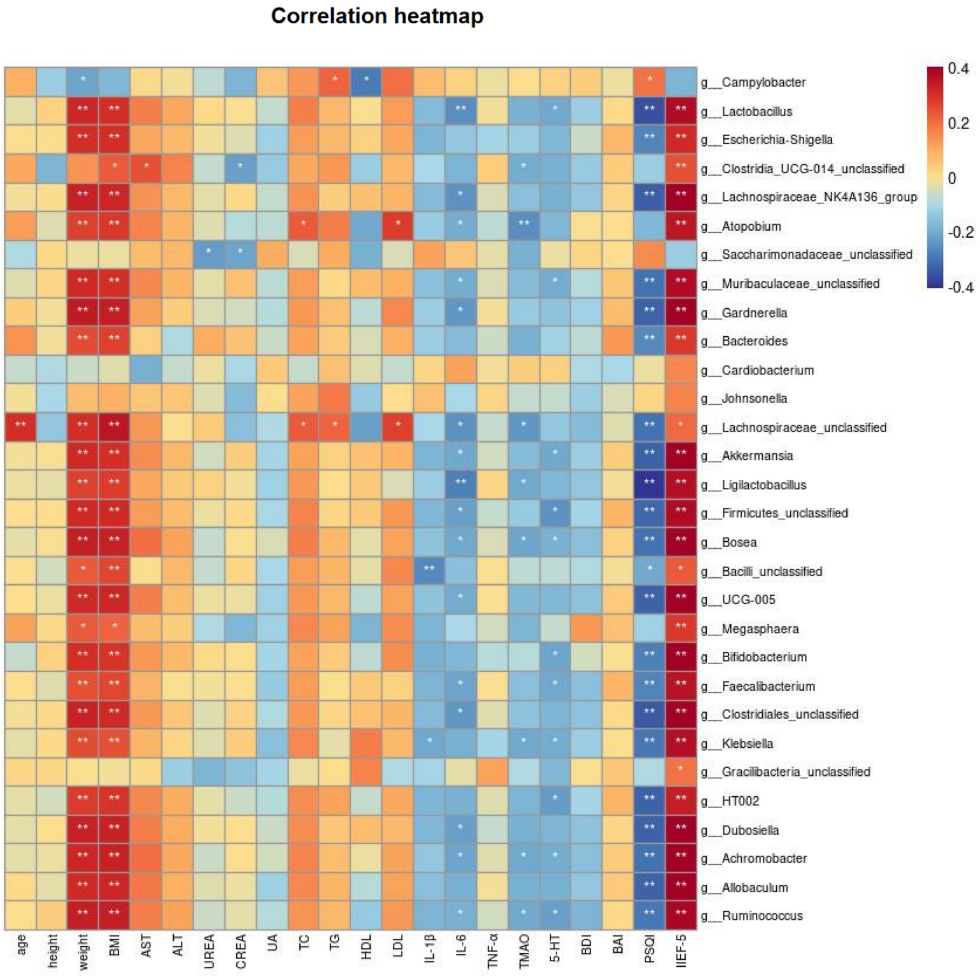

Figure 3 The spearman correlation heatmap between 22 clinical characteristics and the top 30 genus level of oral microbiota in relative abundance.*$P < 0.05$; **$P < 0.01$.

and TNF-α in the blood are correlated with the severity of ED.[17] Animal studies have shown that total nitric oxide synthase activity and cyclic guanosine monophosphate (cGMP) levels in the penile corpus cavernosum tissue of rats with periodontal inflammation were significantly lower than those of normal control group rats.[16] Furthermore, another study demonstrated that chronic periodontitis could induce erectile dysfunction by increasing serum levels of CRP, TNF-α and IL-6 in rats, while simultaneously reducing the p-eNOS/eNOS ratio and NO concentration in the penile corpus cavernosum. This condition also degrades the endothelial glycocalyx in the penile corpus cavernosum and decreases eNOS expression through the action of TNF-α.[21] Inflammatory cytokines, such as TNF-α, can down-regulate eNOS expression by shortening the half-life of eNOS mRNA.[21] Additionally, TNF-α promotes the formation of reactive oxygen species (ROS), leading to endothelial dysfunction.[22] Higher serum levels of TNF-α are associated with moderate to severe ED.[23] Notably, after successful periodontal treatment, a significant decrease in TNF-α levels can improve ED symptoms.[15,23,24] Lactobacillus and Bifidobacterium play a crucial role in regulating the immune system by inhibiting pro-inflammatory cytokines such as IL-6 and producing anti-inflammatory cytokines, thereby alleviating inflammatory responses.[25–28] In this study, we observed that the relative abundance of Lactobacillus and Bifidobacterium in the control group was significantly higher than that in the ED group, and their abundance exhibited a negative correlation with serum IL-6 levels. Furthermore, we noted that the relative abundance of Campylobacter in the ED group was elevated compared to the normal group, and this abundance was positively correlated with serum levels of IL-1, IL-6 and TNF-α. Campylobacter is a pathogenic bacterium associated not only with periodontal infections but also with esophageal cancer, enteritis, sickle cell disease and tumor formation.[29–31] It has been shown that Campylobacter can modulate the immune response by upregulating TLR 4 and MD-2 in HT-29 cells.[30] We hypothesize that Campylobacter may induce penile vascular endothelial dysfunction by triggering inflammatory responses.

Oral microorganisms may influence mental health through the brain-mouth axis and interactions with gut microorganisms.[32] The oral microbiota and their metabolites can invade the central nervous system via the trigeminal nerve and olfactory system. Additionally, they can disrupt the intestinal microecological balance by colonizing the gut, thereby affecting brain function through the

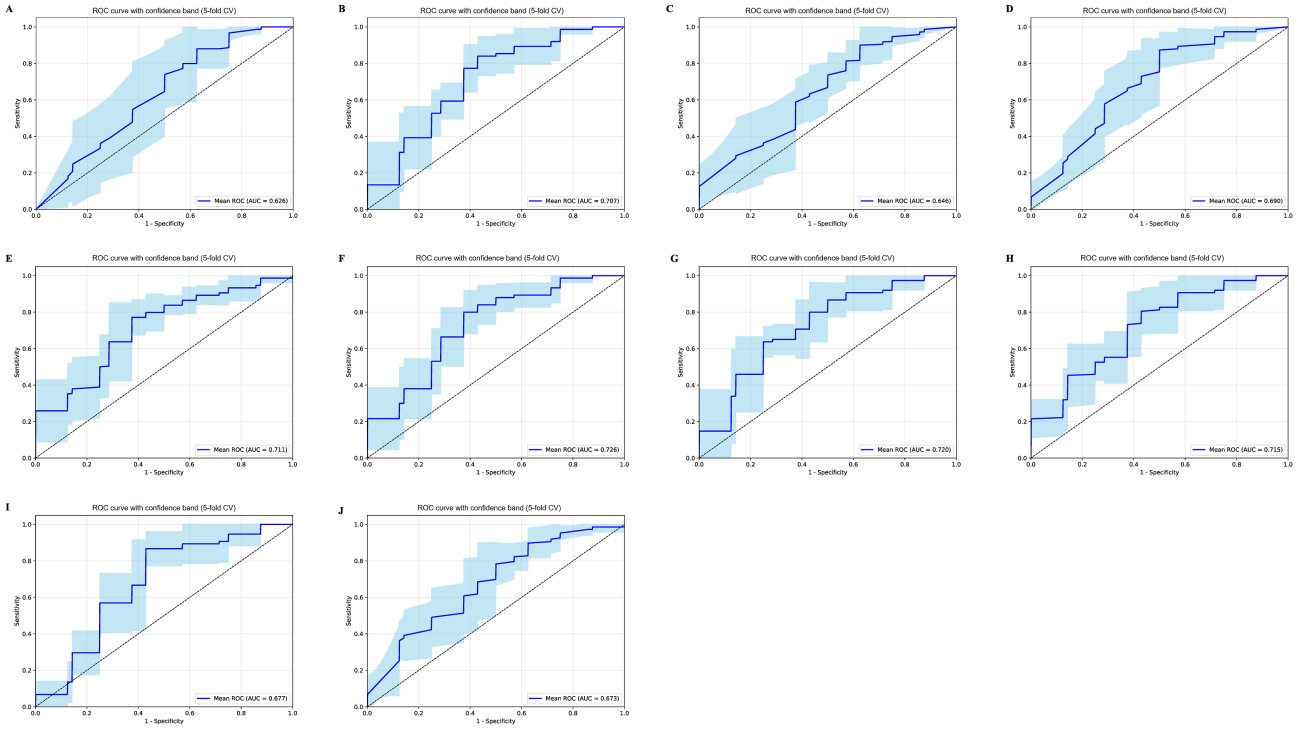

Figure 4 ROC curve analysis of ten ML models. (A) AdaBoost's ROC curve. (B) CatBoost's ROC curve. (C) GBDT's ROC curve. (D) KNN's ROC curve. (E) LGBM's ROC curve. (F) LR's ROC curve. (G) NB's ROC curve. (H) RF's ROC curve. (I) SVM's ROC curve. (J) XGBoost's ROC curve. Abbreviations: CV, cross validation; AdaBoost, adaptive boosting; CatBoost, categorical boosting; GBDT, gradient boosting decision tree; KNN, k-nearest neighbors; LGBM, light gradient boosting machine; LR, logistic regression; NB, naive bayes; RF, random forest; SVM, support vector machine; XGBoost, eXtreme gradient boosting.

gut—brain axis. When the oral mucosal barrier is compromised, oral microorganisms can also access peripheral nerves and the bloodstream, inducing blood—brain barrier dysfunction and neuroinflammation mediated by microglia, ultimately leading to neuronal necrosis and mental health disorders such as depression, anxiety and schizophrenia.[33—35] Animal studies have demonstrated that rats with depression, modeled by chronic unpredictable mild stress, exhibit significant differences in oral microbiota compared to normal rats.[36] Another study indicated that supplementing with Lactobacillus can enhance oral microecology and alleviate anxiety and depression-like behaviors in mice.[37] Furthermore, our findings revealed that the relative abundance of Lactobacillus in the control group was greater than that in the ED group. In our study, the BDI and BAI scores of the ED group were higher than those of the control group, suggesting that patients in the ED group experience a greater degree of anxiety and depression. Additionally, the PSQI score of the ED group was elevated compared to the control group, indicating poorer sleep quality among patients in the ED group. Anxiety and depression can lead to difficulties in falling asleep, and poor sleep quality can exacerbate anxiety and depression, creating a vicious cycle. All these factors may contribute to the onset and progression of ED. Our correlation analysis revealed that the oral microbiota, including Lactobacillus, Akkermansia and Bifidobacterium, exhibited a relatively higher abundance in the normal group and demonstrated a negative correlation with scores on the BDI and the PSQI. In contrast, the relative abundance of Campylobacter, which was elevated in the ED group, displayed a positive correlation with these three scores. Numerous clinical studies have indicated that Lactobacillus and Bifidobacterium can enhance mood and sleep quality.[27,28,38]

The etiology of ED is multifaceted, and the available diagnostic and therapeutic methods remain limited, posing a persistent challenge for andrologists. Oral microbiota serve as an indicator of human health and disease, and we propose that it can be utilized as a non-invasive biomarker for ED. In this study, we established ten machine learning models based on oral microbiota data from ED patients and healthy individuals. By integrating metrics such as AUC, accuracy, sensitivity, specificity and F1-score, we demonstrated that the LGBM model exhibited superior performance. LGBM is a gradient boosting framework based on the decision tree algorithm, characterized by faster training speed, higher efficiency and superior accuracy compared to other boosting algorithms.[39] Our research developed predictive classifiers for ED based on oral microbiota profiles, offering a novel perspective for the prevention, diagnosis and treatment of ED. Given that intervening in the oral microbiota is more feasible than targeting the intestinal microbiota, this approach represents a promising avenue for ED-related treatments through modulation of the oral microbiota. However, this study has certain limitations, including its retrospective design and relatively small sample size. Additionally, there is a scarcity of literature regarding the relationship between oral microbiota and male sexual dysfunction. Future large-scale, multi-center prospective cohort studies may

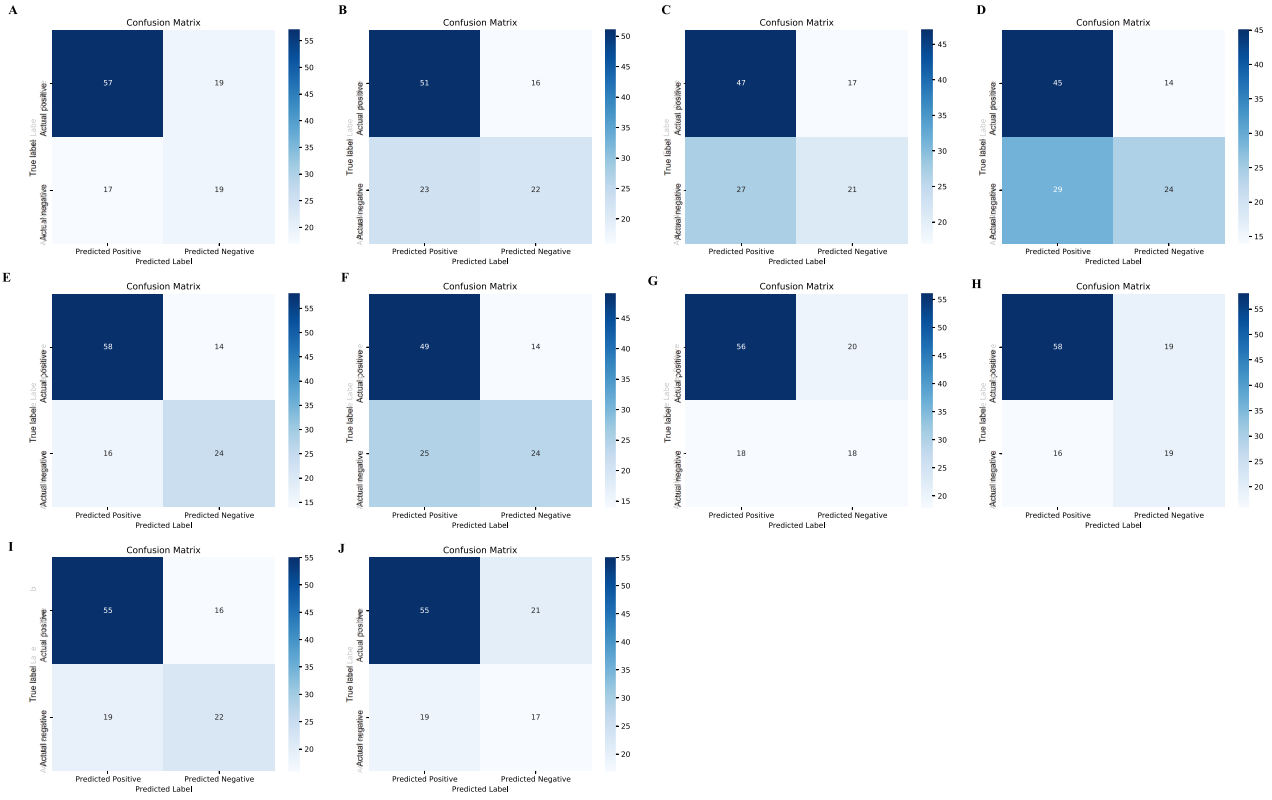

Figure 5 Confusion matrices of ten ML models. (A) AdaBoost's confusion matrices. (B) CatBoost's confusion matrices. (C) GBDT's confusion matrices. (D) KNN's confusion matrices. (E) LGBM's confusion matrices. (F) LR's confusion matrices. (G) NB's confusion matrices. (H) RF's confusion matrices. (I) SVM's confusion matrices. (J) XGBoost's confusion matrices. Abbreviations: AdaBoost, adaptive boosting; CatBoost, categorical boosting; GBDT, gradient boosting decision tree; KNN, k-nearest neighbors; LGBM, light gradient boosting machine; LR, logistic regression; NB, naive bayes; RF, random forest; SVM, support vector machine; XGBoost, eXtreme gradient boosting.

Table 2 Performance metrics of the ten machine learning models.

Models	AUC	Accuracy	Sensitivity	Specificity	PPV	NPV	F1-score
AdaBoost	0.626	0.679	0.770	0.507	0.764	0.531	0.759
CatBoost	0.707	0.653	0.690	0.582	0.763	0.491	0.722
GBDT	0.646	0.608	0.634	0.557	0.747	0.427	0.679
KNN	0.690	0.616	0.612	0.632	0.806	0.502	0.634
LGBM	0.711	0.733	0.784	0.636	0.810	0.598	0.795
LR	0.726	0.652	0.658	0.632	0.786	0.499	0.702
NB	0.720	0.662	0.752	0.468	0.738	0.619	0.726
RF	0.715	0.688	0.782	0.500	0.753	0.552	0.765
SVM	0.677	0.688	0.739	0.579	0.772	0.567	0.746
XGBoost	0.673	0.643	0.743	0.446	0.728	0.460	0.733

Abbreviations: AdaBoost, adaptive boosting; CatBoost, categorical boosting; GBDT, gradient boosting decision tree; KNN, k-nearest neighbors; LGBM, light gradient boosting machine; LR, logistic regression; NB, naive bayes; RF, random forest; SVM, support vector machine; XGBoost, eXtreme gradient boosting.

enhance our understanding of the role of oral microbiota disorders in the pathogenesis of ED.

In summary, oral microbiota dysbiosis is closely associated with the occurrence and progression of ED through mechanisms such as endothelial dysfunction, promotion of atherosclerosis, induction of inflammatory responses and impacts on mental and psychological health. It is essential for men to acknowledge the significance of maintaining oral health. A comprehensive understanding of the relationship between the oral microbiome and ED has the potential to revolutionize prevention and treatment strategies for this condition. Identifying specific microbial characteristics or biomarkers present in the oral cavity could lead to the development of novel prediction tools and personalized

interventions. Consequently, we have established several prediction models utilizing machine learning techniques. In the future, enhancing oral hygiene and supplementing with probiotics may enable us to modify the oral microbiome, thereby preventing and treating ED.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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