BMC Oral Health

RESEARCH

Open Access

Association between oral microbiome and sleep disorders in U.S. adults: analysis of NHANES database 2009–2012

Guihua Hao[1,2], Yiwen Wu[1], Xiaoqiao Mo[3], Xiaomei Zhao[1*], Lili Hou[1*] and Tian Xie[4*]

Abstract

Background The microbiome, especially the gut microbiome, contributes to the regulation, etiology, and pathogenesis of sleep disorder. However, limited evidence regarding the oral microbiome's role in sleep disorder. Therefore, this study aimed to investigate the association between sleep disorder and oral microbial diversity and whether oral microbiota is associated with all-cause mortality in people with sleep disorder.

Methods The study included 4,729 individuals in the National Health and Nutrition Examination Survey (NHANES) from 2009 to 2012 and mortality data until 2019. Sleep disorder was assessed by structured questionnaire. The oral microbiome was characterized by 16 S ribosomal RNA gene sequencing. Logistic regression models were conducted to quantify the association of α-diversity with different sleep status controlling for potential confounding variables, and principal coordinate analysis along with permutational multivariate analysis of variance for β-diversity. The association between the oral microbiome and all-cause mortality was assessed using Cox proportional hazard models.

Results The α-diversity showed that a lower number of operational taxonomic units (OTUs) (adjusted odds ratio [aOR] = 0.996; 95% confidence interval [CI] = 0.994–0.998), less Faith's phylogenetic diversity (aOR = 0.954, 95% CI = 0.934–0.975), and a lower Shannon–Weiner index (aOR = 0.854, 95% CI = 0.772–0.944) were associated with sleep disorder. β-diversity revealed different oral microbiome communities between the two groups, as measured by the Bray–Curtis dissimilarity (R^2 = 0.358%, P = 0.001), unweighted UniFrac distance (R^2 = 0.450%, P = 0.001) and weighted UniFrac distance (R^2 = 0.709%, P = 0.001). Furthermore, the OTUs (odds ratio [OR] = 0.999; 95% CI = 0.998–0.999; P < 0.05), Faith's phylogenetic diversity (OR = 0.987; 95% CI = 0.975–0.998; P < 0.05), Shannon–Weiner index (OR = 0.924; 95% CI = 0.873–0.979; P < 0.05), and the inverse Simpson index (OR = 0.553; 95% CI = 0.306–0.997; P < 0.05) were all associated with a significant increase in the risk of all-cause death in participants with sleep disorder.

Conclusions Intra-population richness, inter-population dispersion, and the phylogenetic diversity of the oral microbiome have all been linked to sleep disorder and all-cause mortality. Overall, these results will help to

*Correspondence:
Xiaomei Zhao
Zhxm58327107@126.com
Lili Hou
pisces_liz@163.com
Tian Xie
tean0523@163.com

Full list of author information is available at the end of the article

better understand the etiology and pathogenesis of sleep disorder. Further studies are required to determine the mechanisms underlying the role of microbiome in the pathogenesis of sleep disorder.

Keywords Oral Microbiome, Sleep disorder, NHANES, Relationship, Mortality, Cross-sectional study

Background

Sleep is a vital physiological process crucial for sustaining and enhancing both physical and mental health [1]. Poor or insufficient sleep is associated with various systematic dysfunctions, including endocrinological, metabolic, and nervous disorders. Sleep disorder may manifest as insomnia, sleep apnea, narcolepsy, or restless legs syndrome [2]. Sleep disorder not only negatively affect enthusiasm and mental health but also linked to adverse health outcomes such as obesity, hypertension, type 2 diabetes, cardiovascular diseases, and increased mortality [3]. In addition, the prevalence of sleep disorder has increased over the past few decades and continues to increase, with a prevalence of 27.1% among adults in the United State [4]. Given the heavy burden and various adverse health outcomes associated with sleep disorder, it is vital to investigate the mechanisms underlying the pathogenesis of sleep disorder.

Recent decades have seen substantial advancements in recognizing that the sleep-wake cycle is regulated by both the central nervous system and signals from peripheral tissues. Emerging evidence suggests that the microbiota-gut-brain axis influences sleep behavior regulation and may be crucial in the etiology and pathogenesis of sleep disorders [5]. The gut microbiota modulates sleep-wake behavior through bacterial metabolites, endocrine signaling, neuronal signaling, and immune responses. Sleep deprivation disrupts gut microbiota, and changes in gut microbiota composition are linked to sleep disorders. Research has investigated novel treatments for sleep disorders by focusing on the gut microbiota [6].

The oral microbiota is the second most diverse microbial community colonizing the human body, exhibits significant diversity and complex ecology, including bacteria, microeukaryotes, archaea, and viruses [7]. The oral microbiota is crucial for maintaining oral health. Dental caries, periodontal disease, and oral cancer, three prevalent oral diseases, are linked to microbial causes [8]. Moreover, oral microbiota, especially periodontal pathogens, are increasingly linked to various systemic diseases, either by directly translocating to other body sites or indirectly by altering host immune and inflammatory responses [9]. Emerging research indicates that the oral microbiota may contribute to the development of various neuropsychiatric disorders, such as Alzheimer's disease, Parkinson's disease, and depression [10, 11]. It is well established that the oral cavity is anatomically closer to the cerebrum than the gut, which has led to a new term known as the oral-brain axis [12, 13]. A recent case-control study found significantly lower relative abundances of *Prevotella*, *Alloprevotella*, *Bacteroides*, *Candidatus saccharimonas*, *and Leptotrichia* in individuals with severe obstructive sleep apnea (OSA) compared to healthy controls [14]. Research on the relationship between microbiota and sleep disorder has predominantly concentrated on the gut microbiota and the gut-brain axis, with limited attention given to the role of the oral microbiota in sleep regulation. Here we tested the hypothesis that there is an association between the oral microbiome and sleep status in a nationally representative sample of individuals. We aimed to determine whether sleep disorder is negatively correlated with oral microbial diversity and whether oral microbiota is associated with all-cause mortality in people with sleep disorder.

Methods

Study design and population

The NHANES, conducted by the National Center for Health Statistics, is a continuous cross-sectional survey designed to evaluate the health and nutrition of the non-institutionalized civilian population in the U.S [15]. This study adhered to the Declaration of Helsinki and received approval and sponsorship from the Centers for Disease Control and Prevention. The survey gathered data every two years through a complex multistage probability sampling method. The NCHS survey weights accounted for selection probabilities and non-response, enabling the production of nationally representative estimates. Informed consent was obtained from all NHANES participants, following approval by the NCHS Research Ethics Review Board. No additional Institutional Review Board approval was required to perform secondary analysis.

The NHANES utilizes a computer-assisted personal interview (CAPI) system for conducting household interviews. Participants were subsequently invited to provide additional information at mobile examination sites, where physical examinations were conducted, blood and urine samples were obtained too [16]. This study utilized data from the 2009–2010 and 2011–2012 NHANES, with unweighted cumulative examination response rates of 77.3% and 69.5%. Eligibility was based on data availability from NHANES 2009–2012. Participants who visited the mobile examination center had comprehensive data on the oral microbiome, sleep disorder, and relevant covariates (Fig. 1).

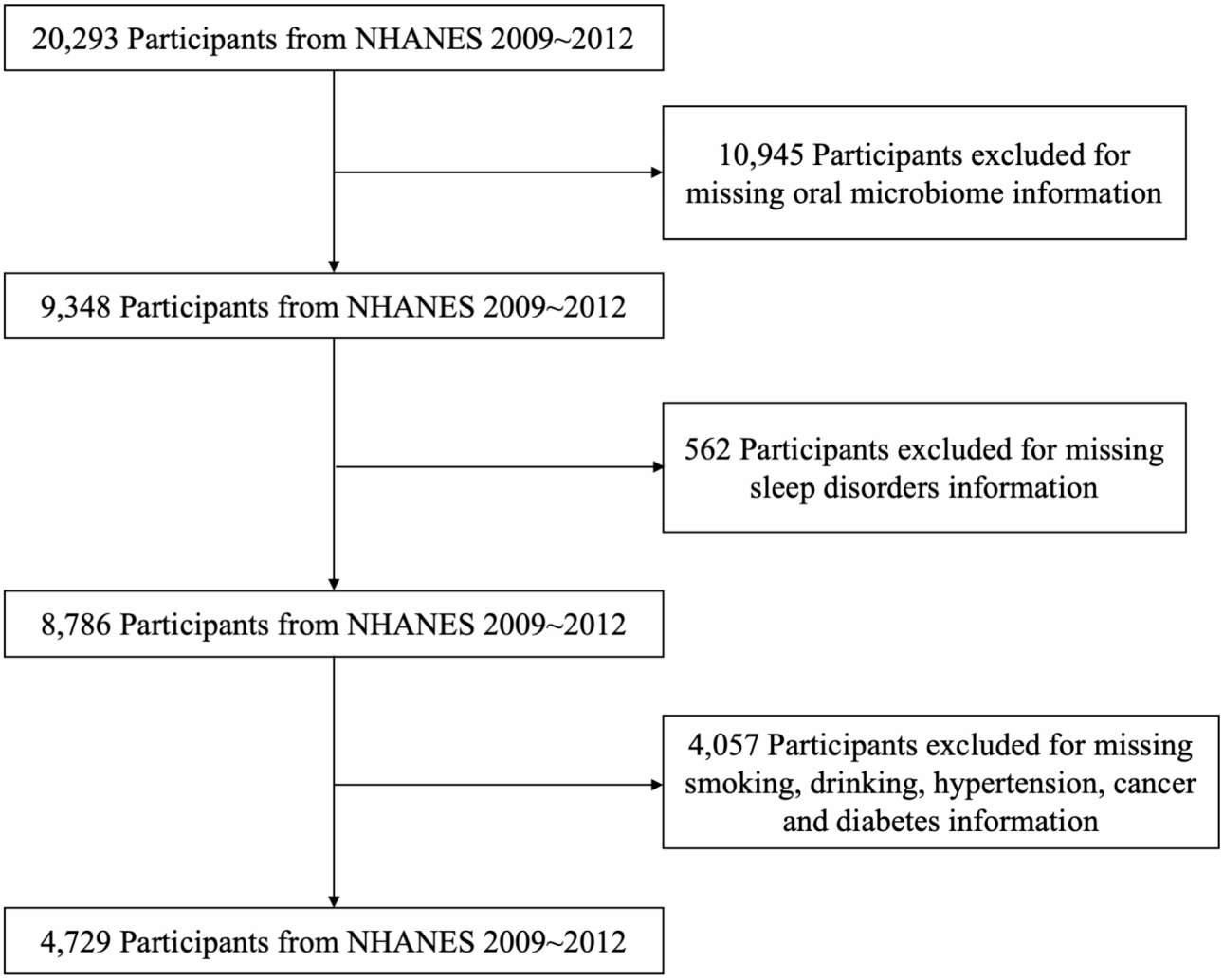

Fig. 1 Flow chart of study population selection

Assessment of the oral Microbiome

For detailed information on the laboratory methods and bioinformatics procedures used to collect oral microbiome data, please refer to the NHANES website (http://www.cdc.gov/nchs/nhanes; accessed February 5, 2024). DNA was extracted from oral rinse samples and sequenced at the Knight Laboratory, University of California, San Diego. The V4 region of the 16S rRNA gene was amplified and sequenced via polymerase chain reaction using the aforementioned DNA, with processing conducted through QIIME1. DADA2 was used to generate amplicon sequence variants (ASVs) from forward and reverse FASTQ files. The analysis identified 2750 operational taxonomic units (OTUs) classified as' Kingdom (Bacteria); Phylum (sample); Class (sample); Order (sample); Family (sample); Genus (sample); Species (sample).' α-and β-diversity describe species variation within and between samples. α-diversity is a measurement of the microbiome diversity within a single sample, is typically measured by species richness and/or evenness. β-diversity highlights variations in microbiome composition across different ecosystems, such as among individuals.

Our study assessed α-diversity using four indicators: operational taxonomic unit (OUT) richness, the Shannon–Weiner index (SWI), the inverse Simpson index, and the Faith's phylogenetic diversity (FPD). The OTU richness and FPD measure richness, while the SWI and inverse Simpson indices assess both richness and evenness. Post-rarefaction data of 10,000 reads per sample were used to measure the alpha diversity. The mean of 10 replications was adopted in this study.

The β-diversity included two measures: Bray–Curtis dissimilarity was used to measure microbiota dissimilarity using bacterial (taxonomic) counts, and UniFrac distance was used to measure phylogenetic distance between microbial communities. The unweighted UniFrac distance assessed species presence or absence by

calculating the fraction of unique branch lengths per sample, while the weighted UniFrac distance incorporated OTU abundance differences into the branch length calculation.

Assessment of sleep disorder

The NHANES evaluated sleep disorder with a single question from the Sleep Disorder Questionnaire: "Has your doctor ever informed you of having a sleep disorder?" [17]. Participants were categorized according to their response (yes or no), while "Do not know" and "Refused" responses were considered missing data.

Assessment of mortality

Mortality status, cause of death, and follow-up duration were ascertained via the National Death Index (NDI) (http://www.cdc.gov/nchs/ndi/about.htm). The NCHS annually publishes the NDI, offering epidemiologists comprehensive mortality data for the entire U.S. population [18]. All-cause mortality was determined using the 10th revision of the International Classification of Diseases and evaluated through the NDI. The main focus of this study was overall mortality. The study's follow-up period was determined from the NHANES 2009–2012 examination data to either the last confirmed date of the patient's survival or until they were censored by December 31, 2019.

Covariates

Variables previously shown to be associated with sleep and potentially linked to the oral microbiome were included in the analysis. During household interviews using CAPI, self-reported sociodemographic variables were collected for adults aged 20 and over. These included age, sex (female or male), race/ethnicity (Mexican American, non-Hispanic White, non-Hispanic Black, and others), educational attainment, marital status, and the family income to poverty ratio (PIR). The PIR represents the family income divided by the poverty threshold for the survey year. Poverty thresholds vary according to the family size and geographic location [19]. This study classified income status into three categories based on the PIR: low (PIR < 1.00), medium (PIR 1.00–4.00), and high (PIR > 4.00). Smoking and alcohol intake was assessed in household interviews via the CAPI for adults aged 20 years and older. Alcohol consumption status was categorized into non-drinker, ever drinker, and current drinker based on questionnaire responses [20]. Smoking status was categorized as never for participants who had never smoked, former for those who had smoked at least 100 cigarettes in their lifetime, and current for individuals who were actively smoking [21]. Additionally, the history of diabetes, hypertension, and cancer was recorded.

Statistical analysis

All analyses accounted for sample weights, clustering, and stratification due to the NHANES's complex sampling design, essential for proper data analysis. Continuous variables are represented by mean and standard deviation, whereas categorical variables are depicted by frequency and percentage. Pearson's Chi-squared test was used to examine the categorical variables.

Summary statistics for α-diversity metrics were analyzed based on demographic and behavioral factors. Univariable and multivariable logistic regression analyses were conducted to examine the association between each α-diversity measure and sleep disorders independently. The multivariate models were adjusted for variables including age, race, sex, smoking, alcohol consumption, diabetes, hypertension, cancer, education level, marital status, and income. We conducted further stratified analyses based on age (20–29, 30–39, 40–49, 50–59, 60–69), race (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, other race/multi-racial), sex (female, male), smoking status (never, former, current), drinking status (never, former, current), diabetes (no, yes), hypertension (no, yes), cancer (no, yes), education (less than high school, high school or equivalent, some college or AA degree, college graduate or above), marital status (never married, married/living with partner, widowed/divorced/separated), and income (low [PIR < 1.00], medium [PIR 1.00–4.00], high [PIR > 4.00]).

To visualize the distribution of oral microbiome communities based on sleep status, β-diversity was assessed using principal coordinate analysis (PCoA) with the *cmdscale* function and survey weights. The *adonis2* function was used to assess the variability (R^2) attributable to sleep disorder status. Its confidence interval was estimated using bootstrap. Sleep disorders status was permuted ($n = 999$) in weighted permutational multivariate analysis of variance (PERMANOVA) using *pairwise Adonis* to test for significant differences in the β-diversity metrics by sleep status. Subgroup analyses were performed stratified by sex due to sex differences in the epidemiology of sleep disorders and hypotheses regarding sex differences in the natural history of sleep status.

Kaplan–Meier survival curves and log-rank tests were used to analyze all-cause mortality, stratified by optimal cut-off values for α-diversity metrics (observed ASVs, Shannon–Weiner index, Inverse Simpson index, and Faith's phylogenetic diversity) determined via X-tile. Cox proportional hazards regression analysis was conducted, which included two models to control for confounding factors. Model 1 was unadjusted, whereas Model 2 included adjustments for age, race, sex, smoking, drinking, diabetes, hypertension, and cancer. Statistical analyses were conducted using SPSS for Windows (version 25.0) and the R statistical package (version 4.3.3). A

two-sided *P*-value of less than 0.05 was deemed statistically significant.

Results

Basic characteristics of the study participants

In total, 4,729 participants were enrolled, and the prevalence of sleep disorder was 26.39%. Participants with sleep disorder were more likely to be female ($P<0.001$). Significant differences were observed between the sleep disorder and non-sleep disorder groups across race, education, marital status, household income, smoking, drinking, diabetes, hypertension, and cancer (all $P<0.001$). Further details are listed in Table 1.

α-diversity tests were conducted to assess the basic features of the oral microbiome. The results indicated significant differences in all variables except diabetes for the observed OTUs and Shannon–Weiner index (both $P<0.01$). The inverse Simpson index showed significant differences for all variables except diabetes, marital status, and hypertension ($P<0.05$). The Faith's phylogenetic diversity showed significant differences across all variables ($P<0.05$). Details are shown in the additional figure files (Additional Fig. 1–Fig. 4).

α-diversity and sleep disorder

In univariable models, the OUTs (OR = 0.994, 95%CI = 0.992–0.995), Faith's phylogenetic diversity (OR = 0.924, 95%CI = 0.906–0.943), Shannon–Weiner index (OR = 0.751, 95%CI = 0.685–0.825), and inverse Simpson index (OR = 0.256, 95% CI = 0.0964–0.679) were all significantly negatively associated with sleep disorder (all $P<0.001$).

In the multivariate models, a significant association was found between oral microbiome α-diversity and sleep disorder. A lower number of the OUTs (adjusted odds ratio [aOR], 0.996, 95%CI = 0.994–0.998), Faith's Phylogenetic Diversity (aOR = 0.954, 95%CI = 0.934–0.975), Shannon-Weiner index (aOR = 0.854, 95%CI = 0.772–0.944) were associated with higher odds of sleep disorder (all $P<0.01$) (Table 2).

The results of subgroup analyses are presented in Supplementary Table 1. There were significant in the association between α-diversity and sleep disorder for age, sex, race, education, marital status, income, smoking, drinking, diabetes, hypertension and cancer subgroups ($P<0.05$), with a few differences in some α-diversity tests.

β-diversity and sleep disorders

The PCoA analysis revealed a shift in the centroids of the microbiome communities when each β-diversity metric was compared by sleep status (Fig. 2). The β-diversity of the oral microbiome was evaluated using PERMANOVA with Bray-Curtis dissimilarity, unweighted UniFrac distance, and weighted UniFrac distance. In the overall population, sleep disorder was significantly associated with Bray–Curtis dissimilarity ($R^2 = 0.358\%$), unweighted UniFrac distance ($R^2 = 0.450\%$), and weighted UniFrac distance ($R^2 = 0.709\%$) (all $P = 0.001$).

In subgroup analysis, both male and female populations, Bray-Curtis dissimilarity ($R^2 = 0.346\%$ Vs. $R^2 = 0.407\%$), unweighted UniFrac distance ($R^2 = 0.512\%$ Vs. $R^2 = 0.33\%$) and Weighted UniFrac distance ($R^2 = 0.715\%$ Vs. $R^2 = 0.656\%$) were still significantly associated with sleep disorder (all $P = 0.001$). The β-diversity of the oral microbiome was higher in males than that in females, except for Bray–Curtis dissimilarity. The details are shown in an additional table (Additional Figs. 5 and 6).

Oral microbiome and mortality

The Kaplan–Meier survival curves revealed significant differences between higher and lower groups for OUTs (cutoff: 111.10), Faith's phylogenetic diversity (cutoff: 13.72), and the Shannon–Weiner index (cutoff: 4.36) (all log-rank $P<0.05$), but not for the inverse Simpson index (Fig. 3).

In the univariable Cox regression, only the Shannon–Weiner index showed that a low level of α-diversity was associated with high risk of all-cause death (OR = 0.939; 95% CI = 0.888–0.993; $P = 0.026$). In the Cox regression model adjusted for age, gender, race, marriage, education, income, diabetes, cancer, hypertension, smoking and drinking, the OUTs (aOR = 0.999; 95% CI = 0.998–0.999; $P = 0.025$), Faith's phylogenetic diversity (aOR = 0.987; 95% CI = 0.975–0.998; $P = 0.026$), Shannon–Weiner index (aOR = 0.924; 95% CI = 0.873–0.979; $P = 0.007$), and the Inverse Simpson index (aOR = 0.553; 95% CI = 0.306–0.997; $P = 0.049$) were all showed significantly negative association with the risk of all-cause death (Table 3).

Discussion

This study is the first nationally representative real-world analysis to establish a link between the oral microbiome and sleep disorder in individuals aged 20–69 years. Our results showed that lower individual α-diversity was related with a higher risk of sleep disorder. The β-diversity analysis indicated a potential link between variations in oral microbiome communities and sleep disorder. Furthermore, our results indicated that the oral microbiome was related to all-cause mortality in population with sleep disorder.

As the fundamental to optimal physical and mental health, sleep disorder as well as its detrimental effects have caused wide public concern and brings global public health challenges to healthcare workers. Some aspects, such as the role of the central nervous system in sleep, are now fully documented. However, the mechanisms of microbiota in different source are still unclear. The

Table 1 Baseline characteristics of the participants according to the sleep status

Characteristics	Total ($n=4729$)	Without Sleep Disorder ($n=3481$)	With Sleep Disorder ($n=1248$)	*P*-value[a]
Age, *N* (%)				< 0.001
20–29 (y)	1152 (24.36)	961 (27.61)	191 (15.30)	
30–39 (y)	1002 (21.19)	764 (21.95)	238 (19.07)	
40–49 (y)	959 (20.28)	674 (19.36)	285 (22.84)	
50–59 (y)	851 (18.00)	557 (16.00)	294 (23.56)	
60–69 (y)	765 (16.18)	525 (15.08)	240 (19.23)	
Sex, **N (%)**				**< 0.001**
Female	2176 (46.01)	1492 (42.86)	684 (54.81)	
Male	2553 (53.99)	1989 (57.14)	564 (45.19)	
Race/Ethnicity, **N (%)**				**< 0.001**
Mexican American	698 (14.76)	571 (16.40)	127 (10.18)	
Other Hispanic	467 (9.88)	368 (10.57)	99 (7.93)	
Non-Hispanic White	2055 (43.46)	1410 (40.51)	645 (51.68)	
Non-Hispanic Black	1046 (22.12)	765 (21.98)	281 (22.52)	
Other race/multi-racial	463 (9.79)	367 (10.54)	96 (7.69)	
Education, **N (%)**				**0.039**
Less than high school	930 (19.67)	703 (20.20)	227 (18.19)	
High school or equivalent	992 (20.98)	735 (21.11)	257 (20.59)	
Some college or AA degree	1510 (31.93)	1072 (30.80)	438 (35.10)	
College graduate or above	1297 (27.43)	971 (27.89)	326 (26.12)	
Marital Status, **N (%)**				**< 0.001**
Never married	1159 (24.51)	883 (25.37)	276 (22.12)	
Married/living with partner	2753 (58.22)	2094 (60.16)	659 (52.80)	
Widowed/divorced/separated	817 (17.28)	504 (14.48)	313 (25.08)	
Income				**< 0.001**
Low income (PIR < 1.00)	1084 (22.92)	761 (21.86)	323 (25.88)	
Medium income (PIR 1.00–4.00)	2262 (47.83)	1725 (49.55)	537 (43.03)	
High income (PIR > 4.00)	1383 (29.25)	995 (28.58)	388 (31.09)	
Smoking Status, **N (%)**				**< 0.001**
Never	2508 (53.03)	1965 (56.45)	543 (43.51)	
Former	993 (21.00)	673 (19.33)	320 (25.64)	
Current	1228 (25.97)	843 (24.22)	385 (30.85)	
Alcohol Drinking Status, **N (%)**				**< 0.001**
Non-drinker	2350 (49.69)	1715 (49.27)	635 (50.88)	
Ever-drinker	272 (5.75)	167 (4.80)	105 (8.41)	
Current drinker	2107 (44.55)	1599 (45.94)	508 (40.71)	
Prescription for Diabetes, **N (%)**				**< 0.001**
No	4154 (87.84)	3141 (90.23)	1013 (81.17)	
Yes	575 (12.16)	340 (9.77)	235 (18.83)	
Prescription for Hypertension, **N (%)**				**< 0.001**
No	3201 (67.69)	2517 (72.31)	684 (54.81)	
Yes	1528 (32.31)	964 (27.69)	564 (45.19)	
Prescription for Cancer, **N (%)**				**< 0.001**
No	4464 (94.40)	3345 (96.09)	1119 (89.66)	
Yes	265 (5.60)	136 (3.91)	129 (10.34)	

Data are shown as the No. (%). The percentages are within-column proportions that apply post-stratification weights

AA, associates in the arts; PIR, family income to poverty ratio

[a] *P* value based on the Rao-Scott-adjusted Pearsonχ^2 test comparing people with and without sleep disorders

Table 2 Univariable and multivariable analysis results of each α- diversity measure associated with sleep disorders

Population	OTUs		Faith's Phylogenetic Diversity		Shannon–Weiner Index		Inverse Simpson Index	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
Univariable								
Overall	0.994 (0.992–0.995)	**< 0.001**	0.924 (0.906–0.943)	**< 0.001**	0.751 (0.685–0.825)	**< 0.001**	0.256 (0.0964–0.679)	**0.006**
Male	0.993 (0.991–0.996)	**< 0.001**	0.919 (0.894–0.945)	**< 0.001**	0.75 (0.653–0.861)	**< 0.001**	0.296 (0.0584–1.5)	0.141
Female	0.995 (0.992–0.997)	**< 0.001**	0.942 (0.916–0.969)	**< 0.001**	0.806 (0.709–0.916)	**< 0.001**	0.429 (0.124–1.49)	0.182
Multivariable								
Overall	0.996 (0.994–0.998)	**< 0.001**	0.954 (0.934–0.975)	**< 0.001**	0.854 (0.772–0.944)	**0.002**	0.377 (0.132–1.047)	0.068
Male	0.997 (0.994–0.999)	**0.004**	0.957 (0.928–0.986)	**0.004**	0.891 (0.767–1.034)	0.128	0.707 (0.121–4.116)	0.699
Female	0.996 (0.993–0.998)	**< 0.001**	0.953 (0.924–0.982)	**0.002**	0.822 (0.717–0.942)	**0.005**	0.267 (0.071–1.004)	0.051

The multivariate models were adjusted for age, race, sex, smoking, drinking, diabetes, hypertension, cancer, education, marital status, and income

OR, odds ratio; CI, confidence interval

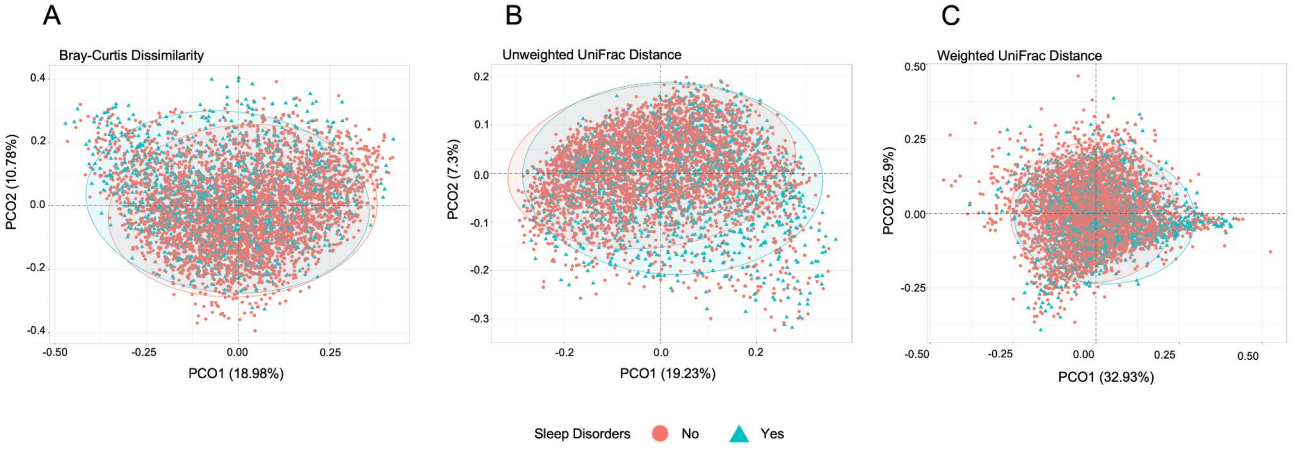

Fig. 2 β-diversity of oral microbiome by sleep status. The β-diversity of Bray-Curtis dissimilarity **(A)**, unweighted UniFrac distance **(B)** and weighted UniFrac distance **(C)** by sleep status using PCoA applying poststratiffcation weights. Each dot represents an individual. The ellipses illustrated the 95% confidence ellipses for sleep disorder and normal group; the triangles represent the centroid of individuals for any sleep disorder and normal group

increasing knowledge of the oral-brain axis has highlighted the significance of oral microbiota in the development of neuropsychiatric disorders. While sleep is recognized as a reversible physiological process within the central nervous system, limited research has independently associated oral microbiome diversity with sleep disorders. In light of this, the present study examined the relationship with sleep disorder in terms of oral microbiota.

The oral microbiome represents one of the most diverse microbial ecosystems in the human body. It is estimated that more than 700 bacterial species can be found in different parts of the oral cavity [22, 23]. Previous studies indicate that the host microbiota significantly influence the development and progression of mental health conditions like insomnia and depression [24, 25]. For example, sleep was shown to affect the microbial abundance in a study designed to assess the dynamics of microbial abundance in the oral cavity of children before and after sleep [26]. However, the evidence to date is disparate. An experimental study revealed that α-diversity, as indicated by the Chao1 index, was significantly greater in buccal and gingival mucosa samples post-sleep compared to pre-sleep. Additionally, the Shannon index showed a significant increase in buccal mucosa samples post-sleep [27]. Moreover, a cross-sectional study found that patients with OSA had lower microbial richness than healthy individuals, as measured by the Chao index and observed species [28]. This is similar to the findings of the present study. Another cross-sectional study of 30 pediatric patients with OSA and 30 controls found that the microbial communities in the oral cavity showed certain differences, suggesting that they may be affected by OSA due to intermittent hypoxia, which may decrease SaO2 and promote propagation of this anaerobic genus [29]. In contrast to these studies with different directions of association, another separate study reported no significant difference in oral microbial diversity between insomnia and control groups [30]. Jia et al. examined the microbial community composition and structure in patients with OSA compared to the controls, revealing no significant differences in the OTUs, the Chao index,

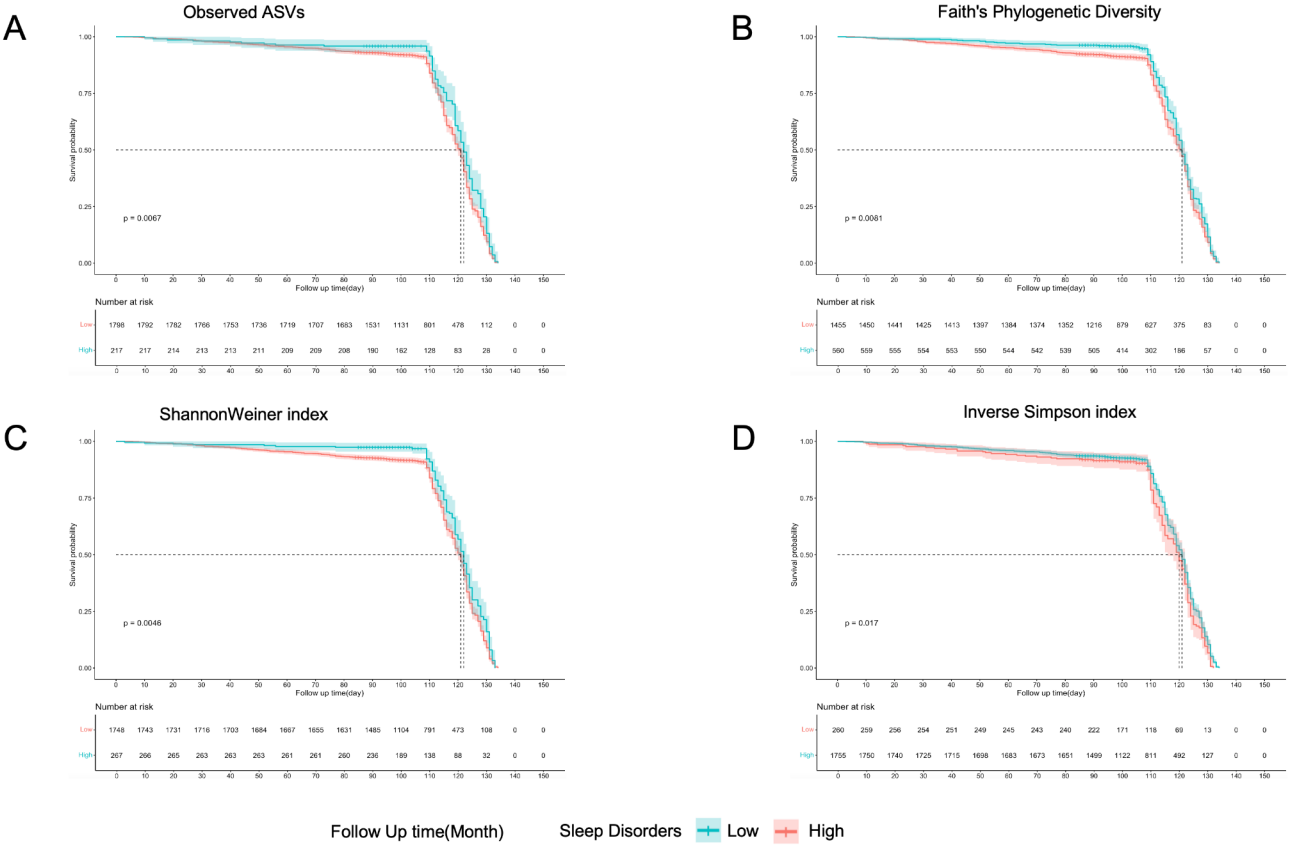

Fig. 3 Kaplan-Meier survival curves for different α-Diversity in sleep disorder groups. **(A)** OUTs, cutoff value = 111.10; **(B)** Faith's Phylogenetic Diversity, cutoff value = 13.72; **(C) S**hannonWeiner index, cutoff value = 4.36; **(D)** Inverse Simpson index, cutoff value = 0.91. All groups were divided according to cutoff value, respectively. Log-rank test, all $p < 0.05$

Table 3 Cox proportional hazards analysis for all-cause mortality according to each α-diversity measure

Alpha Diversity	Univariable		Multivariable	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
OTUs	0.999 (0.998–1.000)	0.072	0.999 (0.998–0.999)	0.025
Faith's phylogenetic diversity	0.989 (0.978–1.000)	0.052	0.987 (0.975–0.998)	0.026
Shannon–Weiner index	0.939 (0.888–0.993)	0.026	0.924 (0.873–0.979)	0.007
Inverse Simpson index	0.632 (0.353–1.132)	0.123	0.553 (0.306–0.997)	0.049

In total, 4729 participants in the NHANES survey (2009–2012) were included in the analyses. Multivariable models were adjusted for age, race, sex, smoking, drinking, diabetes, hypertension, cancer, education, marital status, and income

CI, confidence interval; OR, odds ratio

and the Shannon index [31]. These discrepancies may be attributed to the assessment of results in specific populations or the use of different measures. However, this study found a significant association between α oral microbial diversity and sleep disorder in the population aged 20–69 years, considering demographic, behavioral, and disease factors.

For β-diversity, a prior study identified significant differences in β-diversity and microbial profiles between pediatric OSA patients and controls across five upper respiratory sites, as measured by Bray–Curtis dissimilarity and unweighted UniFrac distance [29]. Similar to that study, the oral microbiome community composition significantly differed between participants with and without sleep disorder, as indicated by both Bray-Curtis dissimilarity and unweighted UniFrac distance in our study. A notable difference in the unweighted UniFract distance based on sleep status was observed, aligning with findings from gut microbiome studies but not previously reported in oral studies [5]. PERMANOVA analysis revealed statistically significant differences in β-diversity based on sleep disorder status, although the effect size (R^2) was small. Statistically significant differences were observed among male, female, and the overall populations (all $P = 0.001$), with substantial overlap in oral microbiome composition distributions in the PCoA plots. This indicates that only

a minor-specific oral microbiome may affect sleep. A prior study noted changes in the salivary microbial community structure in OSA patients, specifically in species richness and trans-habitat diversity, without statistical significance; however, a significant increase in the periodontal pathogen *Prevotella* was observed [28]. Future insights into the variations of biofilm microbiomes across oral cavity sites and their modulation by sleep could aid in devising effective strategies for managing oral biofilms and sleep disorder.

To date, limited evidence was available regarding the interactive effect of the oral microbiome and mortality. Evidence increasingly supports a link between the oral microbiome, cardiovascular inflammation, and cardiovascular disease, potentially contributing to the persistent rise in CVD mortality over the last two decades [32, 33, 34]. Oral dysbiosis contributes to cardiovascular disease through mechanisms such as biofilm formation, endothelial dysfunction, molecular mimicry, platelet aggregation, arterial invasion, and systemic inflammation. These mechanisms operate in a complex manner, either synergistically or independently. Our study identified a significant negative association between oral microbiome and mortality, with the OUTs, Faith's phylogenetic diversity, Shannon–Weiner index, and the Inverse Simpson index were all inversely related to the risk of all-cause death (all $P<0.05$). The mechanism remains unclear, and the interaction between the oral microbiome, inflammation, and the immune system in disease causation is an active research area. Oral dysbiosis may trigger inflammatory responses that result in chronic inflammation, which, combined with environmental factors, can impact systemic organs and worsen diseases such as diabetes, cardiovascular, autoimmune, and neurodegenerative disorders [35]. The impact of bacteria on cytokine production and immune modulation was validated using *Fusobacterium nucleatum* and *Porphyromonas gingivalis* [36].

The strengths of this study are as follows. Firstly, to the best of our knowledge, this is the first study to investigate the association between oral microbiome diversity and sleep disorders with real-world data, and the findings from this study provide insights for future directions and fill the knowledge gap in this area. Secondly, we evaluated a national population with a sufficiently large sample size, making this a national, multi-aged, and multi-racial study. Thirdly, this study employed various α- and β-diversity metrics to explore the relationship between oral microbiome composition and sleep disorder status from different angles. Fourth, this study is the first to reveal the influence of the oral microbiome on all-cause mortality among individuals with sleep disorder. Thus, this study provides new avenues for understanding the pathogenesis of sleep disorder. Finally, we included all participants aged 20–69 years with sleep disorder, and a number of potential confounders were adjusted with sensitivity analyses to ensure the robustness of the results.

Nonetheless, this research possesses certain limitations. First, due to the limitations of published NHANES data, comparisons of specific oral microbial composition at a species level were not possible. Second, the reliance on self-reported sleep disorder during interviews, without objective measurements, may introduce information bias and affect result variability. The analysis might also have been influenced by residual and unmeasured confounding factors. Finally, the NHANES is cross-sectional, therefore, we could not examine the temporal relationship between sleep disorder and oral microbiome diversity. The relationship between sleep disorder and changes in the oral microbiome is uncertain, with possibilities including a causal effect in either direction or a reciprocal interaction. Despite these limitations, our findings provide useful information for future studies on the role of the oral microbiome in sleep disorder.

Conclusions

This study identified a link between oral microbiome diversity and sleep disorder on a population level, potentially enhancing the understanding of their etiology and pathogenesis. Our findings indicate that decreased oral microbiome diversity correlates with higher all-cause mortality, which should attract public attention on the oral health. Further investigations are needed to confirm the potential causal relationship and identify the specific bacterial families, community composition, and structure influencing this association.

Abbreviations

aOR	adjusted odds ratio
ASV	Amplicon sequence variant
CAPI	Computer-assisted personal interview
CI	Confidence interval
NCHS	National Center for Health Statistics
NDI	National Death Index
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
OTU	Operational taxonomic unit
PCoA	Principal coordinate analyses
PERMANOVA	Permutational multivariate analysis of variance
PIR	Family income to poverty ratio

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-05794-w.

Supplementary Material 1

Supplementary Material 2: **fig. 1**. Comparative analysis of OTUs of oral Microbiome by subpopulations among adults (A) Sleep disorders; (B) Smoke; (C) Drink; (D) Race; (E) Income; (F) Cancer; (G) Education; (H) Diabetes; (I) Age; (J) Gender; K. Marriage; L. Hypertension. * $P<0.05$; ** $P<0.01$; *** $P<0.001$. **fig. 2**. Comparative analysis of Faith's phylogenetic diversity of oral Microbiome by subpopulations among adults (A) Sleep disorders; (B) Smoke; (C) Drink; (D) Race; (E) Income; (F) Cancer; (G) Education; (H)

Diabetes; (I) Age; (J) Gender; K. Marriage; L. Hypertension. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **fig. 3**. Comparative analysis of Shannon-Weiner index of oral Microbiome by subpopulations among adults (A) Sleep disorders; (B) Smoke; (C) Drink; (D) Race; (E) Income; (F) Cancer; (G) Education; (H) Diabetes; (I) Age; (J) Gender; K. Marriage; L. Hypertension. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **fig. 4**. Comparative analysis of inverse Simpson index of oral Microbiome by subpopulations among adults (A) Sleep disorders; (B) Smoke; (C) Drink; (D) Race; (E) Income; (F) Cancer; (G) Education; (H) Diabetes; (I) Age; (J) Gender; K. Marriage; L. Hypertension. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **fig. 5**. β-diversity of oral Microbiome by sleep status in male The β-diversity of Bray-Curtis dissimilarity (A), unweighted UniFrac distance (B) and weighted UniFrac distance (C) by sleep status in male using Principal Coordinate Analysis (PCoA) applying post-stratification weights among male and female participants. Each dot represents an individual. The ellipses illustrated the 95% confidence ellipses for with and without sleep disorder group; the triangle represented the centroid of individuals for with and without sleep disorder group. **fig. 6**. β-diversity of oral Microbiome by sleep status in female The β-diversity of Bray-Curtis dissimilarity (A), unweighted UniFrac distance (B) and weighted UniFrac distance (C) by sleep status in male using Principal Coordinate Analysis (PCoA) applying post-stratification weights among female and female participants. Each dot represents an individual. The ellipses illustrated the 95% confidence ellipses for with and without sleep disorder group; the triangle represented the centroid of individuals for with and without sleep disorder group.

Acknowledgements
We would like to thank all university action teams, staff, and participants of the NHANES.

Author contributions
Guihua Hao writing original draft, and Yiwen Wu、Xiaoqiao Mo 、Yayuan Tian 、Bingqian Zhu 、Jingjing Dai 、Xiaomei Zhao do the analysis. Lili Hou and Xie Tian do the supervision.All authors reviewed the manuscript.

Funding
This study was supported by grants from the Shanghai Jiao Tong University School of Medicine, including the Nursing Development Program (SJTUHLXK2024) and the Excellent Nursing Talent Training Program of the Ninth People's Hospital of Shanghai Jiaotong University School of Medicine (JYHRC22-L02), and the Shanghai Shenkang Hospital Research Centre Project (SHDC2023CRS001).

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Consent for publication
Not applicable.

Conflicts of interest
The authors declare that they have no competing interests.

Author details
[1]Department of Nursing, Shanghai Ninth People's Hospital, School of Medicine, Shanghai Jiaotong University, 639 S Zhizaoju Rd, Shanghai 200011, China
[2]Shanghai JiaoTong University School of Nursing, Shanghai, China
[3]Department of Operation Room, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
[4]Department of General Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China

Received: 24 September 2024 / Accepted: 13 March 2025
Published online: 01 April 2025

References

1. Austin-Zimmerman I, Levey DF, Giannakopoulou O, Deak JD, Galimberti M, Adhikari K, et al. Genome-wide association studies and cross-population meta-analyses investigating short and long sleep duration. Nat Commun. 2023;14:6059.
2. Weber FC, Wetter TC. The many faces of sleep disorders in post-traumatic stress disorder: an update on clinical features and treatment. Neuropsychobiology. 2022;81:85–97.
3. Huyett P, Siegel N, Bhattacharyya N. Prevalence of sleep disorders and association with mortality: results from the NHANES 2009–2010. Laryngoscope. 2021;131:686–9.
4. Kase BE, Liu J, Wirth MD, Shivappa N, Hebert JR. Associations between dietary inflammatory index and sleep problems among adults in the united States, NHANES 2005–2016. Sleep Health. 2021;7:273–80.
5. Wang Z, Wang Z, Lu T, Chen W, Yan W, Yuan K, et al. The microbiota-gut-brain axis in sleep disorders. Sleep Med Rev. 2022;65:101691.
6. Lai CT, Chen CY, She SC, Chen WJ, Kuo TBJ, Lin HC, et al. Production of Lactobacillus brevis ProGA28 attenuates stress-related sleep disturbance and modulates the autonomic nervous system and the motor response in anxiety/depression behavioral tests in Wistar-Kyoto rats. Life Sci. 2022;288:120165.
7. Tuganbaev T, Yoshida K, Honda K. The effects of oral microbiota on health. Science. 2022;376(6596):934–6.
8. Baker JL, Mark Welch JL, Kauffman KM, McLean JS, He X. The oral microbiome: diversity, biogeography and human health. Nat Rev Microbiol. 2024;22:89–104.
9. Santacroce L, Passarelli PC, Azzolino D, Bottalico L, Charitos IA, Cazzolla AP, et al. Oral microbiota in human health and disease: A perspective. Exp Biol Med (Maywood). 2023;248:1288–301.
10. Sureda A, Daglia M, Argüelles Castilla S, Sanadgol N, Fazel Nabavi S, Khan H, et al. Oral microbiota and Alzheimer's disease: do all roads lead to Rome? Pharmacol Res. 2020;151:104582.
11. Al Bataineh MT, Künstner A, Dash NR, Abdulsalam RM, Al-Kayyali RZA, Adi MB, et al. Altered composition of the oral microbiota in depression among cigarette smokers: a pilot study. Front Psychiatry. 2022;13:902433.
12. Kapila YL. Oral health's inextricable connection to systemic health: special populations bring to bear multimodal relationships and factors connecting periodontal disease to systemic diseases and conditions. Periodontol 2000. 2021;87:11–6.
13. Parra-Torres V, Melgar-Rodríguez S, Muñoz-Manríquez C, Sanhueza B, Cafferata EA, Paula-Lima AC, et al. Periodontal bacteria in the brain-implication for Alzheimer's disease: a systematic review. Oral Dis. 2023;29:21–8.
14. Gao Y, Wang H, Hu Y, Li J, Xu W, Zhao L, et al. Whole-genome metagenomic analysis of the oral microbiota in patients with obstructive sleep apnea. Sleep Breath. 2023;27:1383–98.
15. Li R, Lin S, Tu J, Chen Y, Cheng B, Mo X, et al. Establishment and evaluation of a novel practical tool for the diagnosis of pre-sarcopenia in young people with diabetes mellitus. J Transl Med. 2023;21:393.
16. Cheng B, Wu X, Li R, Tu J, Lin S, Zhang X, et al. Associations of serum carotenoids with the severity of sunburn and the risk of cancer: A cross-sectional analysis of 1999–2018 NHANES data. Front Nutr. 2022;9:1051351.
17. Li W, Chen D, Ruan W, Peng Y, Lu Z, Wang D. Associations of depression, sleep disorder with total and cause-specific mortality: a prospective cohort study. J Affect Disord. 2022;298:134–41.
18. MacMahon B. The National death index. Am J Public Health. 1983;73:1247–8.
19. Kim JI, Zhu D, Davila J, Lee J, Chubak BM, Melamed ML, et al. Female sexual dysfunction as measured by low sexual frequency is associated with lower socioeconomic status: an analysis of the National health and nutrition examination survey (NHANES), 2007–2016. J Sex Med. 2022;19(1):90–7.
20. Taylor AL, Denniston MM, Klevens RM, McKnight-Eily LR, Jiles RB. Association of hepatitis C virus with alcohol use among U.S. Adults: NHANES 2003–2010. Am J Prev Med. 2016;51:206–15.
21. Sun WJ, Xu L, Chan WM, Lam TH, Schooling CM. Are depressive symptoms associated with cardiovascular mortality among older Chinese: a cohort study of 64,000 people in Hong Kong? Am J Geriatr Psychiatry. 2013;21:1107–15.
22. Wade WG. The oral Microbiome in health and disease. Pharmacol Res. 2013;69:137–43.
23. Deo PN, Deshmukh R. Oral microbiome: unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23:122–8.
24. Liu X, Sun ZM, Liu YN, Ji Q, Sui H, Zhou LH et al. The metabonomic studies of tongue coating in H. pylori positive chronic gastritis patients. Evid Based Complement Alternat Med. 2015; 2015:804085.

25. Krishnan K, Chen T, Paster BJ. A practical guide to the oral Microbiome and its relation to health and disease. Oral Dis. 2017;23:276–86.
26. Carlson-Jones JAP, Kontos A, Kennedy D, Martin J, Lushington K, McKerral J, et al. The microbial abundance dynamics of the paediatric oral cavity before and after sleep. J Oral Microbiol. 2020;12:1741254.
27. Sotozono M, Kuriki N, Asahi Y, Noiri Y, Hayashi M, Motooka D, et al. Impact of sleep on the Microbiome of oral biofilms. PLoS ONE. 2021;16:e0259850.
28. Chen Y, Chen X, Huang X, Duan Y, Gao H, Gao X. Analysis of salivary Microbiome and its association with periodontitis in patients with obstructive sleep apnea. Front Cell Infect Microbiol. 2021;11:752475.
29. Zhang X, Li X, Xu H, Fu Z, Wang F, Huang W, et al. Changes in the oral and nasal microbiota in pediatric obstructive sleep apnea. J Oral Microbiol. 2023;15:2182571.
30. Park SH, Shin NR, Yang M, Bose S, Kwon O, Nam DH, et al. A clinical study on the relationship among insomnia, tongue diagnosis, and oral Microbiome. Am J Chin Med. 2022;50:773–97.
31. Jia P, Zou J, Yin S, Chen F, Yi H, Zhang Q. Analysis of the salivary Microbiome in obstructive sleep apnea syndrome patients. Can J Infect Dis Med Microbiol. 2020;2020:6682020.
32. Nikitakis NG, Papaioannou W, Sakkas LI, Kousvelari E. The autoimmunity-oral Microbiome connection. Oral Dis. 2017;23:828–39.
33. Kholy KE, Genco RJ, Van Dyke TE. Oral infections and cardiovascular disease. Trends Endocrinol Metab. 2015;26:315–21.
34. Tonelli A, Lumngwena EN, Ntusi NAB. The oral Microbiome in the pathophysiology of cardiovascular disease. Nat Rev Cardiol. 2023;20:386–403.
35. Kleinstein SE, Nelson KE, Freire M. Inflammatory networks linking oral Microbiome with systemic health and disease. J Dent Res. 2020;99:1131–9.
36. Pan W, Wang Q, Chen Q. The cytokine network involved in the host immune response to periodontitis. Int J Oral Sci. 2019;11:30.

Publisher's note