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Original Research

Alterations of the oral microbiota in mild Alzheimer's disease and the appropriate application of chlorhexidine gluconate

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ABSTRACT

Objective: This study investigated the effect of 0.2 % chlorhexidine gluconate on oral microbiota dysbiosis in Alzheimer's disease (AD) and explored potential links between oral microbiota and cognition, offering new insights into its role in AD treatment.
Study design: We assessed the impact of 0.2 % chlorhexidine gluconate on the oral microbiota of patients with AD. One hundred patients were divided into two groups based on oral health score (using a cut-off of 8). Subgingival plaque samples were analyzed using 16S rRNA sequencing; no significant differences in bacterial composition were observed between groups at baseline.
Results: Poor oral health correlated with higher oral health scores ($P = 0.000$), fewer teeth ($P = 0.002$), lower cognitive levels ($P = 0.048$), and a higher proportion of patients with diabetes ($P = 0.032$). After 24 weeks of treatment with 0.2 % chlorhexidine gluconate in a randomized controlled trial, subgingival plaques from 66 patients showed changes in *Porphyromonas, Filifactor, Desulfobulbus, Anaeroglobus, Pyramidobacter, Mycoplasma, Dialister, Fretibacterium,* and *Tannerella* ($P < 0.05$). *Treponema* and *Porphyromonas gingivalis* were identified as potential interventional targets.
Conclusion: Chlorhexidine gluconate effectively alters oral flora, reducing harmful bacteria. Targeting specific microbiota disturbances may offer a promising strategy to delay AD onset or slow its progression.
Trial registration: This research was registered with the Chinese Clinical Trial Registry (ChiCTR; Reference: ChiCTR2000032876). Registered: 14th of May 2020; http://www.chictr.org.cn/showprojen.aspx?proj=53555

1. Introduction

An estimated 43.1 % of the global population are affected by neurological conditions, making them a leading cause of disease burden worldwide. Among older adults, Alzheimer's disease (AD) and other dementias account for the highest disability adjusted life-years (DALYs) (1504.2 per 100,000 people) [1]. Approximately 11 million family caregivers provide care for patients with AD or other dementias, contributing an estimated 18.4 billion hours of care valued at $346.6 billion [2]. With a rapidly aging population, 14 % of individuals in China are now aged 65 years or older, posing even greater social and economic consequences than other countries [3].

The levels of inflammatory markers in patients with AD are known to be elevated and have emerged as key players in innate immune functionality [4]. The dysbiosis of microbial composition can exert significant influence on oral and systemic health and the progression of disease [5]. Alterations in oral microecology refers to an association with established risk factors, such as inflammation, poor oral hygiene, dietary habits and host genetics [6]; these factors have been implicated in a range of conditions, including periodontal diseases. Moreover, aberrant

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host-oral microbiome interactions are known to exert systemic implications in diabetes, cardiovascular disease, colorectal cancer and AD [7]. Researchers are actively evaluating disease modifying therapies for AD; in particular, microbiome therapy has been shown to inhibit dysbiosis of the gut microbiota that modulate neuroinflammation [8]. Recent research has emphasized the importance of early prevention and that non-pharmacological interventions play an essential role in the comprehensive care of patients with AD. The current recommendations are that individuals should take appropriate actions to prevent oral diseases or risk factors associated with neurodegenerative disease, including the proactive management of oral health [9].

Mouth rinses, such as chlorhexidine gluconate, povidone iodine, and hydrogen peroxide, exert anti-microbial action and can help to reduce the salivary concentration of microbial organisms [10]. Chlorhexidine gluconate is a broad-spectrum antiseptic and the most frequently used agent against Gram-positive and Gram-negative bacteria. There is some evidence to show that rinsing before and after tooth extraction with chlorhexidine gluconate can reduce the risk of a dry socket without serious side effects or reactions [11]. There is also high-quality evidence that chlorhexidine gluconate can reduce the risk of ventilator-associated pneumonia from 25 % to approximately 19 % for critically ill patients [12]. Chlorhexidine gluconate is also known to adhere firmly to oral surfaces, making it an effective anti-microbial agent [13]. Although the anti-microbial properties of mouth rinsing agents have been investigated in detail, their specific effects on the functionality of oral microbial communities have yet to be fully elucidated [14]. In one study, the odds of low cognitive performance were 41 % lower among those who visited a dentist within the previous year than among those who did not [15]. Although poor oral health is associated with worse cognition, oral health has not been applied clinically to identify impaired cognition.

Thus far, few studies have investigated whether the effects of chlorhexidine gluconate on the oral microbiome could influence AD [16]. Our study is the first clinical trial to evaluate whether chlorhexidine gluconate can influence the oral microbiota in patients with mild AD. Few studies have investigated whether interventions that can target oral health could also be used to assess mental health indications. In our previous research, we showed that oral health interventions could improve cognitive function and mental health [17], and attempted to identify potential therapeutic targets of neuroinflammation in AD [18]. More recently, an evidence-based systematic review showed that the risk of bias was low in our previous study which adopted a randomization process, addressed missing outcome data, and included specific outcome measurements [19]. Therefore, in the present study, we investigated the specific effect of chlorhexidine gluconate on the oral microbiota of patients with mild AD and how this treatment may reduce cognitive decline in AD. The purpose of our study was to further investigate the impact of long-term chlorhexidine gluconate on the oral microbiota in AD.

2. Methods

2.1. Participants and design

We used pre- and post-intervention oral microbiota data collated by an oral health intervention trial. Drawing on previous research [20], 100 patients with mild AD were divided into two groups based on the oral health score (The Kayser-Jones Brief Oral Health Status Examination, BOHSE); there were 41 patients in the BOHSE $\leq$ 8 group and 59 in the BOHSE $>$ 8 group. Of the 100 people who were screened who had not used antibiotics and probiotics over the previous three months, 66 were enrolled in the RCT. This was a 24-week RCT that compared oral health intervention to usual care [21]. The facilitator provided toothpaste and a toothbrush. In addition to regular brushing with toothpaste, participants in the intervention group were also guided to perform oral self-care that involved swabbing the oral cavity with sterile cotton swabs moistened with 0.2 % chlorhexidine gluconate (n = 33) (Fig. 1). The facilitator visited each participant to monitor the implementation of the chlorhexidine compliance.

2.2. Sample collection

Subgingival plaque samples were collected from patients with mild AD (100 individuals at baseline and 66 individuals after 24 weeks). Samples were acquired in the morning after overnight fasting and before the teeth were brushed [22]. All samples were frozen at −80 °C.

2.3. DNA purification and sequencing

DNA extraction and 16S rRNA gene amplicon sequencing were conducted by Sangon Biotech Co. Ltd. (Shanghai, China). Genomic DNA was extracted using the E.Z.N.A Mag-Bind Soil DNA Kit (OmegaBio Tek, USA) in accordance with the manufacturer's instructions. The integrity of the DNA was assessed by 2 % agarose gel electrophoresis, and the concentration of DNA was measured using a Qubit 3.0 fluorometer and a Qubit 3.0 DNA Assay Kit (Invitrogen, USA). Sequencing was performed on an Illumina MiSeq system (2 × 300 bp paired-end reads), targeting the 16S V3-V4 region (341F: CCTACGGGNGGCWGCAG and 805R: GACTACHVGGGTATCTAATCC) [23]. Raw FASTQ files and sample metadata for 16S rRNA sequencing were deposited at the Sangon Biotechnology Database (ftp://ftp.sangon.com:21148) under accession number 16S202084FZ.

2.4. Statistical analysis

Data analyses were performed using a non-parametric Wilcoxon test using IBM SPSS version 19 (IBM Corporation, NY); differences were considered significant when $P < 0.05$. Mothur was used for bioinformatics analysis and R (version 3.6.0) was used for statistical analysis. Multiple comparisons were performed using the Mann-Whitney U test,

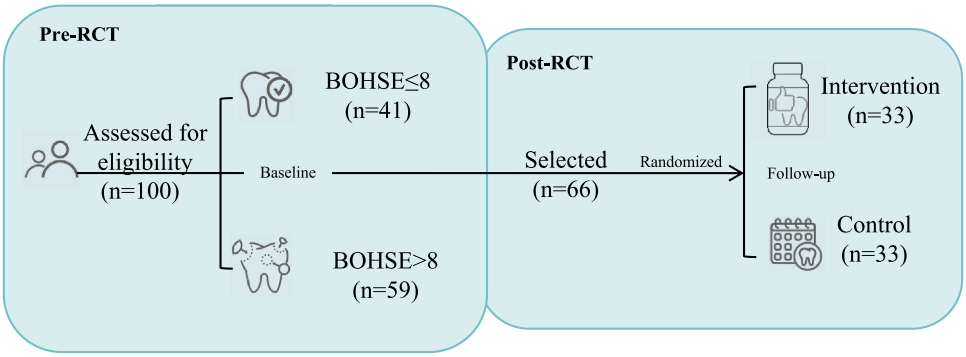

Fig. 1. Study design.Pre- and post-randomized controlled trial (RCT). The measurements were performed before and after intervention.

and adjustments for multiple comparisons were made using the Benjamini-Hochberg-based FDR correction. Beta diversities were visualized by principal coordinate analysis and non-metric multidimensional scaling (NMDS) frameworks. The sums of standardized compositional data of bacteria in different groups were compared using the Wilcoxon signed rank test while correlations within and between the relative abundances of groups of bacteria were determined by Spearman's rho, along with associated adjusted p-values. GraphPad Prism (version 8.0) was used to visualize key data.

3. Results

3.1. Demographic and clinical characteristics

Initially, we screened 100 patients with mild AD aged between 60 and 94 years, with a mean age of 82.27 ± 6.50 years. The maximum and minimum BOHSE values were 12 and 5, respectively, with a mean of 8.75±1.50. There were no significant differences between the two groups in terms of age, education level, gender, marital status, occupation, history of hypertension, body mass index (BMI), and the number of removable dentures ($P > 0.05$). However, there were significant differences in the history of diabetes ($P = 0.032$), the number of natural teeth ($P = 0.002$), the oral health score (BOHSE) ($P = 0.000$), and the Mini-Mental State Examination score (MMSE) ($P = 0.048$).

Sixty-six randomized patients completed the trial, with ages ranging from 66 to 94 years, and a mean age of 82.85 ± 6.00 years. There were no significant differences between the two groups in terms of age, education level, gender, marital status, occupation, history of diabetes, history of hypertension, BMI, the number of removable dentures, and the number of natural teeth ($P > 0.05$). Demographic and clinical characteristics are presented in Table 1.

3.2. RNA sequencing

One hundred subgingival plaque samples were subjected to 16S rRNA amplicon sequencing from 100 patients. Illumina MiSeq sequencing yielded 6455,143 sequencing reads with a mean length of 423 bp. After clustering at a similarity level of 97 %, a total of 25,079 OTUs were obtained, with a community coverage index > 99.9 %. Overall, the OTUs belonged to 17 phyla, 30 classes, 53 orders, 103 families, and 185 genera. Group comparison revealed no significant difference in terms of valid sequences and mean length ($P > 0.05$) but did identify a significant difference in the OTU index ($P = 0.041$). No significant differences were detected between the two groups in terms of each index ($P > 0.05$), indicating that there were no significant differences between the two groups in terms of the diversity and abundance of bacterial flora.

Six months later, 66 subgingival plaque samples were sequenced; Illumina MiSeq sequencing yielded 4075,598 valid sequences with a mean length of 422 bp. A total of 13,683 OTUs were obtained, belonging to 33 phyla, 67 classes, 116 orders, 211 families, and 440 genera. Comparison between the two groups revealed no significant difference in valid sequences and mean length ($P > 0.05$) but did identify a significant difference in the OTU index ($P = 0.041$). There were significant differences in the Shannon ($P = 0.006$) and Simpson ($P = 0.016$) indices between the two groups, indicating significant differences in the diversity and abundance of the bacterial flora between the experimental group and control groups (Table 2).

3.3. Comparison of the oral microbiome between the two groups prior to intervention

The analysis of β-diversity overlapped between the two groups and there was no significant difference in terms of PCA (Fig. 2A). Next, we generated a heatmap which showed that there were no significant differences between the two groups in terms of genera and community composition (Fig. 2B). Genera with a species abundance > 1 % at the genus level in the two groups of 100 subgingival plaque samples included *Streptococcus, Neisseria, Prevotella, Leptotrichia, Veillonella, Fusobacterium, Capnocytophaga, Selenomonas, Porphyromonas, Saccharibacteria_genera_incertae_sedis, Haemophilus, Rothia, Campylobacter, Corynebacterium, Aggregatibacter* and *Treponema* accounting for >83 % of the total bacterial population. The remaining genera were *Granulicatella* and *Lactobacillus.*

3.4. Analysis of the effect of 2 % chlorhexidine gluconate on the oral microbiome

A Shepard plot identified a strong correlation between observed dissimilarity and ordination distance ($R^2 = 0.984$) highlighting a high

Table 1
Baseline clinical characteristics of the Pre-RCT and Post-RCT of mild Alzheimer's disease patients.

Variables	Pre-RCT BOHSE≤8 ($n = 41$)	BOHSE>8 ($n = 59$)	*P*-value	Post-RCT Intervention group($n = 33$)	Control group ($n = 33$)	*P*-value
Age	81.15±6.05	83.05±6.74	0.111	82.70±6.03	83.00±6.04	0.653
Education	11.24±3.97	10.42±4.73	0.542	11.27±4.40	10.67±4.66	0.770
Gender			0.073			0.786
Male	9(22.0)	23(39.0)		10(30.3)	9(27.3)	
Female	32(78.0)	36(61.0)		23(69.7)	24(72.7)	
Marital status		0.599		0.276 [b]	0.135	
Married	25(61.0)	39(66.1)		22(66.7)	16(48.5)	
Widowed	16(39.0)	20(33.9)		11(33.3)	17(51.5)	
Occupation			0.456			0.976
worker	8(19.5)	19(32.2)		8(24.2)	8(24.2)	
leader	7(17.1)	13(22.0)		5(15.2)	7(21.2)	
teacher	10(24.4)	8(13.6)		8(24.2)	7(21.2)	
medical staff	7(17.1)	8(13.6)		5(15.2)	5(15.2)	
employee	9(22.0)	11(1.2)		7(21.2)	6(18.2)	
Diabetes	5(12.2)	18(30.5)	0.032*	9(27.3)	5(15.2)	0.228
Hypertension	24(58.5)	37(62.7)	0.674	21(63.6)	18(54.5)	0.453
BMI	22.37±1.60	22.66±1.55	0.377	22.86±1.50	22.28±1.65	0.190
Natural teeth	21.37±8.07	17.02±6.87	0.002*	20.06±6.83	19.55±8.50	0.938
Denture teeth	3.24±6.51	2.00±3.85	0.802	3.82±5.58	2.91±6.30	0.251
BOHSE	7.37±0.97	9.71±0.95	0.000*	8.45±1.60	8.61±1.30	0.777
MMSE	19.71±1.91	18.90±1.45	0.048*	20.00±1.39	19.36±1.58	0.057

BMI, Body Mass Index; BOHSE, The Kayser-Jones Brief Oral Health Status Examination; MMSE, Mini-Mental State Examination.
Data are analysed and presented as n (%) or mean ± SD. *P*-value calculated using Mann-Whitney U test or Chi-square test. * $p < 0$ 0.05.

Table 2
Clinical characteristics of the Pre-RCT and Post-RCT of mild Alzheimer's disease patients.

Variables	Pre-RCT BOHSE≤8 ($n = 41$)	BOHSE>8 *P*-value ($n = 59$)		Post-RCT Intervention group($n = 33$)	Control group($n = 33$)	*P*-value
SeqNum	66,121.51±7226.82	63,460.36±7160.16	0.140	63,282.09±15,399.53	60,220.88±13,589.04	0.333
SeqNum	423.17±2.92	423.37±2.61	0.933	422.27±2.27	422.12±2.54	0.903
OTU	257.05±26.88	246.44±30.22	0.041*	225.18±90.49	189.45±38.32	0.044*
Shannon	3.36±0.56	3.31±0.49	0.298	3.42±0.51	3.12±0.43	0.006*
Chao	307.77±37.52	291.88±31.44	0.06	239.74±89.82	210.35±44.32	0.228
Ace	300.74±29.65	289.16±30.15	0.08	236.44±89.38	207.3 ± 42.29	0.168
Simpson	0.09±0.10	0.09±0.05	0.255	0.08±0.05	0.10±0.05	0.016*

* $p < 0$ 0.05.

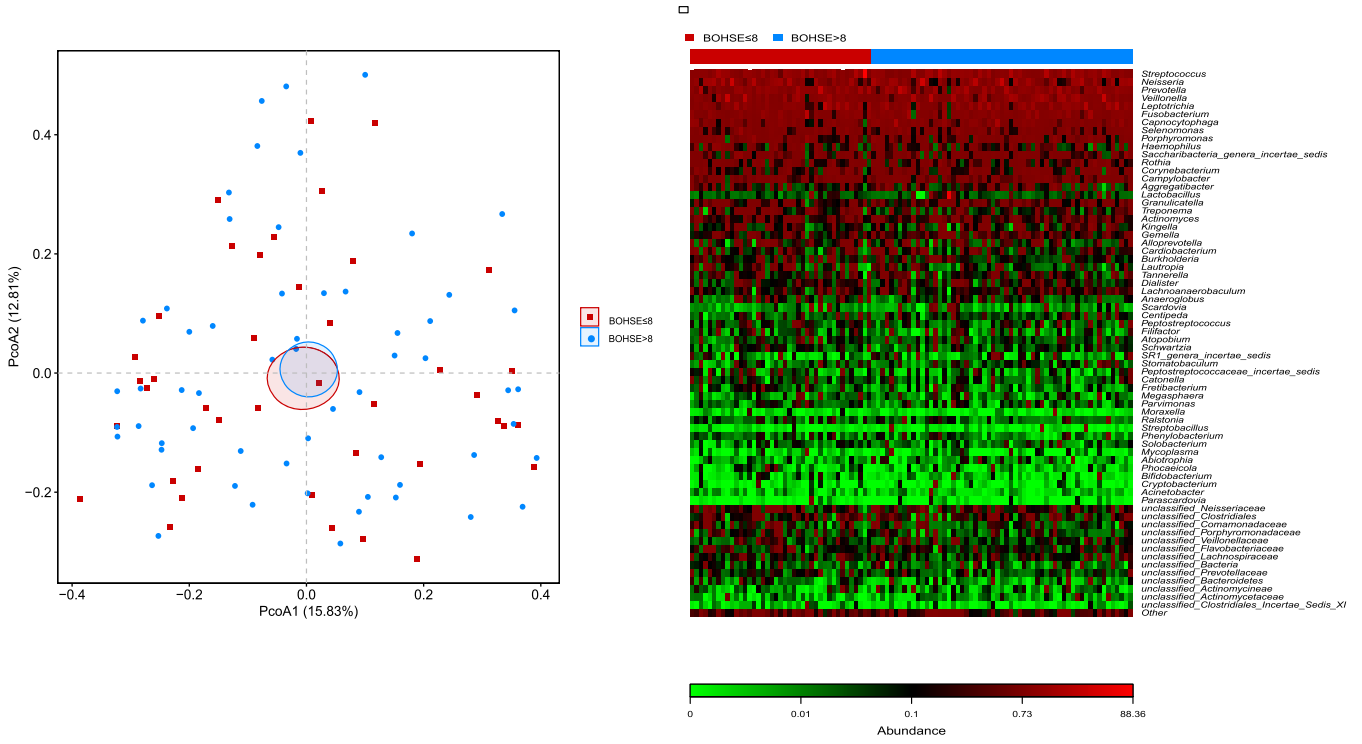

Fig. 2. Comparison of the preintervention oral microbiome of BOHSE≤8 and BOHSE>8 groups. (A) PCoA of oral microbiota in BOHSE≤8 and BOHSE>8 samples. (B) Heatmap of relative abundance between BOHSE≤8 and BOHSE>8 groups at the genus level.

goodness-of-fit of the NMDS (Fig. 3B). Compared to the controls, the intervention group exhibited significantly reduced abundances of certain genera, including *Anaeroglobus, Pyramidobacter, Mycoplasma, Dialister, Porphyromonas, Treponema, Filifactor, Desulfobulbus*, and *Fretibacterium*. In addition, we identified increased abundances of *Bacillus, Aquabacterium, Trichococcus, Haliscomenobacter, Rhodocyclaceae, Lachnoanaerobaculum, Kingella, Leptotrichia, Syntrophorhabdus* and *Enterobacteriaceae* ($P < 0.05$, Fig. 3A and C).

Genera with a species abundance > 1 % at the genus level in the two groups of 66 subgingival plaque samples included *Prevotella, Fusobacterium, Burkholderia, Streptococcus, Neisseria, Porphyromonas, Veillonell, Phenylobacterium, Leptotrichia, Capnocytophaga, Campylobacter, Haemophilus, Selenomonas, Rothia, Treponema, Actinomyces, Aggregatibacter, Lautropia* and *Kingella* accounting for > 83.19 % of the total bacterial population. The remaining genera were *Cardiobacterium* and *Corynebacterium*.

4. Discussion

Neuroinflammation is considered as a potential component in the pathological initiation of AD [24]. Recent analysis of the human oral microbiome has revealed that the microorganisms residing in the oral cavity are a major contributor to the overall health of the host [25]. Dysbiosis of the oral microbiome is closely involved in the pathogenesis of systemic diseases. Comorbidities that can increase the severity of AD include the suboptimal management of diabetes mellitus and inadequate oral hygiene [26].

In our study, we found that harmful oral bacteria were prevalent in patients with mild AD and that their abundance considerably decreased after 24 weeks of 0.2 % chlorhexidine gluconate treatment in the intervention group compared to the control group. A recent meta-analysis identified associations between six oral microbes and unhealthy aging (*Streptococcus, Actinomyces, Desulfovibrio, Campylobacter, Atopobiaceae,* and Veillonella) [27]. However, the definition of a "healthy" microbiome remains controversial in the literature. Some studies have attempted to define a "normal" oral microbiota based on healthy subjects, with *Streptococci, Neisseria, Prevotella, Veillonella,* and *Rothia* identified as the most typical genera [28]. Microorganisms in the oral cavity rarely survive as planktonic cells; instead, they thrive within biofilms attached to tissues [29]. Changes in the oral microbiome of patients often involve reduced alpha diversity, an index commonly used to assess the richness and variety of bacterial taxa and species in the oral

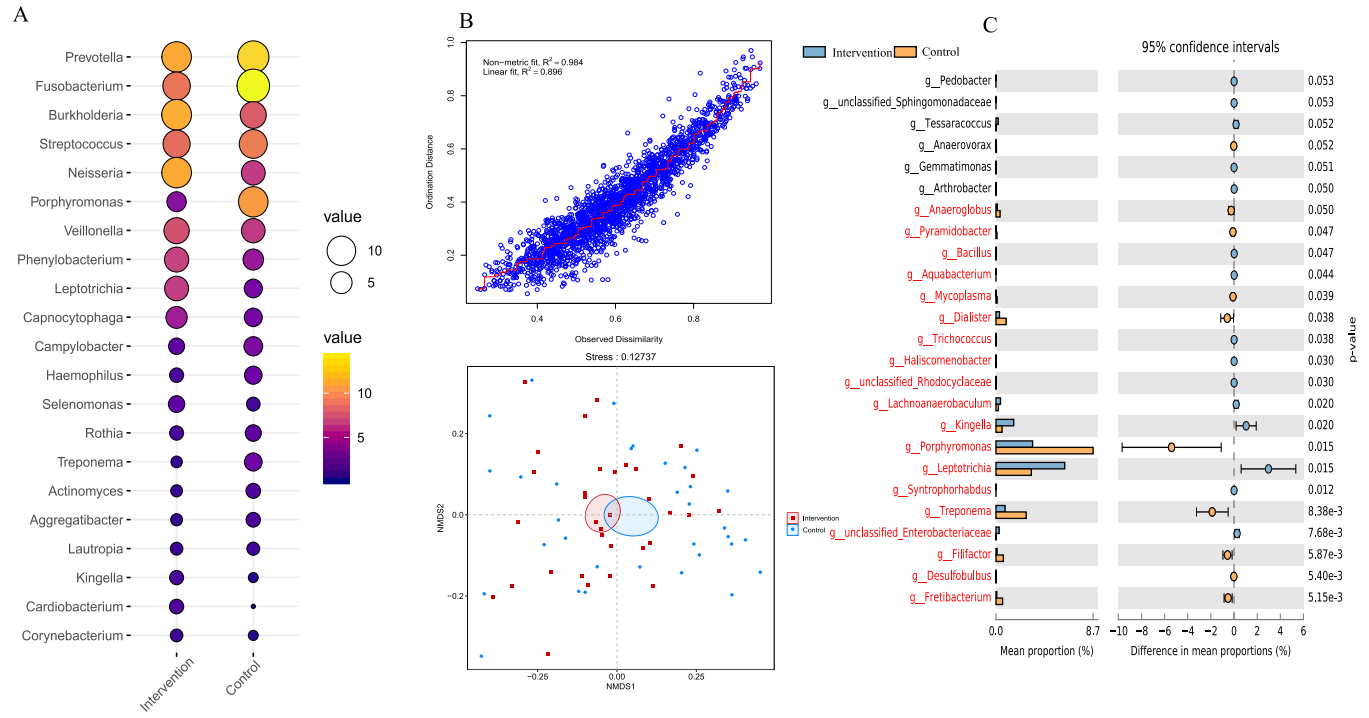

Fig. 3. Comparison of the postintervention oral microbiome of intervention and control groups. (A) Balloon plot showing the results of the oral microbiota in intervention and control groups.(B) Shepard plot and NMDS plot of oral microbiota in intervention and control groups.(C) Extended error bar plot illustrating genera statistically different between intervention and control groups.

cavity [30].

We divided our patients into two groups, based on oral health score and analyzed the oral microbiome; then, we used sequencing technology to provide valuable cut-off values for oral health score (using a cut-off of 8). Our findings suggest that poor oral health is associated with a higher oral health score and fewer teeth. In a previous study, tooth loss was positively associated with the risk of dementia among adults over the age of 60 years [31]. A recent study revealed that periodontal infection and the oral microbiome are associated with AD [32]. Low cognitive performance has been related to an increased odds of periodontal disease and tooth loss [33]. A recent study reported that *Porphyromonas gingivalis* was related to a lower score in the Mini-Mental State Examination (MMSE) [34]. Aβ plaque formation in AD is connected to the innate immune response via activation of the NLRP3 inflammasome in the microglia and the release of ASC specks that drive the assembly and deposition of Aβ [35]. Notably, *P. gingivalis* was the first microbial pathogen shown to induce ASC aggregation specks in *P. gingivalis*–infected primary human monocytes via activation of the NLRP3 inflammasome [36]. Collectively, these findings highlight a possible link between AD and periodontal infection. Thus, there appears to be a bidirectional relationship between the oral microbiome and systemic health; this implies that the development of systemic disease may be associated with the oral microbiome [37]. Type 2 diabetes mellitus resulting in hyper-glycemia can potentially cause periodontitis and lead to vascular complications and the production of proinflammatory molecules [38]. Specifically, patients with diabetes have a higher abundance of *Capnocytophaga* species and *Fusobacterium nucleatum* than patients who are not diabetic [39]. *Capnocytophaga* species are major saccharolytic organisms that have been shown to demonstrate elevated glucose levels, thus indicating enhanced proteolytic potential [40]. *F. nucleatum* has been implicated in anaerobic microenvironments, thus increasing the outgrowth of anaerobic periodontal pathogens [41].

We identified a significant and beneficial change in the microbiome configuration in the intervention group but not in the control group. There was a reduction in the relative abundance of taxa associated with an increased risk of AD in the intervention group. *Prevotella* and *Turicibacter* have been shown to promote the progression of AD [42], and the abundance of these taxa decreased over the course of intervention. These results support our hypothesis that chlorhexidine gluconate intervention may beneficially modify specific microbial groups in the microbiome, thus reducing the risk of AD. *Prevotella* enrichment in patients with AD was previously negatively correlated with cognition and function [43]. Neuroinflammation plays an important role in various neurodegenerative disorders. Microbial dysbiosis, a leaky gut, and cerebral and vascular barriers are also implicated in neurodegenerative disorders [44]. Taati Moghadam et al. [45] previously identified significantly higher levels of *P. gingivalis, F. nucleatum* and *P. intermedia* in an AD group when compared to a control group. These findings identified a potential link between the oral microbiome and inflammation in AD. The higher abundance of specific pathogenic oral bacteria in patients with AD, including *P.s gingivalis, F. nucleatum*, and *P. intermedia*, suggests a potential role for oral microbial dysbiosis contributing to the inflammatory profile observed in such patients. Of these, *Prevotella* is a proinflammatory factor and could exacerbate neuroinflammation [46].

There is an established association between AD and poor oral health, specifically periodontitis [47]. A recent study revealed links between oral cavity-related stressors and neuropsychiatric symptoms in patients with AD [48]. Collectively, the findings of these studies highlight the importance of considering the oral health of patients with neurodegenerative disorders. Furthermore, a recent meta-analysis reported the detection of oral bacteria in the brains of patients with AD [49]. This observation could provide insight into why the spouses of patients with AD are at a higher risk of AD [50]. Overall, it is plausible that the periodontal microbiota could reach the brain, thereby contributing to the progression of AD [51]. Patients with AD exhibit dysbiotic oral microbiota as well as reduction of bacterial diversity and an increase in the prevalence of the *Moraxella, Leptotrichia* and *Sphaerochaeta* genera [52]. In addition, a recent study showed that periodontal treatment had a potential effect on AD-related brain atrophy, as determined by magnetic resonance imaging (MRI) [53]. Oral bacteria can transfer to the

circulation via the periodontal tissues. Subsequently, transient bacteremia can cause inflammatory and metabolic functional complications, which underlie the association between oral disease and systemic comorbidities [54]. For instance, *P. gingivalis* has been detected in the brains of patients with AD and was correlated with AD pathology [55].

Targeting the structural organization of the microbiome may advance treatment strategies by shifting the balance from dysbiosis toward a healthy microbial community [56]. Previous studies have reported that periodontal pathogens such as *Prevotella, Saccharibacteria, Treponema,* and *Selenomonas* are significantly more abundant in AD groups, especially in subgingival plaques [57]. Another study observed increased relative abundances of *Atopobium, Saccharibacteria, Treponema,* and *Selenomonas* in patients with AD, all strongly associated with periodontitis [58].The major pathogens responsible for periodontitis not only stimulate proinflammatory responses in the oral cavity but may also contribute to systemic inflammation [59]. Simple oral hygiene measures could help interrupt this pathogenic pathway. It is important to investigate the specific mechanism of action of chlorhexidine gluconate on oral microorganisms, as its targeted application may offer therapeutic benefits for AD [60].

Our study identified several oral microbes as potential targets for intervention, including *Treponem*a and *Porphyromonas gingivalis*. In addition, intervention with 0.2 % chlorhexidine gluconate had a significant effect on *Filifactor, Desulfobulbus, Fretibacterium*, and *Tannerella*; these findings are consistent with previous studies (59). The oral-brain axis refers to specific communication between the oral cavity and the brain through the oral microbiome; thus, the development of periodontitis may not be the direct result of a single type of pathogenic bacteria [61]. At the genus level, higher abundances of several microbiota have been detected in AD cases, including *Prevotella, Leptotrichia, Actinomyces, Streptococcus, Veillonella, Selenomonas, Porphyromonas, Treponema* and *Kingella*; in contrast, there were lower abundances of *Fusobacterium, Rothia, Actinomyces, Porphyromonas, Cardiobacterium, Prevotella, Aggretibacter* and *Lautropia* [62].

Brain functionality changes with age; thus, alleviating pathological damage represents a promising strategy for improving cognition. The dysbiosis characterized by an increased abundance of harmful microbes, the reduced diversity of the microbiota and the abundance of beneficial microbes can lead to a persistent inflammatory reaction [63]. Advancing age is also associated with an increased abundance of *Prevotella* and a reduced abundance of *Bacteroides* [64]. The primary taxa of the oral cavity in healthy subgingival plaques consist of *Streptococcus, Prevotella, Capnocytophaga, Corynebacterium, Neisseria Methanobrevibacter, Entamoeba* and *Trichomonas* [65]. When the balance of the microbiota is disrupted, harmful bacteria can invade the gum tissue, thus causing inflammation and ultimately, tooth loss [66]. A previous study reported an overall oral bacterial infection rate of 83.75 % in brain samples acquired from patients with AD [49]. *Porphyromonas gingivalis, Treponema, Streptococcus* and *Actinomycetia* have also been detected in post-mortem brain samples from patients with AD [67].

Our result findings are in agreement with a recent study that showed that a lower diversity of bacteria and chlorhexidine gluconate led to a higher abundance of *Neisseria* a reduced abundance of *Actinomyces* [68]. Despite our participants had not used antibiotics and probiotics, confounding variables (e.g., medication, oral dryness, diet) could not be ruled out in our study may bias the oral microbiota results. The diversity of the oral microbiome is known to be lower in patients with AD, thus suggesting an overgrowth of specific microbiota in these patients [69]. Poor oral health leads to detrimental brain structure changes in middle-aged persons [70].

The oral bacteria is not only associated with inflammation; evidence exists to link oral health with systemic disease [71]. The interpretation of microbiome genetic data could identify effective therapies to rebuild the resident microbiome in patients [72]. Our findings suggest that poor oral health and a lower cognitive level is particularly common in patients with diabetes. Diabetes is known to be associated with an increased risk of dementia [73]. Individuals with diabetes may have an increased the risk of Aβ accumulation, thereby increasing the risk of AD [74]. Diabetes appears to act as a catalyst in the progression of AD, accelerating atrophy in the nucleus accumbens, reducing gray matter volume, and further promoting the conversion of cognitive impairment to AD [75]. Inflammation might represent a common pathway for many risk factors associated with dementia. Notably, interventions or lifestyle changes at any stage of life can alter the risk of dementia [76].

In this study, we conducted an RCT to investigate the impact of eradicating chronic infections and specific periodontal bacteria on AD. Preventing poor oral health and screening for various pathogens may help mitigate their potential contribution to AD. By targeting harmful bacteria, effective strategies can be developed to reduce periodontal pathogens across the life course, promoting better oral health at all stages of life. Although targeting secondary microbiome changes is unlikely to yield sustained disease remission, addressing specific disturbances that exacerbate disease severity may still result in meaningful therapeutic benefits [27]. Ultimately, studying oral microbial communities aims to develop tools for engineering their composition toward a state compatible with oral health.

Although the management of AD involves a comprehensive approach that addresses not only cognitive symptoms but also behavioral, psychological, and functional aspects of the disease. The influence of chlorhexidine gluconate as a disinfectant still has several limitations that need to be considered. First, the long-term abuse of oral disinfectants can disrupt the homeostasis of the oral microbiota with possible negative consequences for oral health. Another limitation is that the use of 0.2 % chlorhexidine gluconate did not lead to a significant improvement in the cognition score of patients with AD. This may be related to bias, such as potential observer bias in the BOHSE scoring, selection bias from drop-out (we observed a 34 % attrition rate from screening to enrolment) and the absence of a placebo control or blinding. Finally, it is not sufficient for elderly individuals to wipe their oral cavity with cotton wool soaked in chlorhexidine gluconate. Individualized care plans should be tailored to the specific needs and preferences of each patient and their caregivers, with regular monitoring and adjustments as the disease progresses. Researchers should consider longer-term follow-up and the use of more sensitive cognitive metrics (e.g., ADAS-Cog, MoCA and CDR-SB) in future trials. Researchers should also incorporate peripheral inflammatory markers (e.g., TNF-α, IL-6, and CRP) in future research to validate the hypothesized pathway. In addition, future research should involve an extended follow-up duration and the stratified randomization of larger cohorts based on AD biomarker status and comorbidities.

5. Conclusions

This is the first sufficiently powered RCT of an oral intervention for patients with AD and the first to compare the efficacy of chlorhexidine gluconate in improving oral microecology in this population. We found that a 0.2 % chlorhexidine gluconate oral intervention was superior to usual care in improving the oral microecology of patients with AD at 24 weeks post-intervention. However, the swabbing technique may not scalable or clinically feasible. Our study generated a clear hypothesis and outlined next steps for mechanistic and clinical validation. Rational interventions targeting the oral microbiota could offer a promising strategy to delay AD onset or slow its progression.

CRediT authorship contribution statement

Huizhen Cao: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jiangming Zhong:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Lili Chen:** Writing –

review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Huizhen Cao designed the study. Jiangming Zhong and Huizhen Cao were responsible for data analysis and manuscript preparation. Jiangming Zhong and Lili Chen contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content. All authors approved the final manuscript.

Data availability

ftp://ftp.sangon.com:21148 (The 16S rRNA amplicon sequencing data used during the current study are available at under accession number 16S202084FZ. Additional information is available from the author on reasonable request.)

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