Journal of Dental Sciences 17 (2022) 1762–1779

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

Review article

Association of periodontitis and oral microbiomes with Alzheimer's disease: A narrative systematic review

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Received 29 June 2022; Final revision received 2 July 2022
Available online 21 July 2022

KEYWORDS
Alzheimer's disease;
Cognitive impairment;
Periodontitis;
Oral microbiome

Background/purpose: Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia. The etiology for AD includes age, genetic susceptibility, neuropathology, and infection. Periodontitis is an infectious and inflammatory disease which mainly causes alveolar bone destruction and tooth loss. The evidence of a link between AD and periodontitis remains controversial. Thus far, studies reviewing the association between AD and periodontal disease have been insufficient from the viewpoint of the oral microbiome. The aim of this review was to focus on studies that have explored the relationship between the oral microbiome and AD development by using the next-generation sequencing technique.
Materials and methods: A comprehensive electronic search of MEDLINE via PubMed, EMBASE, Scopus, and Google Scholar was conducted. The keywords included dementia, Alzheimer's disease, cognitive impairment, periodontitis, periodontal disease, and oral microbiome.
Results: This review included 26 articles based on the eligibility criteria. Epidemiologic researches and post-mortem studies showed that the presence of periodontitis is associated with cognitive decline, suggesting a possible role of periodontal pathogens in the pathogenesis of

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https://doi.org/10.1016/j.jds.2022.07.001

AD. The reported microbiome was inconsistent with those in gene sequencing studies. Nevertheless, Gram-negative species may be possible candidates.
Conclusion: This review suggests that periodontal infection is associated with AD. The contributing microbiome remains unconfirmed, possibly because of different microbiome sampling sites or methods. Additional large-scale studies with periodontal intervention and longitudinal follow-up are warranted to clarify the relationship between periodontal disease and AD.

Introduction

Over 55 million people live with dementia globally, with Alzheimer's disease (AD) being the most common cause of dementia, contributing to 50%–60% of cases.[1,2] AD is clinically diagnosed based on memory loss, impaired language functions, impaired visuospatial abilities, impaired judgment, and changes in personality.[3,4] Aging is the most obvious risk for the disease, but genetic and acquired factors also play a major role in AD.[5–10]

The histological characteristics of AD include neuron destruction and abnormal deposition of intraneuronal tau neurofibrillary tangles and extracellular amyloid beta (Aβ) protein plaques in the cerebral neocortex.[3] The abnormally hyperphosphorylated tau protein inhibits assembly and disrupts microtubule network in brain cells, causing synapse loss and neuron death.[11] Aβ is generated by the improper cleavage of the amyloid precursor protein. Aβ42 is the dominant variant of senile plaques in brains affected by AD.[12] Aβ induces hyperphosphorylation of tau in neurons and causes neuritic degeneration.[13] Systemic inflammation may also cause neuroinflammation and contribute to AD pathology.[14] Studies have shown that individuals with higher inflammatory state are more likely to develop cognitive decline.[14,15] Inflammatory cytokines such as tumor necrosis factor alpha and interferon gamma increase Aβ production, which suggests a possible pathway by which systemic inflammation accelerates AD development.[16] Animal studies have revealed that neuronal inflammation induced by intraperitoneal lipopolysaccharide (LPS) injection increases Aβ42 production, astrocyte activation, and neuronal death, along with memory impairment.[17]

The establishment of the gut–brain axis connected gastrointestinal microbiota with cognition decline, suggesting a possible association between bacteria and AD development.[18,19] Bacterial products such as LPS and short chain fatty acids (SCFA) can modulate the peripheral and central nervous systems and act as a potential pathogenic link between the gut microbiota and amyloid pathology in AD.[20] SCFA have also been demonstrated to regulate microglia maturation and function in animal studies, which may alter the host's immune response.[21] Post-mortem studies have reported greater levels of LPS and *Escherichia coli* DNA in AD human brains compared to control groups, with LPS colocalized with Aβ amyloid plaque, suggesting that Gram-negative bacteria are associated with AD pathogenesis.[22]

The oral cavity has the second largest distribution of microorganisms after the gut, harboring over 700 microbiome species.[23] The Human Oral Microbiome Database includes 619 oral taxa that belong to 13 phyla, as follows: *Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, Saccharibacteria, Synergistetes, Tenericutes,* and *Absconditabacteria*.[24] The oral–gut–brain axis is considered direct and indirect evidence that oral microorganisms are associated with immunological mechanisms in the brain, particularly periodontal pathogens.[25] Periodontitis is an inflammatory disease of infectious origin, which may progress to a systemic condition and disrupt the immune system and cause dysbiosis of the oral cavity, gut, and other locations.[26] Past epidemiological studies have revealed a correlation between AD and periodontitis occurrence.[27,28] A study on dementia in monozygotic twin pairs also revealed that tooth loss before the age of 35 is a significant risk factor for AD.[29] Because the genetic influence has been isolated from environmental factors, the inflammatory state caused by periodontitis may contribute to the development of AD. Although the mechanism remains to be determined, studies exploring post-mortem brain tissues have shown the presence of various bacteria, including *Porphyromonas gingivalis* and *Treponema denticola,* which are part of the oral microbiome, in AD patients.[30–32] Animal studies have revealed that periodontal pathogens can access the brains of mice and may contribute to the development of AD.[33] Ishida et al. presented an animal model that showed how *P. gingivalis* exacerbates the pathological features of AD.[34] Possible pathways that allow *P. gingivalis* and other microbiomes to influence the brain include (1) the bloodstream and a weakened blood–brain barrier affected by age and ongoing infections or inflammation, (2) the olfactory and trigeminal nerves, and (3) direct access through perivascular spaces.[35] The definitive pathway should be explored for a better understanding of the 2 diseases.

Microbiomes that are difficult to detect using traditional cultivation methods can be identified using 16S rRNA gene clonal analysis.[36,37] An analysis of 16S rRNA gene sequences identified 1179 taxa, of which only 24% were named, 8% were cultivated but unnamed, and 68% were uncultivated phylotypes, which demonstrated how culture-independent molecular biology methods broaden our understanding of the oral microbiome.[24] New

candidate pathogens for caries, periodontal diseases, and endodontic infection have also been identified using 16S rRNA gene amplification.[38–40] However, the high cost and technical sensitivity of 16S rRNA gene amplification, followed by cloning and Sanger sequencing, make analyzing large numbers of samples difficult.[41] Next-generation sequencing (NGS) provides greater sampling depth and detection for low-abundance taxa with a higher throughput compared to the classic Sanger technique. The limitation of NGS is that the short length of reads may be insufficient for bacterial identification, thus most studies using NGS have focused on hypervariable regions of the 16S rRNA gene, which can still be informative despite short length reads.[42,43]

NGS is more sensitive than traditional methods and may provide insight into the oral microbiome. Thus far, the use of the NGS technique has been lacking in studies reviewing the connection between AD and periodontal disease. Hence, this review focused on studies that explored the relationship between the oral microbiome and AD development using the NGS technique.

Materials and methods

Search strategy

A comprehensive electronic search of MEDLINE via PubMed, EMBASE, Scopus, and Google Scholar was conducted for human studies published in English up to November, 2021 by using of keywords such as dementia, Alzheimer's disease, cognitive impairment, periodontitis, periodontal disease, and oral microbiome. The inclusion criteria were as follows: (1) Investigating the prevalence of periodontitis in AD and cognitively normal patients; (2) Investigating the clinical parameters of periodontitis in AD patients and healthy controls; (3) Investigating the composition of the oral microbiome in patients with AD and healthy controls. The exclusion criteria were as follows: (1) Studies that did not specify AD or recognition impairment caused by dementia; (2) Narrative reviews; (3) Articles that were not written in English.

Outcome measures

AD and periodontal measures were included in the review. The AD measures were as follows: (1) Mini-Mental State Examination (MMSE) (2) Hasegawa Dementia Scale-Revised scores, (3) Raven's Coloured Progressive Matrices (RCPM) test, (4) Visual-Paired Associate (VisPA) task, (5) Verbal-Paired Associate (VerPA) task, (6) 2-min Digit Symbol Substitution Test (DSST), (7) Spatial Copying Task, (8) Block Design Test (BDT), (9) Alzheimer's Disease Assessment Scale, (10) East Boston Memory Test, (11) Clock-Drawing Test, (12) Clinical Dementia Rating scale, (13) Sum of Boxes, (14) Aβ deposition. The periodontal measures were as follows: (1) Inflammatory markers, (2) clinical attachment level (CAL), (3) probing depth (PD), (4) teeth number.

Results

Results of the search

A total of 311 potential articles were identified through the electronic search. After screening, 264 articles were excluded based on their title or abstract. The remaining 47 articles were evaluated through full-text evaluation, and 21 were excluded for failing to meet the inclusion criteria. Finally, 26 articles were included in this review. Among the 26 included studies, 21 were human studies and 5 were post-mortem studies. The 21 human studies consisted of 7 cross sectional studies, 7 cohort studies, and 7 case-control studies.

Study outcomes

All included studies were divided into 5 groups for further assessment in Table 1.

1. Database- and survey-based studies

Six studies assessed the association between AD and periodontitis via database analysis. Four of the studies used the National Health and Nutrition Examination Survey as their data source, and the remaining 2 examined data from the Washington Heights-Inwood Columbia Aging Project and National Health Insurance Research Database of Taiwan. The numbers of included patients ranged from 219 to 27,963, and the inclusion criteria ranged from age over 45 years to age over 65 years. Because of the nature of these studies, most of them either diagnosed AD based on ICD code 331.0 or did not mention how they diagnosed AD. Beydoun et al. reported that clinical periodontal parameters, namely PD and CAL, had a marginal association with incident AD risk.[44] Four studies investigated the effect of microbiomes using serum immunoglobulin G (IgG) titers. *Campylobacter rectus*, *P. gingivalis*, and the Red-Green cluster were associated with higher AD risk, whereas the Orange-Red cluster, *P. gingivalis*, *Prevotella melaninogenica*, *Streptococcus oralis*, and *Staphylococcus intermedius* were associated with higher AD mortality risk in one study.[44] The same authors also investigated the relationship between periodontal pathogens and *Helicobacter pylori* and discovered that *P. intermedia*, *C. Rectus*, *P. nigrescens*, *P. melaninogenica*, and *P. gingivalis* interacted synergistically with *H. pylori* with respect to AD incidence.[45] IgG titers to *P. gingivalis* were associated with cognitive test results such as poor delayed verbal recall and impaired subtraction in a dose-dependent matter.[46] Patients with higher *Actinomyces naeslundii* antibody levels had higher risk of developing AD, whereas those with higher *Eubacterium nodatum* antibody levels had a lower risk of developing AD.[47]

2. Clinical periodontal parameters

Five studies focused on the relationship between AD and clinical periodontal parameters. The number of participants

Table 1 Clinical studies investigating the associations between Alzheimer's disease and periodontitis

Cross-sectional and longitudinal studies based on databases or surveys					
Authors	Database and sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	Outcomes	
Beydounet al. (2021)[45]	NHANES (1988–1991) 1439 American patients - Incident AD: 277 - Incident all-cause dementia: 549 - Mean follow-up: 10–11 years - Age: ≥ 65 years at baseline	Diagnosis: - ICD-9 code 331.0: Alzheimer's disease	Parameters: - AL - PD - Serum IgG against 19 periodontal bacteria* Site: Two sites on every tooth in 2 quadrants	- The cumulative incidence proportions of AD were significantly higher in the Hp seropositivity group. - *P. intermedia*, *C. Rectus*, *P. nigrescens*, *P. melaninogenica*, and *P. gingivalis* interacted synergistically with *H. pylori* sero-positivity, particularly with respect to AD incidence.	
Yu et al. (2008) [28]	NHANES (2001–2002) 803 dentate American patients - Age: ≥ 60 years	Parameters recorded: - 2-min Digit Symbol Substitution Test	Diagnosis: - AL > 4 mm in at least 10% of sites - PDL > 3 mm in at least 10% of sites Site: - Three sites on each examined tooth in 2 randomly selected quadrants	- Higher cognitive function was associated with lower odds of periodontal disease.	
Beydoun et al. (2020) [44]	NHANES (1988–1994) linked with National Death Index and Medicare data (2014) 6650 American patients - AD deaths: 52	Diagnosis: - ICD-9 code 331.0: Alzheimer's disease	Parameters recorded: - AL - PD - Serum IgG against 19 periodontal bacteria Site:	AD incident risk - *C. rectus* (55+ and 65+) - *P. gingivalis* (55+ and 65+) - Red-Green cluster AD mortality risk - Orange-Red cluster (55+ and 65+) - *P. gingivalis* IgG (65+)	Inverse AD incident risk+ - S. *intermedius* (marginally among 55+ women) Inverse AD mortality risk - *A. actinomycetemcomitans* (65+)

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Table 1 (*continued*)

Cross-sectional and longitudinal studies based on databases or surveys				
Authors	Database and sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	Outcomes
	- Incident AD: 888 - Incident all-cause dementia: 1737 - Up to 26 years of follow-up - Age: $\geq$ 45 years at baseline		- Two sites on every tooth in 2 quadrants	- *P. melaninogenica* (65+) - *S. Oralis* (men) - *S. intermedius* (men) - Factor 2
Chen et al. (2017) [27]	National Health Insurance Research Database (1996—2013) 27963 Taiwanese patients - Incident CP: 9291 - Control: 18,672 - Mean follow-up: 12 years - Age: $\geq$ 50 years at baseline	Diagnosis: - ICD-9 code 331.0: Alzheimer's disease	Diagnosis: - ICD-9 code 523.4: Chronic periodontal disease	- Patients with 10 years of CP exposure exhibited a higher risk of developing AD than unexposed groups
Noble et al. (2009) [46]	NHANES (1988—1994) 2355 American patients - Age: $\geq$ 60 years at baseline	Diagnosis: - Immediate verbal memory/ registration: Summary score $<$ 4 - Delayed verbal memory: Summary score $<$ 3 - Serial 3 subtraction test: Summary score $<$ 4	Parameters: - Serum IgG against *P. gingivalis*	- Mean *P. gingivalis* IgG was higher among those with impaired performance for each of the 3 cognitive tests. - Individuals in the highest *P. gingivalis* IgG group ($>$ 119 EU) were more likely to have poor delayed verbal memory and impaired subtraction.

Noble et al. (2014) [47]	Washington Heights-Inwood Columbia Aging Project 219 American patients - Incident AD: 110 - Control: 109 - Mean follow-up: 5 years - Age: > 65 years - No AD at baseline	Not mentioned	Parameters: Serum IgG antibody against - *P. gingivalis* - *T. forsythia* - *A. actinomycetemcomitans* Y4 - *T. denticola* - *C. rectus* - *E. nodatum* - *A. naeslundii* genospecies-2	- High anti-*A. naeslundii* titer was associated with increased risk of AD. - High anti-*E. nodatum* IgG was associated with a lower risk of AD.

AD and periodontal clinical parameters in cross-sectional and cohort studies

Authors	Sample size	AD diagnosis or parameters	Periodontal disease diagnosis or parameters	Outcomes
Iwasaki et al. (2015) [63]	291 Japanese patients (101 males/190 females) - Average age: 80.9 years - Age: ≥ 75 years Classification: - No periodontal disease - Periodontal disease - Edentulous	Diagnosis: - MMSE: (≤ 23) - HDS-R scores: (≤ 20)	Diagnosis: - Interproximal AL ≥ 5 mm in ≥ 50% of teeth Parameters: - AL: 6 sites of every tooth - Teeth number	Periodontal disease and edentulism were significantly associated with greater odds of low cognitive performance after controlling for potential confounders.
Moriya et al. (2012) [50]	152 Japanese patients Inclusion criteria: - Age: 70—74 years - Teeth number: > 6	Parameters - RCPM test - VerPA task - VisPA task - BDT	Diagnosis: - Community Periodontal Index of Treatment Needs	Weak but statistically significant negative correlations were established between the RCPM test, the VerPA task, and the VisPA task and periodontal status, but not the Block Design Test.
Kaye et al. (2013) [48]	597 dentate American patients - Followed-up for 32 years - Age: 28—70 years at baseline	Low cognitive statuses: - MMSE: - < 25 points - Age- and education-specific median: < 90% - Spatial Copying Task	Parameters: - Alveolar bone loss progression - Probing pocket depth progression - Tooth loss rate - PD	- Each tooth lost per decade since the baseline dental examination increased the risk of low MMSE and Spatial Copying Task scores by 9% to 12%.

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Table 1 (*continued*)

AD and periodontal clinical parameters in cross-sectional and cohort studies				
Authors	Sample size	AD diagnosis or parameters	Periodontal disease diagnosis or parameters	Outcomes
		- $<$ 10 points	- Caries and restorations	- Each tooth that had progression of alveolar bone loss or probing pocket depth increased the overall risk of low scores by 2% to 5%. - Development of new caries or restorations was associated with an increased risk of a low Spatial Copying Task score.
Iwasaki et al. (2016) [64]	85 Japanese patients - MMSE $>$ 24 at baseline - Age: $>$ 75 years at baseline	Parameters - Difference between MMSE score in 2010 and 2013	Diagnosis: Severe periodontitis: - AL: $\geq$ 6 mm at $\geq$ 2 interproximal sites - PD: $\geq$ 5 mm at $\geq$ 1 interproximal sites Parameters: - Teeth number	- Severe periodontitis was significantly associated with an increased risk of cognitive decline. - Participants with severe periodontitis had a 1.8-point greater decrease in MMSE score than those without severe periodontitis.
Kamer et al. (2012) [49]	152 Danish patients - People born in 1914	Parameters: - DSST - BDT	Diagnosis - Modified Community Periodontal Index score: $\geq$ 3 for at least 10% of the remaining teeth Parameters: - PD - Teeth number	- Patients with periodontal inflammation obtained lower mean DSST and BDT scores. - Patients with many missing teeth had lower mean DSST and BDT scores. - Patients with periodontal inflammation had significantly lower adjusted mean DSST scores compared to patients without periodontal inflammation. However, for adjusted BDT, the significance held only for patients with few missing teeth.

Clinical studies on AD and inflammatory markers or IgG

Authors	Sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	Serology sampling site	Inflammatory markers	IgG	Outcomes
Sochocka et al. (2017) [51]	128 Polish patients (45 males/83 females) - Age: 55—90 years	Diagnosis: - DSM-V and NINCDA-ADRDA criteria Parameters: - MMSE	Parameters: - Teeth number - PD - CAL - BoP - Plaque index Record site: - Six sites on all teeth	Sampling: - Peripheral blood leukocytes (PBL)	Inflammatory markers: - IL-1β - IL-6 - IL-10 - TNF-α	Not mentioned	- Worse periodontal health status as well as cognitive decline were associated with higher Inflammatory state.
Kamer et al. (2009) [52]	34 American patients - AD: 18 - Control: 16	Parameters: - MMSE	Not mentioned	Sampling: - Frozen whole blood	Inflammatory markers: - APOE ε4 - TNF-α - IL-1β - IL-6	IgG against - *A. actinomycetemcomitans* serotype b (ATCC 43718), - *T. forsythia* (ATCC 43037) - *P. gingivalis* (ATCC 33277)	- Plasma TNF-α and antibodies against periodontal bacteria were elevated in AD patients compared with controls and independently associated with AD.
Ide et al. (2016) [53]	59 English patients (30 males/29 females) - Mean age: 77.7 years - Followed-up for 6 months - Mild to moderate dementia - Excluded smokers	Parameters: - Alzheimer's Disease Assessment Scale (ADAS-cog) - MMSE	Diagnosis: - CDC/AAP criteria Parameters: - PD - BOP - PI Record site: - Six sites of each tooth	Sampling: - Venous blood	Inflammatory markers: - CRP - TNF-α - IL-10	IgG against *P. gingivalis*	- Periodontitis was associated with an increase in cognitive decline in patients with Alzheimer's disease, independent of baseline cognitive state.

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Table 1 (*continued*)

Clinical studies on AD and inflammatory markers or IgG							
Authors	Sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	Serology sampling site	Inflammatory markers	IgG	Outcomes
Stein et al. (2012) [54]	158 American patients - Incident MCI or AD: 81 - Control: 77 - Mean age: 70.0 years (Control) 72.1 years (MCI) 74.1 years (AD) - Cognitively intact at baseline	Diagnosis: - AD: McKhann et al. criteria - MCI: Petersen et al. criteria	Not mentioned	Sampling: - Venous blood	Not mentioned	IgG against - *A. actinomycetemcomitans* - *P. gingivalis* - *C. rectus* - *T. denticola* - *F. nucleatum* - *T. forsythia* - *P. intermedia*	- Antibody levels of *F. nucleatum* and *P. intermedia* were significantly increased at baseline serum drawing in the AD patients compared to the controls.

Studies on AD and the oral microbiome using PCR						
Authors	Sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	AD markers	Microbiology	Outcomes
Leblhuber et al. (2020) [55]	20 Austrian patients (11 males/9 females) - Mean age: 78.1 $\pm$ 2.2 years - Probable AD	Parameters: - MMSE - Clock-Drawing Test - Magnetic resonance imaging	Not mentioned	- Neopterin - Tryptophan - Kynurenine Sampling site: - Serum	Sampling site: - Alveolar fluid Method - RNA-based analysis (PerioPOC) Bacteria: - *T. denticola/ T. forsythia/ P. gingivalis/ P. intermedia / A. actinomycetemcomitans*	- *P. gingivalis* was associated with lower MMSE and Clock-Drawing Test scores.
Laugisch et al. (2018) [56]	40 German patients	Diagnosis: - MMSE $\geq$ 19	Diagnosis: - PD of $\geq$ 4 mm	- Aβ1-42 - Total tau	Sampling site: - Subgingival dental biofilm and GCF	- None of the investigated bacteria were

		- AD: 20 - Other forms of dementia: 20 - Age: 30–70 years - Caucasian origin - Recently diagnosed with dementia		- AL $\geq$ 3 mm Parameter: - PI PD - AL - BOP Site: - Six sites of each tooth	Sampling site: - CSF Inflammatory markers in GCF and serum: - IL-1 - MCP-1/CCL-2	- Serum - CSF Method: - Real time PCR (High Pure Template Preparation Kit) - Antibodies Bacteria: - *A. actinomycetemcomitans/ P. gingivalis/ T. forsythia/ T. denticola/ T. socranskii*	detected in the CSF or serum samples. - No significant difference was observed in antibody levels against specific bacteria in the CSF or serum between groups. - In patients with dementia aged up to 70 years, periodontal pathogens did not act as a trigger for developing AD.

Studies on AD and the oral microbiome using NGS or the third-generation technique

Authors	Sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	AD markers	Microbiology sampling site and method	Outcomes	
Holmer et al. (2021)[57]	195 Swedish patients - AD: 46 - MCI: 40 - Subjective cognitive decline: 46 - Control: 63 - Age: 50-80 years	Diagnosis: - NIA-AA diagnostic guidelines	Diagnosis: - PD $\geq$ 6 mm Parameters: - PD - BOP - Radiograph Site: - Six sites of each tooth	Not mentioned	Sampling site: - Subgingival dental biofilm Method: - V3-V4 regions of the 16S rRNA gene - Illumina MiSeq	- Cognitive dysfunction was a significant determinant of subgingival microbial composition and was associated with higher microbial richness and evenness using either alpha or beta diversity measures. More abundant in AD group - S. exigua *(Gram-positive, anaerobic coccobacillus)* - Lachnospiraceae *bacterium (Gram-negative, obligately anaerobic)* - P. oulorum *(Gram-negative, anaerobic)*	More abundant in controls - R. aeria (Gram-positive, aerobic) - C. durum (Gram-positive, aerobic) - Actinomyces genus (Gram-positive, facultatively anaerobic)
Wu et al. (2021) [59]	35 Taiwanese patients - AD: 17	Parameters: - Clinical Dementia Rating Scale	Not mentioned	Not mentioned	Sampling site: - Supra gingival dental plaque	Overall oral microbial diversity in the AD group tended to be lower than that in the control group. More abundant in AD group	More abundant in controls

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Table 1 (*continued*)

Studies on AD and the oral microbiome using NGS or the third-generation technique							
Authors	Sample size	Alzheimer's disease diagnosis or parameters	Periodontal disease diagnosis or parameters	AD markers	Microbiology sampling site and method	Outcomes	
	- Control: 18	- SOB - MMSE			Method: - Full-length 16S rDNA sequencing - PacBio single-molecule real-time sequencing	Order: - *Lactobacillales* (aerotolerant anaerobes) - *Actinomycetales(anaerobic)* - *Veillonellales (anaerobic)* Family: - *Lactobacillaceae* - *Streptococcaceae*(aerobic) - *Actinomycetaceae* - *Veillonellaceae* Genus: - *Lactobacillus* - *Streptococcaceae* - *Actinomycetaceae* - *Veillonella*	Order: - *Fusobacteria* - *Bacteroidetes* - *Cardiobacteriales* Family: - *Fusobacteriaceae* - *Cardiobacteriaceae* - *Porphyromonadaceae* Genus: - *Fusobacterium* - *Cardiobacterium* - *Porphyromonas*
Yang et al. (2021) [58]	68 older American patients (27 males/41 females) - MCI: 34 - Control: 34	Diagnosis - ADRC consensus diagnosis Parameters - Uniform Data Set - CSF assays for Aβ42, total-Tau, and phospho-Tau	Not mentioned	Sampling site: - Blood (CRP and LPS) - CSF (91 proteins on the Olink INFLAMMATION panel)	Sampling site: - Oral swab collection from the dorsal tongue, hard palate, buccal mucosa, and keratinized gingiva Method: - V4 regions of the 16S rRNA gene - Illumina MiSeq	- MCI did not appear to shift the taxonomic composition of the oral microbiome. - No difference was identified in alpha or beta diversity between MCI and control groups. - Levels of CRP and LPS in blood were not significantly different between groups. More abundant in AD group - Amplicon sequence variants of *Pasteurellaceae*	More abundant in controls - Amplicon sequence variants of *L. mirabilis.*

Liu et al. (2019) [60]	78 Chinese patients (39 males/39 females) - AD: 39 - Control: 39	Diagnosis: - Mild: MMSE $\geq$ 20 - Moderate: 10 $\leq$ MMSE $<$ 20 - Severe: MMSE $<$10	Not mentioned	- APOE ε4 -	Sampling site: - Saliva - QIAamp DNA Investigator Kit Method: - V3-V4 regions of the 16S rRNA gene - Illumina Hiseq2500	- Alpha diversity analysis showed that there was lower richness and diversity in AD patients. - No bacteria were found to be associated with the severity of AD. More abundant in AD group Genus: - *Moraxella* (Gram-negative bacteria) - *Leptotrichia* (Gram-negative bacteria) - *Sphaerochaeta* (Gram-negative bacteria) In AD and APOEε4 (+) patients: - Increase: *Abiotrophia* and *Desulfomicrobium* - Decrease: *Actinobacillus* and *Actinomyces* More abundant in controls - *Rothia*

Post-mortem studies on AD and microbiomes

Authors	Sample size	Microbiology sampling site and method	Outcomes
Emery et al. (2017) [61]	26 patients - AD: 14 - Control: 12 Inclusion criteria: - Age: 62—98 years	Sampling site: Temporal cortex Right hemispheres: - Formalin fixed for neuropathological assessment and for immunohistochemical analysis Left hemispheres - Sliced and frozen at -80°C Method - V3 regions of the 16S rRNA gene - Life Technologies Ion Plus Fragment Library Kit (ThermoFisher Scientific)	- Many more bacterial 16S reads were yielded from AD samples, strongly suggesting that contamination was not a major issue for these data. - Contamination: *Rhizobiales; Methylobacteriaceae* More abundant in AD group - Largest component: *Actinobacteria* Family: - *Actinobacteria; Propionibacteriaceae (P. acnes)* - *Actinobacteria; Corynebacteriaceae* More abundant in controls - *Proteobacteria*

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Table 1 (*continued*)

Post-mortem studies on AD and microbiomes				
Authors	Sample size	Microbiology sampling site and method	Outcomes	
Siddiqui et al. (2019) [62]	20 patients (5 males/15 females) - AD: 10 - Control: 10 - Age: 63—103 years	Sampling site: - Brain tissue adjacent to the lateral ventricle of the parietal lobe - Average storing interval: 16 years Method: - V3-V4 regions of the 16S rRNA gene - Illumina MiSeq	- A wide spectrum of bacteria was detected in samples from both groups, containing both oral and gastrointestinal tract microbiome species. More abundant in AD group Phylum - *Firmicutes* Family: - *Pseudomonadaceae*: dominating family Order: - *Actinomycetales* Species - *Prevotella* - *Treponema* - *Veillonella*	More abundant in controls Phylum - *Proteobacteria* Genus - *Fusobacterium*
Dominy et al. (2019) [30]	58 patients - AD: 29 - Control: 29	Sampling site - Middle temporal gyrus AD markers: - Tau (non-phosphorylated/ phosphorylated) - Ubiquitin Methods: - Real-time PCR analysis of *P. gingivalis* Antibodies to gingipain:	- Both RgpB and Kgp antigens in brain tissue independently demonstrated a significant correlation with AD diagnosis, tau load, and ubiquitin load. More abundant in AD group - *P. gingivalis* strains W83, ATCC33277, and FDC381	More abundant in controls

		- Lysine-gingipain (Kgp) - Arginine-gingipain A (RgpA)		
Poole et al. (2013) [31]	20 patients - AD: 10 - Control: 10	Sampling site: - Brain tissue adjacent to the lateral ventricle of the parietal lobe Method: Antibodies against - *P. gingivalis* (LPS and gingipains) - *T. forsythia* - *T. denticola*	- Human brain tissue sections with mouse anti-*P. gingivalis* revealed strong cellular surface membrane labeling in 4 out of 10 AD cases but not in the non-AD age-matched controls. - No immunolabeling was observed with anti-*T. forsythia* antibodies nor with anti-*T. forsythia*. - Four out of 10 AD brains exhibited bands characteristic of LPS.	
Riviere et al. (2002) [32]	Group 1 34 patients (18 males/16 females) - AD: 16 - Control: 18 Group 2 5 patients (2 males/3 females) - AD: 3 - Control: 2	Group 3 4 patients (2 males/2 females) Group 4 33 living patients - AD: 17 - Control: 16	Sampling site: - Frontal lobe cortexes (Group 1) - Frozen trigeminal ganglia (Group 2) - TG, pons, and hippocampus (Group 3) - Saliva (Group 4) Method: - *Treponema* species-specific DNA PCR - *Treponema* species-specific antigens	- *Treponema* may infect the brain via branches of the trigeminal nerve.

Amyloid β plaques (Aβ), Attachment loss (AL), Apolipoprotein E (APOE), Approximal plaque index (API), Bleeding on probing (BoP), Block Design Test (BDT), Clinical attachment level (CAL), Chronic periodontitis (CP), Cerebrospinal fluid (CSF), Hasegawa Dementia Scale-Revised (HDS-R), Immunoglobulin G (IgG), Interleukin (IL), Mild cognitive impairment (MCI), Mini-Mental State Examination (MMSE), National Health and Nutrition Examination Survey (NHAHES), Next- generation sequencing (NGS), Periodontal inflammation (PI), Polymerase chain reaction (PCR), Raven's Coloured Progressive Matrices (RCPM), Subjective cognitive decline (SCD), Verbal Paired Associates (VerPA), Visual Paired Associates (VisPA)

ranged from 85 to 591. Four studies included elderly patients only, and one cohort study included patients who were over 28 years old at baseline. The MMSE was used as either the AD diagnosis criterion or cognitive state parameter in 3 studies, and the remaining 2 used the BDT and other exams. The 3 studies that administered the MMSE reported that low cognitive MMSE test scores were associated with tooth loss, probing pocket depth, and alveolar bone loss. Kaye et al. compared patients under 45 years and patients over 45 years and found that the older group has higher alveolar bone loss at baseline and experiences more tooth loss per decade.[48] MMSE scores are also consistently lower in older men. Kamer et al. found that patients with periodontal inflammation have lower mean DSST and BDT scores.[49] Moriya et al. discovered significant negative correlations between the RCPM test, the VerPA task, or the VisPA task and periodontal status, but no correlation was found between the BDT and periodontal status.[50]

3. Serology-based studies

Serum inflammatory markers or IgG were investigated in 4 studies to determine the relationship between AD and periodontal disease. The number of participants ranged from 34 to 158. Of the 3 studies that assessed the correlation between inflammatory markers and cognitive states, 2 reported that inflammatory markers are elevated in AD patients compared with cognitively normal patients. Sochocka et al. reported that interleukin-1β (IL-1β), IL-6, IL-10, and tumor necrosis factor-α (TNF-α) are elevated in AD patients. Moreover, Kamer et al. reported that TNF-α is elevated in AD patients but IL-1β and IL-6 are not.[51,52] In contrast, Ide et al. did not find any significant relationships between baseline systemic inflammatory markers and cognitive measures.[53] Regarding the influence of periodontal pathogens on cognitive impairment, Kamer et al. reported that AD patients are more likely to have positive tests of IgG antibodies against *Aggregatibacter actinomycetemcomitans, P. gingivalis*, and *Tannerella forsythia*.[52] A retrospective study revealed that AD and mild cognitive impairment (MCI) patients exhibit significantly elevated *P. intermedia a*nd *Fusobacterium nucleatum* antibody levels at baseline, before neurological changes are diagnosed.[54] However, conflicting results from a 6-month cohort study showed no significant relationship between the baseline IgG antibody titer to *P. gingivalis* and the rate of cognitive decline.[53]

4. Microbiology-based studies

Among 6 studies that focused on the relationship between AD and the oral microbiome, one assessed specific periodontal pathogens via RNA-based analysis, one detected specific microorganisms via DNA real-time polymerase chain reaction, 3 analyzed the V3-V4 or V4 regions of the 16S rRNA gene via NGS, and one investigated the full-length of 16S rDNA using single-molecule real-time sequencing. The number of patients ranged from 20 to 195. The 2 studies that specified testing periodontal pathogens yielded different results. Leblhuber et al. found that *P. gingivalis* and *T. denticola* are associated with AD but *T. forsythia* is not.[55] On the other hand, Laugisch et al. compared patients with AD and patients with other forms of dementia (DEMnoAD), and the results showed that periodontal pathogens do not act as a trigger for developing AD.[56]

The 4 gene sequencing studies yielded conflicting results in terms of diversity and abundant species. Holmer et al. reported that the AD group has higher microbiome diversity, Yang et al. did not identify any difference in diversity between MCI and the control group, and Wu et al. and Liu et al. found that the MCI group has lower diversity.[57–60] In regards to species abundance, Holmer et al., Yang et al., and Liu et al. all found that the most predominant microbes are similar in the control groups and study groups. Holmer et al. discovered that *Actinomyces* and *Rothia* are more common in the control group, whereas *Slackia exigua, Lachnospiraceae,* and *Prevotella oulorum* are more common in the AD group.[57] Wu et al. found that *Firmicutes, Lactobacillales, Actinomycetales,* and *Veillonellales* are more common in the MCI group, whereas *Fusobacteria*, *Bacteroidetes,* and *Cardiobacteriales* are more common in the control group.[59] Yang et al. found that *Pasteurellaceae* is more common in the MCI group, whereas *Lautropia mirabilis* is more common in the control group.[58] Liu et al. reported that *Moraxella*, *Leptotrichia*, and *Sphaerochaeta* are more common in the AD group, whereas *Rothia* is more common in the control group.[60]

5. Post-mortem studies

Five post-mortem studies compared the microbiome of the brain tissue of AD patients to that of cognitively normal patients. The number of patients ranged from 20 to 58. One used antibodies against specific pathogens, one used real-time polymerase chain reaction (PCR) against *P. gingivalis*, one used both *Treponema* species-specific antibodies and PCR, and 2 analyzed the V3-V4 or V3 regions of the 16S rRNA gene via NGS. Both genetic sequencing studies revealed that *Actinobacteria* is more abundant in the AD groups, whereas *Proteobacteria* is more abundant in the control groups.[61,62] Dominy et al. focused on *P. gingivalis* and gingipain in brain tissue, and reported that *P. gingivalis* DNA is present in brain tissue in AD patients.[30] Gingipain was also found to be correlated with AD diagnosis. Poole et al. focused on periodontal pathogens in brain tissue with antibodies against *P. gingivalis, T. forsythia*, and *T. denticola*, and reported *P. gingivalis* antigen expression in AD brain tissue but not in the control group.[31] Riviere et al. focused on the detection of *Treponema* species in brain tissue and reported a higher rate of *Treponema* species detection in the AD group compared to the control group.[32]

Discussion

This systematic review aimed to determine the relationship between periodontitis or periodontal pathogens and AD. Large-scale studies based on databases and surveys agree that the presence of periodontitis is linked to cognitive decline.[27,28] This is consistent with the results of clinical studies focusing on the relationship between AD and clinical periodontal parameters.[48–50,63,64] However, although an association between periodontitis and cognitive impairment

has been confirmed, whether AD leads to periodontitis or periodontitis is a contributing factor of AD remains controversial. A previous study comparing AD with other forms of dementia showed that dementia affects a patient's ability to maintain oral hygiene, thus patients with AD are prone to have periodontal disease.[56] Post-mortem studies of AD patients have revealed the presence of *P. gingivalis* LPS in AD patients but not in control patients.[31] Dominy et al. also reported that *P. gingivalis* DNA and gingipain are present in the brain tissue of AD patients.[30] Riviere et al. discovered a higher rate of *Treponema* species detection in the AD group.[32] These findings suggest periodontal pathogens may travel from the mouth to the brain and cause inflammation and eventual destruction of brain tissue. However, brain tissue may be tested for bacteria years after a patient's death, and this may cause bacterial contamination of brain tissue that was not present during the patient's lifetime.[61] Therefore, obtaining data from clinical experiments is imperative.

Serology findings suggest that AD is related to higher inflammatory state and elevated periodontal bacteria antibody levels.[51,52,54] Ide et al. reported that periodontitis is not related to the baseline cognitive state but is related to cognitive decline over a 6-month follow-up period.[53] However, contrary to other studies, no significant association between baseline serum *P. gingivalis* antibody levels and the rate of cognitive decline was found. This may indicate that *P. gingivalis* is not the sole determining factor of cognitive decline. In addition, tooth loss and a history of periodontitis were not found to be related to cognitive decline, which may indicate that active periodontitis plays a crucial role in cognitive decline. None of the 4 studies have compared serological findings to the severity of AD or the onset timing of AD, which can be discussed in future studies for further understanding of the disease.

Although epidemiology studies have proven that AD is related to periodontitis, the linking mechanism remains uncertain. One study focusing on periodontal pathogens reported that *P. gingivalis* levels are related to lower MMSE scores, and that *T. denticola* and *T. forsythia* levels are related to the concentration of immune biomarkers.[55] These findings hint at the possibility of synergistic effects of different microbiomes, which may alter the host's immune response. Another study by Laugisch et al., which compared microbiomes in patients with AD and patients with other forms of dementia, did not reveal any significant difference in bacteria antibody levels between groups. However, they discovered elevated levels of anti-pathogen antibodies in the cerebrospinal fluid (CSF) compared to serum in both groups.[56] This highlights the possibility that intrathecal immune response may be triggered by periodontal pathogens. Interestingly, despite the elevated levels of anti-pathogen antibodies in the CSF, none of the investigated bacteria were detected in the CSF or serum samples. Because only specific antibodies were detected, only portions of bacterial components may have entered the brain or these bacteria may reside only in brain tissue. These results differ from those of Dominy et al., who reported that *P. gingivalis* DNA is present in AD brains and CSF.[30] This may be because Laugisch et al. mostly included early-onset AD, which is primarily associated with genetic factors rather than inflammatory factors such as periodontitis. The inclusion of early-onset AD might also influence bacteria antibody levels, which showed no difference between patients with AD and patients with other forms of dementia in the study by Laugisch et al. Future studies may have to differentiate between early-onset and late-onset AD because they may have different immune responses to pathogens, thus leading to different conclusions.[65]

The 4 studies using either NGS or single molecule real-time sequencing had conflicting results in terms of microbial diversity and the relative abundance of bacterial taxa between groups. This difference may be largely caused by the different sampling sites in the 4 studies, rendering the data of these 4 studies incomparable. Holmer et al. sampled the subgingival dental biofilm, and the 3 operational taxonomic units (OTUs) that were more abundant in patients with AD were all anaerobic species, whereas the 3 OTUs that were more abundant in controls were aerobic or facultatively anaerobic species.[57] *Lachnospiraceae*, which was more common in the AD group, was also found to be more abundant in the gut of the AD group in another study.[66] In addition, *Lachnospiraceae* is also related to inflammatory diseases, such as metabolic syndrome, obesity, diabetes, inflammatory bowel disease, and liver diseases including chronic liver disease.[67] Wu et al. sampled the supragingival dental biofilm and reported that the cariogenic bacteria *Lactobacillale* and *Streptococcaceae*, along with *Actinomycetaceae* and *Veillonellaceae*, are increased in patients with AD.[59] Interestingly, *Streptococcus mutans* is an amyloid-forming organism and is more abundant in the feces of patients with AD compared with normal individuals, and may therefore be a potential contributor to the development of AD during oral dysbiosis.[68,69] Yang et al. collected samples from soft tissue surfaces and reported that the opportunistic pathogen *Pasteurellaceae* is abundant in the MCI group.[58] Although this study cannot draw conclusions regarding the relationship between MCI and periodontal condition because of the lack of periodontal records and representative sampling sites of periodontal pathogens, inflammatory markers such as IL-1α, IL10RA, IL13, and TSLP are positively associated with *Pasteurellaceae*, indicating that the oral microbiome may affect inflammatory states. Liu et al. studied the saliva samples of AD patients and found the 3 taxa such as *Moraxella*, *Leptotrichia*, and *Sphaerochaeta*, which are all Gram-negative bacteria and may induce Aβ plaque formation via LPS, are more abundant in the group of AD patients.[22]

In conclusion, database studies focusing on epidemiologic research and post-mortem studies show that periodontal pathogens may play a role in the pathogenesis of AD. However, the reported microbiomes are inconsistent in the gene sequencing studies, which may be due to different microbiome sampling sites and different cognition standards. To gain a better understanding of the role of the oral microbiome in AD, a standardized sampling site and cognitive test should be implemented. In addition, early-onset AD and late-onset AD should be discussed separately because they have different etiologies. Another limitation of these studies is that the control patients may have already had undiagnosed cognitive impairments, which may require long-term longitudinal studies to test. Future studies that provide periodontal treatment as an intervention are also required to clarify the relationship between periodontal disease and AD.

Acknowledgements

This study was supported by grant (109-2314-B-002-044) from the Ministry of Science and Technology of Taiwan and grant (109-S4549) from National Taiwan University Hospital.

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