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Research Report

Porphyromonas Gingivalis as a Risk Factor to Alzheimer's Disease: A Systematic Review

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Abstract.
Background: Alzheimer's disease (AD) is a chronic neurodegenerative disease that accounts for more than 50% of all dementia cases worldwide. There is wide consensus on the risk factors of AD; however, a clear etiology remains unknown. Evidence suggests that the inflammatory-mediated disease model, such as that found with periodontal disease due to *Porphyromonas gingivalis* (*P. gingivalis*), plays a role in AD progression.
Objective: This study aims to systematically review the literature on the association between *P. gingivalis* to AD, and to identify the homogeneity of the methods used across studies to measure *P. gingivalis* involvement in AD.
Methods: We systematically searched studies on Cochrane library, Ovid Medline, PubMed, Web of Science, WHOLIS, Google Scholar databases, and reference lists of identified studies.
Results: 6 studies out of 636 identified records fulfilled all eligibility criteria. Results showed no clear pathophysiology of AD due to *P. gingivalis* and its various virulence factors. No consensus was found in the literature pertaining to the method of measurement of AD or *P. gingivalis* and its virulence factors.
Conclusion: The included studies suggest that *P. gingivalis* bacteria play a role in the process of systemic inflammation which leads to cerebrospinal fluid inflammation and indirectly cause hastening of AD onset and progression. Our included studies revealed heterogeneity in the methodologies of measurement of AD and/or *P. gingivalis* and its virulence factors, which opens discussion about the benefits and weakness of possible standardization.

Keywords: Alzheimer's disease, dementia, geriatrics, *P. gingivalis*, periodontitis, systematic review

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive chronic neurodegenerative dementing disease [1–3]. AD is the most common cause of dementia, a broad term that describes memory loss and other cognitive ability impairments serious enough to interfere with a person's daily life. Women seem to be more susceptible to AD than men [2]. AD is associated with neuronal degeneration and atrophy in the brain leading to death. In a normal non-AD diagnosed brain, amyloid-β (Aβ) proteins are found in-and-around the neurons. However, in AD, these proteins aggregate together and form clusters in the brain, called "amyloid plaques". Another feature of AD is the abnormal deposits of the tau protein inside degenerating neurons. It is still unknown whether the plaques are the cause of AD or if they represent the brain's response to neurodegeneration [4].

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The complex-multifactorial causes of AD are still unknown [2, 4]. However, there is consensus in the literature that AD is an interaction between genetic and environmental elements, with age considered the main risk factor for AD [3, 5].

AD patients show signs of generalized inflammation and neuronal degeneration that are consistent with bacterial infections. These signs include complement, inflammasome, and microglial activation as well as altered cytokine profiles [6]. Recently, several studies have discovered periodontal pathogens in the brain and suggested their involvement in AD-associated inflammation [7–12].

Porphyromonas gingivalis (*P. gingivalis*) is a periodontopathic Gram-negative non-motile bacterium that is implicated in periodontal inflammation and periodontitis [13]. *P. gingivalis* is found in about 86% of subgingival plaque samples from patients with chronic periodontitis [13, 14]. It is also associated with several diseases and conditions, e.g., atherosclerosis, rheumatoid arthritis, non-alcoholic steatohepatitis, and squamous cell carcinoma due to the complex nature of its virulence factors [15]. The inflammatory nature of *P. gingivalis* and its virulence factors lead to stimulation of the inflammatory response which causes blood vessel damage [11, 16, 17]. One virulence factor highly associated with periodontal and inflammatory disease progression is gingipains. Gingipains are enzymes secreted by *P. gingivalis* that play a significant role in *P. gingivalis* survival, virulence, host invasion, and colonization [18]. Gingipains play a crucial role in degrading host immune response and tissue damage by the degradation of collagen, which in turn impede wound healing [19, 20]. Olsen and Singhrao explain that this happens through gingipains destroying complement through proteolytic degradation, and by inhibiting complement activation by binding to a complement inhibitor [21]. Moreover, *P. gingivalis* expression of gingipains also allows its persistence in the periodontal tissue and further complicates its elimination by the immune response [22]. Using tissue microarrays containing matched brain samples, Dominy et al. identified gingipains in the brains of AD patients. In the study, the levels of gingipains identified were found to be correlated to the extent of AD pathology [23].

Aside from the inflammation caused by *P. gingivalis* [24], blood vessel damage due to periodontal disease may act as a gateway for further pathogenic microorganisms to enter directly into the bloodstream, and subsequently to systematic circulation [20, 25]. Recently, *P. gingivalis* was shown to be present in the periodontium of AD patients [26, 27]. Another cross-sectional study reported finding *P. gingivalis* intracerebrally in four out of the ten AD-diagnosed brain samples, while none for all non-AD diagnosed brain samples [10].

An inflammatory-mediated model explaining the progression of AD has been described in the literature previously [28]. Innate immune inflammatory activity in the AD brain can result from the deposition of Aβ protein as well as from specific bacterial infections that tend to possess weak immunostimulatory responses [29]. Recently, numerous studies have discussed that periodontal inflammation may result in hastening of AD and/or dementia onset even though the mechanism is unclear [11, 30–35]. In a large cohort with follow-up of more than 10 years, subjects with periodontal disease were found to have a higher risk of developing AD and dementia [36]. Periodontal disease involvement in AD is further supported in a recent review by Kamer et al. that stated that an increased incidence of AD is evident in patients with periodontal disease [37].

The present study aims to systematically review and analyze the literature on the association between the periodontal pathogen, *P. gingivalis,* and the dementing condition of AD. The objectives of the study are to identify the risk of AD associated with *P. gingivalis* and its virulence factors and to explore the different tools and methods used across studies to measure *P. gingivalis* involvement in AD.

METHODS

This systematic literature review adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines [38].

We applied the following inclusion-exclusion criteria: 1) Original research and studies using human populations and/or human specimens and published in peer-reviewed journals. Editorials, viewpoints, policy reviews, summaries, or discussion papers were excluded. 2) Studies with control groups. 3) Studies focusing on AD that has been diagnosed using clear definitions and/or criteria. 4) Studies including non-human sources or that focused only on specific populations such as HIV patients or psychiatric patients were excluded.

Two researchers independently conducted the literature search for studies published in English in Cochrane, OVID, PubMed, Web of Science, and

WHOlis until 31 August 2020. The authors also used Google Scholar to search for relevant articles.

We used the following search terms to identify relevant studies: "*Porphyromonas gingivalis*" and "*Alzheimer*". The authors AE and CI screened title-abstract and full texts of the references independently against the inclusion-exclusion criteria. In cases of disagreement, the issues were discussed among all authors.

We assessed the quality of the included studies by using the National Heart, Lung, and Blood Institute (NHLBI) Study Quality Assessment Tools [39]. Use of randomization, the calculation of sample sizes, and the size of the unit of allocation impacted the quality assessment score given to the individual studies. A comparative analysis was done using analytical categories developed based on the methodology used to measure both AD and its progression, and the presence of *P. gingivalis* and its virulence factors. These categories were: 1) Mini-Mental State Examination (MMSE) average score of samples, 2) *P. gingivalis* antibody immunoglobulin (*P. gingivalis* Ab IgG) measures, 3) tumor necrosis factor alpha (TNF-α) measures, 4) *P. gingivalis*, virulence factor lipopolysaccharide (LPS), or gingipains measures or presence and 5) further analysis. As for 5), 'further analysis' is for the purpose of this review defined as variables the included-studies' authors have reported to have a possible influence on the relation between AD and *P. gingivalis*. Some authors suggest that these variables may have also affected the obtained results or acted as confounders. We extracted the data using a data extraction sheet pre-designed and pre-tested for the objectives of our study. Information extracted included study aim, sample size, sampling strategy, statistical measures, and results.

RESULTS

In total, the search yielded 6292 records from which, 46 articles were kept after duplicate removal and abstract screening. Applying full inclusion and exclusion criteria on these articles, 6 met the eligibility criteria. Detailed study selection process and reasons for exclusion are described in Fig. 1 (PRISMA Flowchart).

Characteristics of selected studies

This systematic review comprised of four case-control and two cohort studies. A descriptive analysis of the included articles is presented in Table 1.

Five out of the six studies [40–43] enrolled AD patients as case samples. From those, Ide et al. [40] recruited AD patients with periodontitis as case samples. In the remaining study by Poole et al. [10], postmortem AD-diagnosed brain samples were used.

For controls, three studies [42–44] enlisted patients with the criteria of not having cognitive impairment. Poole et al. [10], however, similar to the case samples, used brain samples diagnosed to be AD-free. In the remaining two studies, Ide et al. [40] recruited controls with the criteria of AD patients without periodontitis while Laugisch et al. [41] recruited non-AD dementia patients as controls.

Risk of bias within studies

All articles were found to be of an average grade or above, and thus, none have been excluded. Quality Assessment scores of included studies are presented in Table 1.

Possible predictors of AD

Our primary analysis was built on the following: 1) MMSE average score of samples, 2) *P. gingivalis* antibody immunoglobulin (*P. gingivalis* Ab IgG) measures, 3) TNF-α, and 4) *P. gingivalis* or virulence factor LPS or gingipains measures, presence, or absence. Table 2 presents an overview of the reported results.

Mini-Mental State Examination average score

In the recruited AD cases, the highest MMSE score of 28.8 was found in the study by Sparks Stein et al. [43], while two studies reported the same lowest score: 19.5 [40, 44]. In controls, the highest MMSE scores of 29.3 and 29.4 were reported by Kamer et al. [44] and Sparks Stein et al. [43], respectively. However, none of the studies claimed a statistically significant association between MMSE and *P. gingivalis* presence or prevalence. Only one study, by Poole et al. [10], did not report MMSE scores for participants.

P. gingivalis antibody immunoglobulin (P. gingivalis Ab IgG)

All studies, except one by Poole et al. [10], included measuring of *P. gingivalis* Ab IgG. However, three studies [41, 42, 44] only reported the number of participants that tested positive for this

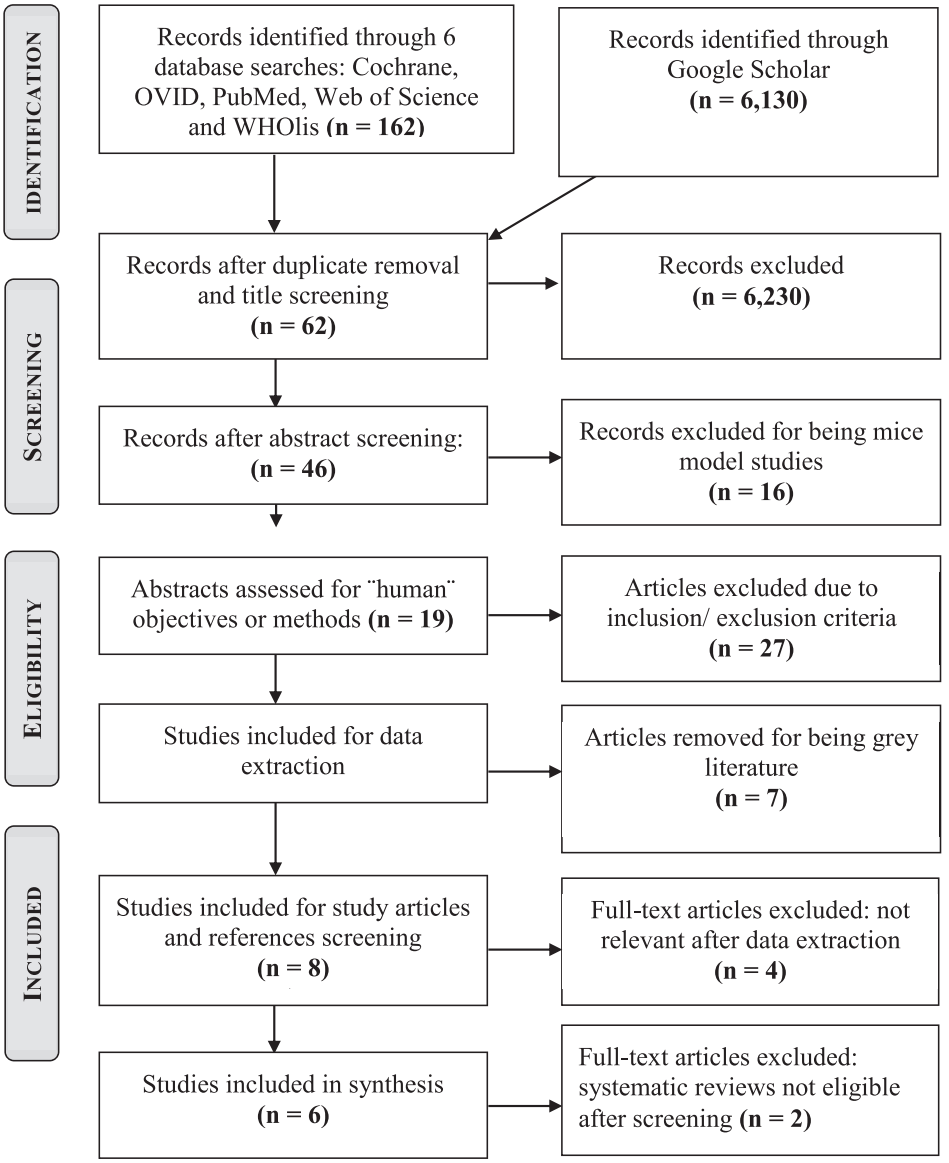

Fig. 1. PRISMA flow chart showing the detailed study selection process.

antibody. The study by Noble et al. [42] found that high levels of *P. gingivalis* Ab IgG were observed in only 23% of the participants while the other 77% had other periodontal bacteria antibodies other than those of *P. gingivalis* as the antibodies with highest levels measured. The association between *P. gingivalis* Ab IgG and AD were statistically insignificant in all five studies.

Although all studies, except Poole et al. [10], reported *P. gingivalis* AB IgG measurement, only the study by Noble et al. [42] reported the positive thresholds used in their investigations.

Tumor necrosis factor-alpha

Only the two articles by Ide et al. [40] and Kamer et al. [44] investigated TNF-α measures. Ide et al. [40] reported higher TNF-α rates among cases when compared to controls. The study by Kamer et al. [44] found similar results (TNF-α level reported for cases was 13.0 pg/ml, while in controls it was 8.2 pg/ml).

P. gingivalis or virulence factor lipopolysaccharides or gingipains

Out of the six studies, only Poole et al. [10] and Laugisch et al. [41] performed testing for presence

Table 1
Characteristics of Included Studies

Study	Country	Case Samples	Controls	Baseline Mean-Age	Gender	Study type	Measure used for *P. gingivalis*	Measure used for AD	Follow-up	Quality Assessment
(Noble et al., 2014) [42]	USA (Manhattan)	110 incident AD cases	109 without incident CI at last follow-up	Cases: 79 Controls: 72	67.8% Female	Case-cohort study	Serum IgG AB levels by checkboard immunoblotting	Series of neurological tests CARE- Diagnostic Interview	5 years	11/14 (79%)
(Ide et al., 2016) [40]	UK (South Hampton)	22 mild/ moderate AD Patients with PD	37 mild AD Patients w/out PD	Cases: 74.9 Controls: 79.4	49.2% Female	Observational cohort study for 6 months	Venous blood sample for CRP, pro-cytokine TNFα, IL10, and AB to PG. Number of teeth and full periodontal chart for presence or absence of periodontitis	NINCDS-ARDRA[1] ADAS-cog[2] sMMSE	6 months	10/14 (71%)
(Poole et al., 2013) [10]	UK (New Castle)	10 AD brain samples	10 Non-AD brain samples	Cases: 80.2 Controls: 74.8	N/A	Random case-control	Immunolabelling and immunoblotting with anti-PG	Confirmed AD brain samples	N/A[3]	7/11 (64%)
(Sparks Stein et al., 2012) [43]	USA (University of Kentucky)	35 developed AD. (46 developed MCI, 81 in total)	77 cognitively intact	Cases: 74.1 (for AD) Controls: 70	Cases: 74.3% Female Control: 58.4% Female	case-control study nested within a cohort study.	Venous blood evaluation for levels of IgG AB to the oral bacteria	NINCDS-ARDRA[1] McKann et al. Criteria: MMSE	Cases: 9.6–9.8 years Controls: 12.5 years	8/12 (67%)
(Laugisch et al., 2018) [41]	Germany	20 AD patients	20 DEM-noAD[5] patients	Cases: 58.3 Controls: 61.1	Cases: 55% Female Control: 40% Female	pilot observational study	CSF by lumbar puncture Blood serum Full clinical periodontal examination Subgingival biofilm sample and gingival crevicular fluid	AD by:NIAA (2011) guideline[4], MMSE CSF for biomarkers MRI CERAD	N/A[1]	8/12 (67%)
(Kamer et al., 2009) [44]	USA (New York)	18 AD patients	16 cognitively normal	Cases: 40–65 ($n=2$), 66–79 ($n=6$), > 80 ($n=10$) Controls: 40–65 ($n=7$), 66–79 ($n=6$),>80	Cases: 78% Female Control: 94% Female	Longitudinal case study	Fasting plasma for IgG AB and cytokine levels APOE genotyping using frozen whole blood.	NINCDS-ARDRA[1] DSM-IV MMSE	N/A[3]	8/12 (67%)

IgG, immunoglobulin; AD, Alzheimer's disease; TD, *Treponema denticola*; TF, *Treponema forsythia*; PG, *Porphyromonas gingivalis*; AB, antibody; sMMSE, standardized Mini-Mental Examination; LPS, lipopolysaccharides; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; *APOE*, Apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DSM-IV, Diagnostic and Statistical Manual for Mental Diseases- 4th Edition. [1]NINCDS-ADRDA National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. One of the most commonly used criteria in the diagnosis of Alzheimer's disease [70]. [2]ADAS-COG, Alzheimer's Disease Assessment Scale-cognitive subscale. [3]Not Applicable or available. [4]National Institutes of Health and the Alzheimer's Association Guidelines published in 2011. It includes new revised diagnostic clinical criteria for Alzheimer's disease. The new guidelines include a deeper understanding of the early stages of the disease [71]. [5]In the study by Laugisch et al. [41], the controls used were patients diagnosed with dementing diseases other than Alzheimer's disease.

Table 2
Main outcome measures reported by studies

	MMSE Average (at baseline)		PG AB IgG		TNF-α		PG or vir. factors	
Study	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
(Noble et al., 2014) [42]	–	–	Found in 25 ($n = 110$)	Found in 25 ($n = 109$)	–	–	–	–
(Ide et al., 2016) [40]	19.5	21.0	Mean average: 0.38 Units	Mean average: 0.37 Units	0.29 pg/ml difference	0.16 pg/ml difference	–	–
(Poole et al., 2013) [10]	–	–	–	–	–	–	Found in 4 ($n = 10$)	Found in 0 ($n = 10$)
(Sparks Stein et al., 2012) [43]	28.8	23.8	Mean average: 0.38 Units	Mean average: 0.22 Units	–	–	–	–
(Laugisch et al., 2018) [41]	22.1	23.8	Found in 2 ($n = 20$)	Found in 3 ($n = 20$)	–	–	Not detected in serum or CSF	
(Kamer et al., 2009) [44]	19.5	29.3	Found in 5 ($n = 18$)[1]	Found in 6 ($n = 16$)[2]	13.0 pg/ml	8.2 pm/ml	–	–

AD, Alzheimer's disease; PD, periodontal disease; CI, cognitive impairment; MMSE, Mini-Mental State Examination; PG AB IgG, *Porphyromonas gingivalis* antibody immunoglobulin; TNF-α, tumor necrosis factor alpha; CSF, cerebrospinal fluid. [1]In the study by Kamer et al. [44], antibody immunoglobulin titers found were reported cumulatively. No separate results for AB IgG were presented.

Table 3
Additional Analysis and Data Extracted from Studies

	(Noble et al., 2014) [42]	(Ide et al., 2016) [40]	(Poole et al., 2013) [10]	(Sparks Stein et al., 2012) [43]	(Laugisch et al., 2018) [41]	(Kamer et al., 2009) [44]
Education	Cases: 7.8 years Controls: 11.9 years	–	–	Cases: 16 years Controls: 15.9 years	–	Cases: 14.4 years Controls: 15.6 years
Race	47% Hispanic 26.5% Black (no difference in cases against controls)	–	–	–	All Caucasian	Cases: 83% white Controls: 81% white
APOE Status	Cases: 24.1% Positive Controls: 26.4% Positive	–	*(Siddiqui et al.) [68]*[1]	Cases: 37.1 % Positive Controls: 15.6% Positive	– –	Measured
Smoking	Cases: 7.6% Controls: 11.5%	Only non-smokers recruited	–	Cases: 38.9% Controls: 47.8%	All non-smokers	
Diabetes	Cases: 21.8% Positive Controls: 15.6% Positive	–	–	Cases: 8.3% Controls: 11.6%	All non-diabetic	No medical confounders[2]
Hypertension	Cases: 57.3% Positive Controls: 56.9% Positive	–	–	–	–	
Stroke History	Cases: 15.5% Positive Controls: 2.8% Positive	–	–	–	–	
Oral Health Measures	Oral health status measured	Oral health status measured	–	–	Oral health status measured	
Participant Matching	By ethnicity and from the same community	From same community	By age	By age and gender	From same mental health unit	

APOE, Apolipoprotein. [1]Although not mentioned in the study by Poole et al. [10], *APOE* genotypes of samples have been detected in a follow-up study by Siddiqui et al. [68] in 2019. The study states that 40% (4/10 samples) of the AD-diagnosed samples where positive of *APOE* 4 while 10% (1/10) of the control non-AD diagnosed samples was *APOE* positive [68]. [2]The study states that all participants went through extensive diagnosis to rule out confounding medical, neurological, and psychiatric conditions.

of *P. gingivalis* or *P. gingivalis* virulence factors such as LPS and gingipains. Poole et al. [10] reported that four out of the ten AD-diagnosed samples tested positive for LPS, while all controls tested negative. On the other hand, gingipains were not detected in all control and AD-samples. The other study, conducted by Laugisch et al. [41], investigated the presence of *P. gingivalis* in the serum or cerebrospinal fluid of the participants. The authors reported that no *P. gingivalis* was detected in the serum or cerebrospinal fluid of both the cases and the controls.

Further results

In our analyses, we found that some authors considered several other characteristics (education, race, non-communicable diseases, smoking, and oral and medical history) that may also play a role in the relation between AD and *P. gingivalis*. Table 3

presents an overview of these variables and the results obtained from the included studies.

Education

The educational level of participants was reported as years of schooling completed in three of the included studies [42–44]. Out of the three studies, only the study by Noble et al. [42] reported that AD cases had a lower average of education years completed. They also reported that this was a statistically significant association between education and AD.

Race and ethnicity

Three studies presented the ethnicity of their participants [41, 42, 44]. Laugisch et al. [41] only recruited samples of Caucasian origin while Kamer et al. [44] reported that 81% of the control subjects and 83% of the AD case subjects were of white ethnicity. Noble et al. [42] reported that 47.7% of the control subjects and 48.2% of the cases were Hispanic, while 27.5% of the control subjects and 25.5% of the cases were of non-Hispanic black ethnicity. Kamer et al. [44] and Noble et al. [42] reported no difference between populations affected by AD across the recruited ethnicities.

Apolipoprotein E Status (APOE)

APOE genotyping was performed in three of the six studies included [42–44]. Sparks Stein et al. [43] reported a significant difference due to *APOE* status between controls and AD-diagnosed cases while Noble et al. [42] reported no difference. Kamer et al. [44] did not report their findings regarding *APOE*.

Smoking status

Four studies reported the smoking status of participants [40–43]. The studies by Ide et al. [40] and Laugisch et al. [41] only recruited non-smokers and non-smokers for the past five years, respectively. The two other studies by Noble et al. [42] and Sparks Stein et al. [43] reported the smoking status of the participants as non-significant.

Diabetes, hypertension, and stroke history

Four out of the six studies investigated various general health conditions in their participants [41–44]. Noble et al. [42] included diabetes, hypertension, and stroke history of their participants. However, Sparks Stein et al. [43] only included and analyzed the diabetic condition. Laugisch et al. [41] reported that for their study, they only recruited non-diabetic participants. Kamer et al. [44] did not report any findings related to these conditions. In the four studies that mentioned general health measures, only Noble et al. [42] reported a statistically significant association between stroke history and AD progression and diagnosis.

Oral health measures

Three out of the six studies include a dental/oral health examination. Ide et al. [40] and Laugisch et al. [41] reported the mean scores of the number of teeth, bleeding on probing, measurement of pocket depth, and full-mouth plaque score. Ide et al. [40] and Laugisch et al. [41] reported significant association between oral health conditions and AD, while Sparks Stein et al. [43] found no difference.

DISCUSSION

Discussion of key findings

Several studies have identified an association between PD and AD; however, the nature of this relation remains unclear [23, 45]. Recent published articles suggest that *P. gingivalis*, which was recently found in AD-diagnosed brain autopsy specimens, is the link between those two inflammatory conditions [10, 41, 46]. Our study synthesized the evidence on the relationship between *P. gingivalis* and AD and contrary to hypothesis of direct causation between *P. gingivalis* and AD, we found that the presence of *P. gingivalis* virulence factors or antibodies has no proven direct association with AD. We also found that antibody levels detected in the included studies did not differ significantly between cases and controls. Moreover, this systematic review found no homogeneity in the methodology used across studies (besides the use of MMSE scale for AD progression). The results also highlight that there are divergent approaches with regards to *P. gingivalis* assessment in humans. Some of the included studies analyzed *P. gingivalis* AB IgG, yet they employed different threshold values.

Our review also found several inflammatory measures used across the studies that may indicate association between *P. gingivalis* and AD and in line with the hypothesis of AD being a direct cause of systemic inflammation [6, 28, 29, 47–50]. This is consistent with recent published literature that suggests that periodontal bacteria and their virulence factors, such as *P. gingivalis* and gingipains, caused neuronal

impairment and inflammatory responses common to the pathological processes found in AD [23, 51, 52].

Risk of AD development due to P. gingivalis exposure

When establishing the risk of AD development due to *P. gingivalis* and its virulence factors, Ide et al. [40], Laugisch et al. [41], Noble et al. [42], and Kamer et al. [44] reported that there is an observed association between PD and AD; however, no proven direct associations between levels of *P. gingivalis* or *P. gingivalis* AB IgG in serum and AD were found. This may be deemed contrary to the results found in the study by Sparks Stein et al. [43], where the authors reported significantly elevated levels of *P. gingivalis* AB IgG in AD patients at baseline. Papapanou et al. [26] found *P. gingivalis* in 100% of periodontitis patients and 80% of the healthy controls. Thus, *P. gingivalis* AB IgG presence or prevalence may also be indicative of many other factors. One way to avoid such a drawback is to match participants according to oral condition or baseline *P. gingivalis* and *P. gingivalis* AB IgG levels. Several studies included in our analysis controlled for potential confounders, e.g., Ide et al. [40] differentiated between cases and controls using periodontitis status, while Kamer et al. [44] claimed that participants were matched according to several medical and dental diagnoses. Ide et al. [40] reported no significance between levels of *P. gingivalis* or *P. gingivalis* AB IgG in serum and AD. Kamer et al. [44] reported the cumulative level of antibody immunoglobulin to six different periodontal pathogens. In their study, Poole et al. [10] suggested that AD-affected brains are at a higher risk of secondary *P. gingivalis* infection. As memory typically deteriorates during AD, it may be harder for AD patients to maintain oral hygiene measures, which would also explain poor oral health conditions as AD advances [2, 3, 32].

Our findings suggest that no sufficient evidence in the literature is available that supports the hypothesis of direct connection between AD and *P. gingivalis* bacteria. These findings support previous statements by Leira et al. (2017) and Ranjan et al. (2018) that suggest that the current literature lacks evidence on the causal relation between *P. gingivalis* and AD [8, 11]. Regardless of these statements, we still found several inflammatory measures across the studies that may indicate association and in line with the hypothesis of AD being a direct cause of systematic inflammation [6, 28, 29, 47–50].

Inflammatory process hypothesis

Consistent with our results, other molecular and biological studies also emphasized the importance of inflammatory processes and infectious agents in developing localized inflammation in the brain [4, 24, 28, 29, 47, 49] as possible mechanisms that give rise to the landmark feature of AD in the brain, the amyloid plaques [4]. This hypothesis is specifically viable after the classification of these plaques as an antimicrobial peptide and an immune response [53]. Other studies have also identified *P. gingivalis* virulence factor, gingipains, in AD-diagnosed brain samples, where its intracranial levels have been found to be correlated with tau and ubiquitin pathology [23]. However, this was not supported by the findings of one of our included studies, by Poole et al. [10], which has not detected the presence of gingipains in all tested AD-diagnosed brain samples.

Oral health status

Recent studies have also investigated the association between periodontal disease such as periodontitis, and *P. gingivalis*, to AD and several inflammatory diseases [16, 20, 51, 54–57]. One of those studies conducted on the Nun Study, a continuing longitudinal study that examines the onset of AD [58], found that periodontal disease almost doubles the risk of AD onset [59]. By exclusively examining the risk of AD associated with the periodontal pathogen, *P. gingivalis*, and its virulence factors in humans, this study provides a recent and comprehensive analysis of available evidence.

Oral health measures were not fully explained in all studies included in this review. Several studies reported higher incidences of AD in participants with deteriorating oral health [7, 30, 34, 60–62]. Some authors suggest that inflammatory processes due to oral health infections cause immune system activation and release of cytokines in the bloodstream. The theory adds that some of these changes affect the permeability of the blood-brain barrier. This, the studies propose, leads to the passage of *P. gingivalis* virulence factors into the central nervous system. These factors then lead to plaque formation as a defensive mechanism in the brain [4, 20, 54]. Some longitudinal studies have shown a significant association between AD and poor oral health [7, 32, 52, 54, 63]. Whether the relationship is causal remains a question. Including the oral health status and parameters when investigating oral pathogens rather than merely

focusing on specific bacteria, such as *P. gingivalis*, may provide the sought-after results regarding AD pathophysiology and diagnosis.

Sample characteristics

Our analysis found that age and gender are of high relevance when researching AD. This is due to AD being a disease that commonly appears in the elderly population [2, 3, 64]. Studies also show that AD is more prevalent in females than males [1–3, 64, 65]. We found that race is also seen as a risk factor for AD. According to a survey by Mayeda et al. in 2016, AD was found most prevalent in the Black race, followed by Hispanics, and then Caucasians [66].

Apolipoprotein ε4

Evidence in literature shows that one risk factor linked to increased incidence and earlier onset of AD is presence of apolipoprotein *APOE* ε4 [67]. Three of our included studies have mentioned performing *APOE* genotyping to their samples and only one, by Sparks Stein et al. [43] reported a significant difference due to *APOE* status between controls and AD-diagnosed cases. Although not reported in their study and mentioned in a subsequent study by Siddiqui et al. [68], *APOE* genotyping of the samples recruited in the study by Poole et al. [10] showed that 40% of AD-diagnosed samples and 10% of non-AD diagnosed samples were profiled as positive of *APOE* ε4 gene [68].

Limitations

Several limitations might have influenced the results obtained in our study. We included studies published only in the English language and therefore may have missed relevant articles published in other languages. All included studies were conducted either in the USA, the UK, or Germany demonstrating lacking evidence from other regions. Most of the included studies applied hospital-based and community-based sample selection methods; this may also lead to bias. Due to the high degree of subjectivity and different tools used to measure and diagnose AD in the included studies, we were not able to perform a formal meta-analysis. Furthermore, AD diagnosis requires demonstrated amyloid deposits and neurofibrillary tangles, either post-mortem or by a positron emission tomography examination, along with the presence of the clinical signs, which were not mentioned in the diagnosis criteria used for the AD cases in the studies included in this review [69]. Standardized diagnostic measures for AD would allow for better comparability across studies. Lastly, human error and subjected bias cannot be omitted.

CONCLUSION

This study analyzed available literature on the association between *P. gingivalis* bacteria and AD and its progression. Our findings show insufficient data to evaluate the association between *P. gingivalis* and AD and its development. However, all included studies suggest the probability of *P. gingivalis* bacteria, especially through its virulence factors, in playing a role in the process of systematic inflammation. Moreover, our results show a lack of homogeneity in AD and *P. gingivalis* diagnosis and measuring across the included studies.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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