

Review

Oral Inflammation, Tooth Loss, Risk Factors, and Association with Progression of Alzheimer's Disease

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Abstract

Periodontitis is a polymicrobial chronic inflammatory disease of tooth-supporting tissues with bacterial etiology affecting all age groups, becoming chronic in a subgroup of older individuals. Periodontal pathogens *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are implicated in the development of a number of inflammatory pathologies at remote organ sites, including Alzheimer's disease (AD). The initial inflammatory hypothesis proposed that AD hallmark proteins were the main contributors of central nervous system (CNS) inflammation. This hypothesis is expanding to include the role of infections, lifestyle, and genetic and environmental factors in the pathogenesis of AD. Periodontal disease (PD) typifies a condition that encompasses all of the above factors including pathogenic bacteria. These bacteria not only are the source of low-grade, chronic infection and inflammation that follow daily episodes of bacteremia arising from everyday tasks such as brushing, flossing teeth, chewing food, and during dental procedures, but they also disseminate into the brain from closely related anatomical pathways. The long-term effect of inflammatory mediators, pathogens, and/or their virulence factors, reaching the brain systemically or otherwise would, over time, prime the brain's own microglia in individuals who have inherent susceptibility traits. Such susceptibilities contribute to inadequate neutralization of invading agents, upon reaching the brain. This has the capacity to create a vicious cycle of sustained local inflammatory milieu resulting in the loss of cytoarchitectural integrity and vital neurons with subsequent loss of function (deterioration in memory). The possible pathways between PD and AD development are considered here, as well as environmental factors that may modulate/exacerbate AD symptoms.

Keywords: Alzheimer's disease, inflammation, oral health, periodontal disease

Introduction

Alzheimer's disease (AD), a form of dementia, is the most common neurodegenerative disease worldwide [1]. The prevalence of AD increases exponentially with age, rising from 3% among the 65 to 74 year age group and to almost 50% among those around 85 years and older [2, 3]. Mental deterioration is slow but progressive, contributing to poor memory, disorientation, confusion, and eventually profound dementia. Susceptible individuals can take decades before clinically presenting with the disease. This implies that the etiology of AD is heterogeneous and that the importance of finding new risk factors for development in the case of late-onset AD remains a priority.

Although partial efficacy of non-steroidal anti-inflammatory drugs in some AD patients [4, 5] gave rise to the inflammatory hypothesis that accounts for the intrinsic elements of CNS inflammation [6], support also exists for the extrinsic inflammatory mediators. The elderly, for example, having suffered multiple episodes of recurrent infections, can present with dementia like clinical symptoms in likely late-onset AD cases [7] as well as those subjects with confirmed clinical diagnoses of AD [8, 9].

The innate immune responses suggest extrinsic inflammatory cytokines are involved in exacerbating neurocognition [9] and cytokine-related genes are being implicated in the susceptibility to inflammation in late-onset AD [10, 11, 12]. Furthermore, human brain tissue specimens from postmortem AD patients demonstrate evidence for neuroinflammation via the activated complement system as C1q, C3b, and reactive oxygen species are all involved in the amyloid fibril formation [11,13, 14]. These observations are strengthened by genome-wide studies supporting the role of innate immune components such as complement receptor 1 (CR1) and a fluid-phase regulatory protein clusterin [15, 16].

Pathological Hallmarks of Alzheimer's Disease

In AD, marked neuronal loss is observed in the hippocampus where high densities of the classical hallmark lesions are initially observed [17]. An accumulation of intraneuronal neurofibrillary tangles (NFTs) and extracellular amyloid- β ($A\beta$) plaques are the two easily demonstrated histological features of AD brains [18]. Synaptic dysfunction is considered as one of the earliest structural defects [19]. Specifically, NFTs indicate the severity of disease with $A\beta$ plaques depicting disease progression [17]. NFTs in neurons constitute hyperphosphorylated tau protein that alters the polymerization and stability of microtubules [20]. The loss of synapses between neurons correlates well with cognitive decline [19]. The cumulative knock-on effect of these cytoarchitectural changes is compromised protein-protein interactions with other cytoskeletal elements eventually leading to further synaptic loss and the demise of the NFT-bearing neurons [17, 18, 20, 21].

Amyloid plaques are largely made up of fibrillary $A\beta$ peptides $A\beta_{40/42}$ amino-acid residues and are the result of α -, β -, and γ -secretase enzymes cleaving the transmembrane amyloid- β protein precursor expressed by all CNS cells. The proteolytic fragment consisting of the last 28 residues of the amyloid- β protein precursor ectodomain prior to the membrane and including the first 12 to -14 residues of the transmembrane region generate $A\beta_{40/42}$ amino-acid residues that lead to deposition of extracellular, insoluble fibrillar $A\beta$. Clearing insoluble $A\beta$ peptides from the brain involves phagocytosis by microglial cells [22, 23], an activity that invariably fails with the consequences of further accumulation of fibrillary $A\beta$.

$A\beta$ peptides and insulin are known substrates of an insulin-degrading enzyme [24, 25]. Both of these proteins are important in the pathogenesis of AD and type II diabetes mellitus, respectively. Literature supports the risk-factor relationship between type II diabetes with

increased risk of cognitive impairment and dementia via its potential pro-inflammatory toxicity from perturbed glucose metabolism [26]. However, it is not clear if A β itself promotes insulin resistance in AD with the generation of subsequent oxidative stress, reactive oxygen species, and related pro-inflammatory cascades [27]. Streptozotocin used for generating experimental diabetic rats as a model for investigating late-onset AD in relation to insulin resistance is a toxin derived from *Streptomyces* species of bacteria [28]. It is, therefore, plausible to suggest that an initial microbial trigger may be responsible for insulin resistance and the subsequent deposition of A β in late-onset AD.

A β plaques can be observed in the brains of cognitively intact individuals, but they are fewer and are generally of the diffuse (A β_{40}) type, which so far appear to have little pathological significance. Of the two forms of amyloid, fibrillary A β is regarded as being neurotoxic [29] and *in vitro* studies have shown it to lyse all types of cells by apoptosis [30]. Evidence from neuroradiological and neuropathological investigations link this hallmark (A $\beta_{40/42}$) to the initiation of intracerebral inflammatory response in the inherited forms of AD as well as late-onset AD [14, 31, 32].

The inflammatory element of the disease may be a significant risk factor for late-onset AD, specifically as innate immune components such as C1q and C3b are involved with plaque maturation [11, 13]. The relevance of detecting C1q and C3b within A $\beta_{40/42}$ also draws attention to the cellular mechanisms involved with regulating inflammation in neurodegeneration and in clearing A $\beta_{40/42}$. The inflammatory components corroborate data from genetic studies proposing a relationship between AD and cytokine polymorphisms [12, 33], as well as the complement proteins such as clusterin and CR1 in its pathogenesis [15, 16]. With respect to the complement activation cascade, CR1 is a membrane-associated complement inhibitory protein that binds C4b

and C3b acting at the C3/C5 convertase stage of the alternative and classical complement pathway, and clusterin is a fluid-phase protein that interferes with the assembly of the lytic membrane attack complex. Decrease in clearance of the lesion by the innate immune system contributes to bystander damage that promotes a vicious cycle of chronic intra-cerebral inflammatory (high endogenous levels of inflammatory mediators) response [14, 34].

What is Intra-Cerebral Inflammation?

In the past the brain was considered as an “immunoprivileged organ,” as elements of the immune system [such as neutrophils, naive T cells (adaptive immune system), plasma proteins, and extra-cerebral-toxins to prevent inappropriate glial cell activation] cannot cross the blood-brain barrier (BBB). However, it is now understood that the circumventricular organs are not subject to the BBB [35], a region of the brain where infections (both systemic and direct via the trigeminal and olfactory nerve pathways (see Figure 1) and inflammatory mediators can access the brain [36-38].

The brain deals with inappropriate toxins derived locally (A β deposits) or from extracerebral sources (infectious agents) using its own innate immune system consisting of ependymal cells, microglia, astrocytes, and oligodendrocytes [39]. Normally these cells regulate the production and uptake of endotoxins and secrete trophic factors that nurture the CNS cells and protect their functions [40]. However, following physical damage and/or invasion by foreign agents (lipopolysaccharide, LPS), glial cells (specifically microglia) bearing the LPS receptor (CD14) and the highly conserved toll-like receptors 2 and 4 (TLR 2 and 4) undergo a number of phenotypic (resting to activated state) and functional changes. Key morphological changes include thickening and shortening of branching processes attached to a hypertrophic glial cell

body [41]. Once activated, microglia upregulate the expression of MHC class II molecules along with the secretion of pro-inflammatory cytokines (TNF- α and IL- β), complement proteins, quinolinic acid, arachidonic acid and its metabolites, nitric oxide, platelet activating factor, α and β chemokines excitatory amino acids, and free radicals [14]. When these innate factors are secreted by microglia, a local CNS inflammatory response is mounted.

In AD brains, A β is recognized as a nidus for intracerebral inflammation placing chronic neuroinflammation downstream of this primary hallmark [13, 14]. However, this view is changing, especially with late-onset AD cases as the importance of the innate immune molecules is being uncovered by genome-wide studies [11]. If nerve cell death and chronic CNS inflammation are common precursors of the development of dementia, explaining equivalent numbers of A β deposits and NFTs in clinical and subclinical AD in the very elderly who bypass dementia is important [42-45] and adds further complications to the etiological nature of AD. Even A β in subclinical AD subjects, which initiates intracerebral inflammation [11, 46, 47], appears not to lead to clinical decline in cognition. One possible explanation for the lack of cognitive decline could lay in the inflammatory genetic traits of these and the non-impaired individuals with vital neuronal cells being rescued from death and so providing a “cognitive reserve.” The inflammatory signals that initiate phagocytosis by microglia are driven by A β that involve the CD14 and TLR 2 and 4 signaling [22, 23, 48, 49], a pathway also used by microglia for bacterial LPS recognition [50]. Spontaneous loss-of-function mutation in the TLR 4 gene has been demonstrated to have an inhibitory effect on microglial cell activation. For example, presence of A β in the microglia with mutated gene expression [49] demonstrated reduced secretion of inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor- α

(TNF- α) and nitric oxide. These findings further strengthen the cognitive reserve hypothesis in very old subjects in the presence of classical AD hallmark lesions.

Using an *ex-vivo* experimental model to examine the expression of pro-inflammatory cytokine profiles in whole blood from the healthy middle-aged offspring of patients affected by late-onset AD, van Exel et al. [10] reported higher levels of specific cytokines than were found in the siblings from non-AD parents. This finding also demonstrates that inflammation-related risk factors are present in currently healthy subjects who may have a genetic ‘susceptibility profile’ a phrase, coined by McGeer and McGeer [33] to late-onset AD. Despite the inheritance factors, the brain’s response to inflammation is slow as supported by animal models of AD [51]. Currently, the greater accumulation of A β deposits in late-onset AD is considered to be the result of defects in the clearance system [52]. Therefore, the pertinent question would be, “what initially triggers A $\beta_{40/42}$ release in patients with late-onset AD?”

Infections are common in elderly individuals and are the main cause of death in a majority of neurodegenerative conditions. One hypothesis is that the elderly, *per se*, are immunocompromised and this correlates with their susceptibility to an increased incidence of infection [53, 54]. Recurrent bacteremia from common infections in the elderly, due to, for example, chronic periodontitis [55, 56], intra-abdominal [57], and urinary tract infections [58], will contribute to systemic circulation infections. In addition, chronic periodontitis demonstrates a promising link that encompasses environmental influences, susceptibility profiles, infectious agents, and a multitude of host’s factors that affect its episodic re-occurrence [59]. Numerous studies show that tooth loss due to PD to correlate with cognitive impairment in AD [60-63]. Further studies have demonstrated systemically derived immune components such as antibodies to the periodontal pathogens circulating in the blood plasma of AD subjects [64]. In addition, AD

patients with antibodies to the periodontal pathogens in their blood have displayed inflammatory mediators (cytokines) in the systemic circulation [64]. As Holmes et al. [9] suggest, these cytokines and those from alternative sources in the peripheral circulation have the potential to reach the brain parenchyma, and subsequently prime microglia to mount a local immune response with appropriate stimuli, and impair memory.

An alternative hypothesis for the role of A β in subclinical and/or clinical AD individuals is that A β is acting as an antimicrobial peptide [65] to counteract infections by functioning as part of the early innate immune defense mechanisms that mediate innate and adaptive immune responses [66]. Traditionally, antimicrobial peptides act as look-outs for invading microorganisms to maintain the balance between commensals. The main target for antimicrobial peptides is the pathogen cell membrane, as most antimicrobial peptides are cationic [67]. Antimicrobial peptides undergo electrostatic interactions with negatively charged molecules to penetrate bacterial cell walls, including anionic lipids and LPS [67]. They then invade the lipid bilayer, creating trans-membrane pores through which leakage of ions and metabolites, cytoplasmic components, dissipation of electrical potentials, and cell death of microbes takes place [68]. This hypothesis suggests the involvement of a pathogenic precursor in the initiation of A β release before inflammation becomes detectable in the presence of this hallmark. We support this hypothesis and propose a suite of susceptibility traits and immunosuppressive (stressed or rundown) episodes during life that give way to chronic bacterial infections. These bacterial elements in the individuals with susceptibility profiles may trigger release of A β to neutralize their effect. Over time A β will accumulate in the brains of healthy but susceptible individuals and initiate neuroinflammation that may cross the threshold from subclinical to late-onset AD.

The etiological hypothesis suggests that viruses and bacteria and/or their virulence factors access the brain and thereby contribute to AD pathogenesis. A review by Holmes and Cotterell [69] provides a range of infective agents consistently being linked to AD. These include viruses such as Herpes simplex virus type I [70], *Chlamydomphila pneumoniae* [71], *Treponema* spp., [72] *Borrelia burgdorferi* [73], and more recently LPS from *P. gingivalis* [74], one of the key bacteria causing PD in humans.

Age-Related Personal Hygiene Changes as Risk for Infections

Advancing age is the greatest risk factor for all forms of AD. Some consequences of advancing age are a compromised immune system [53, 54] and a neglect of general and oral personal hygiene [61, 75, 76], and such conditions are associated with recurrent, chronic infections. Recurrent, chronic infections enhance systemic hyperinflammatory profile that may lead to confusion and other dementia-like clinical features [7-9] in which the exact structural/cellular changes taking place at the time remain unknown.

Several studies support deterioration in oral health with increasing age [77-80]. The exact reasons are poorly understood, but advancing age is likely to compromise the manual dexterity of senior citizens and this may make cleaning their teeth more difficult, or perhaps it is because as general health concerns and conditions increase with age, maintenance of oral health becomes a lower priority. The elderly are more likely to be on multiple medications, many of which, as a side effect, cause xerostomia and this will inevitably be a factor in deteriorating oral health [81]. Furthermore, if the elderly suffer from physical impairments, accessing the dentist may become more difficult. Elderly people resident in care institutions are, to a certain extent, dependent on the level of care within the establishment for the level of oral hygiene and dental health they

receive. These factors were supported in a large-scale survey carried out in the US by Griffin et al. [79], which found that older age groups were more likely to be edentulous or have untreated dental disease and root caries. Those who were either residents in institutions or homebound had higher levels of untreated cavities, gingivitis (a marker of poor oral hygiene), and poorer overall oral health than the elderly living independently. The study shows that cost, lack of transportation, and limited mobility were key barriers to accessing dental care for nursing home residents [79]. Other groups of elderly that show higher untreated dental disease and lower levels of oral health are those from ethnic minorities and low-income families [79].

Periodontal Disease

PD is a polymicrobial inflammatory disease that has been estimated to affect 10-15% of the developed world population and is a major cause of tooth loss. The prevalence of PD increases with age, affecting around 50% of people over the age of 55 years [82]. The disease affects tooth supporting tissues wherein the interaction of specific bacteria and the host's immune responses play a pivotal role [59]. The host's response to bacteria and their products is an important factor in determining the extent and severity of PD [83]. The acute bacterial challenges stimulate junctional transformed pocket epithelium to produce a broad range of inflammatory mediators to guard against gingival tissue damage. The inefficient clearance of subgingival pathogens by the innate immune system compromises the integrity of periodontal tissues and eventually results in the formation of periodontal pockets.

Periodontitis, involving specific bacteria coupled with the host's response, initiates an acute phase receptor-mediated cytokine production by epithelial cells and simultaneous neuropeptide release, resulting in vasodilation of local vessels. Chemokines mobilize neutrophils from blood

vessels for migration to the area of bacterial invasion. Gingival bleeding, swelling, and redness together with the presence of neutrophils/macrophages within the inflamed gingivae indicate clinical signs of inflammation. The infection is both confined to, and subsequently cleared by, neutrophils and macrophages, or expands to include other cells and structures [84]. In the high susceptibility profile group of individuals, the acute phase responses fail to clear the infection, and chronic inflammatory lesions develop within a matter of weeks. The pathogenic consortium consisting of *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* (*T. forsythia*), and *Treponema denticola* (*T. denticola*) appear to be the main organisms involved in the development of chronic PD [85, 86]. The subgingival sulcus serves as a niche enabling a cyclic chronic inflammatory process which in turn facilitates recurrent bacteremia following routine oral health regimes [55, 56, 87]. A number of inflammatory pathologies are said to develop in this way, including cardiovascular diseases [88, 89], rheumatoid arthritis [90-92], diabetes mellitus [93], and others as well as AD [60, 61, 64, 72, 74].

Anatomical Relationship of Facial Nerves and the Blood Supply to the Brain

The position of the oral cavity, serving the need for speech and food consumption, connects with the brain via series of nerves. Cranial nerve 1 (CN1) is the special sensory nerve for olfaction and contributes not only to our sense of smell but also to that of taste. CN1 has complex pathways that trigger visceral responses (salivation and nausea or accelerated peristalsis in the intestinal tract and increased gastric secretion) to various odors. Although CN1 is recognized and named as the olfactory nerve, the majority of the olfactory tract comprises of secondary, rather than primary sensory axons; thus it is really not a “nerve” but rather a bulb and tract. There is a physical connection between the oral and nasal cavity, extending onto the

superior nasal conchae and nasal septum and contains neurosensory cells and olfactory glands, which keep the mucosa moist and in which the dissolution of inhaled scents (aromatic molecules) occurs. The peripheral processes of the primary sensory neurons in the epithelium perform as sensory receptors and transmit sensation centrally, which congregate into around 20 bundles, which, in turn, pass through foramina of the cribriform plate of the ethmoid bone. The cribriform plate of the ethmoid bone is the porous barrier between the nasal passages and the brain itself. Once they have passed through the cribriform plate, the central processes synapse on the secondary sensory neurons in the olfactory bulb itself, which houses the nerve cell bodies. Behind this area is the olfactory tract and trigone; the nerve cell bodies travel to the three olfactory areas, located in the anterior part of the entorhinal cortex area, encompassing the hippocampal gyrus and all ultimately lead to the hippocampus [94].

Cranial nerve V (CN V) or the trigeminal nerve, arising from the mid-lateral surface for the pons, is primarily a general sensory nerve with smaller motor component. There are three divisions of the CN V which are ophthalmic (V₁), maxillary (V₂) and mandibular (V₃) where, the motor root of CN V travels with the mandibular branch. The ophthalmic division (V₁) exits the neurocranium through the supraorbital fissure, the maxillary division (V₂) through the foramen rotundum in the sphenoid bone and the mandibular (V₃) branch through the sphenoid's foramen ovale. CN V is a general sensory nerve to the scalp, face, nasal and oral cavities (including the teeth and tongue), and brachial motor nerve to the muscles of mastication (temporalis, masseter, medial pterygoid, and lateral pterygoid), tensor tympani, tensor (veli) palitini, mylohyoid, and the anterior belly of the digastric. When dental or periodontal therapy is performed using local anesthesia (e.g., novocaine, xylocaine), the drug is injected into the oral mucosa covering the bony foramina where the sensory branches of the CN V exit into the oral

cavity. For the maxillary dental arcade, the injection is aimed toward the pterygopalatine ganglion; for the mandibular teeth, this is directed toward the mandibular foramen. In the case of the pterygopalatine ganglion, this supplies sensation via branches of V₂ from the nasal cavity, plate, nasopharynx, and maxillary teeth. The lingual and inferior alveolar nerve branches carry sensation from the entirety of the lower jaw, mandibular teeth, gums, and anterior two thirds of the tongue as shown in Figure 1. As with most nerves, the branches of the trigeminal nerve are accompanied by veins and arteries along the peripheries of their pathways [94].

The olfactory and the trigeminal nerve(s) pathways are also exploited by periodontal pathogens as a means of bypassing the BBB for direct entry into the CNS [72, 95, 96], an observation supported by studies in immunosuppressed animal models using *T. denticola* [97]. The animal model study allows some insight into the virulence of the organism and the host's immune defenses as being important for this occurrence.

The systemic route as an alternative mode of bacterial entry into the brain is favored due to bacteremia as mentioned earlier, association of periodontal pathogens with atherosclerotic lesions and in particular *P. gingivalis* having the ability to adhere to erythrocytes for innate immune evasion [87, 98, 99] as well as gaining advantage for transportation to remote body organs [99].

The brain is supplied by three paired blood vessels: the right and left internal carotid arteries, arising from the common carotid artery at the base of the neck. It has three divisions that enter the cranium, anteriorly through the carotid canal of the temporal bone and through foramen lacerum in the middle cranial fossa. The vertebral arteries arise from the subclavian arteries, bilaterally and both enter the cranium via the foramen magnum. The vertebral and internal carotid arteries unite on the base of the brain at the Circle of Willis, via a series of

interconnecting smaller arteries. The basilar artery is created when the vertebral arteries join. The Circle of Willis itself is composed of the posterior cerebral, posterior communicating, internal carotid, anterior cerebral and anterior communicating arteries; all these arteries branch to supply the brain itself [94] including the circumventricular organ regions where bacteria and bacterial products access the brain.

The Association between Periodontal Disease and Alzheimer's Disease

Longitudinal studies have shown that people with PD who progressed to the development of AD had poorer oral health [61, 76, 79, 100-102]. Does poor oral health always mean that the pathogens will disseminate to the brain even in AD patients? Both our unpublished data from controlled experiments using animal models, and that of Foschi et al. [97], indicate that the presence and motility of the low virulence strains of periodontal pathogens may not be sufficient for them to access the brain. However, animal models of oral diseases (periodontitis and endodontic) may require an adjustment for the optimization of dosage and/or duration of infection to allow for bacteria to translocate to the brain. Our unpublished data demonstrates that *P. gingivalis* (FDC381) accessed the brain of ApoE^{null} mice following an oral infection while *P. gingivalis* (ATCC 33277) failed, even in SCID mice mono and poly infections [97]. It therefore appears that the greater virulence of *P. gingivalis* (FDC 381) due to having acquired fimbriae, likely allowed its adherence to erythrocytes for innate immune evasion, a process that has gained the bacterium an advantage for dissemination [99], to the brain [Poole et al. unpublished results]. When the duration of active infection supersedes the virulence of the bacteria, there remains a high possibility that the host harboring the periodontal pathogens will demonstrate these bacteria disseminating to the brain. Immunocompromised status seen in AD patients will also enhance the

infection process. This begs the question as to what causes some individuals to harbor periodontal bacteria rather than other species? Could there be as yet unknown genetic factors/inflammatory traits, lifestyle driven environmental stressors?

However, it should be noted that patients suffering from AD cognitive impairment are poor at managing their personal oral health. In addition, a caregiver or dentist may face a marked decrease in co-operation from the AD patient, making management of oral health more challenging. With patients having increased cognitive impairment, such as reduced adaptation to change, dentists often choose not to carry out the dental treatment that would give optimal oral health. For example, patients with AD may well be unable to cope with extensive, potentially unpleasant dental procedures, leaving them with fewer teeth, which could have a detrimental impact on their eating ability.

Nutritional deficiencies are documented in the elderly as well as in the dementia subjects, especially with regard to lessened intake of B-vitamins and folic acid in the diet. The marker that indicates these deficiencies also correlates with cognitive decline, but as consequence of disease rather than a cause [103]. The mechanism of cognitive decline is suggested via synaptic dysfunction, which is one of the earliest structural defects associated with decline in memory [19]. Diet provides the essential B-vitamins, phospholipids, and other micronutrients, which are required for the formation of new synapses [104].

Epidemiological Evidence for the Association between Chronic Periodontitis and Alzheimer's Disease

Clinical/epidemiological studies so far, all agree on loss of teeth leading to poor memory [60-63]. Further studies have examined possible inflammatory biomarkers in an attempt to link

and/or to find new diagnostic makers of AD. Others have, however, used more specific measures including IgG levels to *P. gingivalis* and other specific periodontal pathogens [64, 105]. A study by Sparks Stein et al. [105] used cohort methodology analyzing levels of serum antibodies to periodontal disease. At the start of the study period, all participants were cognitively intact, but higher levels of serum antibodies to periodontal pathogens at baseline led to some individuals developing AD [105]. As baseline measures were taken years before diagnosis of AD, the elevation in serum antibodies cannot be attributed to secondary effects of AD (for example, poor oral hygiene). Although clinical measurements of oral health were not taken in the Sparks Stein et al. [105] investigation, periodontal bacterial species are generally accepted as being specific enough to PD and assessing serum antibody levels to these pathogens may prove to be a true indicator of PD in AD patients.

Possible Confounders

Several environmental, epidemiological, and risk factors show similar trends and patterns in both PD and AD. Whether or not this is coincidental or arises through shared developmental pathways remains unclear. The impact of these associations is the potential for these factors to act as confounders, influencing the true relationship between PD and AD.

The incidence of both PD and AD increases with age; this has already been mentioned in previous sections. Gender related trends exist between AD and periodontal disease. The incidence of AD has been shown to be higher in women after 85 years of age. This is thought to be due to the protective effect of pre-menopause estrogen [106]. Men, however, have been shown to have a greater incidence of PD (up to 50%) than women overall [107]. Interestingly, this study also showed that men with PD also had increased incidence of coronary artery disease,

suggesting fewer men than women survive to old age. Physical activity has been shown to have a positive effect on cognitive function and those aged 70-79 years with high levels of activity show lower levels of inflammatory markers such as IL-6 and C-reactive protein [106]. Likewise obesity has shown similar trends, in that those who are obese suffer a greater incidence of AD. Although physical activity and the incidence of PD have not been investigated directly, many studies have shown an indirect relationship, highlighting a greater incidence of PD with obesity and diabetes [107, 108]. It has been suggested that obesity may be the second highest risk factor for PD after smoking [109]. The underlying mechanism for this association is thought to be related to proinflammatory cytokines, including IL-6 and C-reactive protein that are released by adipose tissue, along with hormones adipokines or adipocytokines. With the increasing levels of evidence supporting inflammatory processes in AD development, it is possible that the relationship between AD and obesity may follow similar mechanisms [109]. Poorer general health may be associated with PD due to a compromising immune system and therefore, the ability of the host to defend against periodontal bacterial infections.

It is generally accepted that smoking is the major risk factor in periodontal disease. Smokers are 2-7 times more likely to present with PD than non-smokers and this is unrelated to oral hygiene. Disease progression is more rapid and response to treatment is poorer [109]. Smoking is thought to affect neutrophil function, reducing the host's ability to eliminate periodontal pathogens. Smokers with PD have distinct patterns of pathogenic microbial profile than non-smokers with PD [110-112]. Smoking is also thought to lead to release of reactive oxidative species and oxidative stress mediated tissue damage. Inflammatory cytokine and chemokine expression in smokers compared to non-smokers show many differences reflecting the

immunosuppressant effect of smoking, which may contribute to an enhanced susceptibility to periodontitis [113].

The relationship between smoking and AD is less clear; with some studies suggesting smoking has a beneficial effect due to nicotine treatment improving cognitive performance in age associated memory impairment [114], while others suggesting that smoking increase the risk of AD. In the proposed theory, an increased risk of AD is similarly related to the factors causing periodontal disease, whereby smoking increases free radical generation leading to high oxidative stress, or affects the inflammatory immune system leading to phagocyte activation and further oxidative damage.

PD would appear to have an increased prevalence in both ethnic minority groups and lower socioeconomic status. This has been attributed to a complex combination of social, psychological, and structural factors including nutrition, oral hygiene, healthcare utilization, and access to care. It is thought that having lack of resources to pay for care, not having a regular source of care, or availability of transportation to healthcare centers contribute to these trends [115-117].

The relationship between race and socioeconomic status and AD appears more complex. Meta-analysis assessing the relationship between education level and AD showed that overall those with low or no education were more likely to develop AD, but this was not shown in all studies. This relationship was stronger in developed, compared to developing countries possibly due to life-expectancy being shorter in developing countries. Individuals with less education appear to have lower cognitive function compared to those with higher education levels. Education-dementia relationship appears to vary according to age, gender, and race/ethnicity and the suggestion is that the relationship ties in with a person's life events beginning prior to and

carrying on beyond years of formal education [118]. These epidemiological trends associated with both PD and AD demonstrates the importance for excluding the impact of confounders when investigating a true relationship between PD and AD.

Genetic Risk Factors for Late-Onset Alzheimer's Disease and Periodontitis

The apolipoprotein E (ApoE) gene is a known genetic risk factor associated with late-onset AD, and more recent investigations suggest some further genetic risk factor associations with innate immune molecules and inflammatory traits in late-onset AD [10, 15, 16]. In particular, cytokine-related genes appear to be involved in the susceptibility to inflammation in late-onset AD [10, 12, 33] as well as in PD [119-121].

As the immune system plays an important role in PD pathogenesis [59], it is thought that PD itself may have genetic associations. Polymorphisms in IL- α , IL-1 β , IL-6, and TNF- α genotype are reported for periodontitis [119-121] and similarly IL-1 α , IL-1 β , IL-6, TNF- α , α 2-macroglobulin, and α 1-antichymotrypsin are all upregulated in AD [12, 33] suggesting commonalities between susceptibility profiles in these two disease conditions. As mentioned earlier, offspring of parents with AD have higher inflammatory cytokines in their blood than those who are descendants of non-AD parents [10]. Similarly, parents with poor oral health tend to have children with poor oral health; however, it is difficult to conclude that the poor oral health trait is a result of the genetic makeup of the individual and not simply an environmental influence [122].

Stress Environmental and Genetic Factors

Stress: In health

Acute stressors in the environment, such as facing a dangerous situation, activate physiological systems designed to enhance self-preservation [123]. These include the sympathetic-adrenal-medullary and the more slowly responding hypothalamus pituitary adrenal (HPA) axis. This response prepares the body to cope with threat [124]. The two response systems work by increasing certain hormones, such as adrenaline and cortisol. These, in turn, act on the biological functioning of the individual, such as increased heart rate, inhibition of the digestive system, or increased glucose supply to the muscles. Although the physiological changes enhance our ability to mount a physical response to threat, the associated neurochemical changes can lead to cognitive failures, which may present as dementia-like even within a cognitively normal population. For example, elevations in cortisol as a result of activation of the HPA axis can lead to impairments in attention [125], working memory and inhibitory control [126], and declarative memory [127]. Cognitive impairments due to acute stressors are reversible. Further acute stress responses may actually have some benefits for health with enhancements in immune function being reported [128]. However, when short-term beneficial adaptations designed to maintain homeostasis during acutely stressful events become excessive or prolonged, problems can occur [129].

Stress: Periodontal disease

Psychological stress may affect periodontal disease. Such stressors can lead to a change in health behaviors, which in turn may lead to associated increased risk of PD. For example, poorer oral hygiene coupled with increased smoking [130] and alcohol intake [131]; visiting the dentist less regularly, and eating less healthily with higher fat and sugar diets [132] encourage bacterial growth and worsen periodontal status.

Stress impairs the balance between pro- and anti-inflammatory responses. Alterations in inflammatory polymorphic gene function, in particular of IL-1 and IL-6 (119-121), will affect polymorphonuclear leukocyte chemotaxis. The net effect will be reduced lymphocyte proliferation and this may increase the vulnerability of periodontal tissue to microorganisms leading to further tissue destruction [133]. Inhibition of T cell responses by glucocorticoids appears to explain, in part, susceptibility to PD and pro-inflammatory cytokines are potent activators of the HPA axis [134, 135]. Patients with PD who are stressed show increased IL-6 and IL-1 β levels in gingival crevicular fluid [133]. Results of several studies were reviewed and demonstrated a correlation between psychological stress and salivary and blood stress markers relating to inflammatory response and progression of PD [134]. However, a cause and effect relationship has not, so far been found, therefore stress is considered a risk factor for PD rather than a cause.

Stress: Alzheimer's disease

High levels of environmental stressors could lead to impairments in cognitive processes, which are important for maintaining oral hygiene. For example, acute and chronic activation of the HPA axis can lead to elevated basal cortisol levels. High levels of circulating cortisol causes hippocampal damage and so impair hippocampus-dependent memory processes [125]. In a cognitively intact elderly population, higher cortisol levels were indicative of impairments of declarative memory and executive functioning [135], both of which are needed to maintain good oral hygiene.

The hippocampus is the area of pathology in AD and is vulnerable to effects of stress and trauma [136]. Chronic stress can impair immune responses and so compromise the body's ability

to resist disease [137]. The brain attempts to compensate cellular stress, by regulatory mechanisms, involving upregulation of heat shock proteins [138]. Loss of heat shock proteins, *in vitro*, was shown to contribute to accumulation of hyperphosphorylated tau, a component of NFTs, and a hallmark of AD pathology [139]. In addition, stress activated protein kinases, for example, mitogen-activated protein kinase 38 and the c-jun N-terminal kinases are activated in AD [140, 141]. These two stress pathways can also be activated by oxidative stress [142, 143], a common denominator of environmental, pathological, and habitual factors.

Critical Remarks about the Present State of the Research to Relationship between Alzheimer's Disease and Chronic Periodontitis

Clinical observational studies thus far all correlate with loss of teeth leading to poor memory. However, direct evidence is lacking to support a causal association between periodontal bacteria and progression of AD. The clinical studies have been performed on elderly cohorts where overlapping features of the aging process, such as deposition of A β in the brain is likely to have already begun. When assessing periodontal status clinically, levels of caries may not have been accounted for. Oral hygiene studies were based on retrospective questionnaire surveys, which may introduce selection bias depending on the response rate. People who are motivated to complete questionnaires may be more likely to visit the dentist than those who do not. Genetic factors have not given importance in some of the studies. Both the Riviere et al. [72] and the Poole et al. [74] studies lacked information concerning the periodontal status of the cases analyzed.

Future Research

How PD contributes to impaired memory remains intriguing and future studies should be directed towards addressing these mechanisms. There is paucity of information relating to the exact risk factor(s) for the development of deteriorating memory from missing teeth in the prodromal phase of AD. Are these factors downstream of co-morbidities such as diabetes affecting periodontal status of the individual? Or common susceptibility profiles play a key role in loss or gain of function in future generations of AD parents. Further evidence to support an association between PD and AD, research would need to demonstrate that the inflammatory and immunological responses that the bacteria and their virulence factors induce may subsequently lead to the onset of AD. Perhaps associations between parents with AD and their children at a much younger age should be monitored for oral health as that should eliminate any overlapping confounders that may be masking the true links in these two conditions.

This review clearly indicates significant gaps in our current understanding of the causal association of pathogens (spirochetes, bacteria, viruses) in a slowly progressive and debilitating disease such as AD. Although various types of neurotropic spirochetes including oral (*Treponema* spp) and non-oral spirochetes (*B. burgdorferi*) have been detected directly in association with the pathological hallmarks of AD [73] but, a causal relationship has not been tested in animal models. To the best of our knowledge, there is no single *in vivo* animal model study that has examined the role of periodontal bacteria during chronic infection (9-12 months of exposure times) to study the sequence of neuropathological events associated with the development of AD pathology. Even though infectious agents have been implicated in relation to AD hallmark pathology for over a century ago, several clinical and molecular studies strongly support an association between PD and AD. However, to date, there is a paucity of reports

supporting a causative relationship between periodontal pathogens and AD cases and *in vivo* transgenic mice.

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Legends

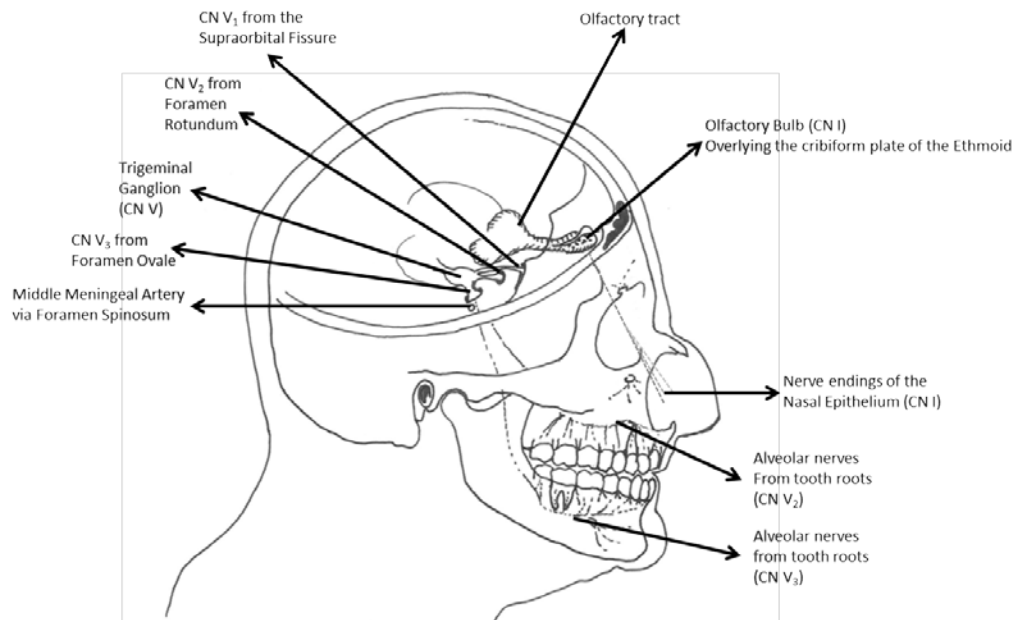


Figure 1. Nerve pathways from the oral and nasal cavity to the brain, showing the 2nd and 3rd branches of the Trigeminal (CN V) and the Olfactory nerve (CN I). The middle meningeal artery enters the brain at the foramen spinosum in the lateral portion of the greater wing of the sphenoid, and then follows the cranial base and lateral portions of the vault, supplying the dura and bones of the calvarium.