

## Hypothesis

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# Can *Porphyromonas gingivalis* Contribute to Alzheimer's Disease Already at the Stage of Gingivitis?

Ingar Olsen\*

Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway

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**Abstract.** Alzheimer's disease (AD) has been associated with periodontitis, which starts as gingivitis. Similar to periodontitis, gingivitis bacteria, bacterial products, and inflammatory mediators can travel to the brain via the blood stream and promote brain inflammation. Periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, both associated with AD, have been found in dental plaque of children already at the age of 3. It is suggested that these bacteria during long-term exposure may drive microglia (brain resident macrophage cells) into a pro-inflammatory M1 phase where they contribute to AD rather than protect against it. This notion comes from studies in mice showing that microglia actually can “remember” previous inflammatory challenge and become “trained” or “tolerant” to toxins like lipopolysaccharide. If gingivitis has an impact on AD, which should be verified, AD prophylaxis should start already at this pre-periodontitis stage with removal of supragingival plaque.

Keywords: Bacteria, bacteremia, inflammation, microglia, periodontitis, systemic

## GINGIVITIS

Periodontitis starts as gingivitis. While some cases of gingivitis never progress to periodontitis, periodontitis is always preceded by gingivitis. Gingivitis is a non-destructive disease that causes inflammation of the gingiva. The most common form of gingivitis occurs in response to supragingival bacterial biofilms (plaques) accumulating on tooth surfaces and gingiva. According to Stamm [1], there is a general consensus that marginal gingivitis begins in early childhood, increases in prevalence and severity

to the early teenage years, thereafter subsiding slightly and leveling off for the remainder of the second decade of life. The general prevalence of adult gingivitis varies from approximately 50 to 100% for dentate subjects [1].

What is generally overseen is that subjects with gingivitis can also experience intermittent bacteremias. Since the clinical diagnostic features of gingivitis, i.e., redness, swelling, and bleeding of the gingiva are based on vascular changes [2], a bacteremia can easily occur. Indeed, dental plaque accumulation and gingival inflammation significantly increased the prevalence of bacteremia following tooth brushing [3]. Of note, periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* have been detected already in 3-year-old children. The colonization of

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\*Correspondence to: Ingar Olsen, Department of Oral Biology, Faculty of Dentistry, University of Oslo, P.O. Box 1052 Blindern, 0316 Oslo, Norway. Tel.: +47 90777482; E-mail: ingar.olsen@odont.uio.no.

these bacteria was associated with the onset and severity of gingivitis [4]. Interestingly, not only *P. gingivalis*, but also *A. actinomycetemcomitans*, has been associated with Alzheimer's disease (AD) [5]. Besides, *Actinomycetales* and *Prevotella* (bacterial markers of gingivitis) have been detected in AD autopsy brains together with periodontal bacteria by high throughput sequencing [6].

In a study profiling the local and systemic inflammatory responses to experimentally induced gingivitis in eight females, it was demonstrated after 21 days that gingivitis could be added to the systemic inflammatory burden of an individual since dental plaque accumulation caused a significant systemic sICAM response [7]. There were no changes in the levels of cytokines in the gingival crevicular fluid (GCF) (exudate secreted by the crevices located at the point where the gingival line meets the teeth), although individual variations in the cytokine levels were observed. This agreed with the mild nature of gingivitis and its short duration of endotoxemia. The study was supported by Alzahrani et al. [8] who found a significant reduction in the mean CRP and TNF- $\alpha$  levels after treatment of severe gingivitis, but not after mild gingivitis in otherwise systemically healthy individuals. In another study, experimental gingivitis in humans caused subtle degrees of complement activation [9]. Furthermore, when subjects with good oral hygiene ceased tooth brushing, the release of IL-1b increased in the GCF with increasing plaque accumulation and exacerbation of gingival inflammation [10, 11]. It cannot be excluded that plaque bacteria, bacterial products, and inflammatory mediators from gingivitis established early in life provoke pro-inflammatory innate responses that weaken the blood-brain barrier (BBB), allowing these products to spread and quietly promote the pathogenesis of AD. Cytokines, chemokines, signal transduction molecules, and lipopolysaccharide (LPS) are implicated in BBB dysfunction in response to systemic inflammation [12], and a global BBB leakage has been demonstrated in patients with early AD associated with cognitive decline [13].

## PERIODONTITIS

Periodontitis is an infectious disease damaging soft tissue and bone that support the teeth. If not treated, periodontitis can cause teeth to loosen and lead to tooth loss. Periodontitis is very common in the adult population [14], all over the world. Usually, it is the

result of poor dental hygiene where specific bacterial and viral pathogens [15] have been allowed to accumulate in subgingival plaque.

Although this biofilm can contain a multitude of different bacteria, the keystone bacterium in "chronic" periodontitis is believed to be *P. gingivalis* [16–18]. Another bacterium of particular importance is *A. actinomycetemcomitans* that has been associated with rapid periodontal destruction in juveniles. Careful tooth brushing, daily flossing, and regular dental checkups can greatly improve the chances of successful periodontitis treatment.

## RELATIONSHIP BETWEEN PERIODONTITIS AND ALZHEIMER'S DISEASE

A number of studies have reported an association between periodontitis and AD [19–28]. Several reports have suggested how virulence factors of *P. gingivalis* can contribute to AD, particularly LPS, gingipains, PPAD (*P. gingivalis* peptidyl-arginine deiminase), Mfa1 fimbrial protein, BCAT (branched-chain amino acid aminotransferase), and capsular polysaccharides [29–34]. *P. gingivalis* and its gingipains were detected in the brains of AD patients [22]. Noteworthy was also the observation that small-molecule gingipain inhibitors blocked gingipain-induced neurodegeneration in the mouse brain. Currently, clinical studies are going on to see if this beneficial effect can be reproduced in AD patients.

In periodontitis, subgingival plaque bacteria, bacterial products, and inflammatory mediators travel to the brain through the blood stream and cause brain inflammation [35, 36]. Bacteremia can occur each time the patient brushes his/her teeth, chews, flosses, or receives treatment by a dentist or dental hygienist [37]. It has been estimated that a patient with periodontitis experiences several occasions of bacteremia each day lasting for a total up to 3 hours [3]. Furthermore, a patient with periodontitis can have a wound bed estimated to cover a surface area of 8–20 cm<sup>2</sup> [34] from which bacteria have easy access to the blood stream [20]. Bacteria from periodontitis can also be transferred to the brain via other routes such as the trigeminal nerve, circumventricular organs, perivascular spaces, and by olfactory unsheathing cells acting as Trojan horses [20].

Inflammation is increasingly being considered as a hallmark contributor to AD development and

exacerbation [38]. Pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 are increased in the brains of persons with AD. This initiate accumulation of amyloid- $\beta$  (A $\beta$ ) plaques and tau hyperphosphorylation (hallmarks of AD) which cause loss of neurons [39]. Chronic inflammation is considered as one of the most important factors in amyloid diseases [40]. Brain inflammation is also mediated by microglia and astrocytes (brain resident macrophages) which express inflammatory mediators after having become activated by bacteria and LPS. Increasing evidence implicates sustained glial-mediated inflammation as a major contributor to the neurodegenerative processes and cognitive deficits in AD [41].

### **TIME DELAY BEFORE PERIODONTITIS PROMOTES ALZHEIMER'S DISEASE DEVELOPMENT**

It has been suggested that it may take 10–20 years before periodontitis promotes manifest AD development [42–44]. Tzeng et al. [45] examining 2,207 patients with gingivitis and “chronic” periodontitis found after adjusting for sex, age, monthly income, urbanization level, geographic region, and comorbidities, that the hazard ratio for dementia was 2.54 (95% CI 1.297–3.352,  $p=0.002$ ). An interval of 10–20 years could also be the time it takes for A $\beta$  to reach a plateau sufficient to induce mild cognitive impairment [27]. A possible time delay for gingivitis in this respect has not been considered. Interestingly, studies on the effect of gingival inflammation on stroke showed that not only extended periodontal disease but even gingivitis might pose a threat [46].

### **AMYLOID- $\beta$ OCCURS IN THE BRAIN OF BOTH HEALTHY AND AD PEOPLE**

Accumulations of A $\beta$  can be seen in the brains of healthy old people without AD. Therefore, the presence of A $\beta$  in the brains of cognitively healthy older people has been interpreted as an indication against a causative role of A $\beta$  in AD [47]. It was held that if A $\beta$  is crucial to the development of AD, it should be associated with other AD-like neurological changes. There is increasing concern that A $\beta$  accumulation in AD could be caused by inflammatory processes. Over many years *P. gingivalis* may spread slowly from neuron to neuron along anatomically connected pathways causing brain inflammation. Therefore, brain inflammation due to *P. gingivalis* could be an early

event that explains the pathology found in middle-aged individuals before cognitive decline appears [22]. It is also likely that *P. gingivalis* contributes to intracerebral and systemic A $\beta$  production [33, 48].

Data published during the last 6 years by several groups have revealed that A $\beta$  peptides are antimicrobial in *in vitro* assays against some common and clinically relevant microorganisms (reviewed by [49]). If occurring also *in vivo* this may imply that A $\beta$  detected in persons with preclinical AD could have been initiated by early exposure to *P. gingivalis*.

It is also possible that A $\beta$  in healthy older people gradually contributes to neuroinflammation and neurodegeneration when microglia activation develops as a protective response to *P. gingivalis* [50]. Microglia have a dual role in the pathogenesis of AD. On one hand, they can play a beneficial role in generating anti-A $\beta$  antibodies and stimulating clearance of amyloid plaques [51]. On the other hand, microglia can release inflammatory mediators such as inflammatory cytokines, complement components, chemokines, and free radicals that are all known to contribute to A $\beta$  production and accumulation. Activation of microglia in the central nervous system involves two opposing phenotypes, M1 and M2. Depending on the trigger of activation, M1 can exert cytotoxic (pro-inflammatory cytokine release) and M2 neuroprotective (immune resolution) effects [52]. The chronic nature of low-level infections such as gingivitis/periodontitis and associated by products, e.g., endo/exotoxins and cytokines could affect the capacity of susceptible brains' defense to a point where microglia, “remembering” previous *P. gingivalis* exposure, turn into the M1 phase [50, 53].

### **CONCLUDING REMARKS**

It is intriguing that periodontitis should take 10–20 years to promote AD. It must be realized, however, that periodontitis is a chronic, low-grade infection releasing intermittent bacteremias. It seems plausible that the brain inflammation of AD could start already with the development of gingivitis, at least with generalized severe gingivitis, although the abundance of *P. gingivalis* in gingivitis may be low. Typical of *P. gingivalis*, though, is that it has dysbiotic effects even at low concentrations. Bacteria, bacterial products (e.g., toxins and proteolytic enzymes), and inflammatory mediators from gingivitis could reach the blood stream before those of succeeding periodontitis. Microglia can “remember” previous

inflammatory challenge and become “trained” or “tolerant” to toxins like LPS and thus have become sensitized to *P. gingivalis* during later exposure to this bacterium. This could bring microglia into a pro-inflammatory phase where they might promote AD rather than protect against it. If gingivitis has an impact on AD, prophylaxis of AD should start already at this pre-periodontitis stage by removal of supragingival plaque. The possible impact of gingivitis on dementia, however, needs to be studied.

It should be emphasized that *P. gingivalis* is not the sole organism that may be associated with AD. Even bacteria from the gastrointestinal tract, virus, and yeasts may be involved. Therefore, *P. gingivalis* in this context should be thought of as a model oral pathogen with possible effect on AD pathogenesis.

## CONFLICT OF INTEREST

The author has no conflict of interest to report.

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