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DE CIÊNCIAS DA SAÚDE

Association of *Porphyromonas gingivalis*, a major periodontopathic bacteria, in patients with Alzheimer's disease

Caroline Blanc

Dissertação conducente ao Grau de Mestre em
Medicina Dentária (Ciclo Integrado)

Gandra, 23 de maio de 2020

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Trabalho realizado sob a Orientação de "Prof Doutora Marta Relvas"

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O Orientador

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RESUMO

O objetivo desta dissertação é fazer uma revisão integrativa sistemática da literatura publicada para perceber a associação entre *Porphyromonas gingivalis* (*P. gingivalis*) e a doença de Alzheimer. Foi realizada uma pesquisa na PUBMED e PUBMED CENTRAL. Dos 79 artigos encontrados, apenas 12 foram considerados por ser mais relevantes. A bactéria *P. gingivalis* foi identificada no cérebro de pacientes com doença de Alzheimer: componentes tóxicos (como gengipinas e lipopolissacarideo) da *P. gingivalis* foram identificados e propostos como causa da produção de placa amilóide e inflamação no cérebro. Esta bactéria, fundamental para a periodontite, tem a capacidade de invadir a corrente sanguínea e de se propagar das bolsas periodontais até ao cérebro e causar inflamação, participando assim no desenvolvimento da doença de Alzheimer. A *P. gingivalis* pode ser um alvo terapêutico para os doentes com doença de Alzheimer, podendo assim, reduzir a incidência e a gravidade da patologia. A sua inibição ou a prevenção da dissiminação da *P. gingivalis* desde as bolsas periodontais até ao cérebro poderia ser benéfica para os doentes com doença de Alzheimer.

Palavras-chave: "*Porphyromonas gingivalis*" ; "doença de Alzheimer" ; "demência vascular" ; "periodontite".

ABSTRACT

The aim of this dissertation is to make a systematic integrative review of published literature to assess possible association between *Porphyromonas gingivalis (P. gingivalis)* and Alzheimer's disease. A research was realized on PUBMED and PUBMED CENTRAL. On 79 articles found, only 12 were of interest between 2008 and 2020. This bacterium, fundamental to periodontitis, has the ability by the bloodstream to spread from periodontal pockets to the brain and cause inflammation, thus participating in the development of Alzheimer's disease. Part of *P. gingivalis* have been identified in the brain of patients with Alzheimer's disease: its toxic components (as gingipains and lipopolysaccharides) have been identified and proposed as cause of production of amyloid plaque and abnormal protein tau, source of neuroinflammation and neurodegeneration. *P.gingivalis* could be a therapeutic target for patients with Alzheimer's disease and could thus reduce the incidence and severity of the pathology. Prevention (dental health care) of *P.gingivalis* dissemination from site of periodontitis to the brain and inhibition (recent therapeutic approach) of its neurotoxicity could be beneficial for patients with Alzheimer's disease.

Key-words: "*Porphyromonas gingivalis*"; "Alzheimer's disease"; "vascular dementia"; "periodontitis"

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ABREVIATURAS

P.gingivalis – *Porphyromonas gingivalis*

LPS – Lipopolysaccharide

Aβ – Amyloid β

NTF - intraneural neurofibrillary tangle

CSF - Cerebrospinal fluid

IgG – Immunoglobulin G

BBB - Blood-brain barrier

CNS - central nervous system

Kgp - lysine gingipain

Rgp - arginine gingipain

CatB - Cathepsin B protease

TLR – Tool-like receptor

PAR2 - Protease activated receptor 2

PAMPs – pathogen-associated molecular pattern

I. INTRODUCTION

Alzheimer's disease is the most common cause of dementia in elder population (1,2). Due to ever-increasing life expectancy, Alzheimer's disease is a great public concern. This disease is characterised by a brain disorder which can alter the memory and cognitive function (3). It can be an early form or a sporadic form, which is the case in 95% of Alzheimer's disease patients (4,5). Inflammation is detected in disease's brain through presence of pro-inflammatory cytokines such as IL1-β, TNF-α and IL-6. Two hallmark proteins are representative of disease and present in the brain of Alzheimer's disease: amyloid-beta (Aβ) plaque and hyperphosphorylated Tau (2,6,7). Exact onset of Alzheimer disease, except for age-dependent risk, is not entirely known but in recent years, theory of bacterial infection playing a role in the progression of the disease has been put forward. Peripheral inflammation is largely considered to contribute to pathogenesis of Alzheimer's disease, as it can increase brain inflammation (8). In the past few years, a link between periodontitis and Alzheimer's disease was established by a growing numbers of clinical studies (9–11). But one question remains to be answered: how can the periodontitis be implicated in the Alzheimer's disease?

Periodontitis, an oral multi-bacterial disease, is a periodontal low-grade chronic inflammation (10). This periodontal disease is presumed to aggravate systemic inflammation (5) and is caused by a dysbiosis with some oral bacteria, one of them being *Porphyromonas gingivalis (P. gingivalis)*. Therefore this review is focused on a major periodontal pathogen *P. gingivalis* which have been proposed, as risk factor, to contribute in development of Alzheimer's disease (1,5,12,13). His dissemination from area of periodontal inflammation to systemic circulation can be induced by common task, such as brushing, flossing and masticate, as well as dental procedure like scaling or teeth extraction (11,13,14). *P. gingivalis* can lead to important systemic alteration and is implicated in onset of different systemic pathologies, such as cardiovascular pathologies, rheumatoid arthritis and neurodegenerative pathologies (15). The main hypothesis leading this review is that there exist an association between *P. gingivalis* and Alzheimer's disease. Understanding possible *P. gingivalis* ways of action on brain could shed new light on oral management of Alzheimer's patients.

II. METHODS

PICO model was used: Patient (with Alzheimer's disease), Intervention (positive to the periodontal bacteria*, P. gingivalis*), Control (patient without the Alzheimer disease) and Outcome (association of *P. gingivalis* in patients with Alzheimer's disease). The built question is the follow: is *P. gingivalis*, a periodontal bacterium, associated with Alzheimer's disease? Methods has been inspired from PRISMA protocol by its use of a flow diagram.

1. Research methodology

A bibliographic search was performed on electronic platform via PUBMED. Different combinations of Keys words were used: "*Porphyromonas gingivalis*" AND ("Alzheimer's disease" OR "vascular dementia") AND ("periodontite" OR "periodontitis")

2. Eligibility criteria

Several kinds of publication were taken in consideration: meta-analyzes, experimental studies, study cases, clinicals trials, *in vitro* and *vivo* experimentations and integrative/systematic reviews. Only articles dealing with the role of *P. gingivalis* in association with Alzheimer's disease and published, de January 2008 until Abril 2020, in English or Portuguese were selected. Election of article was focus on the last 20 years, because there was no researcher interested in this topic before. Only the ones published in full and in electronical form were elected. Title, resume and abstract were analyzed in order to assess relevance of each articles. Then potentially eligible articles were examinated by a full text analyzing to check utility for our study.

Inclusion and exclusion criteria were the following:

- Studies explaining association of *P. gingivalis* in the Alzheimer's diseases were included
- Studies describing influence of *P. gingivalis* on the onset of the Alzheimer's disease were included
- Studies conducted on human and mice were included
- Article not written in English or Portuguese, before January 2008 were excluded
- Finally, literature reviews were excluded for results

3. Screening methodology

After identification of eligibly articles through several databases and platforms, selection of relevant articles was performed in order to investigate influence of *P. gingivalis*.

Total of articles found was compiled in Mendeley citation manager, where duplicates were removes. Evaluation of each publication in its entirety was realized to elect accurate publication which will meet the purpose of the study. Screening included analyze of title and abstract in order to remove non-relevant article for this review. After screening phase, all articles were read in full-length, to assess eligibility for qualitative analysis. Different factors were chosen to evaluate articles selected: name, authors, years, country, type of study, sample size, part tested, technic used, result and conclusion.

Elected articles needed to respond to the outcome of this study which is association between *P. gingivalis* and Alzheimer's disease.

III. RESULTS

A total of 79 articles were identified on the PubMed database, between 2008 and 2020. With application of study eligibility criteria (studies about relation of *P. gingivalis*, major pathogen of periodontitis with Alzheimer's disease), 32 articles were taken into consideration. After screening and application of inclusion and exclusion criteria, 12 articles were included for qualitative analysis. Studies identified but not selected were used later to deepen and extend knowledge on this review subject matter and to enrich discussion. All the procedure is explained in a flow chart procedure (Figure 1). On 12 studies selected, 4 were conducted on humans, 7 were conducted on mice and 1 on both.

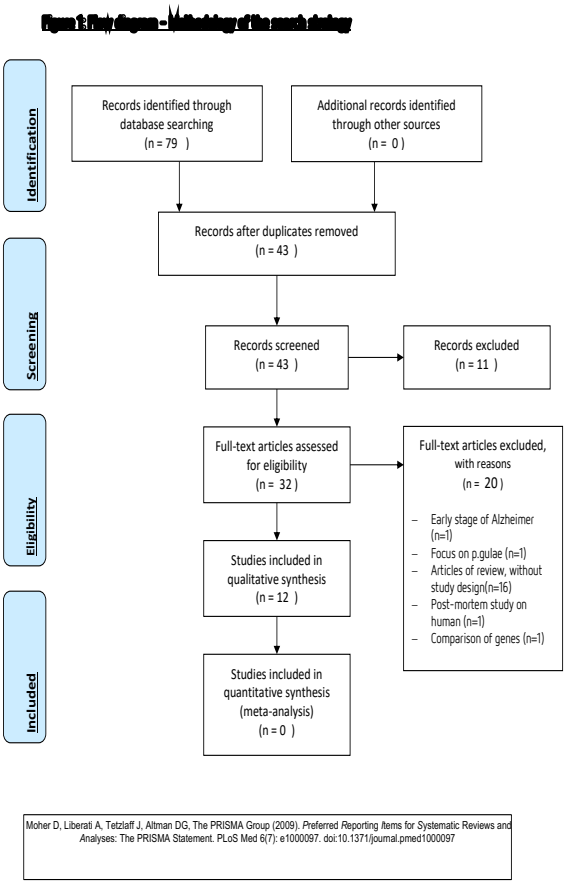


Figure 1

Extracted data were compiled in tables: authors, years, ways of *P. gingivalis* inoculation, sample size, technic used for evaluation, result and conclusion in Table 1 and authors, years, study design, sample size and tested anatomic structure, used technic, result and conclusion for Table 2. Results were collected and organized in following section:

1. Effect of *P. gingivalis* on mice

In 50% of surveys, *P. gingivalis* or his products lipopolysaccharide-pg (LPS-pg) and gingipains (trypsin-like cysteine proteinases produced by *P. gingivalis*) were found in the brain tissue of mice after oral inoculation of *P. gingivalis* or induction of periodontitis (3,13,16,17) . Three studies demonstrate that expression levels of pro-inflammatory

cytokines TNFα, IL-6 and IL-1ß in the brain tissues of mice infected orally with *P. gingivalis* were increased in comparison with experimental groups (3,16,18). A study conducted on the liver of mice also proved that infection with *P. gingivalis* promote the rise of IL-1β laud in liver macrophages of *P. gingivalis* contaminated mice (19).

In 62,5% of the researches on mice, infection by *P. gingivalis* also induced increase of amyloid β in the brain (3,13,16,19,20). In one study, Dominy et al colocalized hyperphosphoryled Tau protein with gingipain and showed that Tau was a target of gingipain protelolysis capacity (13).

Mice's behavior was evaluated in four trials: 3 of the studies pointed a cognitive function impaired in the *P. gingivalis* infected mice (3,9,18). But again all expectations, one study showed that continuous brain exposure of Pg-LPS didn't enhance cognitive impairment in Alzheimers's disease model mice (12).

Table 1.1: Relevant data gathered from the selected studies on mice

Authors [YEARS]	Country	Via of *p.gingivalis* contamination	Sample size	Part tested	Tecnic used for evaluation	Result	Conclusion
S. Dominiy et al. [2019] (13)	USA	Experimental periodontitis was induced by ligature placement.	40 43- to 44-week-old female or 100 8-week-old female	Brain tissue (hippocampus)	ELISA PCR	• Higher number of degenerating neurons were found in mice inoculated with gingipain than mice injected with saline • Oral *P. gingivalis* infection in mice lead to brain colonization and higher production of $A\beta_{1-42}$ • Gingipains are neurotoxic in vivo and in vitro, providing detrimental effects on protein tau, needed for normal neuronal function	• *P. gingivalis* and gingipains in the brain have a primordial role in the pathogenesis of Alzheimer's disease • *P. gingivalis* DNA and gingipain antibodies were found in AD brains • Small-molecule gingipain inhibitors administrally orally blocks neurodegeneration induced by gingipains, lead to *P. gingivalis* nivel in mice brain, and significantly decreases the host $A\beta_{1-42}$ response to *P. gingivalis* brain infection.
Kenyu Hayaski [2019](12)	Japan	Intra-cerebro-ventricular (ICV) injection of Pg-LPS	19 mice: 6 young and 13 middle age	Entire brain (injection ICV)	Y-maze and Morris water maze test Immuno-histochemical	• Acute and continuous brain exposure of Pg-LPS didn't lead to positive findings on AD model mice • Mice with vehicule or Pg-LPS did not show significant difference of Y-maze and Morris water maze tests • Pg-LPS induced cardiac atrophy in both young and middle- aged mice	• Continuous brain exposure of Pg-LPS initiated sarcopenias and cardiac injury without intensify cognitive impairment in Alzheimer's disease model mice • Continous brain exposure pf pg-LPS is not deleterious in healthy individual but can deteriorate prognosis od AD patients • Acute brain exposure of pg-LPS has little positive effect in AD patients
Ye Ding [2019](18)	China	Oral inoculation of *P. gingivalis* using feeding needles (0.1 ml of *P. gingivalis* in 2% carboxy-methyl cellulose	60 mices: 30 aged of 4 week and 30 twelve-month-old.	Behavior Brain tissues (cerebral cortex)	Morris water maze qRT-PCR ELISA Immuno-histochemistry	• Learning and memory abilities of the middle-aged *P.gingivalis* infected mice were decreased • Laud of the pro-inflammatory cytokines TNF α, IL-6 and IL-1ß in the brain tissues of middle-aged mice *P.gingivalis* infected mice were higher.	*P.gingivalis* periodontal infection could lead to cognitive impairment via the increase of the pro-inflammatory cytokines TNF- α, IL-6 and IL-1ß in cerebral tissues of middle-aged mice

Table 1.2 (cont.)

Authors [YEARS]	Country	Via of *P.gingivalis* contamination	Sample size	Part tested	Tecnic used for evaluation	Result	Conclusion
Ran Nie [2019](19)	China	Injection of *P.gingivalis* intra-peritoneally	20 females mice aged of 12 months	Liver	PCR Immuno-fluorescence Western blotting	• Chronic systemic *P. gingivalis* infection induces a quantity-independent increase in TLR2 and IL-1β levels in liver macrophages of middle-aged mice • Chronic systemic *P.gingivalis* infection increases the Aß PP, CatB and Aβ production in liver macrophages of middle-aged mice	• Chronic systemic *P. gingivalis* infection caused the Aβ deposit in inflammatory monocytes/macrophages via the stimulation of CatB/NF-κB signaling, implying that monocytes/macrophages serve as mean of transport of Aβ in patients with periodontitis. • CatB might be a n new therapeutic target for preventing the periodontitis-related Alzheimer disease onset and development
Vladimir Ilievski [2018](16)	USA	100 µl of Pg in CMC containing 10^9 Pg was applied (2 applications of 50 µl 3x/week) in the oral cavity	20 mice aged of 8 weeks	Hippocampus	Immuno-fluorescence microsocpy Confocal microscopy Quantitative PCR	• Pg/gingipains was detected in the hippocampi of mice in the experimental group, localized intra-nuclearly, peri-nuclearly and extracellularly • Significant higher level of IL6, TNF α and IL1 β in the experimental group • Greater number of degenerations of neuron in the experimental group • Phosphoryled Tau protein was detected in experimental group	• After several *P. gingivalis* oral infection, neurodegeneration and formation of extra-cellular $A\beta_{42}$ was found • Chronic oral infection of Pg can be an initiator of the development of Alzheimer's disease
Naoyuki Ishida [2017](3)	Japan	Experimental periodontitis by inoculation of P. ginigivalis mixed with carboxy-methyl cellulose, which was delivered orally	Transgenic mice model	Behavior Brain tissues (hippocampus, cortex)	The novel objection test ELISA	• Cognitive function was significantly impaired in periodontitis-induced mice • Levels of Aβ, Aβ40, and Aβ42 accumulation in the hippocampus and cortex were increased in *P. gingivalis* infected APP-Tg mice • Brain levels of IL-1β and TNF-α were increased in infected mice	Periodontitis induced by *P. gingivalis* may intensify brain Aβ accumulation, causing cognitive impairments, by a mechanism that engage neuroinflammation.

Table 1.3 (cont.)

Authors [YEARS]	Country	Via of *P.gingivalis* contamination	Sample size	Part tested	Tecnic used for evaluation	Result	Conclusion
						• LPS Level were higher in serum and brain of *P. gingivalis*-infected mice. • *P. gingivalis* LPS lead to production Aβ in neural cell cultures and augmentation of TNF-α and IL-1β production in a culture of microglial cells primed with Aβ.	.
Zhou Wu [2017](1)	Japan	systemic exposure to PgLPSI daily (1 mg/kg/day, intraperi-toneally; for 5 week	18 mice: 6 adults, 6 middle-aged and 6 Cat-/-	Hippocampus	Passive avoidance test Locomotor activity RT-PCR Immuno-blotting Immuno-fluorescence ELISA	Chronic systemic exposure to PgLPS: • Leads to CatB-dependent learning and memory impairments mice of middle-age • induces CatB production in neuron and microglia in the middle-aged mice • increases CatB-dependent IL-1β synthesis in microglia in mice of middle-age CatB-dependent TLR2 and TLR4 are increased in microglia in the middle-aged mice • induces CatB-dependent Aβ deposition in neurons in mice of middle-age	• Chronic systemic exposure to PgLPS lead to Alzheimer's disease -like features, as learning and memory impairment, microglia-mediated neuroinflammation and A β deposition, in mice of middle-age • These observations strongly imply that CatB plays a primordial role in the link between periodontitis and Alzheimer's disease. • CatB may be use as therapeutic tool for preventing periodontitis-associated cognitive decline in Alzheimer's disease.
Poole [2014](17)	UK	oral infection of ApoE−/− mice with periodontal pathogens for a chronic infection period of 24 week	12 mices	Brain tissues (hippocampus, dentate gyrus, lateral ventricle))	PCR Immuno-blotting	• Oral infection to *P. gingivalis* in ApoE−/− mice was able to enter the brain • At 12 weeks, 6 out of 12 ApoE−/− mice brains presented *P. gingivalis* DNA	• Complement cascade, activated in response to *P. gingivalis* brain infection supports the hypothesis that chronic local inflammation plays a role in development of Alzheimer's disease • *P. gingivalis* in the brain induced damage complement mediated in absence of Aβ deposition

2. Effect of *P. gingivalis* on humans

As shown on Table 2, five studies with human subjects were compared in this survey. In a prospective pilot study performed by Dominy et al (13) , authors took samples of cerebrospinal fluid (CSF) in patients with clinical Alzheimer's disease and collected matching saliva of each patient. All of the 10-saliva samples were positive to *P. gingivalis*

and 7 of the 10 samples of CSF were significatively positive with *P. gingivalis*. In the most recent study performed on human, authors found a possible association between *P. gingivalis* and Alzheimer's disease: 5 of 7 patients with Alzheimer's disease and periodontitis stage III or IV presented strain of periodontal bacteria *P. gingivalis*. These positives to *P. gingivalis* patients manifested lower score (with p<0,05) in cognitive test (5).

Three studies (75% of researches conducted on human) had concerned themselves with levels of *P. gingivalis* in serum IgG antibody (21–23). They found that periodontitis pathogen, *P. gingivalis*, is linked to poor cognitive performance. Subjects with a higher load of *P. gingivalis* IgG were more likely to have a bad delayed verbal memory and impaired subtraction, with apparent dose-response (the more *P. gingivalis* in IgG, the poorer cognitive performance) (22). Stein et al (23) found out that antibodies level to *P. gingivalis* were elevated in Alzheimer's disease patients (same level before and after onset of Alzheimer's disease): 39 µg/ml against 22 in control patients (without cognitive disease). Antibodies high level in cohort of patients with Alzheimer's disease were similar to serum antibodies level in population of patients with clinical periodontitis: 43,1 µg/ml±6,4 in comparison with healthy patients: 8,2 µg/ml ±0,9.

Table 2: Relevant data gathered from retrieved studies on humans

Authors [YEARS]	Country	Study	Sample size	Part tested	Technique used	Result	Conclusion
Friedrich Leblhuber(5) [2020]	Austria	Exploratory pilot study	20 AD patients	Alveolar fluid to periodontal bacteria	RNA-based analysis	In 5 out 7 patients with periodontitis, a strain of *P. gingivalis* was found Association between the salivary presence of *P. gingivalis* and lower score in cognitive test was found	Possible association between the most virulent strain of periodontitis, *P. gingivalis*, and the Alzheimer's disease
Stephen S. Dominy [2019](13)	USA	Prospective pilot study	10 AD patients	Matched saliva and CSF samples were taken and analyzed for *P. gingivalis* DNA	PCR	All 10 patients were positive to *P.gingivalis*	Evidence *for P. gingivalis* infection in the brain of Alzheimer's disease patients is provided by CSF result
James N. Noble [2014](21)	USA	Longitudinal and case-cohort study	219 individuals (110 Alzheimer's disease cases and 109 controls without cognitive impairment)	Serum IgG antibody levels to periodontal bacteria: (*P. gingivalis),*	checkerboard immune-blotting	High antibody levels to *P.gingivalis* were found in 23% of subjects	Serum IgG levels to periodontal bacterium are associated with an increasing risk for developing
Pamela Sparks Spein [2012](23)	USA	Retrospective study and case-control study	158 subjects	IgG antibody levels to 7 oral bacteria were analyzed: (*P. gingivalis*)	ELISA	At baseline, similar levels of antibodies to *P. gingivalis* were found between patients with periodontitis and patients with Alzheimer's disease	Oral bacteria associated with periodontitis induce high host responses in patients with Alzheimer's disease
James N. Noble [2009](22)	USA	Cross sectional observational study	2355 participants	Serum *P. gingivalis* IgG	ELISA Three cognitive tests	Mean *P.gingivalis* IgG was higher among those with impaired performance for the 3 cognitive tests	Patients with high levels of *P. gingivalis* IgG significantly tends more to impaired cognitive function Significant dose–response relation between increasing *P. gingivalis* IgG and subtraction test performance

IV. DISCUSSION

1. *P. gingivalis* neurotoxicity

P. gingivalis is a key bacterium of periodontitis, asaccharolytic gram-negative anaerobe. It survives in periodontal pockets, major sign of this periodontal pathology (15,24,25). *P. gingivalis* is part of the red Socransky complex of biofilm's bacteria (10,25). Some systemic pathologies might be associated with *P. gingivalis*, such as cardiovascular disease, degeneration neuronal, diabete, arthritis (12,15,24). *P. gingivalis* was found in brain tissue and in CSF (13), providing definitive evidence of its migration from oral cavity to the brain (17,26). The mechanism of *P. gingivalis* from a peripheral infection (periodontitis) until the brain is a complex process, which involves several pathways: stimulation of peripheral nerves and blood dissemination or by action of LPS (lipopolysaccharide) in site lacking blood-brain barrier (BBB) as in circumventricular organs, either direct action of LPS from systemic circulation on vascular cell of BBB (1,8,12). Once past the BBB (5), *P. gingivalis* is going to induce neuroinflammation through different means, which is going to participate to pathogenesis of neurodegenerative disease as Alzheimer's disease (1,12).

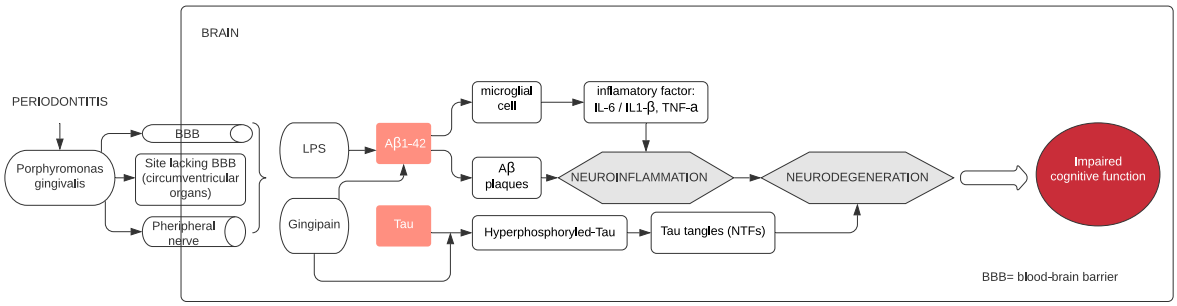
P. gingivalis have major toxic virulence factors localized in its outer membrane, such as gingipain, LPS and fimbria (12–14,25). These toxins induce inflammation by promoting production of pro-inflammatory cytokines (3). Gingipains, which are cysteine proteases, are highly potent modulators of host colonization, inactivation of host defenses and tissues destruction (13). Gingipain have been found in brain of young mice after oral infection at *P. gingivalis* (16) and in brain of individuals with Alzheimer's disease (13). Two types of gingipains are of interest: lysine gingipain (or Kgp) and arginine gingipain (or Rgp). These gingipains contribute, after infiltration in the brain, to cell migration of microglia towards infected site and lead to neuroinflammation. Gingipains activates PAR2, a cell-receptor activated by protease, which plays a role in inflammation. Kgp and Rgp may increase vascular permeability by degrading component of basal membrane and endothelial cell tight junction proteins and so contribute to loss of blood-brain barrier functional integrity (20,27,28).

LPS, components of virulence on *P. gingivalis* outer membrane, also induce increase of pro-inflammatory cytokines, activating CNS innate immune system and therefore contributing to neuroinflammation and neurodegeneration in Alzheimer's disease (3,4) (Figure 2). LPS, using pathogens associated molecular patterns (PAMPs), can activate Toll-like receptor (TLR) present in surface of glia cells, such as microglia. This activation LPS/TLR lead to an antibacterial response (1,4). In a post-mortem study, *p.g*-LPS was identified in Alzheimer's disease brain but not in control case without cognitive troubles (26). *P.g*-LPS were also found in mouse brains and serum after inoculation of *P.gingivalis* in order to create experimental periodontal disease (3), supporting the theory that *P. gingivalis* may play a role in Alzheimer's disease.

Moreover, *p.g*- LPS and gingipains can suppress deposition of opsonons (IgG, C3b, C5b-9) on the bacterial cell surface. Blocking C3, directly implicated in elimination of pathogens, and on which all complement pathway converges, could allow for infection to take hold (6).

However, one study did not show a positive association between an injection of LPS and onset of Alzheimer's disease in healthy individual. This outcome could be due to too short exposure of p.g-LPS. It was concluded, however, that a continuous injection of LPS may worsen the prognosis of a patient already suffering from Alzheimer's disease (12)

Figure 2: Model for *P. gingivalis* contribution to Alzheimer's disease



2. Pathogenesis of Alzheimer's Disease due to *P. gingivalis*

In one of the first studies conducted in 2009, authors concluded that patients with a concentration of IgG 's *P. gingivalis* in serum had poorer verbal memory and subtraction performance test scores than individuals without *P. gingivalis* (22). A longitudinal study confirmed the previous one by identifying an association between serological markers of

periodontitis (of which *P. gingivalis* is a part) and Alzheimer's disease, via the study of IgG antibody serum (21).

The patients studied by Sparks Spein et al expressed, before the clinical diagnosis of Alzheimer's disease, a significant level of *P. gingivalis* antibodies, supporting hypothesis of an association between *P. gingivalis* from periodontitis and onset of Alzheimer's disease (23).

In a study conducted in 2020 on 20 patients with possible Alzheimer's disease, a significant association was observed between *P. gingivalis* and low mini mental state examination (MMSE) score and clock drawing test (CDT) score, meaning that *P. gingivalis* may play a role in cognitive function impairment. Although this study is limited by the small number of subjects, it pointed like the previous studies to an association between *P.gingivalis* and Alzheimer's disease (5).

Two types of lesion are characteristic of Alzheimer's disease on a histologic plane: one lesion extracellular, known as amyloid plaque or dense deposits of beta-amyloid peptide ($A\beta_{1-42}$) and hyperphosphorylated protein Tau, intracellular, which aggregated in intraneural neurofibrillary tangle (NTF). Ultimately these two typical features lead to cerebral dysfunction and loss of neurons (2). Gingipains, which happens to fragment Tau were colocalized with Tau tangles (NTF) and ubiquitin. It turns out that fragmentation of Tau may play a role in formation of hyperphosphorylated Tau, participating in neurodegeneration (13) (Figure 2). In the same study, conducted by Dominy et al, *P. gingivalis* oral exposure in mice conducted to induction of the stereotypical Alzheimer's disease marker: $A\beta_{1-42}$. Aβ, which has antibacterial effect, accumulated itself in reaction to gingipain leading to an $A\beta_{1-42}$ deposition in the brain (13).

Following induction of experimental chronic periodontitis by oral chronic application of *P. gingivalis*, extracellular plaques of $A\beta_{42}$ were detected in non-transgenic C57BL/6 mice. In this study authors also show that oral *P.gingivalis* infection lead to neuroinflammation, NTF production and neurodegeneration in wild-type mice (16).

Ishida et al. used transgenic mouse model of Alzheimer's disease (APP-Tg mice) to find out whether periodontitis, via *P. gingivalis*, exacerbates or not features of Alzheimer's disease. Cognitive functions were significantly altered in mice with periodontitis compared with

control mice. Amyloid β and pro-inflammatory cytokines IL-β and TNF- α load were higher in APP-Tg mice than in control mice: p.g-LPS could therefore exacerbate Aβ accumulation, promoting neuroinflammation (3). In the same study, an in vitro neuron cell culture experimentation showed that LPS induce an increase in $A\beta_{42}$ deposition and promotes production of TNF- α and IL-1β (3).

After exposition at p.g-LPS, wild type middle age mice suffered from Aβ accumulation, augmentation of Cathepin B protease (CatB) in microglia and neuron and increase IL-1β production in comparison with young mice and CatB-/- middle aged mice. Wu et al concluded that chronic exposure to pg-LPS induce microglia-mediated neuroinflammation, Aβ accumulation and impairment of cognitive function in middle -aged mice, in a CatB-dependant manner (1). Cat B was also showed to have a critical role in study conducted by Ran Nie et al. The authors found out that chronic systemic *P. gingivalis* infection induces accumulation of Aβ in inflammatory macrophage via the activation of CatB and increases of IL-1β and inflammatory response in liver macrophages of middle-age mice. This suggested that *P. gingivalis* infection induces production of Aβ in inflammatory macrophages which may serve as vehicle for Aβ entrance in the brain. Even if this study was conduct in the liver, it exposes induction of Aβ in inflammatory macrophages via CatB after P. gingivalis infection (19).
In another survey on middle age mice, oral infection with *P. gingivalis* induced an increase of TNF- α, IL-6 and IL-1 β laud, cause of brain inflammation. *P. gingivalis* can also impair spatial learning and memory abilities in these mice population. However no significant impact of *P.gingivalis* was demonstrate on the young mice in this study (18).

In a resume, different studies as much on mice as on humans support association of P. gingivalis, and its components on the two hallmark proteins that can be found in the brain of Alzheimer's disease: amyloid-beta (Aβ) plaques and hyperphosphorylated Tau (6) leading to neuroinflammation and neurodegeneration .

3. Role of oral health in Alzheimer's disease and possible alternative treatment targeting the *P. gingivalis*

Alzheimer's patients, due to their impaired cognitive functions, have more difficulties to adhere to daily dental hygiene, thus creating a favorable environment for *P. gingivalis* (29). Removing a bacterial focus by stabilizing the periodontitis and thus decreasing possible systemic exposure to *P. gingivalis*, can help to reduce progression of disease (12,29).
Beside daily oral hygiene, Alzheimer's disease patient should be advised of importance of dental healthcare. Indeed, periodontal health will prevent induction of inflammation and gum diseases such as periodontitis, ambient in which bacteria *P. gingivalis* is prosperous.

Oral health decreases as cognitive functions deteriorate (30). In a study conducted by Ide et al, poor oral hygiene and particularly periodontitis are associated with increase in cognitive decline (31). Failure to adhere to rules of daily oral hygiene could increase risk of developing dementia by 22% to 65% compared to individuals with normal hygiene (32). Thus, poor oral hygiene can lead to oral commensal microbial dysbiosis and trigger periodontitis, leading to virulence of pathogen *P. gingivalis*. Dental care should therefore not be neglected, and maintenance of oral hygiene should be acquired in order to decrease severity of the disease.

As shown in different investigations*, P. gingivalis* plays a fundamental role in pathogenesis of Alzheimer's disease. Nie et al propose CatB as a potential Alzheimer's disease therapeutic target, given its implication in Aβ accumulation (19). Another study (Dominy et al) implicates gingipains from *P. gingivalis*. Indeed, if gingipains participate to progression of sporadic dementia, its inhibition should participate to an amelioration of Alzheimer's disease patient's state. A small molecule, COR388, targeting lysin-gingipains of the *P. gingivalis* was tested successfully in decreasing the *P. gingivalis* bacterial load. In other words this study demonstrates that a treatment with this gingipains inhibitors will reduce accumulation of amyloid β , *P. gingivalis* brain infection, and slow and even prevent neurodegeneration in the brain (13), providing new lead for possible Alzheimer's disease treatment.

V. CONCLUSIONS

P. gingivalis is one of the main bacteria implicated in periodontitis and is a perfect example of oral health influence on the human body. A major part of studies is in favor of a relation between Alzheimer's disease and *P. gingivalis* confirming the role of bacterial infection in this pathology. *P. gingivalis* virulence factors such as LPS and gingipain appear to have a major role in induction of Aβ and phosphorylated Tau archetypal markers of Alzheimer's disease, thus causing increase in pro-inflammatory cytokines, source of neuroinflammation leading to neurodegeneration. But even if data collected through this review point to a consistent causative role of *P. gingivalis* in Alzheimer's disease, it will be relevant to effectuate a prospective study with a larger population to completely and definitively confirm pathogenesis of *P. gingivalis* in Alzheimer's disease.

Despite the fact that periodontitis varies according to individual susceptibilities, it seems primordial to prevent this infection in order to avoid dissemination of periodontal pathogens to the brain. Although not everyone who suffers periodontal inflammation will develop Alzheimer's disease and not all patients with Alzheimer's disease will develop periodontitis, there is a strong relation of the both diseases. It's therefore vital to involve in dental care as much the patient as its healthcare staff and family.

In such a way, it will be interesting to lunch information campaign on oral health's positive impact on Alzheimer's disease and on necessity to maintain healthy periodontium exempted of periodontitis.

But a door was open with Dominy et al, with the seducing idea of possibly cure when prevention fails. Their striking study was of great value, by demonstrating involvement of *P. gingivalis* in pathogenesis of Alzheimer's disease. Their precursor treatment opens the way to other means of countering neurotoxicity *of P. gingivalis* and thus prevent or improving health status of patients. Therefore, further studies are necessary to validate efficiency, utility, innocuity and then possibility to diffuse this treatment at public scale.

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