# *Porphyromonas gingivalis* Induces Alveolar Bone Loss and Brain Lesions in Rabbit

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**Abstract**

The aim of this study was to determine whether oral challenged with *Porphyromonas gingivalis* (*P. gingivalis*) mimicking periodontitis-associated with central nervous system (CNS) inflammatory lesions in rabbit.

Rabbits were divided into two groups, Group1 as control group and Group2 as experimental group, the rabbits in Group2 continuously challenged orally with *P.gingivalis* five times a week for 12 weeks. The whole brain taken out carefully. Abnormal changes that seen grossly were recorded. Samples were then sectioned into serial specimens and stained with H&E stain to evaluate the histopathological changes inside the brain. Blood sample were taken from each group to be tested for brain derived neurotrophic factor (BDNF) level. The maxilla and mandible were separated from the remaining skull and defleshed. Alveolar bone loss was evaluated morphometrically by using cone beam computed tomography (CBCT).

The gross pathology of the experimental group showed brain oedema, cerebral vascular congestion and haemorrhage. Histological analysis of infected group with *P. gingivalis* showed lesions in the cerebral cortex & meninges, with perivascular cuffing. Non-caseating granuloma observed in the cerebral cortex. BDNF level in experimental group appears to be higher as compared to the control group. CBCT images demonstrated significantly higher alveolar bone loss in infected group compared to the control group.

The present study provides evidence that infection with a periodontal pathogen, considered as primary culprit of periodontal disease *P. gingivalis* may play a role in the pathogenesis of CNS inflammatory disorders.

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## Introduction

Periodontitis is a worldwide disease. Susceptibility to periodontal diseases was virtually universal. The national surveys of the National Centre of Health Statistics and the National Institute of Dental and Craniofacial Research reported that only 5% to 15% of the population suffers from severe generalized periodontitis, even though moderate disease affect a majority of adults[1]. Periodontal diseases are a group of inflammatory diseases that affect the supporting tissue of the dentition. It is influenced by a complex set of differences of host susceptibility and various virulence among harboured organism. Researchers have proven the clear relationship of *P.gingivalis* as a causing pathogen of periodontitis [2]. Individual colonize by *P.gingivalis* generally respond with a humoral immune response against it. Its virulence factors, especially fimbriae promote both bacterial adhesion and invasion in subgingival regions [3].

Recently it had been found that this bacterium involves in causing extraoral infection where they are up to the stage of playing a role in developing systemic medical problem such as coronary heart disease, stroke, diabetes mellitus as well as pre-term delivery of low birth weight infants[2]. There are positive associations between periodontitis and artherosclerotic plaque

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formations[4]. It had been proven that patients with periodontitis have a ratio of 1.47 higher risk to get stroke[5]. Hypothetically, periodontal-derived-cytokines could reach the brain by both systemic and neural pathways and amplify brain cytokine pools. In addition, several bacteria associated with severe or progressive periodontitis are capable of invading tissues including *A. actinomycetemcomitans, P. gingivalis, and T. denticola.* In fact, Treponema species have been detected in trigeminal ganglia, brainstem, and cortex of human brain, and Alzheimer's Disease donors were more likely to have Treponema and more Treponema species than controls, suggesting that oral bacteria are capable of invading brain tissue perhaps via peripheral nerve fibres [6.]

Chronic inflammation in periodontal disease leads to bacterial dissemination into the blood stream then penetrates different organs and tissues and induces tissue destruction. Individual with periodontitis had 1.14 times higher risk of developing coronary heart disease than those without periodontitis. While others have found that there are significant relationship between periodontal status with myocardial infarction, and periodontitis with cerebrovascular event[7]. *P.gingivalis* found in subgingival tissue suggesting that it may pass through the epithelial barrier. It has been shown that it can passes into a deeper layer, where it could be a mechanism that play a role in systemic disease of the organism**.** *P.gingivalis* can actively invade endothelial cells and replicate intracellularly, suggesting that it has the capacity to persist within this host cell and alter the integrity of endothelial cells [8].

Several researchers carried out many studies and concluded that periodontal inflammatory process is associated with an increased risk of the development of hyperlipidemia and ischemic stroke [9,10]. Increased serum concentrations of immune active substances also influence the atherosclerotic process in cerebral veins and an acute infection can be triggering mechanism for an ischemic stroke. On the other hand, several published case reports stated that *P.gingivalis* was detected in the spinal fluid and with a brain abscess, this abscess was caused by a dental infection [11.12.13.14] . The condition was manged by extraction of the infected tooth with antibiotic coverages, they concluded that the brain abscess is caused by periodontitis which sourced by *P.gingivalis*. The aim of this study was to determine whether orally challenged *P. gingivalis* mimicking periodontitis-associated with CNS inflammatory lesions in rabbit.

## Materials and methods

The protocol was approved by the Committee on the Ethics of Animal Experiments of Faculty of Dentistry, Universiti Teknologi Mara (Ethic no. 28/05/2013. 600-FF (PT. 5/2)).

### Experimental animal design

Twelves healthy male White New Zealand rabbits (3–3.5 kg) were kept in wire-bottomed cages at 25±2 °C, given tap water and standard pellet diet, and exposed to a 12/12-h light/dark cycle at 50– 60 % humidity in an animal room. Rabbits were divided into 2 groups. Group 1 control were continuously fed with the standard pellets for 12 weeks. Group 2 experimental, rabbits were continuously challenged orally with *P. gingivalis* ATCC 33277 (0.2 mL of $1.5\times10^{12}$ bacterial cells/mL in 2 % CMC with PBS) five times a week for 12 weeks [10]. Rabbits were sacrificed, the whole brain were taken out carefully, abnormal changes that seen grossly were recorded. Also, fresh blood sample were taken to evaluate the Brain Derived Neurotrophic Factor (BDNF) level in serum by using commercially available enzyme-linked immunosorbent assay (ELISA) kit.

### Histological assessment

Brain samples were then sectioned into serial sections. Tissues fixed and embedded in paraffin. Serial sections of 5µm thickness were prepared and stained with Haematoxylin & Eosin (H&E). The prepared slides were studied to evaluate the pathological changes inside the brain histologically.

### Evaluation of alveolar bone

The maxilla and mandible were separated from the remaining skull, defleshed to remove completely the soft tissue from the sample. Alveolar bone loss was evaluated firstly morphometrically and secondly by segmental view on the buccal, lingual, mesial and distal surfaces of the maxilla and mandible teeth by using Cone-beam computed tomography (CBCT) images which provide three-dimensional (3-D)

information as well as segmental image by slice of coronal and sagittal view. The exposure time was 12 second with 84kV, with radiation dosage in the range of 650-800 mGy x $cm^2$.

### Statistical analysis

All values are presented as means ± standard error of the mean (SEM). Significant differences were determined by the use of an unpaired, two-tailed Student's *t*-test. Differences were considered significant when $P < 0.05$.

## Results

### Morphometric evaluation by Cone-beam computed tomography

Morphometric evaluation of the cone beam image showed that there were significant differences of the alveolar bone loss between the control and experimental groups. The experimental group shows clear marginal irregular continuity of alveolar bone as compared to the control group (Figure 1).

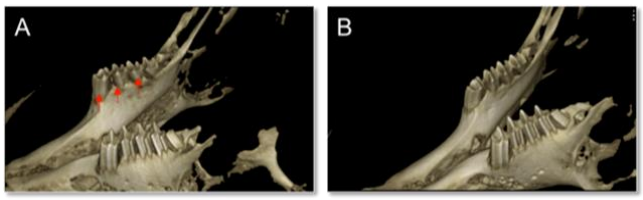


**Figure 1.** Morphometric view in cone beam computed tomography image of controlled group (B) and experimental group (A). Notice that there is prominent bone loss in experimental group where the continuity of the alveolar bone shows some destruction as compared to the controlled group.

### Crown root ratio

A percentage ratio comparison between mean average of clinical crown length over mean average of root length for controlled groups and experimental groups were evaluated according to the following formula: mean of clinical crown/mean of root length X100. Therefore, the percentage ratio for mean average of clinical crown length over mean average of root length for controlled and experimental groups were (3.138/10.728 X100 = 29.3%, 3.344/10.325 X100 = 42%) respectivly. These results reflect that the experimental group had more alveolar bone loss compare to the control one. Mann-Whitney U test demonstrated a significant difference of bone loss in of the experimental group compare to control (Figure 2). These results confirm that, there is bone loss in experimental group which is challenged with *P.gingivalis.*

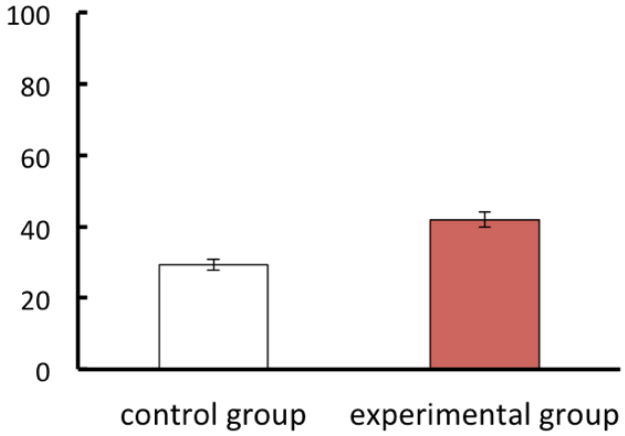


**Figure 2.** Histogram of percentage ratio of mean clinical crown over mean of root length by percentage between control group and experimental group. Here experimental group appears to have higher percentage ratio of mean clinical crown over mean of root length as compared to the control group. Differences were determined by the use of an unpaired, two-tailed Student's *t*-test. *$P < 0.05$.

### Evaluation of brain derived neurotrophic factor (BDNF) in serum

Brain derived neurotrophic factor level in serum concentration were evaluated by using Elisa kit, results showed lower level in control group (52.314pg/ml) compared to the experimental group (93.568 pg /ml). Statistical analysis demonstrated a significant difference (Figure 3).

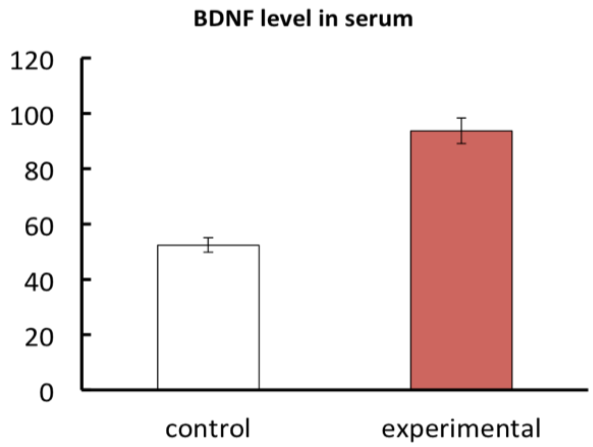


**Figure 3.** The level of Brain Derived Neurotrophic Factor (BDNF) in serum for control and experimental group 52.314pg/ml and 93.568 pg/mlrespectively. Differences were determined by the use of an unpaired, two-tailed Student's *t*-test. *$P < 0.05$.

### Morphological and microscopic appearance in rabbit's brain tissue

The brain (Cerebrum, cerebellum, and spinal cord) from group 2 (challenged) showed a remarkable cerebral oedema and vascular congestion with haemorrhage compared to group 1 (control). The cerebellum showed flattening of the vermis due to the oedema (Figure 4).

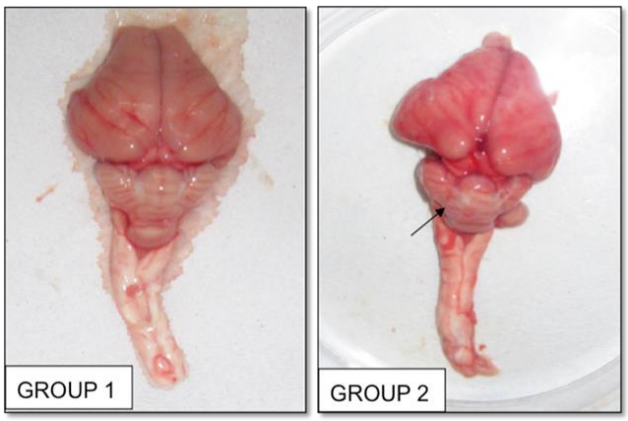


**Figure 4.** Dorsal view of the brain (Cerebrum, cerebellum and spinal cord) from group 2 (challenged) showed a remarkable cerebral oedema and vascular congestion with haemorrhage compared to group 1 (control). The cerebellum showed flattening of the vermis due to the oedema (arrow).

Histopathology of the meninges and brain of rabbits infected (challenged) orally with *P.gingivalis (*12 weeks post infection) Group 2, showed leptomeningitis with infiltration of chronic inflammatory cells (arrows), lymphocytes, and macrophages, the inflammation is not limited to the meninges but also is extended to the brain parenchyma causing meningoencephalitis (Figure 5). A remarkable pathognomonic response showed a typical feature of multifocal perivascular cuffing consist of lymphocytes, macrophages and some neutrophils within the cortex and white matter (Figure 6). The most significant histopathological finding was the focal area of non- caseating granuloma in the brain tissue. The neuropil showed fragmentation, and neurons are not visible, instead there is cell debris and neutrophils with lymphocytes and Gitter cells (foamy macrophages). The non-caseating granuloma was intensely surrounded by cellular infiltration (Figure 7).

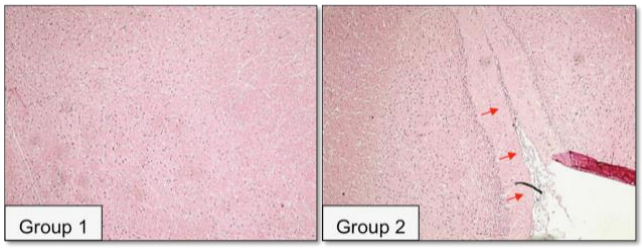


**Figure 5.** Histopathology of the meninges and brain of rabbits infected (challenged) orally with *P.gingivalis (*12 weeks post infection). Group 1 Control non-challenged rabbit. Group 2, showed leptomeningitis with infiltration of chronic inflammatory cells (arrows), lymphocytes, and macrophages, the inflammation is not limited to the meninges but also is extended to the brain parenchyma causing meningoencephalitis, H&E, 10 X.

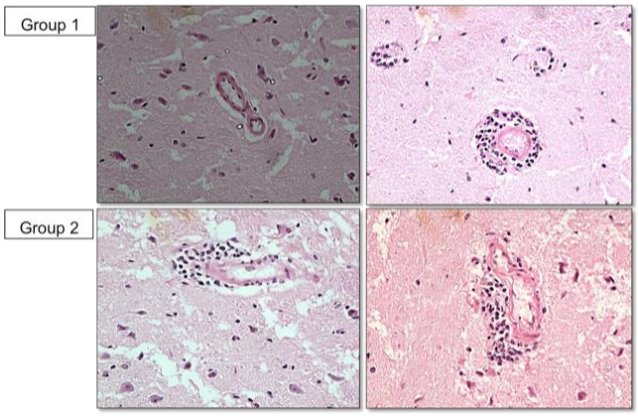


**Figure 6.** (control) Group 1 shows normal brain and cerebral vessels. Group 2 Note the perivascular cuffing in the brain. (Arrows) (H & E, 40 X).

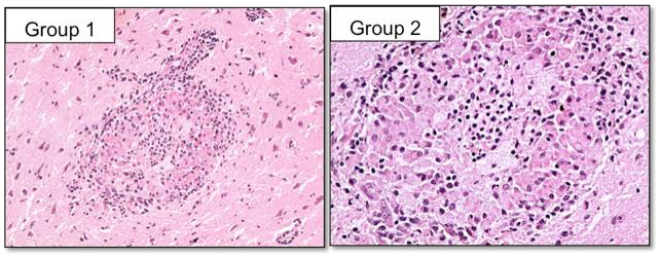


**Figure 7:** Brain tissue, non-caseating granuloma with neutrophils, lymphocytes and Gitter cells (foamy macrophages) shown with centre of non-caseating granuloma, group 2, H&E, X40.

## Discussion

MSC can be found in a variety of adult tissues, such as adipose tissue, periosteum, synovial membrane, muscle, dermis, pericyte, blood, trabecular bone and bone marrow. [8] Bone marrow is the largest and most accessible source of MSC. The number of MSCs in the Periodontal diseases are mainly chronic infectious diseases result from response to a complex dental biofilm microbiome containing various periodontopathic bacteria species[15.16]. Periodontal diseases destroy the tooth-supporting tissues and lead to tooth loss if not adequately treated[17,18]. Epidemiological evidence suggests that periodontal infection is associated with an increased risk of a variety of diseases such as atherosclerotic vascular diseases (7), type 2 diabetes, and non-alcoholic fatty liver disease. Bacteria from the dental biofilm can invade the gingival tissue through the ulcerated sulcular epithelial lining of periodontal pockets and then disseminate into the systemic circulation [19].

Among the various periodontopathic bacteria, considerable research has focused on the role of *P. gingivalis* in possible mechanisms linking periodontal diseases and other human diseases due to its unique pathogenicity [2], and its association with these various diseases. It can also enter the systemic circulation, increase the circulating inflammatory mediators and potentiate an inflammatory reaction in other tissues or organs [7, 8].

The first objective of this research was to evaluate the differences in the bone loss between controlled group and experimental group which is induced by *P.gingivalis* the second does *P.gingivalis* can capable of invading and causing the histopathological changes in the brain tissue. Alveolar bone resorption is a pivotal sequela of periodontal disease which is induced by oral infection with *P. gingivalis* [20,21]. Crown-root ratio were used to assess the amount of bone loos [22]. Results showed that the percentage ratio of control and experimental groups were 29.3 % ,42%. respectively, which could be due to the challenges with *P. gingivalis*. Statistical analyses showed that there is significant difference between controlled group and experimental group.

Brain derived neurotrophic factor play an important role in protecting the central nervous system in any insult situation for example bacterial invasion, traumatic damage, or even a simple triggering circumstances for example in high level of caffeine intake as well as in active and sympathetic response after during physical exercise. It supports the new regeneration of new neural stem cells and neurons in a process known as neurogenesis. Besides, when there is Central Nervous System (CNS) insult, the BDNF level will absolutely increase. The BDNF level is increase at the level of 93.568pg/ml in experimental group challenged with *P. gingivalis* compared to control group (52.314pg/ml), Statistical analysis demonstrated a significant difference. These results could demonstrate the destruction of the brain by dissemination and infection with *P. gingivalis*. While grossly, group 2 (challenged) showed a remarkable cerebral oedema and vascular congestion with haemorrhage compared to group 1 (control). The cerebellum showed flattening of the vermis due to the oedema These findings were in agreement with others studies carried out on human brains autopsy done on patients who had acute necrotizing encephalopathy and meningitis [23,24]. Therefore, based on these findings, we can speculate that the brain haemorrhage might be caused by the bacterial invasion of *P. gingivalis*.

Histological analysis of the challenged group showed leptomeningitis with infiltration of chronic inflammatory cells (arrows), lymphocytes, and macrophages, the inflammation is not limited to the meninges but also is extended to the brain parenchyma causing meningoencephalitis. These findings may be due to the passage of the *P. gingivalis* through the ultimate selective layer of blood brain barrier (BBB) and invasion of the brain tissue inducing inflammatory response.

This highly selective permeability layer only allows passage of water, some gases, and lipid soluble molecules by passing diffusion, as

well as the selective transport of molecules such as glucose and amino acid that are important for neural function. *P. gingivalis* initially might be one of the causes to induced inflammatory response at the meningeal layer which might compromised the Blood Brain Barrier [25]. The perivascular cuffing of vessels with lymphocytic infiltration demonstrated the presence of inflammation and justified the haemorrhage appearance in gross changes of the experimental group brains.

Histological examination of the brain tissue from group challenged with *P. gingivalis* showed presence of non- caseating granuloma intensely surrounded by cellular infiltration. The neuropil showed fragmentation, and neurons are not visible, instead there is cell debris and neutrophils with lymphocytes and Gitter cells (foamy macrophages). It is proven that there is a chronic inflammatory response of the rabbit brain in response to the challenge by *P. gingivalis*.

These findings disagree with many studies which showed the formation of caseating granuloma especially in infectious disease such as tuberculosis [26]. However, in our study the inflammatory changes are very clear in experimental group compared to the controlled group where the histological findings are all totally normal and free from any pathological signs even both of these group are in the same genome species being kept under careful observation of human care.

## Conclusions

*P. gingivalis* is proven to have the ability to cross blood brain barrier and cause histopathological changes to the brain tissue of the rabbit besides causing destruction to the alveolar bone of the maxilla and mandible.

## Declaration of Interest

The authors report no conflict of interest.

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