BRIEF REPORT

Expression of *TNF*, *IL1B*, and *NOS2* in the neural cell after induced by *Porphyromonas gingivalis* with and without coating antibody anti-*Porphyromonas gingivalis* [version 1; peer review: 1 approved, 1 approved with reservations]

Endang Winiati Bachtiar¹, Citra F. Putri¹, Retno D. Soejoedono², Boy M. Bachtiar¹

¹Oral Biology and Oral Science Research Center, Faculty of Dentistry Universitas Indonesia, Jakarta, DKI, 10430, Indonesia

²Department of Infectious Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, IPB University, Bogor, Indonesia

V1 First published: 23 Dec 2020, 9:1499
<https://doi.org/10.12688/f1000research.26749.1>
 Second version: 17 Mar 2021, 9:1499
<https://doi.org/10.12688/f1000research.26749.2>
 Third version: 28 Apr 2021, 9:1499
<https://doi.org/10.12688/f1000research.26749.3>
 Latest published: 28 Jun 2021, 9:1499
<https://doi.org/10.12688/f1000research.26749.4>

Abstract

Porphyromonas gingivalis has virulence factors such as gingipain and lipopolysaccharide, causing bacteremia to reach the brain and activate neuroinflammatory release cytokines. This study analyzed the effect of the co-culture of neuron cells with *P. gingivalis* coated with anti-*P. gingivalis* antibodies against cytokines produced by neuron cells. The gene expressions of the *TNF*, *IL1B*, *NOS2* in neurons was evaluated using RT-qPCR. The results showed that *P. gingivalis* coated with anti-*P. gingivalis* antibody before co-culture with neuron cells could decrease the gene expression of *TNF*, *IL1B*, and *NOS2* of neuron cells.

Keywords

Porphyromonas gingivalis, Blocking Antibody, Neuroinflammation, *TNF-α*, *IL-1β*, *iNOS*

This article is included in the Pathogens gateway.

Open Peer Review

Approval Status

	1	2
version 4		
(revision)		
28 Jun 2021		
version 3		
(revision)		
28 Apr 2021		
version 2		
(revision)		
17 Mar 2021		
version 1		
23 Dec 2020		

1. **Hadhimulya Asmara**, University of Calgary, Calgary, Canada

2. **Widya Lestari**, International Islamic University Malaysia (IIUM), Kuantan, Malaysia

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Endang Winiati Bachtiar (endang04@ui.ac.id)

Author roles: **Bachtiar EW:** Conceptualization, Data Curation, Funding Acquisition, Methodology, Resources, Writing – Review & Editing; **Putri CF:** Data Curation, Investigation, Software, Visualization, Writing – Original Draft Preparation; **Soejoedono RD:** Methodology, Resources; **Bachtiar BM:** Formal Analysis, Methodology, Supervision, Validation

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by a research grant by The Ministry of Research and Innovation, The Republic of Indonesia number NKB 2782 PDUPT 2020.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2020 Bachtiar EW *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Bachtiar EW, Putri CF, Soejoedono RD and Bachtiar BM. **Expression of *TNF*, *IL1B*, and *NOS2* in the neural cell after induced by *Porphyromonas gingivalis* with and without coating antibody anti -*Porphyromonas gingivalis* [version 1; peer review: 1 approved, 1 approved with reservations]** F1000Research 2020, 9:1499 <https://doi.org/10.12688/f1000research.26749.1>

First published: 23 Dec 2020, 9:1499 <https://doi.org/10.12688/f1000research.26749.1>

Introduction

Periodontitis is an infectious disease that causes inflammation of the tooth-supporting tissue, loss of bone adhesions, initiated by the main pathogens, *Porphyromonas gingivalis*. These bacteria are Gram-negative and have virulence factors such as fimbriae, gingipain, and lipopolysaccharide (LPS), which play a critical role in inducing periodontitis. With this virulence factor, *P. gingivalis* and its products not only damage the periodontal tissue but can also enter the blood circulation or bacteremia and cause systemic spread^{1,2}. *P. gingivalis* can move to other organs such as the heart and brain. Sophie's research found the presence of LPS *P. gingivalis* in the brains of Alzheimer's patients³. The mechanism for invading *P. gingivalis* bacteria into brain tissue is by penetrating the blood-brain barrier and damaging neuron cells⁴. When entering the central nervous system, these bacteria will first activate defense cells in the brain, namely the microglia, and astrocytes. Activation of both then releases neuroinflammatory mediators such as TNF- α and IL-1 β . Several studies have stated that neuron cells themselves can also release the neuroinflammatory mediators TNF- α and IL-1 β triggered by foreign bodies such as bacteria. This excessive release of neuroinflammation is toxic to neuron cells and can cause their damage and death^{5,6}. Besides, the excessive release of inducible nitric oxide synthase (iNOS) molecule due to antigen by neuron, microglia, and astrocyte cells, may induce human brain neurodegeneration⁷.

As a form of defense against bacterial attack, the body will naturally produce antibodies to eliminate bacteria. The antibodies produced by the host can specifically recognize certain bacterial species. Either monoclonal or polyclonal antibodies can recognize the lipid A region of the LPS of Gram-negative bacteria, such as *P. gingivalis*⁸. Animal studies by Barekzi *et al.* stated that pooled human polyclonal antibodies that are injected locally in the area of injury in mice have broad-spectrum antimicrobial effects against Gram-negative bacteria⁹. This study aims to evaluate the effect of anti-*P. gingivalis* antibodies on *TNF*, *IL1B*, and *NOS2* gene expression when bacteria interact with neuron cells. We hypothesized that there are differences in the gene expression of *TNF*, *IL1B* and *NOS2* in SHSY-5Y cells that have been exposed to *P. gingivalis* with and without antibody coating.

Methods

Cell lines

This research is an experimental laboratory study with post test only control group design. This study used the neuron cell line SHSY-5Y (Elabscience, USA), originating from a four-year-old human's bone marrow neuroblastoma. The cell culture medium was DMEM High Glucose with L-glutamine (Caisson Labs, USA), 15% FBS (Gibco, South America), and 1% Antibiotic-Antimycotic (Gibco, USA). The cultured condition was 5% CO₂ at 37°C incubator until 90% confluency was achieved (Figure 1)¹⁰.

Porphyromonas gingivalis ATCC 33277 was cultured in Brain Heart Infusion (BHI) agar as a growth medium and incubated under anaerobic conditions with a temperature of 37°C for 24 hours. Then cultured into BHI broth and incubated again

under anaerobic conditions with a temperature of 37°C for 24 hours. Then stored at 4°C until ready to use.

This study also used *P. gingivalis* ATCC 33277 bacterial culture. The multiplicity of infection (MOI) used was 1:100, the number of bacteria was 3.6×10^7 CFU/mL, and the number of neuron cells was 8×10^5 cells/well. In addition, this research used serum anti-*P. gingivalis* antibodies obtained from rabbits after immunization of killed *P. gingivalis*. *P. gingivalis* antisera were obtained from one-month-old rabbits that have been immunized with 1 mL of 1.7×10^8 CFU/mL of *P. gingivalis* culture. The bacteria were inactivated at 60°C for 30 min before being injected intravenously to the rabbit for 8 weeks with two boosters in intervals of 2 weeks. The animals were euthanized by anesthetic ether inhalation and injection by overdose of anesthetic drug (ketamine 50 mg/kg IM and xylazine 10 mg/kg IM), which caused the animal to fall asleep then slowed and eventually stopped the heart. The blood serum was determined by agar gel precipitation test (AGPT) and the antibody was purified using the Qiagen (QIAGEN, Inc., Valencia, Calif.) protein purification kit, following the manufacturer's protocol. Ethical clearance was given by the Ethical Research Committee of Medical Faculty Universitas Indonesia (2020, number 19-11-1402).

Coating of anti-*P. gingivalis* antibodies

The antisera coated *P. gingivalis* (3.6×10^7 CFU/mL) was prepared by 1:300 diluted rabbit antibody serum in 150 μ L growth medium (DMEM High Glucose with L-glutamine (Caisson Labs, USA), 15% FBS (Gibco, South America), and 1% Antibiotic-Antimycotic (Gibco, USA)) for the treatment group; the control was *P. gingivalis* (3.6×10^7 CFU/mL) in 150 μ L growth medium and the growth medium only without addition of bacteria. The tubes were then incubate for 1 hour in an incubator with a temperature of 37°C¹¹.

Experimental design

The experiment design as follows: group A was the neurons plus bacteria with antibody coating, and group B for the neuron group plus bacteria without antibody coating and medium only, with 6 replications of each group.

Harvest of SHSY-5Y neuron cells

Neuron cell cultures that had reached 80% confluence were harvested using 0.25% trypsin-EDTA (Gibco, Canada). The number of cells harvested was counted using a hemocytometer (number of cells 8×10^5 cells/well). The cells were then transferred to a 15 mL tube and resuspended in 2 mL growth medium and then divided into well plates that have been designed with each well containing 100 μ L (4×10^5 cells/well) of SHSY5Y cells. The neuron cell line SHSY-5Y (Elabscience, USA) is a cell that has epithelial-like cell and neuronal-like cell morphology and has a cell density of more than 1×10^6 cells/cm². In this study, cell culture was carried out with two subcultures in January 2020 and February 2020 until the number of cells reached 8×10^5 cells/well. Observation with a microscope was carried out every 2–3 days to identify neuron cells and determine the stage of neuron cell differentiation (Figure 1)

P. gingivalis exposure to SHSY-5Y cells

Each well of 96 well culture plate filled with SHSY-5Y cells and antibody-coated *P. gingivalis* bacteria and incubated for one hour were added. Group A was filled with 30 μ L (1×10^5 CFU/mL) *P. gingivalis* coated with antibody, while group B was filled with 30 μ L of bacterial *P. gingivalis* without antibodies. After that, cells were incubated for 24 hours at 37°C. All cells present in the well plate were evaluated for the morphological features, then harvested for RNA extraction.

RNA extraction and RT-qPCR

The neural cell culture was harvested, and RNA extracted for cDNA synthesis using a Reverse Transcription Kit (ReverTra Ace®, Toyobo, Japan) in line with the manufacturer's instructions. The cDNA sample is ready for use in the Real-Time PCR tool, with the selected primers as Table 1. RT-PCR was performed using the SYBR Premix Ex Taq™ kit. Relative expression of the target gene normalized to GAPDH, gene expression was analyzed using the $2^{-\Delta\Delta C_t}$ method and compared

Figure 1. The morphology of the cultured neuron cell line SHSY-5Y; it appears that the SHSY-5Y cells have a neuronal-like cell shape. (A) Cell image of 3 days culture (40x enlargement). (B) Cell image after 7 days, showing elongation of neuron cell bodies and cells growing in groups (40x magnification). (C) There is an increase in cell proliferation and group cell growth (20x magnification). (D) The cells have reached 80% confluence and are ready to be harvested (20x magnification), (E and F) SHSY-5Y cells have undergone differentiation, seen the presence of axons from cells and the cell proliferation process begins to decline (40x magnification). Underlying data shows raw, unprocessed images used to generate this figure¹².

Table 1. Primers used in this study.

Primer Name	Sequences	Reference
<i>TNF</i>	Forward: 5 'CTG AAC TTC GGG GTG ATC G 3' Reverse: 5 'GCT TGG TGG TTT GCT ACG AC 3'	13
<i>IL1B</i>	Forward: 5'-TAT TAC AGT GGC AAT GAG G-3 Reverse: 5'-ATG AAG GGA AAG AAG GTG-3'	13
<i>NOS2</i>	Forward: , 5'-GCA GAA TGT GAC CAT CAT GG-3' Reverse: 5'-ACA ACC TTG GTG TTG AAG GC-3	14
<i>GAPDH</i>	Forward: 5'-CTG CAC CAC CAA CTG CTT AG-3' Reverse: 5'-AGG TCC ACC ACT GAC ACG TT-3'	13

to control. The gene expression of *TNF*, *IL1B* and *NOS2* were evaluated by RT-qPCR as previously reported¹⁵

Results

Figure 2 shows the microscopic morphology of the SHSY-5Y cells that were not exposed to *P. gingivalis*, and those exposed to *P. gingivalis* and coated with anti-*P. gingivalis* antibodies. From these figures, it is known that cells not exposed to *P. gingivalis* grew more than cells exposed to *P. gingivalis*, both with and without antibodies.

From qPCR analysis, it was observed that there are differences in the gene expression of *TNF-α*, *IL-1β* and *iNOS* in SHSY-5Y cells that have been exposed to *P. gingivalis* with and without antibody coating. Based on this analysis, it can be concluded

that the research hypothesis is accepted. This is shown in Figure 3, where the expression of *TNF-α* and *IL-1β* genes in the antibody-coated group was lower than in the antibody-coated group. Ct values are available as *Underlying data*¹⁶.

Discussion

The SHSY-5Y neuron cell line (Elabscience, USA) is a cell derived from human neuroblastoma and taken from bone marrow tissue. These cells have epithelial-like cell and neuronal-like cell morphology. During culture, SHSY-5Y cells can grow into two types of cells, namely adherent cells and floating cells, both of which are viable. However, in this study, adherent cells were used because they were clearer in morphology and proliferation development, and were easy to evaluate after a routine medium change^{17,18}.

Figure 2. SHSY-5Y cell morphology. (A) SHSY-5Y cell morphology before exposure to *P. gingivalis*, (B) After exposure to *P. gingivalis* without antibodies, (C) After exposure to *P. gingivalis* with antibodies. (Light microscope, 20x magnification).

Figure 3. The level of *TNF*, *IL1B* and *NOS2* gene expression in the antibody-coated group, without antibody coating and neuron cells only.

Microscopy images of SHSY-5Y cells (Figure 2) showed significant growth changes over time. According to Kovalevich and Langford, one of the considerations for the success of SHSY-5Y cell culture is the growth medium used¹⁸. In this study, DMEM growth medium containing L-glutamine was used. Glutamine can help increase neuron cell viability and increase neuron cell density, so that it can be seen on microscopy images that neuron cell cultures grow well. However, the number of cells collected until the end of cell culture is 8×10^5 , where this number is limited for the study sample. This may occur because cells have started to enter the differentiation stage, so that the cell proliferation process tends to decrease¹⁷.

TNF- α and IL-1 β are inflammatory mediators released by immune cells when a stimulus triggers the cells. In the nervous system, TNF- α and IL-1 β are usually released by astrocytes and microglia cells. However, a number of studies suggest that these inflammatory mediators are also released in large numbers by neuron cells when there are intrinsic or extrinsic triggers¹⁹. Extrinsic triggers such as LPS presence from *P. gingivalis* bacteria can trigger the expression of TNF- α and IL-1 β by neuron cells so that it can damage neuron cells^{20–22}. In the incidence of Alzheimer's disease, the release of this inflammatory mediator can cause neuronal cell death, according to a study by Janelsins *et al.*, which stated that the inflammatory mediators TNF- α and IL-1 β appears to be directly proportional to Alzheimer's disease severity^{22–24}.

This study is in line with the research of Janelsins *et al.*, who found that neuron cells can express TNF- α in brain injury in experimental animals. This is evidenced by the detection of the molecules NeuN and TNF- α in the brain of six-month-old mice. In this study, SHSY-5Y neuron cells can also express TNF- α . In addition, Janelsins *et al.* also found that TNF- α contributed to neuron cell death in the brain with Alzheimer's condition. The signaling mechanism is still unknown, but Janelsins *et al.* stated that there was an increase in the expression of TNFR2 and Jun transcript as pro-apoptotic signals mediated by TNF- α ^{24,25}.

The expression of iNOS has been characterized in various cell types as an inflammatory mediator during infection, disease, or tissue damage. iNOS is expressed by astrocytes, microglia, and a small portion of endothelial cells in the brain. However, under conditions of increased inflammatory activation in neuron cells, neurons can also express these cytotoxic agents and other reactive oxidative species. The main component that regulates the signaling pathway of iNOS in neurons is the transcription factor NF- κ B. The results of this study indicate that anti-*P. gingivalis* antibodies can suppress iNOS expression in neuron cell cultures exposed to *P. gingivalis*. Blocking carried out by antibodies to *P. gingivalis* LPS was thought to suppress bacterial pathogenicity so that iNOS expression in neurons was lower than that of the control group. We assume the antibody use reduced neuronal damage. This is in line with Heneka and Feinstein's research, which states that increased expression of iNOS in neurons can affect neurodegeneration and inflammation in the brain^{7,26}.

P. gingivalis have secreted and non-secreted virulence factors. Secreted virulence factors, for example, gingipain, are virulence factors secreted by bacteria to carry out their activities. Meanwhile, non-secreted virulence factors are virulence factors that are not secreted by bacteria, usually attached to the bacterial structure, such as LPS. In this study, the antibodies used were from injections of killed *P. gingivalis* in rabbits. This will result in the formation of polyclonal antibodies against non-secreted virulence factors, namely LPS, because when it is turned off, the bacteria are unable to secrete other virulence factors such as gingipain. The anti-*P. gingivalis* polyclonal antibodies can recognize *P. gingivalis* bacterial cells and these bacteria's LPS structure^{8,9,25}. Therefore, coating this antibody with *P. gingivalis* bacteria for 1 hour before exposure to neuronal cells is thought to block LPS *P. gingivalis* bacteria not to infect neuron cells.

In contrast to the control group that did not use antibodies, *P. gingivalis* was exposed to neuron cells, infecting neuron cells with secreted and non-secreted virulence factors. This occurs because there are no antibodies that block the two types of *P. gingivalis* virulence factors. Therefore, in qPCR analysis results, neuron cell culture with anti *P. gingivalis* antibody showed lower TNF- α and IL-1 β expression than the control group. The study (Figure 3) show that the use of antibodies can suppress the expression of TNF- α and IL-1 β . The low expression of TNF- α and IL-1 β with the use of antibodies is thought to prevent neuronal damage and is expected to prevent the occurrence of Alzheimer's disease or other cognitive disorders. However, different research results may occur because of the MOI value used. In this study, the MOI used was 1:100.

This study's findings indicate that there is good potential for the development of the anti-*P. gingivalis* vaccine. The anti-*P. gingivalis* antibody used in this study was able to block the development of bacteria *in vitro* so that the neuroinflammatory response can also be minimized. Further research at the *in vivo* level and clinical trials can be developed to see the positive effects of administering antibodies locally or systemically. In the case of local infection of *P. gingivalis* in the oral cavity, the local administration of antibodies may have more potential to suppress bacterial development.

In addition, long-term research involving the role of neuron cells and damage to the central nervous system also needs to be done. With this research, it is hoped that it can become a reference to increase the level of research so that in the future, the prevention of *P. gingivalis* infection can be done so that it can prevent neurodegeneration in the incidence of Alzheimer's disease.

Conclusion

The cultured SHSY-5Y neuron cells exposed to *P. gingivalis* bacteria after anti-*P. gingivalis* antibody coating exhibited a reduction in the expression of the *TNF*, *IL1B*, and *NOS2*. Further research to see the effectiveness of anti-*P. gingivalis* antibodies still needs to be developed, especially *in vivo*. The success of anti-*P. gingivalis* antibodies in suppressing factors that can

damage neuronal cells can be used as a guideline for developing a *P. gingivalis* vaccine, since it is one of the oral bacteria that triggers Alzheimer's disease.

Data availability

Underlying data

Open Science Framework: Expression of TNF- α , IL-1 β , and iNOS in the neural cell after induced by Porphyromonas gingivalis with and without coating antibody anti-Porphyromonas gingivalis. <https://doi.org/10.17605/OSF.IO/Q5CVW>¹⁶.

This project contains the following underlying data:

- Beta actin GAPDH 2506202_data (1).xls. (qPCR data for housekeeping gene *GAPDH*.)
- IL1b TNFa_data(1).xls. (qPCR data for *IL1B* and *TNF*.)

- iNOS 23062020_data.xls. (qPCR data for *NOS2*.)

Open Science Framework: Expression of TNF, IL1B, and NOS2 in the neural cell after induced by Porphyromonas gingivalis. <https://doi.org/10.17605/OSF.IO/JFG3T>¹².

This project contains the raw images used to produce Figure 1.

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Acknowledgments

We thank Vivi, Asti, and Anissa for their help in the laboratory work.

References

- Ding Y, Ren J, Yu H, et al.: *Porphyromonas gingivalis*, a periodontitis causing bacterium, induces memory impairment and age-dependent neuroinflammation in mice. *Immun Ageing*. 2018; **15**: 6. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kamer AR, Craig RG, Dasanayake AP, et al.: Inflammation and Alzheimer's disease: possible role of periodontal diseases. *Alzheimers Dement*. 2008; **4**(4): 242–50. [PubMed Abstract](#) | [Publisher Full Text](#)
- Poole S, Singhrao SK, Kesavalu L, et al.: Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. *J Alzheimers Dis*. 2013; **36**(4): 665–77. [PubMed Abstract](#) | [Publisher Full Text](#)
- Armbrust F, Colmorgen C, Pietrzik CU, et al.: The Alzheimer's disease associated bacterial protease RgpB from *P. gingivalis* activates the alternative β -secretase meprin β thereby increasing A β generation. *bioRxiv*. 2019; 11. [Publisher Full Text](#)
- Wang RPH, Ho YS, Leung WK, et al.: Systemic inflammation linking chronic periodontitis to cognitive decline. *Brain Behav Immun*. 2019; **81**: 63–73. [PubMed Abstract](#) | [Publisher Full Text](#)
- Singhrao SK, Chukkapalli S, Poole S, et al.: Chronic *Porphyromonas gingivalis* infection accelerates the occurrence of age-related granules in ApoE $-/-$ mice brains. *J Oral Microbiol*. 2017; **9**(1): 1270602. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Heneka MT, Feinstein DL: Expression and function of inducible nitric oxide synthase in neuron. *J Neuroimmunol*. 2001; **114**(1–2): 8–18. [PubMed Abstract](#) | [Publisher Full Text](#)
- Lipman NS, Jackson LR, Trudel LJ, et al.: Monoclonal versus Polyclonal antibodies: Distinguishing Characteristics, Applications, and Information Resources. *ILAR J*. 2005; **46**(3): 258–68. [PubMed Abstract](#) | [Publisher Full Text](#)
- Barekzi NA, Felts AG, Poelstra KA, et al.: Locally Delivered Polyclonal Antibodies Potentiate Intravenous Antibiotic Efficacy against Gram Negative Infections. *Pharm Res*. 2002; **19**(12): 1801–7. [PubMed Abstract](#) | [Publisher Full Text](#)
- Bachtar EW, Dewi FS, Yusuf AA, et al.: Transplantation of dental pulp stem cells in experimental bone defect. *Journal of Biomimetics, Biomaterials and Biomedical Engineering*. 2017; **34**: 94–100. [Publisher Full Text](#)
- Bachtar EW, Dewiyani S, Akbar SMS, et al.: Inhibition of *Candida albicans* biofilm development by unencapsulated *Enterococcus faecalis* cps2. *J Dent Sci*. 2016; **11**(3): 323–330. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bachtar EW: Expression of TNF, IL1B, and NOS2 in the neural cell after induced by Porphyromonas gingivalis. 2020. <https://www.doi.org/10.17605/OSF.IO/JFG3T>
- Bhat IA, Naykoo NA, Qasim I, et al.: Association of interleukin 1 beta (*IL-1B*) polymorphism with mRNA expression and risk of non small cell lung cancer. *Meta Gene*. 2014; **2**: 123–33. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- McAdam E, Haboubi HN, Forrester G, et al.: Inducible nitric oxide synthase (iNOS) and nitric oxide (NO) are important mediators of reflux-induced cell signalling in esophageal cells. *Carcinogenesis*. 2012; **33**(11): 2035–43. [PubMed Abstract](#) | [Publisher Full Text](#)
- Bachtar BM, Bachtar EW: Proinflammatory MG-63 cells response infection with *Enterococcus faecalis* cps2 evaluated by the expression of TLR-2, IL-1 β , and iNOS mRNA. *BMC Res Notes*. 2017; **10**(1): 401. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bachtar EW: Expression of TNF- α , IL-1 β , and iNOS in the neural cell after induced by Porphyromonas gingivalis with and without coating antibody anti-Porphyromonas gingivalis. 2020. <https://www.doi.org/10.17605/OSF.IO/Q5CVW>
- Shipley MM, Mangold CA, Szpara ML: Differentiation of The SH-SY5Y Human Neuroblastoma Cell Line. *J Vis Exp*. 2016; (108): 53193. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kovalevich J, Langford D: Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods Mol Biol*. 2013; **1078**: 9–21. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Park KM, Bowers WJ: Tumor Necrosis Factor-Alpha Mediated Signaling in Neuronal Homeostasis and Dysfunction. *Cell Signal*. 2010; **22**(7): 977–83. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- DiSabato DJ, Quan N, Godbout JP: Neuroinflammation: the devil is in the details. *J Neurochem*. 2016; **139** Suppl 2(Suppl 2): 136–53. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hewett SJ, Jackman NA, Claycomb RJ: Interleukin-1 β in Central Nervous System Injury and Repair. *Eur J Neurodegener*. 2012; **1**(2): 195–211. [PubMed Abstract](#) | [Publisher Full Text](#)
- Zhao J, Bi W, Xiao S, et al.: Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. *Sci Rep*. 2019; **9**(1): 5790. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Singhrao SK, Olsen I: Assessing the role of *Porphyromonas gingivalis* in periodontitis to determine a causative relationship with Alzheimer's disease. *J Oral Microbiol*. 2019; **11**(1): 1563405. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Janelins MC, Mastrangelo MA, Park KM, et al.: Chronic neuron-specific tumor necrosis factor-alpha expression enhances the local inflammatory environment ultimately leading to neuronal death in 3xTg-AD mice. *Am J Pathol*. 2008; **173**(6): 1768–82. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Zhang J, Yu C, Zhang X, et al.: *Porphyromonas gingivalis* lipopolysaccharide induces cognitive dysfunction, mediated by neuronal inflammation via activation of the TLR4 signaling pathway in C57BL/6 mice. *J Neuroinflammation*. 2018; **15**(1): 37. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sonar SA, Lal G: The iNOS activity during an immune response controls the CNS pathology in experimental autoimmune encephalomyelitis. *Front Immunol*. 2019; **10**: 710. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 16 April 2021

<https://doi.org/10.5256/f1000research.29536.r76420>

© 2021 Lestari W. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Widya Lestari

Department of Oral Biology, Kulliyah of Dentistry, International Islamic University Malaysia (IIUM), Kuantan, Malaysia

This report provides new insight into dentistry. *P. gingivalis* is not only related to Periodontitis but also express in neuron cells, which may give additional knowledge to clinicians. Pathways of expression of TNF alpha and IL-1B area clearly explained. Methodology and results are well established and, we believe these findings will benefit clinicians and also researchers as well.

The methodology:

This is an in vitro study using a nerve cell culture. The experimental design has used a treatment group and a control group which, in my opinion, is good enough to observe the effect of administering anti-*P. gingivalis* antibodies on the expression of neuroinflammatory cytokines.

The results:

The results have been presented clearly. I suggest analyzing them descriptively without using statistics. This is a preliminary study, in my opinion, it has described how the expression of neuroinflammatory cytokines in nerve cell cultures after exposure to *P. gingivalis* which has been treated with anti-*P. gingivalis* antibody.

Overall, a minor revision is needed.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 25 January 2021

<https://doi.org/10.5256/f1000research.29536.r76422>

© 2021 Asmara H. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Hadhimulya Asmara

Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada

The manuscript by Bachtar *et al.* presents some potentially interesting findings on preventing the neurons from damage and death by reducing excessive release of neuroinflammatory mediators such as TNF- α and IL-1 β and other molecules such as iNOS. This paper highlights the potential success of using antibody specially anti- *P. gingivalis* antibodies for suppressing factors that can damage neuronal cells can be used as a guideline for developing a *P. gingivalis* vaccine since it is one of the oral bacteria that triggers Alzheimer's disease.

Overall, the work presents an interesting idea regarding possible antibody use to decrease the release of neuroinflammatory agents and other molecules that are toxins and cause harm to the neurons. If this finding is rigorously proven, it could be a great contribution to the prevention and treatment of bacterial infection in Neurodegenerative disease. At this stage, however, there are several comments that need to be clarified in the manuscript that substantively influences the significance of the findings.

Specific comments:

1. In the paper, the authors did not mention the efficacy of other treatments such as antibiotic treatment for *P. gingivalis* infection and its effect on the release of neuroinflammatory agents. It is important to highlight the antibiotic's effect since if the antibiotic is effective enough to kill the bacteria and prevent the infection on neurons then this paper must add more reasons why this paper or the antibody approach is better than the antibiotic treatment.

2. In the figure 2. The authors used the word “morphology” of the neurons, but the authors did not explain the morphology aspect of the neurons (such as elongation axons or shape of the cell body, etc). It only described the change of the growth or number of the viable cells that was decreased by the exposure of *P. gingivalis* with or without antibody coating. It is better to describe how the exact morphological changes of the neurons as clearly shown in figure 1 before the exposure *P. gingivalis*. Even better if the authors can add the qualitative and quantitative analysis of those differences between the groups.
3. The correlation between figure 2 and figure 3. The reducing number of viable cells in figure 2 and the reducing the expression of TNF- α , IL-1 β , and iNOS in figure 3 seems does not fit with the hypothesis in the paper. If the expression of TNF- α , IL-1 β , and iNOS were decreased on the neurons that were exposed by coating antibody *P. gingivalis* compared to the ones without coating (figure 3) then the number of viable neurons in the figure 2c (expose with antibody) must be higher than figure 2b (without antibody) since the antibody will decrease TNF- α , IL-1 β , and iNOS and it means to prevent the neuronal damage and death on figure 2b. What is the clarification or the explanation of this confusion?
4. It is necessary to state the *P* values or statistical significance of the differences between the three groups in figure 3 (with coating antibody, without coating anti, and neuron only). It is important to conclude that one group is higher or lower than the others groups based on statistical significance or *P* values.
5. As a minor comment, I think there is a typo on the first line of the second paragraph in the discussion section. In my opinion, I think the authors want to show figure 1 instead of figure 2 (as stated in that line) for describing the growth changes of neurons over time.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neuroscience (any works with neurons involvement), Molecular Biology (any works related to DNA and protein).

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Mar 2021

Boy Muchlis Bachtiar

Dear Reviewer,

Thank you for the valuable suggestion in improving of the quality of our manuscript. Here we would like to respond to your feedback.

Specific comments:

1. In the paper, the authors did not mention the efficacy of other treatments such as antibiotic treatment for *P. gingivalis* infection and its effect on the release of neuroinflammatory agents. It is important to highlight the antibiotic's effect since if the antibiotic is effective enough to kill the bacteria and prevent the infection on neurons then this paper must add more reasons why this paper or the antibody approach is better than the antibiotic treatment.

Author's responses:

We thank for this valuable comment. We have inserted the argumentation in the manuscript of this suggestion as follows:

P. gingivalis reside in a structured community of biofilm attached to surfaces embedded in the extracellular matrix which they produce themselves and they are difficult to eradicate due to their resistance to antimicrobials and the body's defense mechanisms¹⁰. The passive immunization approach using polyclonal antibodies to inhibit *P. gingivalis* adhesion to the periodontium tissue is a strategy to prevent biofilm formation and periodontium tissue damage which can lead to deeper tissue invasion so that *P. gingivalis* can enter the systemic circulation.

1. In the figure 2. The authors used the word "morphology" of the neurons, but the authors did not explain the morphology aspect of the neurons (such as elongation axons or shape of the cell body, etc). It only described the change of the growth or number of the viable cells that was decreased by the exposure of *P. gingivalis* with or without antibody coating. It is better to describe how the exact morphological changes of the neurons as clearly shown in figure 1 before the exposure *P. gingivalis*. Even better if the authors can add the qualitative and quantitative analysis of those differences between the groups.

Author's responses:

We have deleted 'morphology'. In Figure 2, it can be seen that there is a decrease in the number of neuron cells after being exposed to *P. gingivalis* bacteria.

1. The correlation between figure 2 and figure 3. The reducing number of viable cells in figure 2 and the reducing the expression of TNF- α , IL-1 β , and iNOS in figure 3 seems does not fit with the hypothesis in the paper. If the expression of TNF- α , IL-1 β , and iNOS were decreased on the neurons that were exposed by coating antibody *P.*

gingivalis compared to the ones without coating (figure 3) then the number of viable neurons in the figure 2c (expose with antibody) must be higher than figure 2b (without antibody) since the antibody will decrease TNF- α , IL-1 β , and iNOS and it means to prevent the neuronal damage and death on figure 2b. What is the clarification or the explanation of this confusion?

Author's responses:

Qualitatively, from the microscope image (Figure 2), there was no difference between the antibody group and the non-antibody group. In our opinion, the number of cells seen on a microscope does not necessarily indicate the expression of neuroinflammation. Based on the results of the real time PCR analysis, it showed that the expression of neuroinflammation was more in the group without antibodies (Figure 3).

1. It is necessary to state the *P* values or statistical significance of the differences between the three groups in figure 3 (with coating antibody, without coating anti, and neuron only). It is important to conclude that one group is higher or lower than the others groups based on statistical significance or *P* values.

Author's responses:

We used pooled samples (we have added this information in the methods section) as there was insufficient amount of RNA from each individual replication of the experiments. But we think the value of gene expression presented here is equal to the average of 6 replications of experiment. Hence, we couldn't get a statistical significance. Some studies also use this kind of data interpretation (Shu-Dong Zhang, Timothy W. Gant, Effect of pooling samples on the efficiency of comparative studies using microarrays, Bioinformatics, Volume 21, Issue 24, 15 December 2005, Pages 4378–4383)

1. As a minor comment, I think there is a typo on the first line of the second paragraph in the discussion section. In my opinion, I think the authors want to show figure 1 instead of figure 2 (as stated in that line) for describing the growth changes of neurons over time.

Author's responses:

Thank you, we have fixed this typo.

Again thanks

With warm regards

Bachtiar EW

Competing Interests: No Competing interest

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research