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Research Report

IMMUNOHISTOCHEMICAL ANALYSIS OF NF- κ B (P50/P65) IN PATIENT WITH AGGRESSIVE AND CHRONIC PERIODONTITIS

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ABSTRACT

Background: Nuclear factor-kappaB (NF- κ B) is a protein complex that plays a role in transcription factors and in response to inflammation. Periodontitis is a periodontal disorder caused by various bacteria such as *A. actinomycetemcomitans* and *P. gingivalis* whose LPS is closely related to NF- κ B (p50/p65). *Aim:* This study observed whether NF- κ B (p50/p65) played a role in aggressive and chronic periodontitis. *Methods:* Data were obtained from periodontal tissue 40 patients with aggressive periodontitis and 40 patients with chronic periodontitis. Samples were derived from periodontal tissue with abnormalities and NF- κ B (p50/p65) protein expression test was performed by immunohistochemistry. The statistical test used was the t-test. *Results:* In NF- κ B (p50) the t value was -12.041 and significance 0.000, with $\alpha = 5\%$, showing significant difference in protein expression of NF- κ B (p50) between patients with aggressive periodontitis and chronic periodontitis. OR estimation for the value of protein expression of NF- κ B (p50) was 0.64 (sign. = 0.000). It shows that if the protein expression of NF- κ B (p50) of the respondents is incremented by 1 (one) unit, the risk of chronic periodontitis increases 1.64 times. Box plot diagram shows that the distribution of the protein expression of NF- κ B (p50) between patients with aggressive periodontitis and chronic periodontitis patients is significantly different. In NF- κ B (p65) the Z value was -7.137 and significance of 0.000, with $\alpha = 5\%$, showed significant differences in protein expression of NF- κ B (p65) between patients with aggressive periodontitis and chronic periodontitis. OR estimates for protein expression of NF- κ B (p65) was 0.66 (sign. = 0.000). This indicates that if the protein expression of NF- κ B (p65) respondents is incremented by 1 (one) unit, the risk of chronic periodontitis increases 1.5 times. Box plot diagram shows that the distribution of the protein expression of NF- κ B (p65) between patients with aggressive and chronic periodontitis patients is significantly different. *Conclusion:* The protein expression of NF- κ B (p50/p65) has more influence on the incidence of chronic periodontitis patients, so it can be used as a marker for chronic periodontitis.

Key words: periodontitis, inflammation, NF- κ B p50, NF- κ B p65, transcription

ABSTRAK

Latar belakang: Nuclear factor – kappaB (NF- κ B) sebagai protein kompleks yang berperan pada faktor transkripsi dan berperan pada respons terhadap peradangan. Periodontitis sebagai kelainan periodontal yang disebabkan berbagai kuman seperti *A. Actinomycetemcomitans* dan *P. gingivalis* dimana LPS kuman ini berkaitan erat dengan NF- κ B (p50/p65). *Tujuan:* Penelitian ini ingin melihat apakah NF- κ B (p50/p65) berperan pada periodontitis agresif dan kronis. *Metode:* Data penelitian didapat dari jaringan periodontal 40 penderita dengan periodontitis agresif dan 40 penderita periodontitis kronis. Sampel berasal dari jaringan yang mengalami kelainan periodontal dan uji ekspresi protein NF- κ B (p50/p65) dilakukan secara imunohistokimia. Uji statistik yang digunakan adalah uji-t. *Hasil:* Pada NF- κ B (p50) diperoleh nilai t sebesar -12.041 dan signifikansi 0.000, dengan $\alpha = 5\%$ maka terdapat perbedaan bermakna ekspresi protein NF- κ B (p50) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis. Nilai estimasi OR untuk variabel ekspresi protein NF- κ B (p50) adalah 0,64 (sign. = 0,000). Artinya, jika ekspresi protein NF κ B (p50) responden bertambah 1 (satu) satuan, maka risiko terjadinya Periodontitis kronis menjadi 1,64 kali. Menggunakan Diagram Box plot memperlihatkan sebaran ekspresi protein NF- κ B (p50) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis yang tampak sangat jauh berbeda. Pada NF- κ B (p65) diperoleh nilai Z sebesar -7.137 dan signifikansi 0.000, dengan $\alpha = 5\%$ maka terdapat perbedaan bermakna ekspresi protein NF- κ B (p65) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis. Nilai estimasi OR untuk variabel ekspresi protein NF- κ B (p65) adalah 0,66 (sign. = 0,000). Artinya, jika ekspresi protein NF- κ B (p65) responden bertambah 1 (satu) satuan, maka risiko terjadinya Periodontitis kronis menjadi 1,5 kali. Menggunakan Diagram Box plot memperlihatkan sebaran ekspresi

protein NF- κ B (p65) antara penderita Periodontitis Agresif dan penderita Periodontitis Kronis yang tampak sangat jauh berbeda. *Kesimpulan: Ekspresi protein NF- κ B (p50/p65) lebih berpengaruh pada kejadian penderita Periodontitis Kronis, sehingga dapat digunakan sebagai marker untuk periodontitis kronis.*

Kata kunci: periodontitis, keradangan, NF- κ B p50 NF- κ B p65, transkripsi

INTRODUCTION

Nuclear factor-kappaB (NF- κ B) is a complex protein, a transcription factor that plays an important role in the regulation of immune system in response to inflammation and as regulators of gene expression. NF- κ B contributes to the activation of a wide variety of genes, such as proinflammatory cytokines, TNF- α , IL-1 and chemokines. In the inactive state of NF- κ B is located in the cytoplasm with the inhibitory protein NF- κ B (Jimi et al., 2007). The family NF- κ B consists of NF- κ B1 (p50/p105), NF- κ B2 (p52/p100), RelA (p65), RelB and c-Rel. Its classical form is a heterodimer consisting of p50 and p65 subunits.

NF- κ B serves to delay neutrophil apoptosis by altering the levels of Bcl2 protein. When an infection with pathogenic microorganisms, such as that in periodontitis, it activates the transcription factor NF- κ B via TLRs stimulation, which is expressed on innate immune system, including macrophages, dendritic cells (DCs) and mucosal epithelial cells (Beinke and Ley, 2004).

NF- κ B consists of a heterodimer between Rel polypeptides and protein p50, which acts to control the expression of many adaptive genes, such as MHC proteins and genes important for the regulation of the apoptotic process. NF- κ B resides in the cytoplasm in an inactive form along with a regulatory protein I- κ B. The interaction between LPS/TLR activates transcription factor nuclear factor- κ B (NF- κ B) that plays a role in activating the transcription of inflammatory mediators (Ohnishi et al., 2007; Takahashi et al., 2008). NF- κ B is activated by LPS in THP-1 cells, the transcription factor NF- κ B is associated with metabolic and inflammatory responses, including nuclear receptors, activators of protein (AP-1) and early growth response (EGR). Furthermore, it is suggested that IKK/NF- κ B is a target for the treatment of periodontitis disorders (White et al., 2000; Carpenter and O'Neill., 2007, Chen, 2007). As it was believed that the expression of NF- κ B (p50/p65) affects the occurrence of aggressive and chronic periodontitis, this study revealed observations of its expression in patients with those disorders.

MATERIALS AND METHODS

This research was an analytical observational studies, case control study design in patients experienced aggressive and chronic periodontitis. Tissue samples were taken from periodontal tissue affected by periodontitis. The population of the research was patients who came to Periodontics Department in Faculty of Dentistry Airlangga University

and had been diagnosed with aggressive or chronic periodontitis.

Informed consent was obtained from all subjects before commencement of the study. A total of 80 gingival tissue (40 aggressive periodontitis and 40 chronic periodontitis) were included in the present study. Gingival tissue were obtained surgically from periodontal tissue under local anesthesia.

The expression of NF- κ B (p50/p65) was detected by immunohistochemistry test. NF- κ B (p50/p65) was detected with biotin-labeled antibodies were and visualized with DAB-deminobenzidine.

RESULTS

Immunohistochemistry results to observe NF- κ B (p50) protein expression for patients with aggressive and chronic periodontitis can be seen in Table 1.

The differences in NF- κ B (p50) protein expression in patients with aggressive and chronic periodontitis are seen in Table 1. The mean protein expression in patients with aggressive periodontitis was 9.80 whereas in patients with chronic periodontitis it was 21.33. The mean value indicates that the protein expression of NF- κ B (p50) in aggressive periodontitis patients was significantly lower than protein expression in patients with chronic periodontitis. Using independent samples t-test with homogeneous variance data the obtained t value was -12.041 and 0.000 significance, using the $\alpha = 5\%$. Conclusively, there were significant differences in the protein expression of NF- κ B (p50) between patients with aggressive periodontitis and chronic periodontitis.

By using simple logistic regression analysis values, we obtained sign. 0,000, which means that the protein expression of NF- κ B (p50) influences the occurrence of aggressive periodontitis and chronic periodontitis. OR estimated values for the protein expression of NF- κ B (p50) was 0.64 ($p = 0.000$). If the protein expression of NF- κ B (p50) of the respondents increased by one unit, then the

Table 1. NF- κ B protein expression descriptive value (p50), in patients with aggressive (PA) and chronic periodontitis (PK)

Periodontitis	N	Mean	Std Deviation	Std Error Mean	t – test
PA	40	9,80	4,040	.639	t = -12,041
PK	40	21,33	4,509	.713	p = 0,000

risk for the occurrence of aggressive periodontitis would be 0.604 times or risk of chronic periodontitis would be 1.64 times higher than that with aggressive periodontitis. Differences in NF-κB (p50) protein expression is described using box plot diagram, which clearly shows the difference in the distribution of data between the NF-κB (p50) protein expression of patients with aggressive periodontitis and chronic periodontitis.

Figure 1 shows that the distribution of NF-κB (p50) protein expression between patients with aggressive periodontitis and chronic periodontitis were very much different.

Immunohistochemical Examination of Periodontal Tissues

In figure 2, NF-κB (p50) protein expression of periodontal tissues samples in aggressive and chronic periodontitis were analyzed by immunohistochemistry method using peroxidase labeled at 400x magnification, suggesting that NF-κB (p50) is expressed and the picture it appears as having brown color (arrow).

Protein expression of NF-κB (p65) patients with aggressive periodontitis and chronic periodontitis patients can be seen in Table 2.

To see the differences in NF-κB (p65) protein expression in patients with aggressive periodontitis and

chronic periodontitis patients, we obtained values as seen in Table 2. Median NF-κB (p65) protein expression in patients with aggressive periodontitis of 8.0 whereas in patients with chronic periodontitis it was 22.0. After distribution test using Kolmogorov-Smirnov on the data, statistical test value revealed 1.406 with significance 0.038, which means that the data were not normally distributed. To see the difference we used Wilcoxon Mann Whitney, revealing Z values of -7.137 and significance of 0.000. If α=5%, it can be concluded there are differences in the protein expression of NF-κB (p65) between patients with aggressive periodontitis and chronic periodontitis.

By using simple logistic regression analysis obtained value sign = 0.000, which means that NF-κB (p65)

Table 2. NF-κB protein expression descriptive value (p65) in patients with aggressive periodontitis (PA) and chronic periodontitis (PK)

Periodontitis	N	Mean Rank	Median	Wilcoxon Mann Whitney
PA	40	22.00	8	Z = - 7,137
PK	40	59	21	p = 0,000

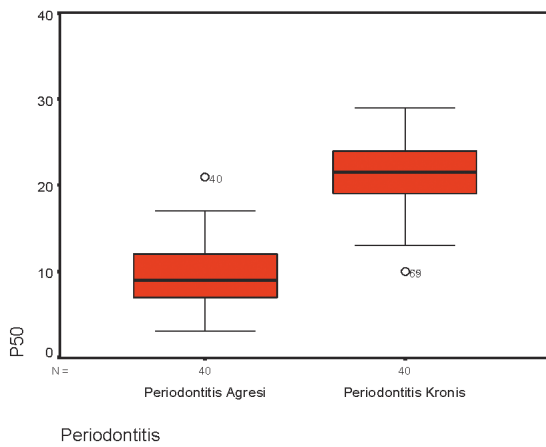


Figure 1. Box plots of NF-κB (p50) protein expression in aggressive and chronic periodontitis

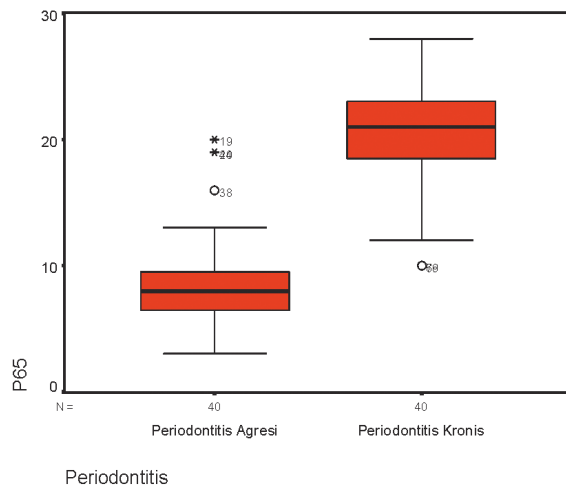


Figure 3. Box plots of NF-κB (p65) protein expression in aggressive and chronic periodontitis patients

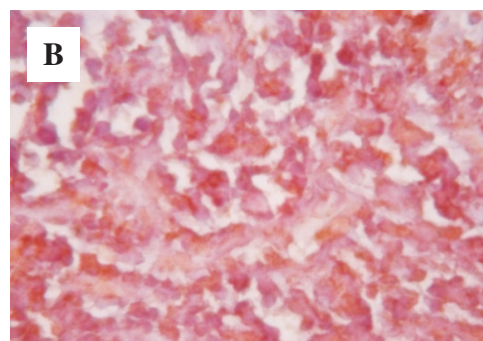
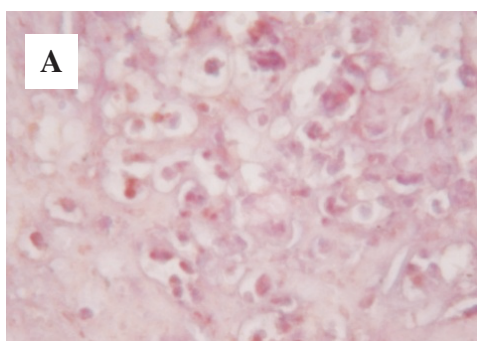


Figure 2. NF-κB (p50) protein expression (arrow) of periodontal tissue with DAB peroxidase immunohistochemical staining, magnification 400x. (A) aggressive periodontitis, (B) chronic periodontitis

protein expression influences the occurrence of aggressive periodontitis and chronic periodontitis. OR estimated values of NF- κ B (p65) protein expression was 0.659 ($p = 0.000$). If NF- κ B (p65) protein expression of the respondents increases by one unit then the risk for aggressive periodontitis increases 0.66 times or the risk of chronic periodontitis to increased 1.5 times higher than that of aggressive periodontitis. Differences in protein expression of NF- κ B (p65) described using Box plot diagram shows clearly data distribution on the difference of NF- κ B (p65) protein expression in patients with aggressive and chronic periodontitis.

Figure 3 shows p65 protein expression distribution between patients with aggressive and chronic periodontitis is very much different.

Immunohistochemical Examination of Periodontal Tissues

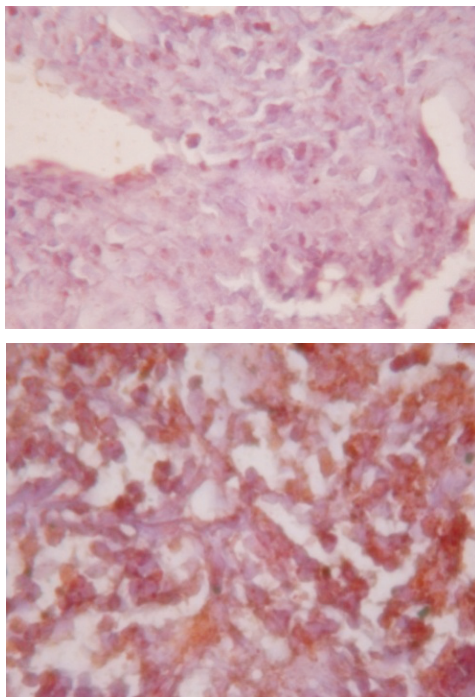


Figure 4. NF- κ B (p65) protein expression (arrows) of periodontal tissue with DAB peroxidase immunohistochemical staining, magnification 400x. (A) aggressive periodontitis, (B) Chronic Periodontitis

OR estimates for NF- κ B (p65) protein expression was 0.738 ($p = 0.03$). This indicates that if NF- κ B (p65) protein expression of the respondents increases by 1 (one) unit, then the risk of aggressive periodontitis would be 0.738 times or if NF- κ B (p65) protein expression of the respondents increases by 1 (one) unit, then the risk of chronic periodontitis will increase to 1,355 times higher.

DISCUSSION

Nuclear Factor- κ B (NF- κ B) is a transcription factor that bridges the innate immune system with the adaptive

immune system, and is essential for the detection of activation of innate immune response because their activation is associated with the TLR2/TLR4 receptors in the innate immune system (Abbas and Lichtman, 2007). NF- κ B activity is very dependent on cytoplasm movement to the nucleus and the cell's response to stimuli. NF- κ B is a homodimer or heterodimer type, consisting of RelA (p65), c-Rel, RelB, NF- κ B1 (p50/p105), NF- κ B2 (p52/p100). The family p65, c-Rel and RelB contain C-terminal transcriptional activation domains (TAD) which plays an important role in the stimulation of the target gene expression, whereas p50 and p52 are formed as large proteins but not have the TAD in which p50 and p52 can act as a factor transcription as homodimers.

The research was conducted on a sample of patients with aggressive and chronic periodontitis due to various periodontal pathogens in the oral cavity, so that tolerant endotoxin may occur for the presence of clinical manifestations. In periodontitis bacteria that play a role are the *P. gingivalis* and *A. actinomycetemcomitans* in which LPS activates germ cells using TLR2 pathway and/or TLR4. The interaction between LPS/TLR will activate NF- κ B which plays a role in activating inflammatory mediators transcription (Ohnishi et al., 2007; Takahashi et al., 2008). Secretion of cytokines TNF- α , IL-1 β and IL-6 will be inhibited only by TLR4 in response to LPS, not by TLR2. Competitive nature between *A. actinomycetemcomitans* and *P. gingivalis* has a role in periodontal tissue damage. When *A. actinomycetemcomitans* is more dominant, there will be aggressive periodontitis. Whereas, when the dominant is *P. gingivalis*, the clinical situation will be chronic periodontitis.

Homodimer complex of NF- κ B (p50) and NF- κ B (p65) proteins will suppress the expression force of TLR responses, thereby inhibiting the production of proinflammatory cytokines (Sun et al., 2008; Jotwani, et al., 2010). The formation of a heterodimer complex of NF- κ B (p50) and NF- κ B (p65) is the most common formation in mammalian cells. NF- κ B proteins present in human cells cytoplasm and in inactive state and bound to a protein known as the NF- κ B. NF- κ B signal plays an important role in several aspects of the activity of osteoclasts, osteoblasts and chondroblast (Boyce et al 2010). NF- κ B transcription factor plays a role in the expression of various genes that play a role in the regulation of immune response and inflammation, proliferation, tumorigenesis and cell survival. NF- κ B acts to regulate genes such as TNF- α and IL-1 β and directly enhances the inflammatory response. Activation of NF- κ B by the B cell receptor and T cells are also required to stimulate antigen proliferation, cytokine production and survival of B and T cells (Jimi et al., 2007).

Improved cascade pathway of NF- κ B kinase results in an increase of NF- κ B (p105/p50) transcription factor. This situation is also found in the research by Kaisho and Akira (2006) and Abbas et al (2007). Immunohistochemical examination on NF- κ B (p50/p65) in patients with periodontitis appears to increase as compared to that in

healthy samples. NF- κ B signaling inhibition can be done through IL-4 inhibition mechanism by osteoclastogenesis process. Most of the existing genes are removed or depreciated due to the presence of inactive IKK/ NF- κ B. The involvement of NF- κ B in patients with arthritis is apparent in differentiation and osteoclasts activity disorders, where NF- κ B as factor that has a major influence on the biological response. (Beinke and Ley, 2004).

The activation of different TLR will also result in the activation of NF- κ B and cytokine products through TLR2 and TLR4 in neutralizing antibodies in that it seems that NF- κ B (p50) and NF- κ B (p65) protein expression is increasing in chronic periodontitis. The results of this study support the research by Jotwani et al., (2010) which states that NF- κ B (p50) and NF- κ B (p65) were significantly higher in patients with chronic periodontitis.

NF- κ B activity is found in NF- κ B (p50/p65) heterodimer present in the cytoplasm in an inactive state and binds to NF- κ B. NF- κ B protein degradation causes the release of p50/p65 heterodimer, and put the protein into the cell nucleus. NF- κ B (p50) homodimer is a repressor for transcriptional process and as a mediator in endotoxin tolerans. Increased NF- κ B (p50) homodimer in inflammatory cytokines with *P. gingivalis* LPS stimulation will suppress immune response stimulation. Such circumstances is in accordance with the results of this study, which found that NF- κ B (p50) differed significantly between patients with aggressive periodontitis and chronic periodontitis. Patients with chronic periodontitis showed a higher value. This situation shows that *P. gingivalis* LPS, which is the cause of chronic periodontitis, activates NF- κ B (p50).

NF- κ B (p50) is stimulated through TLR2 pathway, so what happens is a clinical state of chronic periodontitis in which NF- κ B (p50) serves as an important protein involved in the regulation of different transcription process, while NF- κ B (p65) regulates the activation of NF- κ B. NF- κ B (p50) and NF- κ B (p65) are respectively in the form of homodimer, while the frequently found common form is in the form of heterodimer to become NF- κ B (p50/p65) (Jimi et al. 2007; Espinosa et al., 2008).

Statistical analysis showed that NF- κ B (p50/p65) has effect on the occurrence of chronic periodontitis. This is consistent with the logistic regression analysis on NF- κ B (p50) and NF- κ B (p65) in this study, in which the risk of towards chronic periodontitis was 1.6 times and 1.5 times. According to research conducted by Jotwani et al, (2010), NF- κ B (p50/p65) increased after stimulation with *P. gingivalis* LPS compared with stimulation with *E. coli*. *A. actinomycetemcomitans* has the same potential with *E. coli*. *E. coli* LPS stimulates inflammation through TLR4, whereas *P. gingivalis* LPS via TLR2 and/or TLR4 to activate the cell. The secretion of inflammatory cytokines TNF- α , IL-1 β and IL-6 would be inhibited by TLR4 antibody in response to *P. gingivalis* stimulation. Based on the classification, *P. gingivalis* is a periodontal pathogenic bacteria, which are weak in stimulating the activity of the immune response. NF- κ B (p50) has immunosuppressive

ability, so that when there is a change, it would affect the NF- κ B (p50) homodimer complex that is expressed in tissues from patients with chronic periodontitis. Activation of dendritic cells by *P. gingivalis* LPS will increase p50/p65 ratio in patients with chronic periodontitis. This study showed that increased p50/p65 occurred in chronic periodontitis respondents. In samples of periodontitis patients, it was known that periodontal pathogenic bacteria are varied, so it can be concluded that patients with chronic periodontitis were affected by *P. gingivalis* so that NF- κ B (p50/p65) was activated through TLR4. Good response to treatment was detected in NF- κ B activated through (p65) protein phosphorylation (Espinosa et al., 2008).

CONCLUSION

Based on this study, it appears that patients with chronic periodontitis show increased NF- κ B (p50/p65) protein expression compared with protein expression in patients with aggressive periodontitis. Through NF- κ B pathway, chronic periodontitis is more likely caused by *P. gingivalis* bacteria.

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