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Majalah Kedokteran Gigi



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Research Report

Interleukin-1 β expression on periodontitis patients in Surabaya

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ABSTRACT

Background: Periodontal disease, commonly known as periodontitis is an infectious disease which has multifactorial etiologic factors. It may affect everybody in any ages with no gender nor sex predilection and usually can be detected under routine clinical examination. This disease is a manifestation of local factors, host factor and environmental factors, resulting in periodontal tissue damage which may cause tooth mobility and tooth loss. Interleukin-1 is a pro-inflammatory protein which functions primarily as inflammatory mediator in host innate immune responses. IL-1 is a regulator, affecting many biological activities including proliferation, development, homeostasis, regeneration, repair and inflammation which contribute to tissue damage and alveolar bone resorption.

Purpose: This research was aimed to reveal the basic pathogenesis of periodontitis and could determine the future definitive treatment for patients with periodontitis. **Methods:** Data were obtained from 40 patients with aggressive periodontitis and 40 patients with chronic periodontitis. Samples were collected from periodontal tissue patients and protein expression of IL-1 β was performed with immunohistochemistry. **Results:** Most female patient suffer aggressive periodontitis and chronic periodontitis. The datas were analyzed with t-test. The t values result was -8623, significance 0.001, with $\alpha = 5\%$, which indicated there was significant difference in IL-1 β expression between aggressive and chronic periodontitis. The box plot diagram showed marked difference in distribution of protein expression of IL-1 β between patients with aggressive periodontitis and chronic periodontitis. With a regression equation, it might be concluded that the protein expression of IL-1 β might affect the incidence of aggressive periodontitis and chronic periodontitis. The OR value was calculated for 0.746 (sign. = 0.001), which indicate each increment of one unit protein expression of IL-1 β will lead the risk for Aggressive periodontitis 0.746 times higher or if the protein expression of respondents increased one unit, the risk of chronic IL-1 β periodontitis may be 1.34 times higher. **Conclusion:** This study elucidated that the elevation proteins expression of IL-1 β in patients with chronic periodontitis demonstrated this cytokine as an indicator of inflammation.

Key words: Aggressive periodontitis, chronic periodontitis, interleukin-1 β

ABSTRAK

Latar belakang: Penyakit periodontal yang biasa dikenal dengan periodontitis adalah penyakit infeksi, yang disebabkan oleh berbagai faktor, dapat menyerang setiap orang tanpa membedakan usia dan gender serta mudah ditemukan pada pemeriksaan klinis oleh seorang dokter gigi. Penyakit ini merupakan manifestasi dari interaksi antara faktor lokal dengan faktor lingkungan, yang berakibat pada kerusakan jaringan periodontal, dapat mengakibatkan terjadinya kegoyangan gigi hingga tanggalnya gigi. Interleukin-1 merupakan protein pro-inflamatori dengan fungsi utama sebagai mediator respon inflamasi pejamu pada sistem imunitas innate. Interleukin-1 merupakan regulator, dimana memainkan peranan pada sejumlah aktivitas biologis termasuk proliferasi, pengembangan, homeostasis, regenerasi, repair dan peradangan berperan pada kerusakan jaringan ikat serta resorpsi tulang alveolar.

Tujuan: Penelitian ini bertujuan untuk menentukan dasar patogenesis periodontitis dan dapat digunakan sebagai dasar perawatan penderita periodontitis pada masa mendatang. **Metode:** Data penelitian didapat dari 40 penderita dengan periodontitis agresif dan 40 penderita periodontitis kronis. Sampel berasal dari jaringan yang mengalami kelainan periodontal dan uji ekspresi protein IL-1 β dilakukan secara imunohistokimia. **Hasil:** Penderita yang mengalami kelainan pada penelitian ini sebagian besar perempuan baik periodontitis agresif maupun periodontitis kronis. Uji statistik yang digunakan adalah uji-t diperoleh nilai t sebesar -8.623 dan signifikansi 0.001, dengan $\alpha = 5\%$ maka terdapat perbedaan bermakna ekspresi protein IL-1 β antara penderita periodontitis agresif dan penderita periodontitis kronis. Diagram box plot memperlihatkan sebaran ekspresi protein IL-1 β antara penderita periodontitis agresif dan penderita periodontitis kronis yang tampak sangat jauh berbeda. Ekspresi protein IL-1 β berpengaruh pada kejadian

penderita periodontitis agresif dan penderita periodontitis kronis, dengan bentuk persamaan regresi. Nilai estimasi OR untuk variabel ekspresi protein IL-1 β adalah 0,746 (sign. = 0,00). Artinya, jika ekspresi protein IL-1 β responden bertambah satu satuan, maka risiko terjadinya periodontitis agresif menjadi 0,746 kali atau jika ekspresi protein IL-1 β responden bertambah satu satuan, maka risiko terjadinya periodontitis kronis menjadi 1,34 kali. **Kesimpulan:** Ekspresi protein IL-1 β yang meningkat pada penderita periodontitis kronis menunjukkan bahwa sitokin ini merupakan indikator pada keadaan peradangan.

Kata kunci: Periodontitis agresif, periodontitis kronis, interleukin-1 β

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INTRODUCTION

Periodontal disease, commonly known as periodontitis, is an infectious disease with multifactorial etiologic factors which may cause of periodontal tissue destruction. Periodontitis is a multifactorial disease with bacterial infection as its main etiologic factor, the incidence and severity of illness were determined by the host response to infection, but until now, clinical diagnosis often remains unclear so that treatment often gives unsatisfactory results, which may indicate the necessity of exploration in molecular level.

As a marker of active inflammation, the interleukin-1 (IL-1) plays important role in immunological processes of inflammatory response. Interleukin-1 is a pro-inflammatory protein produced by macrophages and endothelial cells platelets which functions mainly as a mediator of inflammatory responses in host innate immunity system. IL-1 is a multifunctional cytokine which influences most of the inflammatory cell types, is a cytokine that is produced during inflammatory processes and involved in connective tissue destruction from the early phase. IL-1 may function as a regulator, which plays a role in a number of biological activities including proliferation, development, homeostasis, regeneration, repair and inflammation.^{1,2} Interleukin 1 exists in two forms, namely IL-1 α and IL-1 β . IL-1 β is a potent inflammatory cytokine involved in many important cellular functions, such as proliferation, activation, and differentiation and is an important component of the innate immune response moreover it; also induces the chemotactic of leukocytes by stimulating the induction of IL-8 and activating neutrophils for phagocytosis and degranulation. It is also found that IL-1 β not only modulates the inflammation in gingival epithelial cells but also regulates the production of other inflammatory cytokines including IL-8.³

IL-1 β is a potent substance that has catabolic effect on bone 10 times higher compared to IL-1 β . The biological impact of IL-1 depends on the amount of cytokines that are released by the body. At low levels, it functions as a local inflammatory mediators, while in high level, IL-1

may enter into the circulation and accelerates the endocrine activity.⁴⁻⁶ Interleukin-1 may function as a regulator, which plays a role in a number of biological activities including proliferation, development, homeostasis, regeneration, repair and inflammation.^{1,2}

IL-1 β stimulate various cell types to produce connective tissue catabolic and mediators of bone resorption such as IL-1, IL-6, TNF α , PGE₂ and matrix metalloproteinase.⁷ IL-1 β and tumor necrosis factor (TNF) are cytokine that possess the ability to cause bone destruction.^{8,9}

Based on those previous studies above, identification of IL-1 β may be used as a risk factor for periodontitis. This research was aimed to reveal the basic pathogenesis of periodontitis and could determine the future definitive treatment of patients with periodontitis.

MATERIALS AND METHODS

This research was an analytical observational study, case control study design in patients who experienced aggressive and chronic periodontitis. Complete medical and dental histories were taken from all subjects. None of subjects had a history of systemic disease and had received antibiotics or other medication or periodontal treatment within the past 4 months. Informed consent was obtained from the patients and the protocol was approved by the Ethical Committee of Faculty of Dentistry Airlangga University. Tissue samples were taken from periodontal tissue that was affected by periodontitis.

The population of this research was patients who came to Dental Hospital of Faculty of Dentistry Airlangga University for 10 month, and had been diagnosed with aggressive periodontitis (AP) or chronic periodontitis (CP). The patients were diagnosed according to clinical and radiographic criteria as AP (n = 40) and CP (n = 40).

The parameters were shown as mean $\pm$ standard deviation and analyzed by t test. The expression of IL-1 β was detected by immunohistochemistry method. IL-1 β was detected with biotin-labeled antibodies and visualized with DAB-deminobenzidine.

RESULTS

The data that were eligible for analysis consisted of 40 patients with aggressive periodontitis and 40 patients with chronic periodontitis. The following diagram showed patient distribution with aggressive periodontitis and chronic periodontitis during the study which was distinguished by gender (Figure 1).

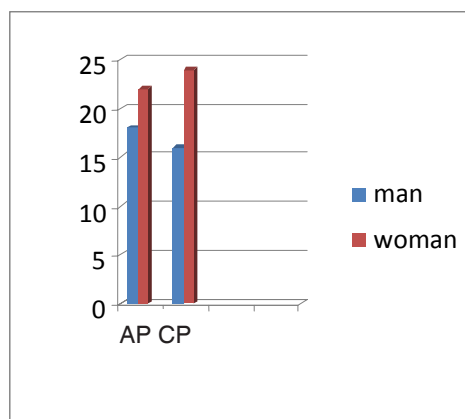


Figure 1. Frequency distribution column, based on gender, aggressive and chronic periodontitis (AP and CP).

Patients who had periodontal disease, either aggressive periodontitis or chronic periodontitis, in this study were mostly women. This situation could be seen from 22 samples (55%) of patients with aggressive periodontitis and 24 samples (60%) of patients with chronic periodontitis were women (Table 1).

Table 1. Descriptive value of protein expression of IL-1 β , people with aggressive periodontitis (AP) and chronic periodontitis (CP) in Surabaya

	Periodontitis	N	Mean	Standard Deviation
Protein Expression IL-1 β	AP	40	9.32	6.12
	CP	40	20	4.87

Protein expression of IL-1 β patient with aggressive periodontitis and patient with chronic periodontitis could be seen in table 1. The following were data values based on examination of 40 patients with aggressive periodontitis and 40 patients with chronic periodontitis. The independent samples t-test with variances homogeneous data was performed to see the difference in protein expression of IL-1 β between the patient with aggressive periodontitis and chronic periodontitis. The t-test values were obtained at -8623 and significance of 0.00. If α value was set on 5%, there was significant difference in protein expression of IL-1 β patients with aggressive periodontitis and chronic periodontitis patients.

The average of protein expression in patients with aggressive periodontitis was 9.32, while in patients with chronic periodontitis was 20. Patient with chronic periodontitis had significantly higher IL-1 β compared to patients with aggressive periodontitis (Figure 2).

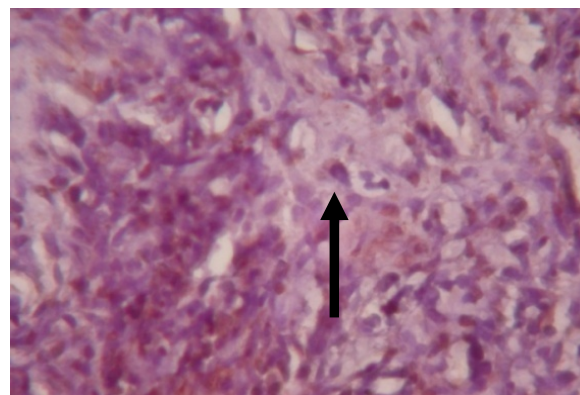


Figure 2. Expression of IL-1 β protein (arrow) in periodontal tissue by immunohistochemistry staining with peroxidase DAB and 400 $\times$ magnification.

The difference of protein expression may be easily explained with the box plot diagrams expression of IL-1 β (Figure 3). The box plots clearly demonstrated the data distribution of protein expression between patients with aggressive periodontitis and chronic periodontitis.

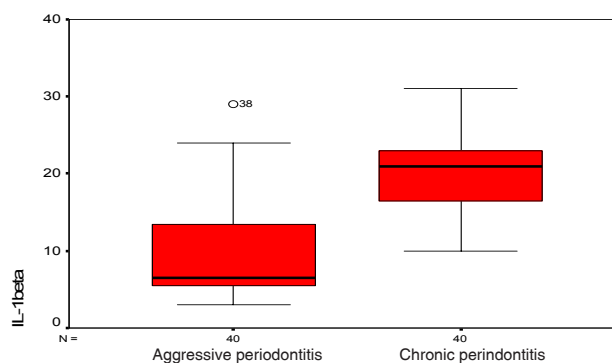


Figure 3. Box plots of IL-1 β protein expression.

Box plot diagram demonstrated the difference of protein expression of IL-1 between patients with aggressive and chronic periodontitis. With a regression equation, it might be concluded that the protein expression of IL-1 β might affect the incidence aggressive periodontitis and chronic periodontitis.

The OR value for protein expression of IL-1 β was estimated 0.746 (sign = 0.001). It meant each increment of one unit protein expression of IL-1 β , the risk for aggressive periodontitis was 0.746 times higher or if the protein expression of respondents increased one unit, the risk of chronic periodontitis may be 1.34 times higher.

DISCUSSION

Epithelial cells are one of the first lines of defense against pathogens, and although these cells are not as specialized as professional phagocytes in dealing with pathogens, they may play a sentinel role. The cells may use the extracellular secretion of IL-1 β to induce other neighboring epithelial cells in autocrine manner to help amplify the release of inflammatory, chemokine, and antimicrobial molecules.

Based on sample characteristics, it seemed that there was no gender predilection, because men and women had the same representation. However, this study showed that periodontitis was more likely to be found in women, possibly because women visit dentists more often than men and other possibilities were the age of puberty was earlier, hormonal changes during menstruation and pregnancy might affect the host and might also aggravate the periodontitis. It was corresponded with the previous studies from Bret *et al.*,¹⁰ and Guzeldemir *et al.*,¹ which found that most of the study sample were woman.

The IL-1 is one of cytokine that was functioned in periodontal tissues, this kind of interleukin is more dominant as IL-1 β which has various pro-inflammatory capabilities, produced during inflammation and involved from the early stage of connective tissue damage and was considered as an important role in the pathogenesis of periodontitis. IL-1 β secretion is induced soon after microbial invasion, there for it could be hypothesized that IL-1 β might play an important role in the induction of other inflammatory cytokines.³ IL-1 β stimulates various cells types to produce connective tissue and catabolic mediators of bone resorption such as IL-6, TNF α , PGE₂ and matrix metalloproteinase. Level of cytokines which was secreted in response to the bacteria may explain individual response differences in sensitivity to and severity of the periodontal disease.^{7,11} The IL-1 β expression is associated with the severity of inflammatory diseases including periodontitis, which then would quickly encode pro-inflammatory proteins by transcription factors like nuclear factor- κ B (NF- κ B).¹²

This research demonstrated an increase in protein expression of IL-1 β in chronic periodontitis group compared with aggressive periodontitis, which was corresponded with the research conducted by Gursoy *et al.*,¹³ who also found that levels of IL-1 β were high in the periodontitis group compared with healthy control samples. Levels of IL-1 β on periodontal tissues might increase according to the severity of illness, which was related to the presence of periodontal pathogen.³ It shall be noted that gingival inflammation may affect the secretion of IL-1 β . Research conducted by Toker *et al.*,⁶ found that IL-1 β was significantly elevated in periodontal tissue and gingival fluid of the inflamed side compared to the healthy side. In vivo and in vitro studies had shown that in patients with periodontitis and other infectious diseases, IL-1 β which was produced was involved in the inflammatory process and aimed to eliminate the periodontal pathogen from host.¹⁴

High level of IL-1 will stimulate the secretion of CD4 + T-cells which will increase the secretion of specific antibodies. IL-1 also will stimulate the production of IL-2 through the Th1 cell, which in turn will stimulate CD8 + T cells, and suppress the activation of polyclonal B-cell and the production of antigen-specific antibodies. T-helper type 1 (Th1), Th2 and monocyte derived cytokines in gingival tissue and gingival crevicular fluid (GFC) was involved in periodontal inflammation. The IL-1 β (cytokine) imbalance may affect the periodontal supporting bone and collagen tissue destruction in patients with periodontitis. Interleukin 1 β may suppress IL-10 production in periodontal ligament cells, which mean that there is interaction between IL-1 β and IL-10, and the dynamic interaction between pro-inflammatory and anti-inflammatory cytokines plays an important role in the pathogenesis of periodontal defects. Cytokines determines the local defense against bacterial endotoxins and maintain homeostasis in periodontal tissues.^{15,16} Periodontopathogen bacteria that cause periodontitis produce lipopolysaccharide, which was potential in stimulating host response and contributing to tissue damage. It also stimulated macrophages to release IL-1 β and TNF. This cytokine had the ability to stimulate bone destruction.^{9,17} While neutrophils and the other cells that serve as protection were not working properly and at the end would cause bone destruction.¹⁸

As a multifunctional pro-inflammatory mediator with a crucial role in the regulation of inflammatory reactions, the observation that IL-1 β can act on large number of cells, like fibroblast, chondrocytes, bone cells, neutrophils and lymphocyte suggests that periodontal destruction and repair in periodontitis may in part be associated with this cytokine. It may be concluded that IL-1 β as a marker of active inflammation will contribute in pathological inflammatory response and periodontitis, as an active inflammation, will elevates the level of protein expression. The results of this study may be can be used as a further reference on therapeutic modality to actively control the inflammation and tissue damage in various diseases.

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