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Research Report

The correlation between immunoexpression of estrogen receptor and the severity of periodontal disease

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ABSTRACT

Background: *The decreased level of estrogen during menopause may be one of the risk factors of periodontal disease. The influence of estrogen to periodontal tissue disturbance is mediated by the presence of estrogen receptor on tissue. The precise mechanism how the estrogens mediate this effect is still unclear.* ***Purpose:*** *The aim of this study was to determine the correlation between estrogen receptor α and β on the periodontal pocket of women who had severe chronic periodontitis measured based on the periodontal pocket depth.* ***Methods:*** *Twenty four periodontitis patients from menopausal and productive women according to the criteria were examined upon her periodontal status and immunoexpression of estrogen receptor α and β on their periodontal pocket wall.* ***Results:*** *The result showed that in the menopausal and productive women, immunoexpression of estrogen receptor α and β was not correlated with the periodontal pocket depth (p>0.05). However, the pocket depth seemed to show higher correlation with immunoexpression of estrogen receptor α than that with estrogen receptor β, r=0.37 vs. r=0.12 for menopausal women, and r=41 vs. r=0.11 for productive women.* ***Conclusion:*** *It was concluded that no significant correlation was found between the estrogen receptor and periodontal pocket depth both on menopausal and productive women, presumed that estrogen has little role in the severity of periodontitis based on periodontal pocket depth. However, the estrogen receptor α has valuable effect on the severity of periodontal disease more than the estrogen receptor β.*

Key words: *Estrogen, estrogen receptor α and β, periodontal disease/severity, periodontal pocket*

ABSTRAK

Latar belakang: *Berkurangnya kadar estrogen pada masa menopause merupakan salah satu faktor resiko penyakit periodontal. Peran estrogen dalam kerusakan jaringan periodontal dimediatori oleh reseptor estrogen α dan β yang terdapat dalam jaringan. Akan tetapi, mekanisme estrogen mempengaruhi efek ini sampai saat ini masih belum jelas.* ***Tujuan:*** *Penelitian ini bertujuan untuk menentukan korelasi antara reseptor estrogen pada poket periodontal wanita menopause penderita periodontitis kronis dengan keparahan periodontitis yang ditentukan berdasarkan kedalaman poket.* ***Metode:*** *Dilakukan pemeriksaan status periodontal dan immunoekspresi reseptor estrogen α dan β pada dinding poket periodontal dari 24 wanita menopause dan belum menopause penderita periodontitis yang sesuai dengan kriteria yang telah ditetapkan.* ***Hasil:*** *Hasil mendapatkan bahwa immunoekspresi reseptor estrogen α dan β tidak berkorelasi dengan kedalaman poket periodontal wanita menopause dan belum menopause (p>0,05). Meskipun demikian, kedalaman poket periodontal tampak lebih berkorelasi dengan reseptor estrogen α daripada dengan reseptor estrogen β, dengan nilai r=0,37 vs r=0,12 pada wanita menopause, dan r=0,41 vs r=0,11 pada wanita belum menopause.* ***Kesimpulan:*** *Dapat disimpulkan bahwa tidak terdapat korelasi yang signifikan antara reseptor estrogen dan kedalaman poket periodontal wanita menopause dan belum menopause sehingga diduga bahwa estrogen mempunyai sedikit pengaruh pada keparahan periodontitis. Meskipun demikian, reseptor estrogen α tampak lebih berperan terhadap keparahan penyakit periodontal dibandingkan reseptor estrogen β.*

Kata kunci: *Estrogen, reseptor estrogen α dan β, keparahan penyakit periodontal, poket periodontal*

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INTRODUCTION

In women, hormonal changes throughout lifetime influence their periodontal tissue health. Estrogen can modulate some cytokines that regulate the host response to infection.[1] A study previously has reported that the periodontal disease of menopausal women is more severe than reproductive women.[2] Estrogen deficiency at menopausal women could be suspected to be an aggravating factor in periodontal disease.[3] Estrogen deficiency is related with the increase of alveolar bone resorption,[3] and periodontal loss attachment leading to severity of periodontal disease that can cause dental loose.[4,5]

The influence of estrogen to periodontal tissue disturbance is mediated by the presence of estrogen receptor on tissue. Estrogen receptor (ER) is a protein mediating estrogen biological effect on cells.[6] Two kinds of ER are identified in human being, ER α and ER β.[7] Distribution of ER α and β is different, although, it is occasionally overlapped.[8]

In oral cavity, the immunoexpression of ER α had been detected on odontoblast, endothelial, Schwan cells of pulp tissue and show no difference based on the age and sex.[9] The periodontal ligament cells showed the immunoexpression of ER β.[10] Leimola-Virtanen *et al*.[11] had demonstrated that there was no significant difference of mRNA expression of ER in the oral mucosa of menopausal women and nineteen year old women. Presently, the presence of ER α and β in periodontitis patients has not been discussed. The precise mechanism by which estrogens mediate these effects is still unclear. It was hypothesized that the decreased of estrogen on menopausal women will decrease the immunoexpression of estrogen receptor so that the severity of periodontitis will increase. The specific of ER which mediate the estrogen effect in periodontitis is unknown. The purpose of this study is to determine the immunoexpression of estrogen receptor α and β on the periodontal pocket of menopausal women which had chronic periodontitis and to know the correlation between ER α and β with the severity of periodontitis measured based on the periodontal pocket depth.

MATERIALS AND METHODS

This study was a cross-sectional observation. The material used was the result of scrapping periodontal pocket in the menopausal women and productive women with periodontitis coming to The Periodontal division in the Oral and Dental Clinic Dr. Hasan Sadikin Hospital Bandung. The patients within the inclusion criteria were provided informed consent. The inclusion criteria of the sample were a) Menopausal women, women having had no menstruation for 12 months respectively, getting no estrogen replacement therapy and having no diabetes mellitus history. b) Productive women (as a control), above 30 years old, getting routine menstruation monthly, not pregnant, not using oral contraception and having no diabetes mellitus history.

Subsequently, the patients were examined upon their periodontal tissue including clinical gingival examination, bleeding on probing, and probing depth. Probing was conducted using periodontal probe inserted in the gingival sulcus in direction with tooth axis. Periodontal pocket depth was determined from the distance to which a tip of periodontal probe penetrates into the pocket. Periodontitis was marked by gingival inflammation, probing depth more than 3 mm and clinical attachment loss.

The periodontal lateral pocket wall was scrapped using tooth pick made of smooth bamboo split into two parts. Periodontal pocket scrapping was conducted twice for each examination of ER α and β. The result of periodontal pocket scrapping was subsequently smeared on the object glass and fixed in 95% alcohol solution. Furthermore, immunocytochemical staining was conducted using antibody of ER α and β in the Laboratory of Pathology Anatomi Dr. Hasan Sadikin Hospital Bandung. Briefly, the rabbit polyclonal antibody MC20 from Santa Cruz Biotechnology (Santa Cruz, USA) was used for detection of ER α, and the chicken polyclonal ER β 503 immunoglobulin Y for ER β.

The slides were immersed in PBS, incubated in 0.3% H_2O_2 to quench endogenous peroxidase. To block unspecific binding of secondary antibodies, slides were incubated in 100µl serum blocking reagent. Primary antibody of ER α antibody MC20 (Santa Cruz Biotechnology, 1:100 dilution in blocking solution, incubated one hour at room temperature) was added. The polyclonal ER β 503 immunoglobulin Y was diluted 1:100. After several washes, slides were incubated with a secondary biotinylated antibody (sc-2040, Santa Cruz Biotechnology, USA). Finally, streptavidin conjugated to horseradish peroxidase (DAKO, LSAB2 kit) was applied and visualized by incubated in 0.5% diaminobenzidine (DAKO). All slides were slightly counterstained with Mayer's hematoxylin, mounted and examined with light microscope. As the positive control of estrogen receptor α, invasive ductal carcinoma of breast was used, and hyperplasia prostate for positive control of estrogen receptor β, while for negative control, slides was incubated with normal serum. The immunostaining was assessed in a quantitative manner by dividing the number of cells which immunoreactivity with the number of total cells.[12] Statistical analysis was carried out using the Regression Analysis test to determine the correlation between immunoexpression of estrogen receptor and probing depth.

RESULTS

A total of 24 periodontitis patients from menopausal and productive women were examined. Menopausal group consisted of 13 women aged 58.08 years old in average (the age range was 50–70 years old) and menopausal duration was 5.85 years in average (the range was 1–16 years). The examination of periodontal pocket depth showed 4.62 mm in average with the deepest was 7 mm. The productive group consisted of 11 women aged 40.73 years old in

Table 1. Periodontal pocket depth and immunoexpression of estrogen receptor of the menopausal women and productive women

Sample groups	N	Age	Periodontal Pocket	Menopause Duration	Immuno-expression ER α	Immuno-expression ER β
		(years)	(mm)	(years)	(%)	(%)
Menopausal Women	13	58.08 (SD=6.06)	4.62 (SD=1.19)	5.85 (SD=4.3)	27.16 (SD=15.69)	14.72 (SD=16.78)
Productive women	11	40.73 (SD=7.04)	4.27 (SD=1.01)	-------	32.11 (SD=18.22)	13.51 (SD=10.48)

average (the range was 30–47 years old). The result of periodontal pocket depth examination was 3-6 mm with 4.27 mm in average. The result of this study is presented in the table 1.

The study showed the presence of immunoexpression of ER with brown-colored in nucleus and or cytoplasm. The intensity of nuclear brown-colored was stronger than that in the cytoplasm. Likewise, the result of immunocytochemical smear using antibody of ER β showed stronger intensity of nuclear brown color than that in the cytoplasm (Figure 1).

The result of regression analysis test between the immunoexpression of ER and periodontal pocket depth on the menopausal and productive group was presented in table 2. It showed that the immunoexpression of ER α and β was not correlated with periodontal pocket depth ($p > 0.05$) both on the menopausal and productive women.

Table 2. Regression Analysis test between the periodontal pocket depth and immunoexpression of estrogen receptor

	Probing depth	
	Menopausal Women	Productive Women
ER α	r = 0,37	r = 0,41
ER β	r = 0,12	r = 0,11

DISCUSSION

Periodontitis is a multi-factorial infectious disease. Although the presence of bacteria on the plaque is considered as the main factor of periodontitis, the systemic factors may play an important role in its initiation and progression. There are some factors that may influence the

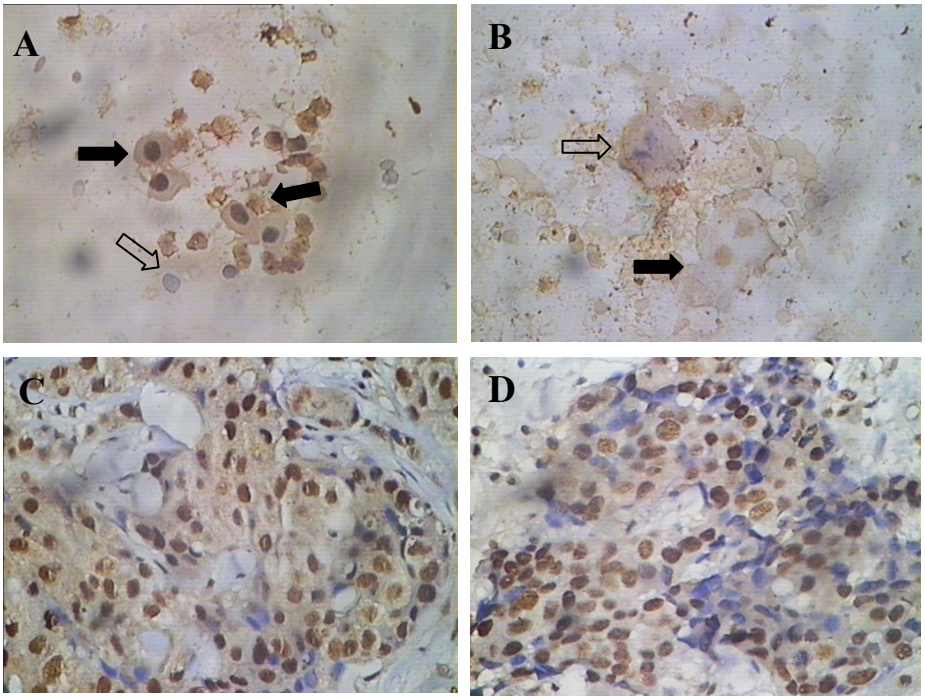

Figure 1. Immunoexpression of estrogen receptor α and β on the periodontal pocket, A) Immunoexpression of estrogen receptor α in the nucleus and or in the cytoplasm (black arrow) and negative cells of estrogen receptor α (blank arrow), B) Positive cells of estrogen receptor β (black arrow) and negative estrogen receptor β, C) Positive control of estrogen receptor α in the breast invasive ductal carcinoma, D) Positive control of estrogen receptor β in the hyperplasia prostate.

extent and severity of periodontitis, including hormonal factors, genetic factors, smoking, emotional stress, nutrition, and degradation of immune response on HIV.[13] In menopausal women, the decrease of estrogen is correlated with the increase of loss attachment leading to the increase of periodontal disease severity.[3,5] This study demonstrated that the periodontal disease of menopausal women is more severe than reproductive women[3] and it correlated with the menopausal periode.[14]

The result of this study showed that cells from periodontal pocket smear expressed the ER α and β both on the menopausal and productive women through immunocytochemical method. Immunocytochemical method has been found favorable since many years ago for routine examination of ER. It was a simple, quick and moderately reliable technique to evaluate ER status.[15] Even though the number of cells examined is small but immunocytochemistry showed high sensitivity and specificity, short procedure time and did not need retrieval antigenic procedure.[16] Furthermore, the samples are simply collected, not invasive nor painful.

The presence of ER α and β in the periodontal pocket cells in this study suggests that estrogen has important role in the periodontal pocket. This result is in agreement with study conducted by Xie and Shu[17] that have found the expression of ER in the gingival tissues that might have relation to periodontitis of females. Periodontal pocket is a main clinical sign of the periodontal tissue destruction on the periodontitis due to specific periodontopathogen bacteria in the gingival sulcular area. These bacteria have ability to invade the gingival through sulcular epithelium and bring out their products into deeper periodontal tissue. This may induce the host response through bringing out inflammatory mediator that initiating inflammatory process on the periodontal tissue. Inflammatory mediator is produced by inflammatory cells, epithelial cells and gingival fibroblast. These mediators may stimulate fibroblast and macrophage to produce matrix metalloproteinase, collagenase and prostaglandin E_2 that cause breakage on collagen, glycoaminoglican and alveolar bone resulted in a periodontal pocket, clinical attachment loss and alveolar bone resorption.[18,19]

Estrogen play role in managing periodontal response to periodontopathogen bacteria.[1] The role of estrogen is to accelerate the wound healing process through increasing matrix deposition, accelerating epithelization, and reducing inflammatory response.[20] Estrogen also has been reported can induce cellular proliferation in gingival fibroblasts.[21]

Estrogen increases cellular proliferation, differentiation and sulcular epithelial keratinization.[22] Sulcular epithelial has a role as gingival defense toward bacterial invasion through proliferation, keratinization degree and continuous epithelial substitution. The continuous substitution on gingival epithelial cells may cause bacteria released from cellular surface of gingival epithelium. Mitotic activity of gingival epithelial cells is once in 24 hours periodically.[23] Estrogen also inhibits the release of inflammatory mediators by decelerating gene transcription through estrogen receptor α and β.[24] Several studies had reported that estrogen inhibits the release of cytokine proinflammatory IL1,[24] IL-I ß, IL-6,[25] PGE2, MMP-9[26] and TNF-α.[27] Consequently, the periodontal pocket formation, clinical attachment loss and alveolar bone resorption can be inhibited.

Although these study have demonstrated the immunoexpression of estrogen receptor α and β on the periodontal pocket, but the correlation between periodontal pocket depth and immunoexpression of ER α and β on the menopausal and productive women exhibited the presence of value, but statistically not significant. This result suggested that the estrogen is not the main factor on periodontal pocket. The effect of estrogen depends on the number of complex binding between estrogen and estrogen receptor on the sulcular epithelial cells. The more complex binding, activity of estrogen is greater. Besides acting indirectly on the gingival tissues, estrogen could affect the gingival tissues directly by ER.[17] This study is not asses the serum estrogen level so it cannot extensively explain the result.

The explanation of these result is may be the estrogen status is more influential to the alteration of alveolar bone density.[4] Osteoporosis on the menopausal women is also a risk indicator for periodontal disease in postmenopausal Caucasian women.[28] A theory reveals that the decrease of mineral in the alveolar bone due to osteoporosis is risk factor of attachment loss during periodontitis.[29] Bone mineral density (BMD) is related to resorption of alveolar crest, and clinical attachment loss on post-menopausal women.[28] Study on the post-menopausal women of Asian-American race showed the relation among bone mineral density, edentulous, and clinical attachment loss, however, there was no correlation between BMD and periodontal pocket depth. BMD is a predictive factor of edentulous and clinical attachment loss.[29] Among postmenopausal women, there were significant trends for increasing prevalence of moderate or severe periodontal disease and tooth lost as the level of BMD decreased.[5]

However, in the study conducted by Lopes *et al*.,[30] that in postmenopausal women, there is no significant correlation between BMD and the increase of periodontitis risk measured based on the gingival index, plaque index and the clinical attachment level. A study in two groups of pre-menopausal women, the group with severe generalized periodontitis and periodontally healthy group, showed that there was no significant correlation between the clinical attachment level and BMD value.[31]

The presence of these differences needs a further study to determine the influence of estrogen on the periodontal tissue either through ER α or estrogen β on the menopausal and productive women with periodontitis. However, this study showed that in the menopausal and productive women, the immunoexpression of ER α seemed to have higher correlation with periodontal pocket depth than that with ER β ($r = 0.37$ vs. $r = 0.12$ for menopausal women, and $r = 41$ vs. $r = 0.11$ for productive women). This confirms the result of previous study that in the gingival tissues of female with periodontitis, the positive rate of ER in the

gingival tissues increased significantly.[17] Nevertheless, detection frequency of estrogen receptor α genotypes in female Chinese population was not statistically different among the aggressive periodontitis, chronic periodontitis and healthy control.[32]

It concluded that the immunoexpression of ER had no correlation with the severity of periodontal disease determined based on the periodontal pocket depth both on menopausal and productive women which presumed that estrogen has little role in the severity of periodontitis based on periodontal pocket depth. However, the ER α was more correlated with the periodontal pocket depth than the ER β. To get better evaluation of the estrogen role on the pathogenesis and severity periodontitis, additional prospective longitudinal studies with further analysis of possible confounding factors for menopause status, estrogen receptor, osteoporosis and periodontal status in larger cohorts of post-menopausal women are needed. The results of the studies will have a practical significance in the diagnosis, prevention and approach for the treatment of periodontal disease in menopausal women.

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