Analysis of Formyl Peptide Receptor 1 Protein Value as an Indicator of Neutrophil Chemotaxis Dysfunction in Aggressive Periodontitis

Prajna Metta, Yanti Rusyanti, Nunung Rusminah, Bremmy Laksono

Abstract-The decrease of neutrophil chemotaxis function may cause increased susceptibility to aggressive periodontitis (AP). Neutrophil chemotaxis is affected by formyl peptide receptor 1 (FPR1), which when activated will respond to bacterial chemotactic peptide formyl methionyl leusyl phenylalanine (FMLP). FPR1 protein value is decreased in response to a wide number of inflammatory stimuli in AP patients. This study was aimed to assess the alteration of FPR1 protein value in AP patients and if FPR1 protein value could be used as an indicator of neutrophil chemotaxis dysfunction in AP. This is a case control study with 20 AP patients and 20 control subjects. Three milliliters of peripheral blood were drawn and analyzed for FPR1 protein value with ELISA. The data were statistically analyzed with Mann-Whitney test (p>0,05). Results showed that the mean value of FPR1 protein value in AP group is 0,353 pg/mL (0,11 to 1,18 pg/mL) and the mean value of FPR1 protein value in control group is 0,296 pg/mL (0,05 to 0,88 pg/mL). P value 0,787 > 0,05 suggested that there is no significant difference of FPR1 protein value in both groups. The present study suggests that FPR1 protein value has no significance alteration in AP patients and could not be used as an indicator of neutrophil chemotaxis dysfunction.

Keywords—Aggressive periodontitis, chemotaxis dysfunction, FPR1 protein value, neutrophil.

I. INTRODUCTION

A P is characterized by a rapid loss of clinical attachment and alveolar bone and normally affects young adults. According to the extent of the periodontal destruction, these infections may be localized and generalized. Diagnosis of AP requires exclusion of systemic diseases that may severely impair host defenses and lead to premature tooth loss [1]. The disease is generally found to have a higher risk in blacks and males, although reports vary between different races and ethnicities, with some populations showing prevalence as high as 28.8% [2]. A study by Timmerman et al. in 1998 reported high prevalence rates of AP in Indonesia ranging between 3% and 10% [3] whereas a study in Faculty of Dentistry, Universitas Padjadjaran, Indonesia reported an estimation of 3.13% AP cases in 3 months in 2010 [4].

As opposed to chronic periodontitis (CP), the amount of plaque accumulation in AP subjects is inconsistent with the severity and progression of the periodontal destruction, and rarely mineralizes to form calculus, but the plaque is highly pathogenic due to the presence elevated levels of bacteria like Aggregatibacter actinomycetemcomitans (Aa) [1], [5]. Inflammation caused by lipopolysaccharide (LPS) from bacteria increase neutrophil activation and therefore increases formyl-peptide-receptor-1 (FPR1) protein synthesis at the cell surface. This perpetuation inflammatory response facilitates greater numbers of receptor (FPR1) on each cell [6]. Constitutively expressed on the surface of quiescent neutrophils, FPR1 receptor expression is rapidly up-regulated in response to a wide number of inflammatory stimuli [6-8]. Studies by Andersson et al. (1987) and Tennenberg et al. (1988) showed increased amount of receptors in neutrophil after stimulation by chemotactic agent [9]-[11].

Neutrophils from some AP patients exhibit abnormal chemotactic responsiveness when challenged with the synthetic formyl peptide, FMLP [12]-[14]. Perez et al. (1994) reported patient in whom abnormal neutrophils chemotactic responses to FMLP were associated with a defective population of neutrophil formyl peptide receptor(s) (FPR) [15]. Those studies explained that the occurrence of periodontal disease is associated with the decrease of neutrophil chemotactic activity or decrease the ability of bacterial killing [6], [15]. Previous work by Van Dyke et al. (1990) has clearly demonstrated that 70% to 80% of patients with the clinical characteristics of AP express a defect of in vitroneutrophil chemotaxis. The study revealed a reduced expression of 2 biomarkers at the neutrophil cells surface, glycoprotein 110 (GP110) and FPR1 [16]. The aim of this study was to assess the alteration of FPR1 protein value in AP patients and whether it could be used as an indicator of neutrophil chemotaxis dysfunction in AP.

II. MATERIAL AND METHOD

A. Subjects

AP patients were located from among the patients of Department of Periodontology, Postgraduate Programme, Universitas Padjadjaran, Indonesia and selected by consecutive sampling. AP was diagnosed clinically and radiographically in clinically healthy patients and the subjects were selected with the exclusion criteria of pregnancy, nursing, menopause, smoking, and long-term medication.

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Normally healthy controls were located among the student and staff population of Universitas Padjadjaran and demonstrated no clinical or radiographic evidence of periodontal disease other than mild gingivitis. According to the inclusion criteria, there were 20 subjects in each group.

B. FPR1 Protein Value Assay

Three milliliters of peripheral blood were drawn from each subject, and then centrifuged for 10 minutes at 3000 rpm. A 100 μ L serum from the centrifuged blood was analyzed for FPR1 protein value using ELISA in Molecular Genetics Laboratory – The Center of Genetic Study, Faculty of Medicine, Universitas Padjadjaran. Each serum was analyzed in a duplo method to avoid bias result. Data was statistically analyzed with Mann-Whitney test (p>0,05).

III. RESULTS

The age of AP group ranges between 24-55 with average of 39.8 and the age of control group ranges between 21-40 with the average of 27.65. The AP group consists of 11 male subjects and 9 female subjects, whereas in control group consists of 7 male subjects and 13 female subjects (Table I).

	TABLE I					
SUBJECTS CHARACTERISTICS						
	Groups		_			
	AP	Control	P Value			
	(n=20)	(n=20)				
1. Age (year)*			0,000			
Mean (SD)	39,8 (8,93)	27,65 (5,59)				
Median	39	27				
Range	24-55	21-40				
2. Sex**			0,204			
Male	11	7				
Female	9	13				

* Mann-Whitney test, **Chi-Square test

Mann-Whitney test was used to analyze the difference in FPR1 protein value between AP and control groups as seen in Table II. Mean of FPR1 protein value in AP group was 0,353 pg/mL (SD=0,29) ranging between 0,11-1,18 pg/mL, and in control group was 0,296 pg/mL (SD=0,22) ranging between 0,05-0,88 pg/mL. Based on statistical examination, P value was 0.878 (P > 0.05) suggested that there was no significant difference of FPR1 protein value in both groups.

TABLE II FPR1 PROTEIN VALUE IN AP AND CONTROL GROUPS						
	Groups		P Value			
	AP (pg/mL)	Control (pg/mL)				
	(n=20)	(n=20)				
Mean (SD)	0,353 (0,29)	0,296 (0,22)	0,787			
Median	0,223	0,26				
Range	0,11-1,18	0,05-0,88				

IV. DISCUSSION

Our study showed the age of AP group ranges between 24-55 with the average of 39.8. This result is different from previous research in 1997 by Albandar et al. in black population of African-American, Hispanic and white population in the U.S. showed that AP patients' age ranges between 13-17 years old [17] and Kowashi in 1988 reported the age ranges between 19-28 years old [18]. This difference may have derived from several factors, such as: different sampling method, inclusion and exclusion criteria, research design, analysis method and compromised clinical condition of AP disease [1], [17], [18]. Diagnosing AP will be difficult if the patient aged more than 30 years old. The insignificant result may be caused by inappropriate sample criteria to the theory denoting that AP happens in younger age [19], [20]. Age will not be bias if each sample is examined the involving gene in AP disease, therefore a further study with sample selection using DNA sequencing, microbiology PCR, and DNA identify Aggregatibacter probes to actinomycetemcomitans may be needed [20].

This research showed no difference in gender between both groups (p=0.204, p > 0.05) therefore there is no tendency of gender affecting AP disease. Studies have shown that higher periodontal disease prevalence are in females. Nassar et al. (1994) mentioned that AP ratio in woman compared to man is 1.88:1 in Saudi subject and that gender difference is significant (x=5.490, P < .05) [21]. Study of Melvin et al. (1991) showed that number of women are more than men in the ratio of 4.3:1 in Caucasian and 1.1:1 in the entire subjects [22]. On the contrary, our research showed that number of women are less than men in the ratio of 1:1.2, corresponding to the study by Cho et al. (2011) in Korea showing that the number of women is fewer which is 1:2.5 and Albandar's study in Uganda which is as big as 1:1.52 [1]. Further research is needed to find the comparison between the number of women and men of AP patients in Indonesia globally. The above researches denote that there is gender ratio difference of AP patients in each ethnic; hence the influence of gender to AP incidence is still not assured.

Our result showed the protein value of FPR1 in AP group does not experience a significant change. This is consistent with the research of De Nardin et al. (1990) and Perez et al. (1991) denoting that there is no difference of formyl peptide (FLMP) receptor protein value between localized aggressive periodontitis (LAP) patients and control group. That study also mentioned the decrease of chemotactic function in LAP patient is not suspected to be entirely caused by the reduction of receptor protein value, but may be derived from changes or damaged within the receptor itself [14], [23], [24]. The above studies are contrary to few others mentioning that there is a reduction of FMLP receptor protein expression in AP patients. Van Dyke et al. (1981, 1983) and De Nardin et al. (1990) said that LAP patients showed a reduction of FMLP, C5a, and LTB₄ receptor expression as big as 50% [23], [25]–[27].

The research done by Van Dyke et al. (1981) revealed FPR protein expression on the surface of neutrophil cell is less in LAP patients compared to normal persons, but its amount is not mentioned [25]. His research in 1985 showed the amount of binding site for FMLP to the neutrophil cell of LAP patients as big as 9200 and in normal person as big as 20.000 [28].

This means the amount of FPR1 protein value toward FMLP in LAP patients is less than normal. Similar study done by Sigusch et al. (2001) showed that chemotaxis toward FMLP is decreased in LAP group compared to chronic periodontitis and control group [29]. Abnormal chemotaxis is associated with the change of FPR1 activity towards FMLP in LAP patients [30].

FPR1 protein value in this research is contrary to the results of Van Dyke et al. (1981 and 1985). De Nardin et al. (1990), and Sigusch et al. (2001) mentioned that there is a FPR1 protein value reduction in AP [25], [28], [29], [31]. This is suspected to be the result of (i) reduction of receptor numbers in cell membrane, (ii) damaged in FMLP receptor in cell membrane or co-receptor for FMLP such as GP110 (glycoprotein 110) or CD38 which facilitates and increases chemotactic response, or (iii) combination of both [32]. De Nardin (1994) mentioned that genetic defects in response to different chemoattractant may occur as the result of (i) their structural arrangements in the plasma membrane and/or their effects on cell activation (i.e.in chemotaxis, respiratory burst, etc.) and (ii) the transmembrane signaling mechanism. Change in cell function may occur if there is damage in one of the receptors. Damage in one of the receptors may have caused a damage to other receptors which have similar function [33].

Previous researches suggested that FPR1 gene is very polymorphic [31], [34]-[36]. Aggresive periodontitis is highly correlated to single nucleotide polymorphism (SNP) within FPR1 gene in few populations [31], [35], [36]. Single nucleotide polymorphisms that occur in one or more nucleotide base of FPR1 gene may alter amino acid synthesis, which then may change the protein value and function. Altered amino acid caused by SNP is a non-synonymous SNP form, whereas if the amino acid does not change even though there is SNP(s) thus it is called a synonymous SNP. Synonymous SNP form does not alter a protein function, but a non-synonymous form can change the function because the amino acid arrangement may or may not affect the protein function [34], [37].

FPR1 receptor function is affected by transcription in FPR1 gene coding region and the number is affected by transcription in promoter area. Mutation or SNP occurred in FPR1 gene promoter or coding region affects the protein transcription and eventually can change amino acid synthesis, thus can affect the protein number of function [35], [37]-[39].

FPR1 protein value of AP group does not experience a significant change because its number in cell surface does not decrease, this is suspected as a result of no interference in the promoter area [35], [39]. Clinically visualized tissue disruption in AP patients is suspected as a result of (i) transcription interference in FPR1 gene coding region, (ii) transduction signaling pathway interference, (iii) decrease in receptor binding affinity, (iv) or three of them which associated with receptor chemotaxis function [40]. Decrease in receptor function resulted from signaling disruption can occur because of abnormal intracellular Ca²⁺ mobilization, elevated diacylglycerol levels coupled with decreased diglyceride kinase activity, and reduced activity of calcium-dependent

protein kinase C activity. Biochemistry studies toward neutrophil damage in LAP has revealed a receptor reduction toward C5a chemotactic peptide and FMLP without significant alteration in binding affinity [25], [41]. Perez et al. (1991) found one LAP patient having normal number of receptor but there is a reduction of high-affinity receptor number [14], [30]. Other research of Daniel et al. in 1993 suggested that FMLP receptor affinity is not different between LAP patients and normal [12], thus the effect of binding receptor affinity on FPR1 protein value in AP has not exactly been known.

Researches in America, Africa, Europe, and Japan have proven the correlation existence between few SNP with reduction of FPR1 protein value in PA patients [30], [31], [34]-[37], [42]. Richard et al. (2009) recorded that there are more than 30 FPR1 gene variations, but has yet been a study recording FPR1 gene variation in Indonesian population, hence there has not been known the SNP location of FPR1 gene in Indonesian in relation to the protein value. Neutrophil chemotaxis function is affected by few chemoattractants such as C5a, LTB4, IL-8 and FMLP from bacteria [43]. Unchanged FPR1 protein value from AP group does not merely prove the return of chemotaxis function, but there was suspected to have an interference in another receptor (receptor toward C5a, LTB₄, and IL-8) which capable of affecting its chemotaxis activity instead.

Polymorphism in promoter area or coding region and receptor affinity difference are not investigated in this research, therefore the SNP effect to the FPR1 number and function, receptor affinity effect, and neutrophil chemotaxis cannot be concluded yet. Few researches supports the existence of neutrophil chemotaxis disruption in AP patients as result of some SNP in FPR1 gene [28], [34], [35], [37]-[39].

This study has limitation in establishing diagnosis, thus can affect the result. Anamnesis and oral hygiene factor have to be considered in selecting the research subject. Clinical examination and radiograph assessment in subjects' family such as: siblings and two generations above can be corroborating factors in determining AP diagnosis. Age limitation also becomes worth-considering aspect in subjects' selection so that the investigated subjects can be more homogenous.

Based on the result, it can be concluded that FPR1 protein value in AP patients does not experience significant alteration. FPR1 protein value cannot be determined as an indicator of neutrophil chemotaxis dysfunction in AP.

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