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ORIGINAL ARTICLE

Periodontal tissue destruction in aggressive periodontitis: Determination of gene or environmental factors

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KEYWORDS

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Polymorphism c576 T > C > G;
c301 G > C;
c348 T > C of FPR1 gene;
Il-8;
Dental plaque;
Clinical Attachment Loss (CAL)

Abstract *Aim:* This study observed the role of defective neutrophil function in aggressive periodontitis through FPR1 gene polymorphism and the level of Il-8 compared with the role of dental plaque presence towards periodontal tissue damage (Clinical Attachment Loss/CAL) in patients in Indonesia.

Methods: Case-control study was used to detect differences in polymorphism expression of FPR1 gene, the level of Il-8, dental plaque, and Clinical Attachment Loss/CAL from 32 Aggressive Periodontitis (AP) and 29 Non-Aggressive Periodontitis (NAP) samples, selected with consecutive sampling method. Polymorphism was identified using polymerase chain reaction (PCR) technique, and the level of IL-8 in the gingival crevicular fluid was identified using the enzyme-linked immunosorbent assay (ELISA) test. The Clinical Attachment Loss was analysed by using William periodontal probe, and the oral environment analysis was performed by using the OHI-S plaque index. Statistical analysis was used to determine the significance of the polymorphism difference of FPR gene, Il-8, Plaque and CAL amongst all subjects and also the control and correlations among these factors.

Results: The results showed that in the Aggressive Periodontitis (AP), the presence of the polymorphism of c576 T > C > G of FPR1 gene caused as much as 5.04 times higher occurrence of aggressive periodontitis ($p = 0.006$; OR = 5.040 (1.51–16.74)). The low level of Il-8 (below 0.064 pg/μl), showed as much as 34.5 times higher occurrence of aggressive periodontitis (OR = 34.5 (6.76–176.08)). The oral hygiene of the AP samples were better significantly ($p = 0.002$), and on the Clinical Attachment Loss (CAL) sample was even more ($p = 0.02$). The polymorphism of c301 G > C of FPR1 gene correlated with the CAL ($r = 0.37$; $p = 0.039$). The

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polymorphism of c576 T > C > G correlated significantly with the Il-8 (r = 0.5; p = 0.0287). The polymorphism of c348 T > C correlated significantly with the dental plaque (r = 0.355; p = 0.049), whereas the dental plaque correlation with CAL was not significant.

Conclusion: The research conclusion showed that in aggressive periodontitis, genetic and environmental factors were correlated with the cause of periodontal tissue injury, and the role of genetic factors was more prominent on the injury.

1. Introduction

The International Workshop for a Classification of Periodontal Diseases and Conditions in 1999 established a diagnosis of Aggressive Periodontitis for periodontitis occurring in individuals of the age group of 14-years-old up to above 30-years-old (Newman et al., 2014). This term replaced the "Juvenile Periodontitis" term since the age factor did not determine Aggressive Periodontitis (Hinrichs and Kotsakis, 2012).

This disease progresses very rapidly, with clinical symptoms of progressive attachment loss and bilateral symmetric arch shape alveolar bone resorption. The oral hygiene of aggressive periodontitis patients look good, with a small amount of dental plaque and unclear signs of inflammation, is not commensurate with the severity of the illness. Several studies have done towards Aggressive Periodontitis patients, and discovered that the most dominant pathogen bacteria was *Aggregatibacter actinomycetemcomitans* with dysfunction of PMN immune function, genetic influence and tend to occurred in the family group (Joshipura et al., 2015; Ishikawa, 2007; Dumitrescu and Kobayashi, 2010; Noack and Hoffman, 2004; Van der velden, 2017).

The complicated state of aggressive Periodontitis caused by many factors involved (a multifactorial disease). Besides of the presence of periodontal pathogens (biofilm plaque), there is strong evidence that genetic, lifestyle and environmental factors also contribute to the occurrence, severity, and progression of the disease (Van Winkelhoff et al., 2003). The role of bacterial virulence in periodontitis is clear enough, but recent findings discovered that bacterial attacks alone were not sufficient enough on destructing periodontal tissue. The host's immune response to such attacks will help the periodontal tissue destruction (Joshipura et al., 2015; Van Winkelhoff et al., 2003; Page and Kornman, 1997).

This new paradigm revealed that the bacterial interaction with the host response was intended for protection, but the same interaction caused the tissue destruction. This condition was known as a double-sided sword phenomenon, that altered the host-microbe homeostasis (Page et al., 1997). One cause of a person's susceptibility towards periodontitis is the neutrophil chemotaxis dysfunction (Van Winkelhoff et al., 2003; Michalowicz and Pihlstrom, 2003; Bartold and Van Dyke, 2013; Gwinn et al., 1999).

Research related to the defected function of neutrophil chemotaxis in Aggressive Periodontitis had evaluated the presence of FPR gene polymorphism. The results of previous studies showed that FPR1 gene polymorphism was the risk factor of Aggressive Periodontitis and varied among different ethnic groups. For a deeper perspective, the condition of the neutrophil cells function needs to be studied in molecular and genetic biology review (Page and Kornman, 1997). The ability of the neutrophil cells to respond towards signals originating from the extracellular environment is the beginning of the inflammatory process.

These signals are in the form of either chemical or physical stimulants and sourced from either bacteria or the body's tissues, which responded biologically by cellular network regulation. Each cell has several receptors according to a specific function. The main signal of neutrophil chemotaxis in Aggressive Periodontitis is formyl methionyl leucyl phenylalanine (fMLP) secreted by bacteria. fMLP is one of the bacterial peptides known as a chemoattractant, a powerful PMN phagocytosis activator and also a strong mononuclear (Maney and Walters, 2009). The presence of fMLP responded by neutrophil-formyl peptide receptors (FPR) on the PMN walls (Gwinn et al., 1999).

Another signal of neutrophil chemotaxis is interleukin-8 (IL-8), which is a chemokine that also acts as an endogenous chemoattractant in the neutrophil chemotaxis (Maney and Walters, 2009; Kumar et al., 2005). The IL-8 helps activate the integrin in endothelial cells that make neutrophils attached to endothelial cells and then extravasate out from the blood vessels (Gwinn et al., 1999). Various studies on the levels of IL-8 in the periodontitis conditions, however, showed several different results. Both fMLP and IL-8 responded with the Formyl Peptide Receptor (FPR) located on the surface of the neutrophil cell. (Kumar et al., 2005; Van Dyke et al., 1981; Gainet, 1999) The changes occurred in these components will disrupt the balance of neutrophil homeostasis (Tsikitis et al., 2004).

Several studies (Gwinn et al., 1999; Maney and Walters, 2009; Zhang et al., 2003; Gunji et al., 2007; Maney et al., 2009; Skvortso and Gabdoulkhakova, 2017) related towards the defected function of neutrophil chemotaxis in Aggressive Periodontitis included, among others, the presence of FPR polymorphism. The results of previous studies on the condition of FPR1 gene polymorphism in Aggressive Periodontitis were varied between different ethnicities and had not studied any Indonesian ethnic group. Besides, the role of IL-8 in chemotaxis associated with the FPR1 gene polymorphism as the risk factor components of neutrophil homeostasis changes in aggressive periodontitis remains unclear.

The description above clearly described the role of a bacterial and genetic factor that alters the neutrophil chemotaxis function homeostasis, as the cause of individual susceptibility towards aggressive periodontal disease. The purpose of this study was to analyze the prominence between the genetic and the environmental factor as an etiopathogenesis of periodontal tissue destruction in Aggressive Periodontitis (Joshipura et al., 2015; Van Winkelhoff et al., 2003; Page et al., 1997).

2. Method

2.1. Material

The samples consisted of the venous blood and crevicular fluid that collected from as much as 32 aggressive periodontitis patients and 29 non-aggressive periodontitis patients, in which both fulfilled the inclusion criteria. All samples were taken from Periodontics Clinic, Dental Hospital of Faculty of Dentistry Universitas Padjadjaran, Bandung, Indonesia. Ethical Clearence No.46/UN6.C2.1.2/KEPK/PN/2012 This study was based on the molecular epidemiology principles with a case-control study that was done in Molecular Biology Laboratory, Health Research Unit of Faculty of Medicine Universitas Padjadjaran/Hasan Sadikin Hospital, Bandung, Indonesia.

2.2. DNA isolation

DNA was isolated from the venous blood of each subject using Pharmacia® DNA isolation kit. As much as 200 ng of DNA template was taken for the Polymerase Chain Reaction (PCR) step. The PCR was performed by using the primers of F5′ TTCACCTCCACTTTGCCATT 3′ and R 5′ TGACAGCAACGATGGACATG 3′ (Zhang et al., 2003) which covered the two exons. The PCR product of exon 2 of FPR1 gene was described as a segment that was obtained from 61 subjects (32 AP subjects and 29 NAP subjects). The size of this PCR product was 439 bp, as seen in Fig. 1. This fragment will be detecting the single-nucleotide polymorphism (SNP) of the variant type of c.301 G > C, c.306 T > C, c.348 T > C, c.546 C > A, c.568 A > T, and c.576 T > C > G.

2.2.1. DNA sequencing

DNA sequencing was performed by using dideoxy Sanger sequencing method in the First Base Laboratory, Malaysia. The sequencing result was aligned afterwards with the reference sequence from the gene bank used the Bioedit® software.

2.3. Interleukin-8 level analysis

The interleukin-8 level analysis was performed by the enzyme-linked immunosorbent assay (ELISA) test with Human Interleukin-8 reagent. The Il-8 level was analysed from the gingival crevicular fluid taken with the paper point.

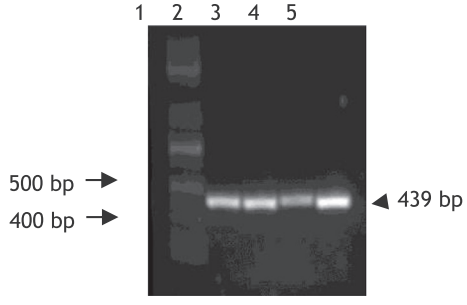

Fig. 1 Description of the electrophoresis of PCR product of FPR1 gene. Lane 1 → Ladder 100. Lane 2–5 → PCR products of 439 base pairs (bp).

2.4. Oral environment analysis simplified oral hygene index, OHI-S (Green and Vermillion)

The measurement of plaque on facial surface #16, #11, #26, #31, lingual surface #36 and #46 resulted on the ordinal values.

2.5. Clinical Attachment Loss (CAL) Analysis

The CAL analysis was performed on tooth #16, #11, #26, #36, #31 and #46 by using William periodontal probe. The level of attachment is determined by subtracting from the depth of the pocket the distance from the gingival margin to the CEJ.

2.6. Statistical method

The statistical analysis was used to determine the significance of differences occurred in the sequence variant frequency among AP and NAP as the control group. The odds ratio (OR) was used to determine the risk factor of the FPR1 gene polymorphism and the Il-8 level, with a p-value less than 0.05 considered to be statistically significant. Analysis of biserial point correlation was performed to determine the relationship between all factors.

3. Results

3.1. Respondent's characteristic

There was no significant difference between patients with AP and NAP examination results on the characteristics of all subjects through anamnesis and clinical examinations, including age, gender, plaque value education, clinical loss attachment.

3.2. Analysis of neutrophil chemotaxis function

3.2.1. Detection of FPR1 gene polymorphism

The examination of FPR1 gene polymorphism in this study was performed as the genetic marker of the conditions of the neutrophil cell function in periodontal disease. Before entering the next examination stage of examination, the PCR product was checked for its existence first by using the electrophoresis method, as seen in Fig. 1 and electropherograms as seen in Fig. 2.

The PCR products of 439 bp. with the SNP variant of c.301 G > C, c306 T.C, c.348 T > C, c546 C > A, c.568 A > T, and c.576 T > C > G. were presented in Table 1.

Fig. 2(a) showed the normal description of the 546 A gene and the polymorphism in the gene region of the 546 A > C; (b) showed the normal description of the 568 A gene and the polymorphism in the gene region of the 568 A > T; (c) showed the normal description of the 576 T gene and the polymorphism in the gene region of 576 T > C and 576 T > G.

Table 1 showed the results of the FPR1 gene polymorphism in the exon 2 region with the PCR sequencing method of 439 bp with variants of SNP c.301 G > C, c306 T.C, c.348 T > C, c546 C > A, c.568 A > T, and c.576 T > C > G. It appeared that the C and G alleles in the c576 T > C > G gene with visible factors related to aggressive periodontitis occurrence, were significantly different in the AP and NAP group

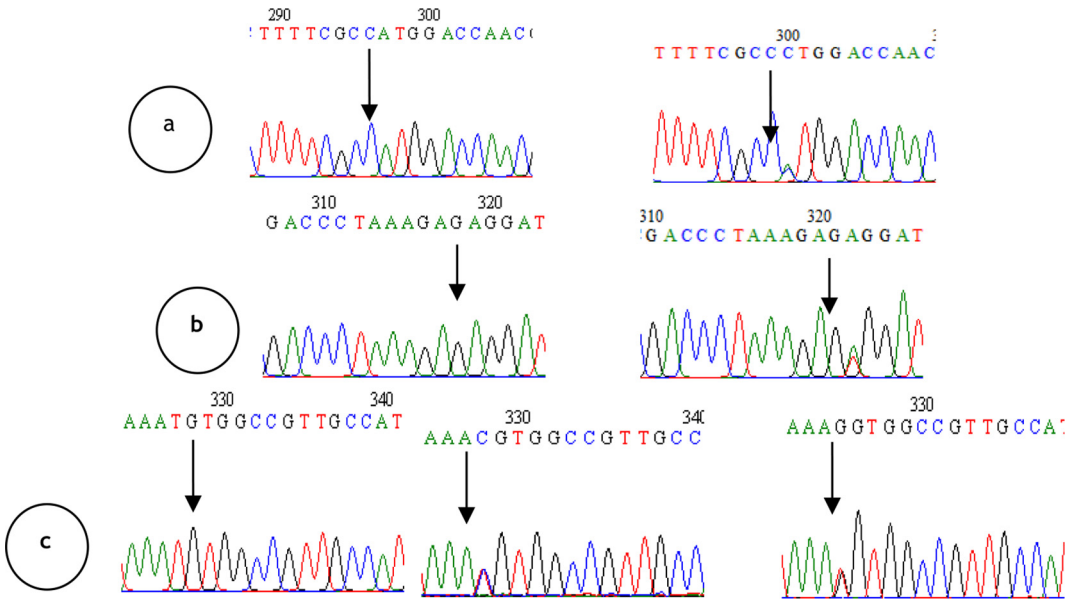

Fig. 2 Description of the research subject's FPR1 gene electropherograms. (a) The 546 A locus was changed to 546 C; (b) the 568 A locus was changed to 568 T; (c) the 576 T locus was changed to 576 C and 576 G.

Table 1 The frequency of FPR1 gene polymorphism on all of the research subjects.

Location	Genotype	Group		p-value	OR (CI 95%)
		AP (n = 32)	NAP (n = 29)		
301 G > C	GC/CC	29 (90.6%)	23 (79.3%)	0.287(2)	2.522 (0.48–14.53)
	GG	3 (9.4%)	6 (20.7%)		
306 T > C	TC/CC	1 (3.1%)	0 (0%)	1.000(2)	–
	TT	31 (96.9%)	029(100%)		
348 T > C	TC	28 (87.5%)	26 (89.7%)	1.000(2)	0.81 (0.13–4.881)
	TT	4 (12.5%)	3 (10.3%)		
546 C > A	CA/AA	14 (43.8%)	16 (55.2%)	0.373 (1)	0.63 (0.23–1.96)
	CC	18 (56.3%)	13 (44.8%)		
568 A > T	AT	15 (46.9%)	9 (31.0%)	0.206(1)	1.961 (0.687–5.599)
	AA	17 (53.1%)	20(69.0%)		
576 T > C > G	TC/TG/GC	27 (52.5%)	15 (47.5%)	**0.006(1)** *	5.040 (1.517–16.741)
	TT	5 (8.2%)	14 (23.0%)		

Notes:
AP = Aggressive Periodontitis.
NAP = Non-Aggressive Periodontitis.
p-value was counted with the chi-square test (1); or the Fisher's exact test (2) untuk ekspektasi sel < 5. OR (IK 95%) = Odds rasio dan interval konfidensi 95%.

(p = 0.006; OR = 5.040 (1.517–16.741)). Polymorphism in the c576 T > C > G gene was occurred 5.04 times often in the Aggressive Periodontitis group compared with the Non-Aggressive Periodontitis group, indicating the abnormalities of the chemotaxis function of the neutrophil cells in aggressive periodontitis.

3.2.2. Interleukin-8 (Il-8) level analysis

The Interleukin-8 level was analysed from the gingival crevicular fluid of the CAL teeth, with the ≥4 mm CAL, both in the AP and the NAP patients, with the ELISA test. The results of the ELISA test were presented in Table 2.

The results of difference test analysis with Mann-Whitney test showed a significant difference in the IL-8 levels between the AP and NAP group (p < 0.001). In the AP group, the mean and the median value of the Il-8 level were lower compared with the NAP group.

Based on the differences of the Il-8 level, a diagnostic test was performed to determine the odds ratio and the Receiver Operating Characteristics (ROC). The cut-off value based on the ROC curve for the Il-8 level was ≤0.064 pg/μl (as seen in the following curve in Fig. 3).

Fig. 3 showed that the intersection point for the Interleukin-8 levels in this research group was 0.064 pg/μl. This

Table 2 Interleukin-8 (IL-8) level on all of the research subjects.

Variable	Group		P-value*
	AP (n = 32)	NAP (n = 29)	
IL-8 level (pg/µl)			< 0.001
Mean (SD)	0.07053 (0.48761)	0.12938 (0.113063)	
Median	0.062	0.06900	
Range	0.051–0.335	0.560–0.476	

Notes:
AP = Aggressive Periodontitis.
NAP = Non-Aggressive Periodontitis.
* Counted with the Mann-Whitney test.

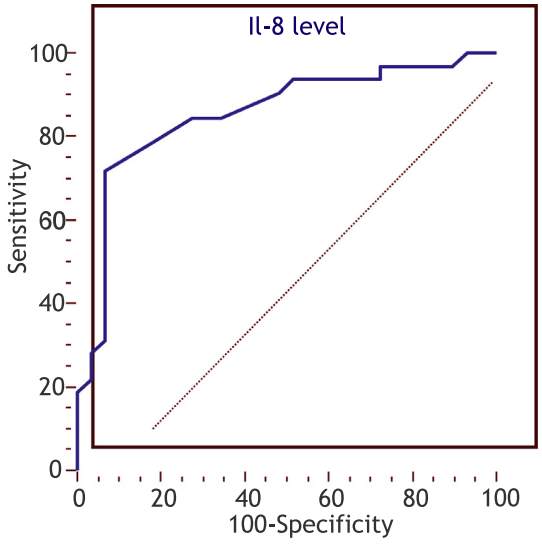

Fig. 3 Intersection point of Interleukin-8 level.

means that in the Il-8 level of ≤0.064 pg/µl was an abnormal (false negative) condition, whilst in the level of ≥0.064 pg/µl was a normal (false positive) condition.

Table 3 showed that if the Il-8 level was ≤0.064 pg/µl, then the aggressive periodontitis occurred 34.5 times often than the non-aggressive periodontitis.

3.3. Oral hygiene analisis

The plaque examination was performed to determine the bacterial-oral environment effect towards periodontal disease by the OHI-S index.

Table 4 showed that the plaque accumulation in Aggressive Periodontitis patients was significantly lower than the control group. Plaque accumulation was represented the oral bacteria factor in periodontal disease.

3.4. Clinical Attachment Loss Analysis

The CAL examination in this research was performed to show the tissue destruction occurred due to the periodontal disease.

Table 5 showed that the mean of the difference of CAL in AP and NAP was significant (p = 0.002), which means that in the AP group, the attachment loss was more progressive than in the NAP group. The CAL value in this study represented the amount of periodontal tissue damage.

3.5. Correlation analysis

Table 6 showed that there was no correlation between the various SNP FPR1 genes with age, although there was a significant correlation between the polymorphism of the 301 G > C FPR1 gene with CAL (p = 0.039; r = 0.37) in aggressive periodontitis. This condition showed that only in the AP group found a correlation between genes and periodontal tissue destruction, whilst age showed no effect on genetic conditions.

Table 7 showed a correlation between the 576 T > C > G FPR1 gene polymorphism with the Il-8 level (p = 0.0287; r = 0.5) in aggressive periodontitis, suggested that the polymorphism of the FPR1 gene, along with the low Il-8 level was a risk factor for the occurrence of aggressive periodontitis.

Table 8 showed that there was no correlation between the Il-8 level with both CAL value and age, which means that the Il-8 level was not influenced by age, and also was not influencing the CAL value.

Table 9 showed that there was a correlation between the polymorphism of the FPR1 gene with dental plaque, which was in the 348 T > C FPR1 gene (p = 0.046; r = 0.355). This condition showed that the polymorphism of the FPR1 gene, along with dental plaque, was a determining factor (r = 0.355) of the tissue destruction in aggressive periodontitis.

Table 3 Interleukin-8 (Il-8) level based on the intersection point value.

Variable	Group		P-value	OR (CI 95%)
	AP (n = 32)	NAP (n = 29)		
Il-8 level (pg/µl)				
≤0.064	23	2	< 0.001	
> 0.064	9	27		34.5 (6.760–76.081)

Notes:
AP = Aggressive Periodontitis.
NAP = Non-Aggressive Periodontitis.
p-value was counted with the chi-square test.

Table 4 Average value of plaque accumulations on all of the research subjects.

Characteristics	Group		
	AP (n = 32)	NAP (n = 29)	p-value
Plaque value			
Mean	1.94 (0.878)	2.03 (0.865)	0.002*
Median	2	2	
0.661 interval	1–3	1–3	

The Mann-Whitney test.

Table 5 Average value (SD) of Clinical Loss Attachment (CAL) on all of the research subjects.

Characteristics	Group		
	AP (n = 32)	NAP (n = 29)	p-value
CAL			
Mean	4.94 (0.948)	4.14 (1.156)	**0.002***
Median	5	4	
Range	4–8	3–7	

Notes:
AP = Aggressive Periodontitis.
NAP = Non-Aggressive Periodontitis.

Table 10 showed that the CAL value was not significantly related to both dental plaque and age in the AP group, but in the NAP group was vice versa. This condition suggested that the periodontal tissue destruction in aggressive periodontitis was caused not only by the plaque bacterial and also the age (disease duration).

4. Discussion

The subjects characteristic data in this study was considered to be able to represent both of the aggressive periodontitis and the non-aggressive periodontitis patients in general, due to no differences found in all of the sample's characters. This condition means that all confounding factors in this study have excluded as an attempt to eliminated any research bias.

In addition towards the oral hygiene examination of oral hygiene, this study also used the blood sample and the gingival crevicular fluid. The assessment of any biomarkers found in the blood sample can be done by various techniques, including the PCR technique to imitate a number of genes as a template initiator in gene sequencing, and the primers used in this study were based on previous research (Gunji et al., 2007).

The results of this study indicated the presence of the FPR1 gene polymorphism as a risk factor for the aggressive periodontitis, which was located in the locus 576 T > C > G of the FPR1 gene. This result was consistent with the results of the previous studies which suggested the presence of the FPR1 gene polymorphism in African-American, Brazilian, Chinese, Japanese, and Turkish aggressive periodontitis patients, although the gene locus was different (Zhang et al., 2008; Maney and Walters, 2009; Gwinn et al., 1999; Benachour et al., 2009). Research conducted by Zhang et al. (2003) discovered that the polymorphism in the 568 A/T and 576 T > C > G region polymorphisms in the African-American patients suspected to be occurred due to the presence of a single nucleotide polymorphism in the second extracellular loop. The analysis of the haplotype relationship of the three SNPs (−12915 C > T, 301 G > C, and 546 C > A) indicated that one of three SNPs (−12915 CT, 301 C, and 546 A) significantly existed in aggressive periodontitis ($P < 0.0001$). Hodge et al. found a significant relation between c.348 T > C with Aggressive Periodontitis in Brazilians, but the result was not significant in African-Americans.

The results of the research conducted by Gwinn et al. (1999) also found the existence of two polymorphisms on FRP Phe110Ser and Cys126Trp. Research conducted by Gunji et al. (2007) towards the Japanese populations also found polymorphisms in the −12915 C > T, −10056 T > C, −8430 A > G, 301 G > C, and 546 C > A locus. The research of Maney and Walters (2009) found that the c.348 T > C FPR1 SNP was related with aggressive periodontitis

Table 6 Correlation of FPR1 gene polymorphism with age and CAL value in all of the research subjects.

Correlation	AP		NAP	
	r_{pb}	p-value	r_{pb}	p-value
301 G > C gene – age	0.2	0.263	−0.29	0.130
306 T > C gene – age	−0.32	0.076	na	Na
348 T > C gene – age	0.17	0.345	−0.16	0.392
546 C > A gene – age	0.1	0.586	−0.28	0.143
568 A > T gene – age	−0.11	0.546	−0.1	0.600
576 T > C > G gene – age	−0.17	0.339	0.01	0.968
301 G > C gene – CAL	0.37	**0.039***	−0.14	0.478
306 T > C gene – CAL	−0.59	0.0004	na	Na
348 T > C gene – CAL	0.23	0.210	0.06	0.766
546 C > A gene – CAL	0.01	0.960	−0.17	0.376
568 A > T gene – CAL	−0.13	0.477	−0.05	0.797
576 T > C > G gene – CAL	−0.16	0.397	−0.06	0.774

Notes:
r_{pb} = point biserial correlation coefficient.
na = not available; The correlation value for the control group data cannot calculated because there was only.
one data for the 306 T > C gene.

Table 7 Correlation of the Il-8 level with polymorphism of the FPR1 gene in all of the research subjects.

	AP		NAP	
Correlation	r_{pb}	p-value	r_{pb}	p-value
301 G > C gene – Il-8	0.15	0.430	0.29	0.188
348 T > C gene – Il-8	–	–	–	–
546 C > A gene – Il-8	–	–	–	–
306 T > C gene – Il-8	−0.024	0.894	–	–
568 A > T gene – Il-8	0.193	0.290	0.027	0.889
576 T > C > G gene – Il-8	**0.5**	**0.0287***	0.176	0.361

Notes:
AP = Aggressive Periodontitis.
NAP = Non-Aggressive Periodontitis.
r_{pb} = point biserial correlation coefficient.
OR (CI 95%); 10.5 (0.46–239.78).

Table 8 Correlation of the Il-8 level with CAL value and age in all of the research subjects.

Correlation	AP		NAP	
	r_s	p-value	r_s	p-value
Il8 – CAL	0.202	0.268	−0.175	0.368
Il8 – age	−0.048	0.794	−0.172	0.373

Notes:
r_s = Spearman's rank correlation coefficient.

Table 9 Correlation of the polymorphism of FPR1 gene, Il-8 level, and dental plaque in all of the research subjects.

Correlation	AP		NAP	
	r_s	p-value	r_s	p-value
301 G > C gene – dental plaque	−0.223	0.220	0.129	0.505
306 T > C gene – dental plaque	0.197	0.279	n.a	n.a
348 T > C gene – dental plaque	0.355	**0.046***	0.021	0.912
546 C > A gene – dental plaque	0.218	0.230	0.087	0.652
568 A > T gene – dental plaque	0.004	0.984	0.094	0.628
576 T > C > G gene – dental plaque	−0.169	0.355	−0.174	0.366
Interleukin-8 level – dental plaque	0.129	0.482	−0.277	0.145

Notes:
r_s = Spearman's rank correlation coefficient.
na = not available; The correlation value for the control group data cannot calculated because there was only.
one data for the 306 T > C gene.

Table 10 Correlation of CAL value with dental plaque and age in all of the research subjects.

Correlation	AP		NAP	
	r_s	p-value	r_s	p-value
CAL – dental plaque	0.095	0.603	0.358	**0.057***
CAL – age	0.44	0.11	0.535	**0.003***

(OR = 18.9) in African-Americans. The results of the FPR1 gene polymorphism in exon 2 region in this study discovered that the FPR1 gene polymorphism in the 576 T > C > G locus was different significantly between the aggressive periodontitis and control group (p = 0.006; OR = 5.040 (1.517–16.741)), means that individuals with FPR1 gene polymorphism were five times more susceptible towards aggressive periodontitis.

The FPR1 gene belongs to the class of G protein-coupled receptors involved in the chemotaxis process. This receptor is

able to bind N-formyl peptides such as N-formyl methionine produced by bacterial degradation as well as the host cells. These receptors are also involved not only as an intermediate response of immune cells towards infection but also able to suppress the immune system under certain conditions. FPR belongs in the classification of receptors that having seven hydrophobic transmembranes. Induction of FPR will stimulate changes in eukaryotic cells, including reorganization of the cytoskeleton that will facilitate the cell migration and chemokine synthesis. However, the polymorphism of the FPR1 gene was not the only factor causes an aggressive periodontitis. In addition to gene factors, environmental factors are another multifactorial as the characteristic of aggressive periodontitis (Listgarten, 1986; Hodge and Michalowicz, 2001; Slots, 2013).

Environmental factors such as microorganisms existence in the gingival sulcus play a role in triggering the host cells producing proinflammatory cytokines. In this study, the level of Il-8 was calculated to ensuring the presence of abnormalities of neutrophil function further. The results showed the low Il-8 levels, which was below 0.0064 pg/μl, and significantly different between the AP and NAP patients ($p < 0.001$; OR = 34.5 (6.760–176.081) with the cutoff point was 0.064 pg/μl. Il-8 is one of the most important chemoattractants for neutrophil chemotaxis. In the chemotaxis process, Il-8 is required as an endogenous chemoattractant (Tsikitis et al., 2004), which is synthesized by both inflammatory and noninflammatory host cells in response to various stimuli. The level will increase in the inflamed region. The ROC curves for the levels of Il-8 in Aggressive Periodontitis (AP) was seen more below the cutoff point (23 of the 32 subjects), and in the NAP group was seen more above the cutoff point (27 from 29 subjects.) At the NAP group, the level of Il-8 was consistent to the theory stated that the inflammatory process began with the presence of pathogenic bacteria located in the gingival sulcus stimulating the gingival epithelial cells to produced Il-8 as the chemoattractant triggering neutrophils to conjugated towards the infected area. Il-8 is also produced by the monocytes and macrophages so that the levels of Il-8 are abundant in the infection, consistent with research conducted by Ertugrul et al. (2013), which discovered the higher level of Il-8 in aggressive periodontitis patients compared to the chronic and healthy periodontitis patients. However, the research of Ladez et al. (2012) showed that the level of Il-8 level in aggressive periodontitis patients was not significantly different from the level in the chronic and healthy periodontitis patients.

Il-8 acts as a messenger for other biological processes such as angiogenesis, cell proliferation, apoptosis, tumor metastases and body defense (Cekici et al., 2014). There are two types of Il-8 based on the function, homeostatic and inflammatory. The low Il-8 level in aggressive periodontitis was suspected as the involvement of the Aa bacteria producing leukotoxin which caused necrosis or apoptosis of the neutrophil cells (Lally et al., 1999). Along with this process, neutrophil will secrete Human Neutrophil Elastase (HNE) to triggered the proteolysis process in the infected area that will convert the initial Il-8 molecules into small undetected (Leavell et al., 1997). The involvement of *P. gingivalis* that inhibited the epithelial cells from producing the Il-8. However, by observing the results of various studies suggested that Il-8 acts as an endogenous chemoattractant in neutrophil chemotaxis (Tsikitis et al., 2004; Kohidai and Csaba, 1998), and Il-8 was a chemotactic cytokine that plays an important role in the neutrophil cell migration to the infected region (Cekici et al., 2014), the decreasing level of IL-8 found in this research was a strong evidence of a decreasing neutrophil chemotaxis ability caused by aggressive periodontitis, indicated by the presence of FPR1 gene polymorphism and low IL-8.

This study discovered a correlation between the polymorphism in the 301 G > C region of the FPR1 gene with periodontal tissue destruction (CAL) ($r = 0.37$; $p = 0.039$). The FPR1 gene polymorphism in aggressive periodontitis caused significant attachment loss in contrast to the non-aggressive periodontitis ($p = 0.002$) as seen in Table 5, and on the condition of less dental plaque accumulation ($p = 0.002$) as presented in Table 4. Although the accumulation of plaque was minimal, the involvement of *Aggregatibacter actinomycetemcomitans* (Aa) producing leukotoxin will accelerate necrosis or apoptosis of the neutrophil cells (Lally et al., 1999). The Human Neutrophil Elastase (HNE) will also secreted, which will lead to the occurrence of proteolysis in the infected area (Leavell et al., 1997). Polymorphism 301 G > C FPR genes related to the oral hygiene factors (plaque accumulation) that cause periodontal tissue destruction such as attachment loss in aggressive periodontitis.

Recent understanding of the pathogenesis of periodontal disease suggested that this disease occurred as a result of the complex interaction between periodontopathic microorganisms and host factors. In 1986, Lisgarten had proposed the concept stated that deficiency of the body or an increase in the proportion of certain pathogenic bacteria indicated an imbalance in the host-parasite equipoise. Bacteria cause virtually all forms of periodontal disease, but the clinical features are modulated by the interaction with the host. The host-parasite interaction is naturally unique for each individual and depends on how individuals anticipate changes towards the environment. This concept developed by Darveau (2010), which stated that periodontitis involved in various homeostatic host chaos in periodontal tissues. However, little was known about how the bacteria modulate the secretion of cytokines causes destructive inflammation. Understanding of interactions between microbes as a community and between microbes with the host continuously studied to explain the interaction results, whether manifested to the healthiness or sickness. The results of this study indicated the interaction between bacteria with genetic factors, and genetic factors were more prominent in the periodontal tissue destruction. This condition suggested that although the bacteria have the potential on causing abnormalities in periodontal tissues, the microflora composition of the oral cavity was highly depended on the oral hygiene and individual dietary habits, especially in supragingival plaques. Thus, the bacteria proportion often varies over time, as well as the proportion of the Aa and Pg bacteria that will be more stable during growth and formed the subgingival (Tinoco et al., 1998). Also, host factors play were more prominent in determining the clinical features of the tissue conditions affected by the presence of microorganisms (Kilian et al., 2006; Uzel et al., 2011).

The results of this study that showed the prominence of the genetic factors were shown in the correlation between the FPR1 gene polymorphisms and the CAL value. Gene polymorphism can be meant as the changes in amino acids, which may further alter the flow of signals that will cause homeostatic disorders in the neutrophil cells of aggressive periodontitis patients. Genetic changes interacted with environmental factors resulting in various inflammatory and immunologic

processes (Diehl et al., 1999). Aggressive Periodontitis showed a strongly familial relation describing the influence of genetical components (Glazier, 2002; Fine et al., 2006). Due to its firmness, gene factors were a determinant risk factor, whilst the environmental factors were the indicating factor (Van Dyke and Dave, 2005).

5. Conclusion

Genetic abnormalities of the defective neutrophil function in aggresive periodontitis represented by polymorphism c576 T > C > G, 301 G > C of FPR1 gene. Correlated with the low Il-8 level, more prominent cause of periodontal tissue destruction compare to environmental factors.

Conflict of interest

The authors declare that there is no conflict of interest.

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