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RESEARCH ARTICLE

Porphyromonas Gingivalis fim A Type *I, II, III, IV, V* and *Ib* Genotypes Distribution among Malay Chronic Periodontitis Patients

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Abstract

Objectives: The aim of the present study was to determine the prevalence of *P. gingivalis* with *fimA I, II, III*, *IV*, *V* and *Ib* genotypes in the Malay ethnic group and to relate their presence to chronic periodontitis. Design: A total of 30 Malay subjects (15 periodontitis and 15 periodontally healthy subjects) aged 25 years and above were selected. Clinical periodontal parameters were recorded for each subject. Subgingival plaque samples were collected from the deep pockets ≥5mm in periodontitis subjects and from subgingival areas in healthy subjects. Presence of *P. gingivalis fimA* types I, II, *III*, *IV*, *V* and *Ib* genotypes were identified by polymerase chain reaction followed by agarose gel electrophoresis. Results: *P. gingivalis* was more prevalent in periodontitis subjects (53.33%) as compared to periodontally healthy subjects (13.33%). In periodontitis subjects, *P. gingivalis fimA* I, II, *IV*, *V* and *Ib* genotypes were detected in of 20%, 33.33% 20%, 6.67% and 13.33% respectively among periodontitis subjects. The frequency of genotype fimA type II was greater where plaque accumulation was >1. However, *P. gingivalis* fimA IV genotype followed by fimA Ib genotype were detected in higher percentages. Conclusion: *P. gingivalis* was detected in periodontally diseased and healthy subjects. *P. gingivalis* fimA genotype II, IV and Ib was identified in diseased subjects only. In conclusion, the prevalence of *P. gingivalis* especially fimA genotype II, IV and Ib can be used as indicator to differentiate between diseased and healthy subjects.

Keywords: *P. gingivalis, Chronic periodontitis, Genotypes.*

Introduction

Chronic periodontitis is a destructive inflammatory process involving the periodontal supporting tissues of teeth which results in alveolar bone loss and eventually in severe cases to tooth loss [1, 2].It is a poly microbial progressive disease of global concern affecting mostly adults over 35 years of age. Its variability and severity is mainly influenced by several local and systemic contributing factors [3]. In about 10-15% of the population severe forms of periodontitis occur without a correlation between the supragingival plaque accumulation and the severity of the disease. The number of these patients increases with age and attains the highest level at the age of 40-50 years [4]. Studies have shown the evidence for the primary role of bacteria in the aetiology of destructive periodontal disease [5]. It is mainly a strict and obligately anaerobic infection. *Porphyromonas ginigivalis* is a Gram negative black pigmented strictly anaerobic bacterium that has been encountered as a major (bona fide) etiopathogenic agent in the onset and progression of chronic destructive periodontitis [6, 7]. It has also been reported that *P. gingivalis* was not only detected at a high frequency in patients with periodontitis but also at a low frequency in periodontally healthy individuals without marked gingival inflammation [8-10].

This was then attributed to the clonal heterogenicity of *P. gingivalis* subpopulation with low and high pathogenicities [11]. Clinical and laboratory strains were first examined using animal models studies [12]. In similar study, It was found that *P. gingivalis* strains have been classified either as being virulent/invasive or a virulent /non–invasive [13].*P. gingivalis* possesses a number of virulence factors such as fimbriae, LPS, capsule, cysteine proteinases (gingipains) which play a significant role in the pathogenesis of chronic periodontitis [14, 15]. Among those factors, fimbriae were found to be major virulence factors. They are hair-like filamentous components on the cell surface of which *fimA*, a structural subunit protein of major fimbriae encodes fimbrillin [16].

Those unique structures were implicated in the adherence of this bacterium to and invasion of various host tissues [6]. The fimbriae of *P. gingivalis* mediate oral colonization by serving as an adhesin for the attachment to pellicle coated tooth surface, Gram positive bacteria & host cells [17-19]. Mutants of *P. gingivalis* defective in the major fimbrial subunit *fimA* have demonstrated to be less virulent in a rat model study [20]. Environmental factors such as temperature, osmotic pressure, and PH have been considered to alter the expression of *fimA* gene [21]. *P. gingivalis fimA* gene that encodes the fimbrillin protein (FimA), a structural subunit of major fimbriae of this microorganism, is a single copy gene available on the bacterial chromosome and no homologous structure has been found in other black-pigmented *Porphyromonas* species [22].

A number of epidemiological studies have demonstrated the prevalence of *fimA* genotypes in non-homogenous population of multiracial origin [23]. Similar studies have been also conducted in various countries to explore the relationship of *fimA* genotypes with periodontal disease [24-28]. Nevertheless, no study has been conducted to investigate the distribution of *P. gingivalis fimA* genotypes in the Malay ethnic group from south East Asia and the current study was conducted to explore this association in Malay ethnic group in Malaysia.

Materials and Methods

Study Subjects and Sample Collection

Thirty Malay subjects, aged 25 years and above, who were first examined in the primary dental care unit and referred to the periodontal unit of the postgraduate clinic, Faculty of Dentistry at the University of Malaya were enrolled in the present study. Informed consent of the patients was obtained according to the ethical guidelines of the medical ethical committee (DF OP0703/0030 (P)) at the Faculty of Dentistry, University of Malaya. Patients were excluded from the study if any of the following conditions applied: patients who had any history of systemic conditions or disease, patients on antibiotics within the last three months or who had received any professional periodontal treatment in the same interval, edentulous subjects or those who had less than 16 teeth and pregnant patients.

Patients were divided into two equal groups (15 with periodontitis and 15 age matched periodontally healthy controls). Those subjects (periodontally healthy) who were selected for the control group were those with probing depths of ≤3 mm, had gingivitis score of ≤20% and no evidence of attachment loss clinically (Tan et al., 2001).

Study Protocol

The study questionnaire form was designed to include the demographic data such as sex, age and income, as well as behavioral/environment information, which included to patient's oral hygiene practice, dental visit frequency and habits such as smoking.

Periodontal Examination and Clinical Specimens

The subjects were examined clinically to assess their oral hygiene and periodontal status. The Plaque [29] and gingival [30] indices were recorded four sites of the teeth excluding the third molars, while bleeding index [31], probing pocket depth and loss of attachment assessments were carried out at six sites of the teeth. All measurements were done by periodontal sensor probe Type C (Straumann® GmbH, Freiburg, Germany). Supragingival plaque was first removed from the sample sites with a hand curette. The sites were then cleaned with cotton pellets and dried before sampling of the subgingival plaque. Subgingival plaque was collected using sterile Gracey curettes applied to the root surface. In the periodontitis group, subgingival plaque was obtained from four

teeth (for each subject) with ≥ 5 mm pockets from different mouth quadrants. In periodontally healthy group, subgingival plaque was also collected from four teeth (for each subject) that did not show any sign of bleeding on probing. Subgingival plaque was pooled and suspended in a tube containing 1ml of 10% phosphate buffered saline (PBS) and stored at -80 ˚C.

Genomic DNA Preparations

The bacterial genomic DNA was isolated using EZ-10 Spin Column Bacterial DNA Mini-Preps Kit according to the manufacturer's instructions (Bio Basic, Ontario, Canada).The isolated DNA was dissolved in 100 ml of TE (10mM Tris HCl [pH 8.0] and 1mM EDTA) buffer and stored at -20ºC. The UV absorption was utilized to measure the DNA quantity at A260 (1.0 OD unit is equivalent of 50ug).

The genomic DNA quality was then assessed by an analytical 0.7% agarose gel. Then after, genomic DNA was isolated which did not contain RNA and the extracted DNA obtained was 50 kb size.

PCR Primers and Amplification

Table 1: Lists of the PCR primers utilized for this study

Primer set	Direction	Sequence	Size bp
Universal primer for positive control	Forward reverse	AGA GTT TGA TCC TGG CTC AG GGC TAC CTT GTT ACG ACT T	3500
P. gingivalis specific 16S r RNA	Forward reverse	TGT AGA TGA CTG ATG GTG AAA ACC ACG TCA TCC CCA CCT TCC TC	197
Type I *fimA*	Forward reverse	CTG TGT GTT TAT GGC AAA CTT C AAC CCC GCT CCC TGT ATT CCG A	392
Type II *fimA*	Forward reverse	ACA ACT ATA CTT ATG ACA ATG G AAC CCC GCT CCC TGT ATT CCG A	257
Type III *fim A*	Forward reverse	ATT ACA CCT ACA CAG GTG AGG C AAC CCC GCT CCC TGT ATT CCG A	247
Type IV *fim A*	Forward reverse	CTA TTC AGG TGC TAT TAC CCA A AAC CCC GCT CCC TGT ATT CCG A	251
Type V *fim A*	Forward reverse	AAC AAC AGT CTC CTT GAC AGT G TAT TGG GGG TCG AAC GTT ACT GTC	462
Type Ib *fim A*	Forward reverse	CAG CAG AGC CAA AAA CAA TCG TGT CAG ATA ATT AGC GTC TGC	271

The fimA genotype-specific forward primers were selected from type specific segments of nucleotide sequences of the five genotypes (I, II *III*, *IV*, *V*, *Ib*). The reverse primer was common for the four *fim A* genotypes {*I*, *II*, *III*, I*V*}, but variant in the other genotypes {*V*, *Ib*}. (Biobasic, Ontario, Canada). The PCR method was utilized in the study using both forward and reverse primers (Bio Basic) and the procedures were as follows: *P. gingivalis* 16S rRNA specific primers were described by [32], and *P. gingivalis* 16S rRNA specific primers type I, II, III, IV, V and Ib which coded for fimA, designed by [16].

PCR amplification was achieved in a total volume of 50 µL consisting of PCR components (Take it-easy PCR kit, Germany) of 31 µL of H2O, 544µL of 10x buffers, 8 µL of 25 mM MgCl2, 1.8 µL dNTPs mixture added into each empty tube (experimental and control groups). 2 µL of *P. gingivalis* 16S rRNA primer and fim A genotypes *I, II, III, IV V, Ib* primers (R, F) that were prepared previously were added into experimental and control groups respectively (each group=15 tubes). 2 µL of specimens taken from healthy subjects were added into control group, whereas the specimens taken from periodontitis subjects were added into experimental group. 0.5 µL of Taq DNA polymerase was then added into each tube. The procedure that was used for PCR amplification to prepare *P. gingivalis* and primers type *I , II, III, IV, V* and Ib in the experimental and control groups was similar for the preparation of *P. gingivalis* species-specific positive control group as discussed previously.

The amplification reaction was performed in a thermal cycler (Eppendrof, Hamburg, Germany) with the following cycling parameters: an initial denaturation at 94°C for 2 min, following 30 cycles consisting of 94°C for 10 s, 55°C for 25 s, and 72°C for 25 s, and a final extension at 72°C for 10 min. Positive and negative controls were included in each PCR set and in the processing of all samples. The PCR products were subjected to electrophoresis in a 1.5 % agarose gel-Trisborate EDTA buffer with 0.2 µL of

ethidium bromide. The gel was photographed under UV illumination. A 100-bp DNA ladder (Amresco Inc solon, OH, USA) was used as a molecular size standard.

Reproducibility Study

All the study measurements were performed by the first author. lntra-examiner reproducibility was conducted by him, two patients diagnosed with chronic periodontitis with a time interval of 4-5 h between the two assessments. This was performed to minimize examiner memory recollection of previous recordings between the indices used which were those that have been described.

Statistical Analysis

Chi-square test was utilized to analyse the distribution of fim A genotypes I, II, III, IV, V and Ib in periodontally healthy and periodontitis subjects in relation with clinical parameters. Fisher's exact test was also used to analyse the relation between *P. gingivalis fimA* genotypes and smoking habit in the *P. gingivalis* positive subjects.

Results

Clinical and Socio-demographic Parameters of the Study Population

Mean and Standard Deviation for the Age of the Study Population

Student ***t***-test was performed to compare the means of age for diseased and healthy subjects. The mean age of healthy subjects was less than that shown in diseased subjects (Table 2).

Table 2: Age distribution of the study population

Group	N	Mean (SD)	Age Range
Diseased	**15**	**39.93 (7.045)**	**27-55 years**
Healthy	**15**	**28.93 (2.520)**	**26-35 years**

Analysis of Socio-demographic Parameters of the Study Population

As shown in Table 3, the Chi-square test (χ^2) was used to analyse the variables with three levels such as income, whereas variables with two levels or rankings i.e. gender, smoking habits and regularity of dental visits were analysed by using Fisher exact test. Both groups of subjects exhibited no significant difference ($p>0.05$) in the income level. With regards to the gender, the gender in both groups showed no significant difference ($p>0.05$). In relation to smoking habits, there was no significant difference in the smoking habits between both periodontally healthy and diseased groups ($p>0.05$). Also there was no significant difference ($p>0.05$) in the dental visits between periodontally healthy and diseased groups.

Table 3: Socio-demographic characteristics of the study population

Characteristic	Diseased	Healthy	Total	p-value
Gender				
Male	9(30%)	12(40%)	21(70%)	0.213
Female	6(20%)	3(10%)	9(30%)	(FE Test)
Income level				
Low	5(16.7%)	5(16.7%)	10(33.3%)	0.525
Middle	8(26.7%)	10(33.3%)	18(60%)	(X^2 test)
High	2(6.7%)	0(0%)	2(6.7)	
Smoking habit				
Smoker	7(23.3%)	5(16.7%)	12(40%)	0.456
Non-smoker	8(26.7%)	10(33.3%)	18(60%)	(FE test)
Dental visit				
Regular	3(10%)	7(23.3%)	10(33.3.7%)	0.121
Irregular	12(40%)	8(26.7%)	20(66.7.3%)	(FE Test)

□□□□□□□^{2} = Chi-square test
FE test = Fisher's Exact test

Distribution of *P. gingivalis fimA* type *I, II, III, IV, V and Ib genotypes* in periodontitis Patients and Periodontal Healthy Individuals

Table 4 show the prevalence of *fimA* type *I, II, III, IV, V and Ib* genotypes of *P. gingivalis* in periodontitis patients and periodontal healthy individuals. Fisher's exact test was utilized to determine the association between

fimA genotypes and the periodontal health status. There was no significant difference in the distribution of the five fimA *I, III, IV, V* and *Ib* genotypes between the periodontally healthy and diseased subjects (p>0.05) but there was a significant difference between healthy and periodontitis individuals for the detection of *fimA* type *II* genotype (p<0.05).

Table 4: Distribution of the *fimA* type *I, II, III, IV, V and Ib* genotypes among *P. gingivalis* positive healthy and diseased subjects in relation to periodontal status

fimA	Healthy n=15	Diseased n=15	p-value
Type I	6.67% (1)	20% (3)	0.299(NS)
Type II	0 (0)	33.33 (5)	0.021*
Type IV	0% (0)	20% (3)	0.313(NS)
Type V	6.67% (1)	6.67% (1)	0.500(NS)
Type I b	0% (0)	13.33% (2)	0.500(NS)

NS=Not Significant
* = Significant

Comparison of the Clinical Parameters of the Study Subjects

The distribution of data was assessed by using Kolmogorov-Smirnov normality test. The parametric student ***t***-test was applied as the data for both plaque and gingival indices were normally distributed. As shown in Table 5, the mean difference in plaque and gingival indices between test and control subjects was statistically significant. Generally, those subjects in the periodontally diseased group showed higher plaque and gingival indices as compared with those in the periodontally healthy group. On the other hand as shown in table 6, Mann-Whitney ***U*** test was used for the data of bleeding index, probing pocket depths, probing attachment loss measurements and missing teeth since these data were not normally distributed. There was a significant difference in the mean values of all the four clinical parameters between the periodontally healthy and diseased group (p<0.05). All values for these parameters were higher in the periodontally diseased subjects rather than the periodontally healthy subjects.

Table 5: Comparison of PI and GI of the study subjects using student *t*-test

				t-test for equality of means		
Variable	Group	n	Mean ± SD	95% CI for mean difference	t-value	p-value
Plaque Index (PI)	Diseased	15	.929 ± .655	.667(.316,1.019)	3.889	**<0.001**
	Healthy	15	.261 ± .114			
Gingival Index (GI)	Diseased	15	.909 ± .442	.743(.505,.991)	6.382	**<0.001**
	Healthy	15	.159 ± .092			

Table 6: Comparison of BI, PPD, PAL and missing teeth between test and control groups by using Mann-Whitney *U*-test

					Mann-Whitney *U* test	
Variable	Group	N	Mean	Mean rank	Z-value	p-value
Bleeding index (BI)	Diseased	15	.524	22.53	4.200	**<0.001**
	Healthy	15	.090	8.77		
Probing Pocket Depth (PPD)	Diseased	15	5.50	23.00	4.764	**<0.001**
	Healthy	15	.067	8.00		
Probing Attachment Loss (PAL)	Diseased	15	5.76	23.00	4.762	**<0.001**
	Healthy	15	.067	8.00		
Missing Teeth	Diseased	15	5.07	20.50	3.140	**0.002**
	Healthy	15	1.53	10.50		

Distribution of *P. gingivalis* in Periodontally Healthy and Disease Subjects

Table 7 shows the presence of *P. gingivalis* in periodontally healthy and diseased groups. The table shows a higher percentage of *P.gingivalis* in periodontally diseased (periodontitis) subjects (53.33%) as compared to the periodontally healthy subjects (13.33%). These categorical or nominal data were analysed using Chi-square test. The results indicated a significant difference in the presence and absence of *P. gingivalis* between the periodontally diseased and healthy subjects (p= 0.02).

Table 7: Distribution of *P. gingivalis* among periodontally healthy and diseased subjects using chi-square test

Periodontal status	N	*P.gingivalis* Positive	*P.gingivalis* negative	p-value
Healthy	15	2 (13.33%)	13 (86.67%)	0.020
Disease	15	8 (53.33%)	7 (46.67%)	0.020

Mean Clinical Scores Related to the Presence or Absence of *P. gingivalis* in the Study Subjects

Data distribution was analyzed utilizing Kolmogorov-Smirnov test. Data for plaque, gingival and bleeding indices, pocket depth and attachment loss measurements as well as missing teeth were all not normally distributed ($p<0.05$). Hence, the Mann-Whitney U test was used. Generally, all these parameters means were detected to be higher in the presence of *P. gingivalis* as compared to its absence. With the exception of plaque index and missing teeth ($p>0.05$), the difference for the rest of the parameters were statistically significant ($p<0.05$) as indicated in Table 8.

Table 8: Comparison of PI, GI, BI, PPD, PAL and missing teeth to the presence and absence of *P. gingivalis* using Mann-Whitney *U* test

					Mann-Whitney U test	
Variable	***P. gingivalis***	**N**	**Mean**	**Mean rank**	**Z-value**	**p-value**
Plaque Index (PI)	Negative	20	.515	13.68	1.606	0.109
	Positive	10	.755	19.15		
Gingival Index (GI)	Negative	20	.363	12.33	2.795	0.004*
	Positive	10	.868	21.85		
Bleeding Index (BI)	Negative	20	.226	13.25	1.985	0.049*
	Positive	10	.469	20.00		
Probing Pocket Depth (PPD)	Negative	20	2.00	13.18	2.088	0.039*
	Positive	10	4.33	20.15		
Probing Attachment Loss (PAL)	Negative	20	2.07	13.00	2.245	0.028*
	Positive	10	4.59	20.50		
Missing Teeth	Negative	10	2.75	13.95	1.377	0.183
	Positive	20	4.40	18.60		

Distribution of *P. gingivalis* in Smokers and Non Smokers

As shown in Table 9 *P. gingivalis* was present in 1.2% of the smokers and in 1.8% of nonsmokers and absent in 2.4% of the smokers and 3.6% of non-smokers. Fisher´s Exact test was used to analysis this association. There was no significant difference between smokers and non-smokers in relation to the presence and absence of P. gingivalis ($p>0.05$).

Table 9: Distribution of *P. gingivalis* in smokers and non smokers using Fisher´s Exact test

	P. gingivalis Not present (20)	*P. gingivalis* present (10)	p-value
Smokers	(8) 2.4%	(4) 1.2%	0.656 (NS)
Non smokers	(12) 3.6%	(6) 1.8%	0.656 (NS)

NS= Not significant

Distribution of the *fimA* type *I, II, III*, *IV*, *V* and *Ib* genotypes among *P. gingivalis* Positive Healthy and Diseased Subjects in Relation to Plaque Index, Bleeding Index, Gingival Index, and Probing Pocket Depth, and Probing Attachment Loss

The relation between the percentages of *P. gingivalis* genotypes *I, II, III*, *IV*, *V* and *Ib* in healthy and periodontitis individuals in relation to probing pocket depth (PPD), plaque (PI), bleeding (BI), gingival (GI), indices, and probing attachment loss measurements is shown in Table 10. Results of this investigation between the individuals who were *P. gingivalis* positive showed that genotype *I* and *II* bacteria were found almost equally in each clinical parameter of probing pocket depth and attachment loss. However, genotype *II* was higher than genotype *I* in both these measurements; both types of bacteria were found only in depths more than 3 mm. For the gingival index, genotype *I* and *II* bacteria were related to < 1 and > 1 indices especially genotype *II*. Similarly, the gingival index (GI) (≥1), probing pocket depth (≥3) and probing attachment loss measurement (≥3) were all found to be higher in *fimA IV* genotype positive subjects (30%) followed by *fimA Ib genotype* positive subject (20%) and *fimA V* genotype positive subjects (10%). In the case of the bleeding index, genotype *I* and

II of *P. gingivalis* were associated with bleeding especially for genotype *I*. The bleeding index (BI) measurement >0 was noted to be higher in *P. gingivalis fimA IV* genotype positive subjects (30%) followed by *P. gingivalis fimA Ib* positive subjects (20%). In addition genotypes *I*, *II* , *III*, *IV*, *V* and *Ib* were found (in both categories of plaque index). Comparison of the presence of *P. gingivalis* genotypes *I*, *II* , *III*, *IV*, *V* and *Ib* in healthy positive and diseased individuals, in relation to PPD and LDA measurements as well as BI, GI and PI was done using chi square test. There was no statistically significant difference (p>0.05) between the presences of genotypes *I*, *II* , *III*, *IV*, *V* and *Ib* on parameters.

Table 10: Distribution of *P. gingivalis fimA* genotypes III, IV, V and *Ib* among *P. gingivalis* positive subjects (n=10) in relation to clinical parameters using Fisher's exact test

	Type I	**p-value**	***Type II***	**p-value**	***Type VI***	**p-value**	***Type V***	**p-value**	***Type Ib***	**p-value**
Parameter	Present		Present		Present		Present		Present	
Plaque Index										
<1	2 (20%)	0.31	4 (40%)	0.31	3 (30%)	0.167	1 (10%)	0.667	1 (10%)	0.667
≥1	1 (10%)		1 (10%)		0 (0%)		1 (10%)		1 (10%)	
Bleeding Index										
0	3 (30%)	0.11	2 (20%)	0.10	0 (0%)	0.083	2 (20%)	0.222	0 (0%)	0.468
>0	0 (0%)		0 (0%)		3 (30%)		0 (0%)		2 (20%)	
Gingival Index										
<1	2 (20%)	0.43	3 (30%)	0.33	0 (0%)	0.700	1 (10%)	0.200	0 (0%)	0.800
≥1	1 (10%)		2 (20%)		3 (30%)		1 (10%)		2 (20%)	
Pocket probing depth (mm)										
<3mm	3 (30%)	0.18	5 (50%)	0.40	0 (0%)	0.083	1 (10%)	0.378	0 (0%)	0.622
≥3mm	0 (0%)		0 (0%)		3 (30%)		1 (10%)		2 (20%)	
Probing attachment loss (mm)										
<3mm	3 (30%)	0.50	5 (50%)	0.10	0 (0%)	0.083	1 (10%)	0.378	0 (0%)	0.622
≥3mm	0 (0%)		0 (0%)		3 (30%)		1 (10%)		2 (20%)	

P. gingivalis fimA type *III* genotype was not considered as it was not detected in all the plaque samples

Distribution of *P. gingivalis of fimA genotypes I, II III, IV, V* and *Ib* genotypes in Smokers and Non smokers

Table 11 illustrates the relation between the distribution of *P. gingivalis fimA I, II III, IV*, *V* and *Ib* genotypes among *P. gingivalis* positive subjects and their smoking habit. Chi square test was used to analysis this association. There was no significant difference between smokers and non-smokers in relation to the presence and absence of *P. gingivalis fimA* types *I* and *II III, IV*, *V* and *Ib* genotypes among *P. gingivalis* positive subjects (p>0.05).

Table 11: Distribution of *P. gingivalis of fimA* type *I, II III, IV, V and Ib* genotypes in smokers and non smokers with analysis done by chi square test

***P. gingivalis* genotypes**		**Smokers (N)**	**Non-smokers (N)**	**p-value**
Type *I*	**Present**	4.8% (16)	3% (10)	0.531
	Absent	0.6% (2)	0.6% (2)	0.531
Type *II*	**Present**	4.5% (15)	3% (10)	0.696
	Absent	0.9% (3)	0.6% (2)	0.696
Type IV	**Present**	0(0%)	3 (10%)	0.201

	Absent	12 (40%)	15 (50%)	0.201
Type V	**Present**	1 (3.3%)	1 (3.3%)	0.648
	Absent	11 (36.7%)	17 (56.7%)	0.648
Type Ib	**Present**	1 (3.3%)	11 (36.7%)	0.648
	Absent	1 (3.3%)	17 (56.7%)	0.648

P. gingivalis fimA III genotype was not considered as it was not detected in all clinical plaque samples

Discussion

Periodontal disease is a multifactorial polymicrobial condition that affects the supporting tissues of the teeth leading to periodontal tissue breakdown, loss of periodontal attachment, alveolar bone resorption and in severe cases, eventual tooth loss [33]. There are many factors associated with periodontal disease such as age, gender, socio-economic status, income level, smoking; and dental visits regularity. Age is one of several socio-demographic factors that is assumed to be associated with oral health status. A number of studies reported the prevalence of periodontal disease among elderly population [34, 35]. In the present study, the diseased population was older than the healthy group and this difference in the mean ages was significant statistically in the Malay population.

This may be due to increased severity of periodontal disease and bone loss with age and is probably related to the length of time the periodontal tissues have been exposed to bacterial plaque and is considered to reflect the individual's cumulative oral history, as well as the increase rate of accumulation of plaque. The differences in lifestyle between different levels of socio-economic status groups contribute to the increasing social inequality observed with respect to general health and oral health indicators.

The results obtained in the current study, indicated that in the Malay population, low socio-economic status, which associates with low income, low educational level, and irregular dental visits were important factors for the prevalence of periodontal diseases. These findings agree with those reported in a Canadian population [36] that showed that socio-economic status has an effect on periodontal condition.

The relationship between smoking and periodontal diseases in oral and periodontal disease has been documented since mid 20th Century. In some studies on the relationship between smoking and some subgingival periodontopathogens such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* it was noted that patients who were smokers and non-smokers largely exhibited the same subgingival microflora, suggesting that smoking has limited influence on the microflora involved in periodontal disease [37-39]. Boström et al (2000) reported the detection rates for *P. gingivalis, P. intermedia, P. nigrescens, B.forsythus, A. actinomycetemcomitans, T.denticola, C. rectus and S. intermedius* to be 10 or more percent units greater in smokers than in non-smokers but the differences between smokers and non-smokers were not statistically significant [40].

The current study is consistent with all previous studies that showed that periodontal disease was common in smokers than non-smokers and that smoking had no influence on the subgingival periodontopathogenic microflora. Plaque indices were correlated with severity of periodontal disease. A number of periodontal studies employed numerous variables including bleeding on probing, presence of calculus, probing pocket depths, clinical attachment levels and radiographic assessment of alveolar bone to assess periodontal disease status.

An increased severity of periodontal disease was indicated in terms of deeper periodontal pockets, greater attachment loss, and alveolar bone loss [41, 42]. In the current study, periodontitis subjects displayed significantly higher pocket depth, attachment loss, as well as the number of missing teeth as compared to healthy subjects as also reported previously [42, 43].

P. gingivalis was reported to be a major predominant pathogen that was enormously associated with periodontal disease [1]. Nevertheless, *P. gingivalis* was rarely detected or at a lower frequency in healthy

periodontal status [9, 44]. In the current study, by using sensitive PCR technology, *P. gingivalis* was found in 53.33% of the plaque

samples of the periodontally diseased group and in 13.33% of the plaque samples of the periodontally healthy group.

All subjects were of Malay ethnic origin, hence *P. gingivalis* was observed in both groups although it was more common in the periodontally diseased subjects. This finding was consistent with the results of many other studies where prevalence of *P. gingivalis* were reported in both periodontally healthy and diseased groups [23, 24, 27, 44, 45].

All previous studies including the present one provided evidence that *P. gingivalis* is truly a major pathogen implicated in the etiology of chronic periodontal disease. The present study also exhibited the association between the existence of *P. gingivalis* and clinical parameters, but it contradicted the findings of [46], whereby there was no association detected between the clinical parameters and the presence of *P. gingivalis*.

This contradiction is possibly attributed to the discrepancy in sample size and methodology. In this study, the prevalence of fimA genotype *I, II, III, IV, V* and *Ib* of *P. gingivalis* in periodontitis patients and periodontal healthy individuals were studied. In periodontitis subjects, *P. gingivalis* genotypes were of 20%, 33.33%, 20%, 6.67% and 13.33% for type *I, II, IV, V* and *Ib* respectively. FimA *III* genotype was not detected in any of the subject subgingival plaque samples. On the other hand, in healthy individuals, *P. gingivalis* genotypes were 6.67%, 6.67% and 13.33% for type I, V and Ib respectively.

But fimA II, III and V genotypes were not detected in any of the healthy samples. The present study showed *P. gingivalis* fimA genotype II which accounted for 33.3% of the periodontitis patients was the most prevalent followed by *P. gingivalis fimA* genotype *IV* which was the next most prevalent type among chronic periodontitis subjects (20%) and then *P. gingivalis fimA* genotype *Ib* was detected in 13.33% of subjects.

These findings were concurred with other studies where *P. gingivalis fimA* genotypes *II* and *IV* were the most predominant genotypes among periodontitis subjects [24, 47, 48]. However, these findings simultaneously were in contrast with Brazilian and European studies where *P. gingivalis fimA* genotypes *IV* and *II* were the most predominant genotypes among periodontitis patients with a higher prevalence of *P. gingivalis fimA* genotype *IV* [28, 45]. Other Brazilian, Japanese and Colombian studies showed the predominant incidence of *P. gingivalis fimA* genotype *II* followed by *P. gingivalis fimA* genotype *Ib* [23, 49, 50].

Furthermore, *P. gingivalis fimA* genotype *V* was equally prevalent (6.67%) among both healthy and periodontitis Malay subjects. Interestingly, *P. gingivalis fimA* genotype *III* was not detected in any plaque sample. This finding was in contrast with Colombian and European studies where *P. gingivalis fimA* genotype *V* was not detected in all samples of the study population [48, 50]. This predominance of *P. gingivalis fimA* genotypes *III* and *V* as well as *fimA* genotype *I* in the periodontally healthy group subjects may possibly indicate a difference in the structure of fimbrillin and thus a difference in the fimbriae composition could have an impact on the variation in the pathogenic likelihood of major fimbriae of *P. gingivalis*.

Overall, the variability in the prevalence of different *P. gingivalis fimA* genotypes in the relevant studies including the current study could be possibly attributed to methodological variation such as dilution of samples and amplification of other regions apart from 16S rRNA [51], but it could also be due to ethnicity with distinguished customs and cultural habits such as diet or it might be due to geographic location of the studied population [10, 52].

Another possible reason for different prevalence of *P. gingivalis fimA* genotypes may be observed in the functional difference among the various fimbriae types leading to better adaptation of a given *fimA* genotype to environmental challenge. Therefore any FimA genotype with a higher affinity to adherence and binding to gingival epithelial cells and with an ability to invade these epithelial cells to a greater extent than any other fimbriae may suggest its superior virulence and explain its higher prevalence than other genotypes [49].

In conclusion, *P. gingivalis* was detected in periodontally diseased and healthy subjects. However it was more predominant in the

diseased subjects. *P. gingivalis* fimA genotype *I* and *V* was detected in both groups. *P. gingivalis* fimA genotype *II, IV* and *Ib* was identified in diseased subjects only. Furthermore, *P. gingivalis* fimA *III* was not detected in any of the plaque samples. *P. gingivalis* was significantly associated with clinical parameters except plaque index and missing teeth. There was no significant association between the prevalence of *P. gingivalis* fimA *I, II, III, IV, V* and *Ib* genotypes and clinical parameters of periodontal disease as well as smoking habits.

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