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ORIGINAL ARTICLE

Prostaglandin-endoperoxide synthase (*PTGS2*) and Defensin beta 1 (*DEFB1)* gene polymorphisms are not associated with periodontitis in Malays

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Abstract

Introduction: In line with the association of prostaglandin-endoperoxide synthase 2 (*PTGS2*) and defensin beta 1 (*DEFB1*) single nucleotide polymorphisms (SNPs) with periodontitis among the Chinese and European populations, the current study was aimed to assess the same association among the Malays in Malaysia. *Methods*: Blood samples of individuals with periodontitis (PD) (n=72) and periodontally healthy (PH) (n=62) donors were obtained from Malaysian Periodontal Database and Biobanking system (MPDBS). Genomic DNA samples were analyzed for three *PTGS2* SNPs (rs5275, rs20417, rs689466,) and one *DEFB1* SNP (rs1047031) using Taqman SNP genotyping assays. Notably, rs20417 and rs689466 were located in the promoter region while rs5275 and rs1047031 were located in the 3′ untranslated region of the transcript. Association between the SNPs and PD were then analyzed using genotypic association analysis (additive, dominant and recessive models). *Results:* The allelic frequency for the rs689466-G was higher in PD group (35.2%) compared that in PH group (29.0%). However, the association of rs689466-G and other SNPs with PD was not statistically significant (at 95% CI). No associations were observed for genotypic associations between the *PTGS2* and *DEFB1* SNPs with PD susceptibility. *Conclusions: PTGS2* (rs5275, rs20417, and rs689466) and *DEFB1* (rs1047031) polymorphism was not associated with PD in Malays, unlike the Chinese, Taiwanese & European population. This suggests that other causal variants might be involved in the development and progression of PD among Malays.

Keywords: Periodontitis*; DEFB1; PTGS2*; Single nucleotide polymorphism

INTRODUCTION

Periodontitis (PD) is a chronic inflammatory disease of the periodontium which results in the destruction of the tooth supporting structures such as the connective tissue and alveolar bone. Severe PD is estimated to affect 11.2% of the global adult population[1] and is a major cause of tooth loss if left untreated. This leads to nutritional compromise, altered speech, low self-esteem, and a lower overall quality of life.

The sub-gingival microbial dental biofilm (dental plaque)[2,3] and both hyper and hypo-responsiveness of the host immune systems against the microbial challenge have been described as the main reasons for the development and progression of PD.[2,3] However, genetic variations, mostly single nucleotide polymorphisms (SNPs) or other polymorphisms were also found to be associated with increased susceptibility and severity to periodontal diseases.[4] Based on a large-scale replication study of 23 genes, *GLT6D1* (Glycosyltransferase 6 Domain Containing 1), *ANRIL* (antisense non-coding RNA in the INK4 locus), *IL-10* (Interleukin 10), *PTGS2* (Prostaglandin endoperoxide synthase 2), and *DEFB1* (Defensin beta 1) genes were considered to carry validated susceptibility variants for PD.[5] Among these susceptibility variants, 2 genes have been reported to show clear association with chronic periodontitis (CP) in different ethnicities: *PTGS2 in* Chinese, Taiwanese

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& European population,[6,7,9-11] and *DEFB1* in European population.[10] Notably, genetic and environmental heterogeneity may cause the genotype to vary between different ethnicities and population.[6-11]

PTGS2, also known as cyclooxygenase-2 plays important roles in converting arachidonic acid to prostaglandins, and in the progression of inflammation and carcinogenesis.[12] Increased expression of PTGS2 is involved in CP[13] while PTGS2 mediated prostaglandin synthesis is associated with bone resorption in CP.[6] In addition, PTGS2 inhibitors such as celecoxib, etoricoxib, loxoprofen and meloxicam have been shown to alleviate periodontal conditions by reducing alveolar bone resorption.[14,15] Defensins are proteins secreted by the immune system which help in maintaining the balance between a healthy state and diseased conditions in the complex environment of the mouth, and have a broad-spectrum antimicrobial activity against bacteria, fungi and some viruses.[10] Human β-defensins (hBDs) are a group of low molecular weight (3 to 5kDa) cationic antimicrobial peptides with cycteine-rich β-sheets.[16] Studies have revealed increased hBD-1 protein levels and up-regulated *DEFB1* mRNA in CP individuals[17,18,19] but showed down-regulated expression in aggressive periodontitis (AgP) and gingivitis participants.[17]

Given the importance of PTGS2 and DEFB1 in the development and progression of PD, we examined common *PTGS2* SNPs namely, rs5275 (8473T>C), rs20417 (-765G>C), rs689466 (-1195G>A) and *DEFB1* SNP namely rs1047031 (c*5G>A) for their associations with susceptibility to PD in Malaysian Malay ethnic group.

MATERIALS AND METHODS

Study Design and Ethics Approval

This is a cross-sectional study to analyse association of *PTGS2* (rs5275, rs20417, and rs689466) and *DEFB1* (rs1047031) polymorphism with PD in Malays. Ethical approval for this study was obtained from the medical ethics committee, Faculty of Dentistry, University of Malaya (DF PE1103/0037(L)) and the National Medical Research Registry (NMRR-12-814012063). Procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2008.

Data and sample collection

All data and blood samples used in this study were obtained from the Malaysian Periodontal Database and Biobank System (MPDBS) situated in the Faculty of Dentistry, University Malaya, storing samples collected at Faculties of Dentistry in University of Malaya, Universiti Sains Islam Malaysia, and Universiti Teknologi Mara as well as at various periodontal specialist clinics under the Ministry of Health Malaysia situated in Penang, Johor, Negeri Sembilan, and Kuala Lumpur. All donors provided written informed consent to have their data and samples banked in the MPDBS to conduct genetic studies on periodontitis.

The sociodemographic data of participants were collected using a questionnaire. Clinical periodontal profiles were recorded after full mouth periodontal examination excluding the third molars to assess visible plaque index (VPI), gingival bleeding index (GBI)[20], probing pocket depth (PPD) and clinical attachment loss (CAL) using the William's periodontal probe. Clinical examination was performed by periodontists, from the respective institutions and periodontal specialist clinics where the samples were collected, who were initially calibrated with inter-operator Kappa scores of 75–92% for PPD and 73–91%, for CAL.[21] Notably, Kappa values of 0.61–0.8 is considered as substantial agreement whilst Kappa values >0.8 is almost perfect agreement.[21] Data banked in the MPDBS were collected prior to the commencement of the new classification for PD in 2018,[22] hence, the diagnosis followed the criteria defined by Eke *et al*.[23] However, for this study, re-diagnosis was performed using the 2018 Classification for Periodontal disease.[22]

Blood samples were collected and banked in the MPDBS in the form of concentrated white blood cells (leukocytes). Briefly, 10 ml of venous blood was collected by venipuncture in sodium EDTA vacutainers. Concentrated white blood cells were obtained by centrifuging whole blood at 2500 × g for 10 minutes at room temperature. After centrifugation, three different fractions were distinguishable: the upper clear layer was plasma; the intermediate layer was the layer containing concentrated white blood cells (leukocytes); and the bottom layer contained concentrated erythrocytes. After the upper plasma layer was removed, the concentrated white blood cell layer was extracted and banked in the MPDBS at -80°C.

Samples from MPDBS were collected from

the donors who are Malay ethnicity and aged 35 years or older having minimum of 12 teeth. In addition, the samples for PD was taken from those who were diagnosed with PD Stage 2-4,[22] and samples for healthy participants were taken from those presenting with pocket depth ≤ 3mm and alveolar bone loss <15%.

SNP Genotyping

Genomic DNA was extracted from the white blood cells (leukocytes) (QIAamp DNA Blood Mini Kit, Qiagen, Germany) according to the manufacturer's protocol. The quality and quantity of extracted DNA were determined by UV spectrophotometry using NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA). The DNA samples with A260/A280 of ~ 1.70–1.90 (considered relatively pure) were stored at -20 °C for further use.

In this study, rs5275 (8473C>T), rs20417 (-765G>C) and rs689466 (-1195G>A) in *PTGS2* and rs1047031 (3'UTR c*5G>A) in *DEFB1* were analysed. Genotyping was carried out using Taqman SNP Genotyping assay (Applied Biosystems, USA). Real time PCR was performed in a total volume of 10 µL containing 2.0 µL of DNA (2 ng/µL), 5 µL of 2× TaqMan Universal PCR MasterMix, 0.5 µL Taqman SNP genotyping assay mix, and 2.5 µL of nuclease free water according to the manufacturer's specifications on the ABI 7500 Real Time PCR System. Genotyping data analysis and genotypes calling were performed using the TaqMan Genotyper software (Applied Biosystems, USA).

Statistical analysis

Statistical analysis was performed using Statistical Analysis System Software (V.22.0: SAS Institute, SPSS Inc., CH, USA) and PLINK 1.9 software.[24] Normality of the quantitative data was assessed using Kolomogorov-Smirnov test. Continuous data were presented as mean ± standard deviation and categorical data were presented as percentages (%). Independent t-test was conducted to compare the clinical parameters (periodontal pocket depth and clinical attachment loss) between PD and PH groups. When appropriate, Mann-Whitney U test was done in order to compare differences between mean of clinical parameters (Visible Plaque Index and Gingival Blood Index) in PD and PH groups, where the data was not normally distributed. Fisher's exact test was used to test each SNP for Hardy-Weinberg equilibrium (HWE) in PH and in combined PD-PH samples. Fisher's exact and Chi-square tests were used to examine the allelic and genotypic association of all the SNPs with PD respectively. The genotypic association were analysed according to additive (A1A1 vs A1A2 vs A2A2), dominant (A1A1 and A1A2 vs A2A2) and recessive (A1A1 vs A1A2 and A2A2) models, where A1 is the variant and effect allele. The strength of association was expressed as odds ratio (OR) with a 95% confidence interval (CI). All statistics were considered statistically significant when $p<0.05$.

RESULTS

Demographic Characteristics and Clinical Data

The data and blood samples from a total of 78 Malay PD participants and 62 Malay PH participants were obtained from the MPDBS.[21] Most of the samples were obtained from female participants for both PH (61.3%) and PD (55.1%) groups ($p>0.05$) (Table 1). Participants in the 35-45 years age group was higher among PH (61.3%) compared that in the PD group (43.6%) (Table 1). The age distribution between PD and PH was significantly different ($p=0.015$). Most participants in the PH (50.0%) and PD (53.8%) groups had received secondary education. Smokers comprised 24.2% in the PH group and 16.7% in the PD group. PD is a multi-factorial disease and smoking is a known risk factor for PD. However, smokers were included to the samples due to the high numbers of smokers present in the MPDBS. There were however no differences in relation to gender, education level and smoking habits between the two groups ($p>0.05$).

Comparisons in Periodontal Parameters Between Case and Control Groups

Figure 1 shows the comparison of means for VPI, GBI, PPD and CAL for the PH and PD groups. As expected, the mean values of all clinical parameters were significantly higher in the PD group compared to the PH group. Means for PPD and CAL were further analysed based on the severity of the disease. In the PD group, only 3.70% participants had PPD > 6 mm while 9.52% participant's had CAL > 6 mm. This indicates that majority of the participants had moderate PD while participants with severe PD had very localised disease.

Table 1: **Demographic characteristics and social habits of the study group**

Characteristics	Groups (N=140)		*p* – value
	Periodontitis (N=62)	**Periodontally healthy (N=78)**	
	N (%)	N (%)	
Gender			
Male	24 (38.7)	35 (44.9)	0.468[a]
Female	38 (61.3)	43 (55.1)	
Age (years)			
35 – 45	38 (61.3)	34 (43.6)	0.028[a]
46 – 55	11 (17.7)	31 (39.7)	
56 – 65	13 (21.0)	12 (15.4)	
66 – 75	0 (0.0)	1 (1.3)	
Education level			
Tertiary	28 (45.2)	24 (30.8)	0.129[a]
Secondary	31 (50.0)	42 (53.8)	
Primary	2 (3.2)	9 (11.5)	
Others	1 (1.6)	3 (3.8)	
Smoking habit			
Smoking	15 (24.2)	13 (16.7)	0.269[a]
Non-smoker	47 (75.8)	65 (83.3)	

[a]Chi-Square test; *p* value<0.05

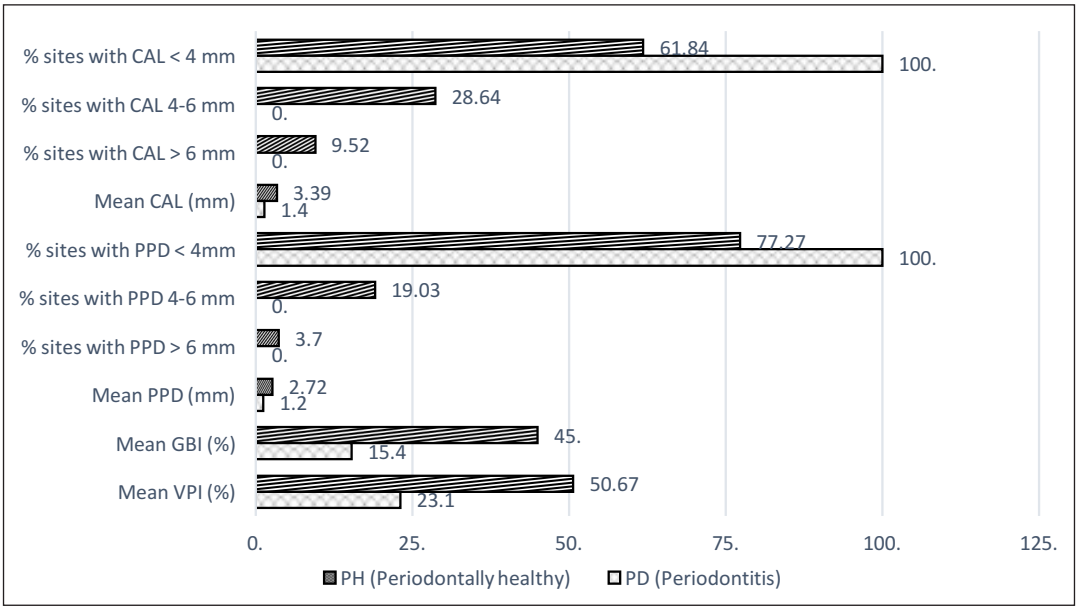

FIG. 1: The comparison of means for VPI, GBI, PPD and CAL for the PH and PD groups. The mean values of all clinical parameters were significantly higher in the PD group compared to PH group. Means for PPD and CAL were further analyzed based on the severity of the disease. Most participants had moderate PD while participants with severe PD had very localised disease.
VPI: visible plaque index, GBI: gingival bleeding index, PPD: probing pocket depth, CAL: clinical attachment level

Genotype and Allele Frequency Analysis for SNPs

Genotype distributions were tested, and all four SNPs were in HWE. The allele frequencies of each SNP were compared between the PD and PH groups. Although the allelic frequency for the rs689466-G allele was higher in the PD group at 35.2% versus the control group at 29.0%, while A allele was lower in the PD group at 64.7% for the PD group versus 71.0% of A allele for the control group, the differences were not statistically significant. Our results did not show any significant differences in the genotype distributions (Table 2) and allelic frequencies (Table 3) of all the SNPs between the PD and PH groups.

We also investigated the genotypic association between the *PTGS2* and *DEFB1* SNPs with PD susceptibility (Table 4). No significant associations ($p>0.05$) were observed for all the SNPs under the additive, dominant and recessive models.

DISCUSSION

Significant associations between *PTGS2* (Chinese, North Indian, & Taiwanese population) and *DEFB1* (European population) polymorphisms with PD have been reported. However, the findings from the current study have not demonstrated similar associations with the Malaysian Malay ethnic population. The Malay people, who generally inhabit the Malay Archipelago, comprising Peninsular Malaysia, Indonesia, Singapore, Peninsular Indo-China and Sri Lanka, comprises a mixed entity of multiple ancestries represented by Austronesian, Proto-Malay, East Asian and South Asian.[25] The Malay ethnic group has been under-represented in human genetic studies and that was the reason for the current study to be commenced.

Previous findings on the association between polymorphisms in *PTGS2* and *DEFB1* genes and CP in different population backgrounds have however also been inconsistent (Table 5).

Table 2: Genotype distribution and frequency analysis of SNPs rs5275, rs20417, rs689466 and rs1047031 in periodontitis and periodontally healthy groups

Genotype	Groups (N=140)		*p – value
	Periodontally Healthy (N=62)	Periodontitis (N=78)	
	N (%)	N (%)	
PTGS2			
rs5275			
CC	6 (7.70)	5 (8.10)	0.329
TC	36 (46.15)	30 (48.40)	
TT	36(46.15)	27 (43.50)	
rs20417			
CC	0 (0)	2 (3.23)	0.641
CG	14 (17.90)	11(17.74)	
GG	64 (82.10)	49 (79.03)	
rs689466			
GG	8 (10.30)	6 (9.68)	0.849
AG	39 (50.00)	24 (38.71)	
AA	31 (39.70)	32 (51.61)	
DEFB1			
rs1047031			
AA	14 (17.95)	9 (14.52)	0.729
GA	37 (47.44)	34 (54.84)	
GG	27 (34.61)	19 (30.64)	

**Chi square, p<0.05 considered significant, SNP: Single nucleotide polymorphism*

Table 3: Allele frequencies of rs5275, rs20417, rs689466 and rs1047031 and association analysis with periodontitis

SNP/allele	A1	A2	Periodontitis n (%)		Periodontally healthy n (%)		*p*-value	OR (95% CI)
			A1	A2	A1	A2		
PTGS2								
rs5275	C	T	48 (30.8)	108 (69.2)	40 (32.3)	84 (67.7)	0.780	0.927 (0.544-1.579)
rs20417	C	G	14 (9.0)	142 (91.0)	15 (12.1)	109 (87.9)	0.406	0.725 (0.339-1.548)
rs689466	G	A	55 (35.2)	101 (64.7)	36 (29.0)	88 (71.0)	0.265	1.343 (0.800-2.255)
DEFB1								
rs1047031	A	G	65 (41.7)	91 (58.3)	52 (41.9)	72 (58.1)	0.963	0.989 (0.607-1.611)

Differences between groups was analyzed by Fisher's exact test and p<0.05 was considered significant
SNP: Single nucleotide polymorphism, OR: Odds ratio, CI: confidence interval, A1-minor allele, A2-major allele n (%): number (percentage), SNP: Single nucleotide polymorphism, OR: Odds ratio, CI: confidence interval, A1-minor allele, A2-major allele

Our study shows that *PTGS2* SNP rs5275 polymorphism was not associated with the risk of PD in Malay participants, which agrees with previous studies conducted in Chinese and North Indian population.[6,26,27] A possible explanation may be due to rs5275 polymorphism being located within the functional region of 3'UTR that affects mRNA stability and translational efficiency.[28,29] This may modulate susceptibility to PD through differential expression of *PTGS2*.

PTGS2 SNP rs20417 polymorphisms showed a higher risk for CP in the Han Chinese population.[8,11] However it has also been reported to have a reduced risk for CP in the Taiwanese population.[7] This was confirmed in a meta-analysis of three studies[6,7,27] conducted by Prakash *et al*.,[27] where rs20417 showed a reduced risk of PD in the Chinese population in subgroup analysis. The reduced risk could be due to the C variant allele of rs20417 causing disruptions in the stimulatory protein-1 binding site, leading to around 30% lower promoter activity.[29] In the current study however, we did not find any significant association between rs20417 polymorphism with the progression of PD. Similar findings were reported by Prakash *et al*.[27] in a North Indian population. No CC genotype was found in PD group, and the frequency of C allele was lower in Malays compared to North Indian population.[27]

The A allele of *PTGS2* SNP rs689466 was associated with an increased risk for severe PD in the Chinese population where they reported a higher risk for AA/GA genotypes compared with GG genotype (adjusted OR: 2.49, 95% CI: 1.33–4.69, *p*=0.005).[7] It was suggested that the A allele at rs689466 site will increase gene expression/ enzymatic activity by creating a C-MYB binding site thus increasing disease susceptibility.[30] However, in the current study we found that the A allele was more prevalent in the Malay PH compared to the Malay PD participants and there was no association with PD disease status. *PTGS2* rs689466 is situated in the 5' flanking region of the *PTGS2* gene where many possible transcription factors binding sites exist.[30] There is a possibility that the interference with the specific binding between transcription factors and the promoter sequences by variant alleles could alter gene expression.

DEFB1 SNP rs1047031 was not associated with the risk of PD in Malays. Schaefer *et al*.[10] reported contrasting results of this SNP in German and Dutch ethnicity, showing significant associations of the rare rs1047031-A allele with an increased risk for PD (OR: 2.2; 95% CI: 1.2–4.3; *p*=0.021), prior and after adjustment for confounders (smoking & gender). The most significant association signal was the homozygous A genotype (OR: 1.3, 95% CI: 1.11–1.57; *p*-value=0.002). A potential

Table 4: Genotype frequencies and association analysis with periodontitis

SNP	Genotype	PD n (%)	PH n (%)	Additive model		Dominant model		Recessive model	
				OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
PTGS2									
rs5275	CC	6 (7.70)	5 (8.10)	0.927 (0.544-1.579)	0.780	0.900 (0.460-1.761)	0.758	0.950 (0.276-3.272)	0.935
	TC	36 (46.15)	30 (48.40)						
	TT	36 (46.15)	27 (43.50)						
rs20417	CC	0 (0)	2 (3.23)	0.725 (0.339-1.548)	0.406	0.825 (0.355-1.913)	0.653	NA	
	CG	14 (17.90)	11 (17.74)						
	GG	64 (82.10)	49 (79.03)						
rs689466	GG	8 (10.30)	6 (9.68)	1.343 (0.800-2.255)	0.265	1.617 (0.825-3.172)	0.162	1.067 (0.350-3.254)	0.91
	AG	39 (50.00)	24 (38.71)						
	AA	31 (39.70)	32 (51.61)						
DEFB1									
rs1047031	AA	14 (17.95)	9 (14.52)	0.989 (0.607-1.611)	0.963	0.835 (0.409-1.704)	0.62	1.288 (0.517-3.210)	0.587
	GA	37 (47.44)	34 (54.84)						
	GG	27 (34.61)	19 (30.64)						

Model association test was analysed by logistic regression and p<0.05 was considered significant**.** PD: Periodontitis, PH: Periodontally healthy, SNP: Single nucleotide polymorphism, OR: Odds ratio, CI: confidence interval, A1-minor allele, A2-major all

Table 5: Studies for association of CP and PTGS2 & DEFB1 SNPs in different population groups

Gene/SNP	Population	Sample size		*p-value	Reference
		Periodontitis (n)	Periodontally healthy (n)		
PTGS2					
rs5275	Chinese	146	148	0.044	Xie et al. (2009)
	North Indian	56	60	0.4166	Daing et al. (2012)
	North Indian	200	200	>0.05	Prakash et al. (2015)
	Malay	78	62	0.780	Present study
rs20417	Taiwanese	343	153	0.006	Ho et al. (2008)
	Chinese	146	148	0.035	Xie et al. (2009)
	North Indian	200	200	0.105	Prakash et al. (2015)
	Malay	78	62	0.406	Present study
rs689466	Chinese	146	148	0.011	Xie et al. (2009)
	German & Dutch	1048	1966	>0.05	Schaefer et al. (2010a)
	North Indian	56	60	0.1535	Daing et al. (2012)
	North Indian	200	200	>0.05	Prakash et al. (2015)
	Malay	78	62	0.265	Present study
DEFB1					
rs1047031	German & Dutch	805	1415	0.0253	Schaefer et al. (2010b)
	Malay	78	62	0.963	Present study

*p-values obtained from a likelihood-ratio test for the different populations are given, $p<0.05$ was considered significant

microRNA-binding site was predicted at the position of rs1047031 which may alter hBD-1 expression although the exact mechanism of *DEFB1* in periodontitis susceptibility is still unknown.[10] There is still debate by researchers on how the rs1047031 variant damages the function of the maintenance of the epithelial barrier and the actual role of *DEFB1* gene in PD progression.[10] Besides that, there was also a possibility of missing out on the true or additional causal variant which is located within *DEFB1* gene. The contrasting results of rs1047031 polymorphism in different studies may be due to different ethnicity populations and individual variation in cytokine production.

A limitation of the current study is the significantly different age distribution between PD and PH participants ($p=0.015$) which may explain our non-significant findings. Aging influences DNA methylation which affects gene activity and expression.[31-36] Aging may also play a role in epigenetics, whereby an alteration in the epigenetic pattern causes variation in gene expression which is associated with PD inflammation & susceptibility.[32] Changes in gene expression is a result of alteration of the chromatin due to DNA methylation and modifications of the histones.[33] In PD, researchers have suggested that epigenetic modifications vary between inflamed and non-inflamed sites of the same individual which happens locally at the crossing point of biofilm-gingiva surrounding the teeth.[32,34] Studies have investigated DNA methylation of PTGS2 gene promoter[35-37] showing increased and decreased methylation in PD participants. Additionally, effects of aging have been found in the inflammatory response (adaptive and innate immunity) by altering the immune-inflammatory status in periodontal tissues resulting in increased PD susceptibility.[38] It is well established that with increasing age, the adaptive immunity starts to decline.[39] The term "inflamm-aging" has been widely accepted in describing the increased risk of chronic inflammation during aging process.[40] Increased macrophage production of PGE2 has been reported to contribute to inflammatory bone loss found in in-vitro aged human gingival fibroblasts and periodontal ligament fibroblasts.[41]

This study is the first to investigate the *PTGS2* and *DEFB1* genetic polymorphism and their possible association with PD in the Malay ethnic group, which is the main ethnic group in Malaysia. The controls were ethnically matched and were examined to have no periodontal disease. Genetic heterogeneity occurs if the ethnic population under study have mixed parentage that can affect their SNP detection.[42] In this study, participants with mixed ethnic parentage were excluded from the study. Majority of the participants included in this study had mild to moderate PD while participants with severe periodontitis had very localised disease. A future study should comprise young participants with severe PD as it has been advocated that the allele frequencies of disease associated variants can possibly be enriched in this group of participants since it is believed that genetic factors largely contribute to their disease development.[42]

Data from the present study could assist researchers and practitioners in downstream studies not limited to the Malaysian Malay population, but also to other Malay population in the Malay Archipelago. The main limitation of this study was the small sample size, as we were unable to retrieve large number of samples that fulfilled our inclusion and exclusion criteria. As PD is a complex disease, a large sample is required to be able to determine the risk variants involved.[42] Besides, complex diseases like PD are multifactorial and polygenic, thus it is influenced by genetic, environmental and lifestyle factors which may have variants which have gene-gene, gene–environment and gene–lifestyle interactions.[42]

CONCLUSION

The current study indicates that *PTGS2* (rs5275, rs20417, rs689466,) and *DEFB1* (rs1047031) polymorphism is not associated with PD in Malays. This suggests that other causal variants apart from *PTGS2* & *DEFB1* are possibly involved in the development and progression of PD. Thus, other PD associated SNPs will need further investigations using a larger sample size with severe PD.

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Authors' contribution: RDV and CCN conceived and designed the study, assisted in data analysis and made worthy corrections of the manuscript. RDV, NAB and SHS were responsible for data collection and revising the manuscript. JKJS researched the topic background, assisted in data collection, performed data analysis and drafted the manuscript. MTR assisted in data analysis and correction of the manuscript.

Conflict of interest: The authors declare no conflict of interests.

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