Original Article

Interleukin 1β (+3954; −511) Genotype Polymorphism and its Association with Severe Chronic Generalized Periodontitis in the Malaysian Population

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Abstract

Introduction: Single-nucleotide polymorphisms (SNPs) in interleukin 1β (IL-1β) gene have been known to be associated with increased susceptibility to chronic periodontitis among various ethnic populations. SNPs are more commonly observed at loci + 3954 and −511. The aim of this study was to evaluate the role of IL-1β gene polymorphism at loci +3954 and −511, and its association with severe chronic generalized periodontitis among the ethnic Malay, Chinese, and Indians within the Malaysian population. **Materials and Methods:** Saliva samples from 120 subjects (60 cases and 60 controls) in the age group of 25–50 years were collected for isolation of genetic material using Norgen technique. Clinical attachment loss of ≥5 mm was considered as severe chronic generalized periodontitis. SNP's at loci +3954 and −511 were identified and analyzed using Kompetitive Allele Specific Polymerase Chain Reaction Genotyping System (KASP™). Differences in the allele/genotype frequencies were assessed by Chi-square test ($P < 0.05$). **Results:** On the comparison between cases and controls of IL-1β genotype polymorphism (+3954 and −511), the difference in the genotype frequencies was statistically insignificant in all the three ethnicities. The genotype frequency in both groups in all three ethnicities of the Malaysian population was similar. **Conclusion:** IL-1β genotype polymorphism at +3954 and −511 was found to be not associated with severe chronic generalized periodontitis among the three ethnicities in Malaysia. Studies with larger sample size should be done to confirm the findings of this study.

Keywords: *Chronic generalized periodontitis, interleukin-1β genotype, Malaysian population, severe, single nucleotide polymorphism*

Introduction

Chronic periodontitis is an inflammatory disease affecting tissues of the periodontium resulting in clinical attachment loss (CAL) and bone loss and is one of the most common causes for tooth loss in developing nations.[1]

The prevalence of chronic periodontitis and severe chronic generalized periodontitis was found to be 48.5% and 18.2% respectively among Malaysian population.[2] Chronic periodontitis is multifactorial in nature with smoking, diabetes, osteoporosis, and hyper-responsive interleukin (IL) (genetic polymorphism) being some of the risk factors.[3] IL-1β is a key regulator of the host response and is a potent bone resorbing factor. It has been reported that single-nucleotide polymorphisms (SNPs) in IL-1β gene are associated with increased susceptibility to chronic periodontitis.[3] Various ethnic populations are known to have varying susceptibility to chronic periodontitis owing to SNPs in IL-1β gene.[4] Around 30% of the Caucasian population has IL-1β genotype polymorphism associated with increased susceptibility to chronic periodontitis.[4]

IL-1 gene complex comprised IL-1α, IL-1β, and IL-receptor antagonist are located on chromosome 2q13–21 and are polymorphic at several locations.[4] Studies have reported SNP's of IL-1β at +3954 and −511 results in increased susceptibility to chronic periodontitis among various populations.[4] Thorough literature search did not reveal any published literature related to SNPs of IL-1β and its association with chronic periodontitis in the Malaysian population. Hence, the aim of this study was to evaluate the role of IL-1β gene polymorphism at loci +3954 and −511, and its association with severe chronic generalized periodontitis among the various ethnicities within the Malaysian population.

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Materials and Methods

The study was conducted from May 2015 to March 2016 at Faculty of Dentistry, SEGi University in collaboration with Sengenics, Malaysia. The study was approved by Institutional Ethics Committee, SEGi University and informed consent was obtained from all the subjects participating in the study. The study comprised 120 subjects (60 cases and 60 controls) in the age group 25–50 years reporting to SEGi Oral Health Centre. Among 120 subjects, 40 each were from Chinese, Malay, and Malay-Indian ethnicities among the Malaysian population. The study subjects were selected from a homogeneous population with both parents being of the same ethnic origin. Controls were subjects with healthy periodontium without any systemic diseases. Cases included patients with CAL ≥5 mm according to American Academy of Periodontology, 1999.[5] All the participants had poor plaque scores (plaque index score of >2.0) and gingival index scores more than 2.0.[6] All examinations were done by a single calibrated examiner using a diagnostic mouth mirror and University of North Carolina-15 periodontal probe. Measurement of probing depth and clinical attachment level was recorded. The exclusion criteria were the presence of systemic diseases such as diabetes mellitus, obesity, acute infections, asthma, cardiovascular diseases, rheumatoid arthritis, and patients under antibiotic or nonsteroidal anti-inflammatory drugs in the past 6 months. Patients were requested to abstain from consuming food 2 h before providing saliva samples. Saliva samples (2–3 ml) were collected from all the patients in a sterile container and were sent to Sengenics lab, Malaysia for IL-1β (+3954; −511) genotype polymorphism analysis.

Norgen's Saliva DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada as per manufacturer advised protocols) was used for the extraction of whole genomic DNA.[7] The extracted DNA was quantified using ThermoFisher Scientific Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, U.S.A). SNP's analysis was completed utilizing Kompetitive Allele Specific polymerase chain reaction (PCR) genotyping system (KASP™).[7] 17.5 μg DNA of every example was aliquoted into every well in 384-well plate separately twice, and the plate was dried in an oven at 60°C for 60 min. After the DNA in the 384-well plate was totally dry, dispense 3 μL of diluted KASP Master mix containing 250 μL concentrated KASP Master mix (LGC Genomic Limited, Middlesex, United Kingdom), 250 μL laboratory grade water and 7 μL of IL-1β (−511) assay mix (rs16944) using Meridian (LGC Genomic Limited, Middlesex, United Kingdom) to the first set of patient's sample and diluted KASP Master mix with IL-1β (+3954) assay mix (rs1143634) to second set of patient's sample. The plate was secured with clear seal and amplified using water bath PCR thermal cycler, Hydrocycler 16 (LGC Genomic Limited, Middlesex, United Kingdom). The PCR cycling condition used by Hydrocycler 16 was 61°C–55°C [Table 1]. PHERAstar (BMG Labtech, Offenburg, Germany) was used to scan the plate. The SNP genotype was analyzed through Kraken™ software and validated by laboratory technologist.

Statistical analysis done

Data obtained was tabulated and statistically analyzed using MedCalc version 12 (Acacialaan 22, 8400 Ostend, Belgium). Paired *t*-test was applied to compare the age distribution among the three ethnicities of the Malaysian population. Chi-square test of proportions was carried out to compare the difference between cases and controls of genotype and allele frequencies distribution. The program package Arlequin 3.0 (Arlequin, Suisse, Institute of Ecology and Evolution, University of Bern) was used to calculate *P* value using Hardy–Weinberg equation (HWE).[8] Differences were considered statistically significant when $P < 0.05$.

Results

This study recruited 120 subjects consisting of 60 healthy volunteers and sixty patients with severe chronic generalized periodontitis (40 Chinese, 40 Malaysians, and 40 Malay-Indians). There were 50 males and 70 females in the age range of 24–50 years (mean age = 35.3 years). There was no significant difference in age distribution of patients between cases and controls under each ethnicity [Table 2]. Table 3 shows the gender distribution in each ethnicity.

In ethnic Malays, IL-1β genotype at +3954 among cases was 17 CC and 3 computed tomography (CT), and in controls, there were 17 CC and 3 CT. Whereas IL-1β genotype

Table 1: Polymerase chain reaction amplification thermal cycling condition used by Hydrocycler 16

Stage	Temperature	Duration	Number of cycles
1	94°C	15 min	1
2	94°C	20 s	10
	61°C (decrease of 0.6°C per cycle to achieve a final temperature of 55°C)	1 min	
3	94°C	20 s	26
	55°C	1 min	

Table 2: Mean age difference between cases and controls

Ethnicity	Mean age		Mean age Difference	*t*-test (*P**)
	Cases	Controls		
Malay	36.7	34.1	2.6	0.352
Indian	35.4	34.8	0.6	0.809
Chinese	38.3	35.5	2.7	0.213

*$P<0.05$=Significant; *t*-test

at −511 in Malay cases was 6 CC, 8 CT, and 6 TT and among controls, there were 7 CC, 6 CT, and 7 TT [Table 4].

Among ethnic Chinese, IL-1β genotype at +3954 in cases was found to be predominantly composed of CC genotype only whereas in controls there were 18 CC and 2 CT genotypes.

At the locus −511 genotype among cases were distributed as 6 CC, 9 CT, and 5 TT whereas, in controls, it was found to be 8 CC, 8 CT, and 4 TT [Table 4].

Among ethnic Malay-Indians, the prevalence of IL-1β genotype at + 3954 in cases was found to be predominantly composed of CC genotype (14) followed by CT (4) and TT (2). Whereas in controls IL-1β genotype at +3954 was distributed as 15 CC and 5 CT. IL-1β genotype at −511 among cases was found to be 4 CC, 8 CT, and 8 CC, whereas in controls it was found to be 4 CC, 6 CT, and 10 TT [Table 4].

The genotype frequency of IL-1β (+3954) and IL-1β (−511) was compared between cases and controls in Malays, Chinese, and Malay-Indians residing in Malaysia. The genotype frequency of both IL-1β (+3954) and (−511) in all the three ethnicities did not deviate from HWE. On the comparison between cases and controls among IL-1β (+3954) as well as IL-1β (−511), the difference in genotype of distribution was statistically insignificant in all the three ethnicities. The pure allelic contribution of TT for IL-1β (+3954) polymorphism was absent in both cases as well as controls in ethnic Malays and Chinese. Thus, the genotype frequency in both groups in all three populations falls within the comparable limits.

Table 3: Gender comparison between cases and controls

Ethnicity	Cases		Controls	
	Male, *n* (%)	Female, *n* (%)	Male, *n* (%)	Female, *n* (%)
Malay	10 (50)	10 (50)	9 (45)	11 (55)
Indian	9 (45)	11 (55)	8 (40)	12 (30)
Chinese	8 (40)	12 (30)	6 (30)	14 (70)

Discussion

Gene polymorphism resulting in increased production of cytokines has been considered as a contributing factor in the pathogenesis of chronic generalized periodontitis.[9] In this study, DNA was isolated from saliva samples of 120 subjects. Noninvasiveness, collection of samples by nontrained individuals and without any specific equipment are the various advantages of using saliva as a source for DNA isolation.[7] In this study, SNP was identified using KASP™ genotyping. SNP genotyping using PCR-RFLP and KASP™ have shown similar results.[10]

Masamatti *et al*. studied 90 subjects (30 chronic periodontitis, 30 aggressive periodontitis, and 30 healthy controls) for IL-1β at + 3954 among the South Indian population.[11] The results showed a positive association between IL-1β genetic polymorphism and chronic periodontitis, whereas no positive association was observed between IL-1β genetic polymorphism and aggressive periodontitis.[12] Archana *et al*. studied sixty patients (15 healthy and 15 each for mild, moderate, and severe chronic periodontitis) for the association between IL-1β polymorphism and chronic periodontitis.[12] The results showed a significant association between severe periodontitis and IL-1β genetic polymorphism.[12] In our study, we have evaluated only

Table 4: Association analysis of genotypes of single nucleotide polymorphism of interleukin 1β gene with chronic periodontitis in ethnic Malays, Chinese and Indians

	Genotype				Genotype			
	Polymorphism in the IL-1β gene at position +3954 (rs1143634) (C/T)				Polymorphism in the IL-1β gene at position−511 (rs16944) (T/C)			
	Case	Control	χ^2	*P*	Case	Control	χ^2	*P*
Malays								
CC	17 (85)	17 (85)	0.056	0.814	6 (30)	7 (35)	0.033	0.856
CT	3 (15)	3 (15)	0.013	0.908	8 (40)	6 (30)	0.303	0.582
TT	0	0	-	-	6 (30)	7 (35)	0.031	0.861
HWE (*P*)	0.716	0.716			0.371	0.073		
Chinese								
CC	20 (100)	18 (90)	0.002	0.965	6 (30)	8 (40)	0.626	0.429
CT	0	2 (10)	0.031	0.861	9 (45)	8 (40)	0.059	0.807
TT	0	0	-	-	5 (25)	4 (20)	0.104	0.748
HWE (*P*)	0.073	0.813			0.662	0.456		
Indian								
CC	14 (70)	15 (75)	0.009	0.924	4 (20)	4 (20)	0.329	0.567
CT	4 (20)	5 (25)	0.007	0.933	8 (40)	6 (30)	0.198	0.657
TT	2 (10)	0	0.579	0.447	8 (40)	10 (50)	0.284	0.594
HWE (*P*)	0.093	0.522			0.456	0.127		

IL-1β: Interleukin 1β; HWE: Hardy-Weinberg equation

severe chronic generalized periodontitis patients. Wu *et al*. studied the association of IL-1 gene variations with moderate to severe chronic periodontitis in four ethnic groups (Caucasians, African Americans, Hispanics, and Asians). The patients were recruited in USA, Chile, and China. The study revealed that IL-1β (+3954) gene variations resulted in the significant effect on the severity of chronic periodontitis in all the four ethnic groups studied.[4]

Armitage *et al*., in 2000, studied 300 subjects of Chinese origin for IL-1β +3954 gene polymorphism and its association to the severity of chronic periodontitis. The study concluded that the prevalence of IL-1B polymorphisms was lower in Chinese subjects. However, these patients were from Chinese heritage and not from China. This study questioned the usefulness of IL-1β polymorphism as a method for determining the susceptibility of Chinese patients to chronic periodontitis.[13] Anusaksathien *et al*. studied 123 Thai subjects categorized into chronic periodontitis, aggressive periodontitis, and healthy groups. The IL-1β (+3954) genotype was studied using polymerase chain reaction.[14] The study concluded that genetic polymorphism of IL-1β gene at +3954 locus was not useful in predicting the severity of periodontal disease in the Thai ethnic group.[14] The results of this study conducted in the South East Asian region is supporting the findings found in our study.

Yücel *et al*. studied SNP's in IL-1β gene at the locus +3954 among 44 chronic periodontitis patients and 47 healthy controls of Turkish origin.[15] The genotype distribution and allele frequencies were not different after stratification of subjects according to CAL. Among chronic periodontitis patients, sites with increased bleeding on probing showed an increased frequency of allele 2. The study concluded that SNP's at +3954 in IL-1β gene could potentially play a significant but not major role in the clinical outcome.[15] Our study considered patients with severe periodontitis only.

Conclusion and Shortcomings of the Study

Our study evaluated the IL-1β genotype polymorphism at loci +3954 and −511 in three ethnic groups residing in Malaysia. The study showed no association between severe chronic generalized periodontitis and IL-1β genotype polymorphism at the above-mentioned loci. The population studied in each group was just 40 subjects. The study should be extended to include a larger sample size for substantiating the results obtained from this study.

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Conflicts of interest

There are no conflicts of interest.

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