

## ORIGINAL ARTICLE

**Interleukin 1 $\beta$  (+3954, –511 and –31) polymorphism in chronic periodontitis patients from North India**RAMESH AMIRISETTY<sup>1</sup>, RITU PRABHA PATEL<sup>1</sup>, SATRUPA DAS<sup>2</sup>, JITENDRA SARAF<sup>1</sup>, AKKA JYOTHY<sup>2</sup> & ANJANA MUNSHI<sup>3</sup><sup>1</sup>Chhattisgarh Dental College & Research Institute, Rajnandgaon, Chhattisgarh, India, <sup>2</sup>Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Begumpet, Hyderabad, India, and <sup>3</sup>Centre for Human Genetics, School of Health Sciences, Central University of Punjab, Bathinda, Punjab, India**Abstract**

**Objective.** Several studies have implicated the role of interleukin-1 in various chronic diseases including periodontitis. The present study was carried out with an aim to evaluate the role of interleukin 1 $\beta$  polymorphisms, namely +3954C/T, –511C/T and –31T/C, in the development of chronic periodontitis. **Materials and methods.** Twenty-nine chronic periodontitis patients and 31 healthy controls of North Indian origin from Chhattisgarh were recruited for the study. The genotypes for the three variants were determined using the PCR-RFLP technique and the strength of association between genotypes and periodontitis was determined by odds ratio with 95% confidence interval (CI) and chi-square analysis. **Results.** Analysis for the +3954 allelic and genotypic frequencies of the polymorphism revealed a significant difference in the CT genotype between periodontitis patients and controls ( $p = 0.03$ ). A significant difference was also observed in the allelic frequencies between the two groups ( $p = 0.02$ ). For the –511 site, TT genotype revealed a significant association with the disease ( $p = 0.01$ ). A significant association was also found following the co-dominant model ( $p = 0.007$ ). However, the –31 polymorphism revealed no significant difference between patients and controls. **Conclusions.** In conclusion, the present study suggests a strong association of the TT genotype of –511 and CT genotype of +3954 variant of interleukin 1 $\beta$  with chronic periodontitis. However, the –31 variant did not show a significant association with the disease.

**Key Words:** chronic periodontitis, interleukin-1 $\beta$ , polymorphism, genetics**Introduction**

Periodontitis is considered as a complex multifactorial disease which becomes chronic as the disease progresses. It is known to be influenced by environmental factors as well as the genetic factors [1]. A large number of studies suggest genes and their variants to be responsible for the disease. Genetic polymorphism causes changes in proteins and their expression might be responsible for the disease development but sometimes these changes are also protective in nature [2]. Studies have suggested cytokines to play a pivotal role in the disease development and act as a potential diagnostic marker of the disease [3]. Among the different cytokines, interleukin 1 (IL-1), a pro-inflammatory cytokine, has been implicated in both acute and chronic inflammatory disease. It is also the

main cytokine responsible for osteoclastic activity in periodontitis [4]. Earlier studies document polymorphisms within the IL-1 gene cluster to be the risk factors for various diseases including adult periodontitis [5]. The master cytokine IL-1 is also known to influence essential functions such as immune cell recruitment, cell proliferation, tissue destruction and vascular smooth muscle cell contraction [6].

IL-1 is a cluster of three genes comprising of IL-1A, IL-1B and IL-1RN that code for IL-1 $\alpha$ , IL-1 $\beta$  and IL-1ra (receptor antagonist), respectively. Their location has been mapped to chromosome 2q13-21 and there are several known polymorphic sites within these loci [7]. IL-1 $\alpha$  and IL-1 $\beta$  are reported to be produced by many cells and an increase in the levels of these cytokines has been well documented in gingival cervical fluid of periodontitis subjects [8]. Several

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studies have been carried out to evaluate the association of interleukin 1 $\beta$  variants with the development of periodontitis, but with conflicting results [9–12]. Therefore, there is a need to evaluate these variants in association with the development of the disease in different ethnic groups to confirm their prognostic value in a disease.

The present study was carried out with an aim to elucidate the role of interleukin 1 $\beta$  (+3954 C/T; –511C/T and –31T/C) polymorphisms and specific haplotypes with chronic periodontitis recruited in a North Indian population hailing from Chhattisgarh.

## Materials and methods

### Subjects

Twenty-nine periodontitis patients (males:females = 23:6) presenting with new cases of chronic periodontitis, evaluated in the Department of Periodontology, Chhattisgarh Dental College and Research Institute, Rajnandgaon, were included in the study. The study was approved by the ethical committee of the study hospital and all the patients were examined by a qualified dentist. Patients with a history of severely compromised immune function, systemic diseases, on periodontal therapy, pregnant females, smokers and those with a history of bleeding disorder were excluded from this study. As a control group, 31 healthy individuals matched for sex and age (males:females = 28:3) were recruited from the same geographic area with no clinical evidence of any disease. Chronic periodontitis was defined as ‘an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss’ Flemmig et al. (1999) [13] and diagnosis was based on both periodontal pocket and as well as radiographic findings. Information on demographic characters and risk factors were collected using a structured questionnaire. All the subjects included in the study were within the age group of 30–55 years and samples were collected only after obtaining the written informed consent.

### DNA isolation and genotyping

Five milliliters of blood was collected in EDTA tubes and genomic DNA was extracted from blood samples using the standard phenol-chloroform method. The polymorphisms in interleukin 1 $\beta$  gene (+3954, –511 and –31) was detected using PCR-RFLP technique. The primers used for the amplification of the +3954, –511 and –31 polymorphism are Forward: 5' AAT TTTGCCACCTCGCCTCA 3' Reverse: 5' CGGA GCGTGCAGTTCAGTGAT 3', Forward: 5' GCC TGAACCCTGCATACCGT 3' Reverse: 5' GCCA ATAGCCCTCCCTTCT 3' and Forward: 5' AGAA GCTTCCACCAATACTC 3' Reverse: 5' ACCACC

TAGTTGTAAGGAAG 3', respectively. The amplified PCR products were separated on 1.5% agarose gel. The amplified 152bp for +3954 site, 155bp for –511 site and 252bp for –31 site PCR products were digested with TaqI, AvaI and AluI restriction enzymes (Fermentas Fast digest), respectively, by incubating at 37°C for 5 min followed by separation of fragments on 2.5% agarose gel. For +3954, the most common homozygous wild (CC) genotype shows the presence of fragment 88bp and 64bp; heterozygous (CT) shows the 152, 88 and 64bp fragment and mutant genotype (TT) was detected as 152bp fragment. For –511, the most common homozygous wild (CC) genotype shows the presence of fragment 92bp and 63bp; heterozygous (CT) shows 155bp, 92bp and 63bp fragment and mutant genotype (TT) was detected as 155bp fragment. For the –31 site, the most common homozygous wild (TT) genotype shows the presence of fragment 200bp and 52bp; heterozygous (TC) shows 252bp, 200bp and 52bp fragment and mutant genotype (CC) was detected as 252bp fragment.

### Statistical analysis

Hardy–Weinberg equilibrium was tested for all the three gene polymorphisms and association between genotypes and chronic periodontitis was examined by Odds ratio with 95% confidence interval (CI) and chi-square analysis using Open EPI6 software (Open Epi Version 2.3.1 from Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA). Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. Statistical significance was defined as  $p < 0.05$ .

### Haplotype analysis

The haplotype analysis was carried out using online software SNPstat.

## Results

Twenty-nine generalized chronic periodontitis patients (male:female = 23:6) and 31 controls (male:female = 28:3) from the same demographic area were included in the study. All the patients belonged to a North Indian population from Chhattisgarh (Rajnandgaon). The clinical characteristics and parameters of the patients and controls have been given in Table I. The mean age was 41.3 years for chronic periodontitis patients and 41.8 years in controls.

The genotypic distribution and the allelic frequencies for the three polymorphisms have been given in Table II. There was a statistically significant difference in the genotypic distribution between

Table I. Clinical characteristics and parameters of periodontitis patients and controls.

Characteristics	Periodontitis patients (n = 29)	Controls (n = 31)	p-value
Age	41.3	41.8	
Male:female	23:6	28:3	
Probing depth (mean ± SD)	4.9 ± 0.87	2.19 ± 0.75	< 0.001
Gingival recession (mean ± SD)	1.65 ± 0.77	0.22 ± 0.43	< 0.001
Clinical attachment level (mean ± SD)	6.5 ± 0.18	2.5 ± 0.76	< 0.001

Probing depth, gingival recession and clinical attachment level are given as mean (SD). *p*-values were calculated using Student's paired *t*-test (SPSS 18).

chronic periodontitis patients and controls for the +3954 C/T variant and -511 C/T variant. The frequency of CT genotype in +3954 was significantly high in patients in comparison with the controls. For CT vs CC,  $\chi^2 = 4.3$ ;  $p = 0.03$ , Odds ratio = 3.2 (95% CI = 1.05–9.6). A significant difference was also observed between the C and T alleles in patients and controls. For T vs C,  $\chi^2 = 5.2$ ;  $p = 0.02$ , Odds ratio = 2.7 (95% CI = 1.13–6.1) (Table III). For the -511 C/T variant the frequency of CT and TT genotypes was significantly high in patients in comparison with the controls. For CT vs CC,  $\chi^2 = 4.3$ ;  $p = 0.03$ , Odds ratio = 0.23 (95% CI = 0.06–0.94); for TT vs CT,  $\chi^2 = 6.3$ ;  $p = 0.01$ , Odds ratio = 4.9 (95% CI = 1.4–17.7) and for CT vs CC+TT,  $\chi^2 = 7.3$ ;  $p = 0.007$ , Odds ratio = 0.21 (95% CI = 0.07–0.7) (Table IV). However, no difference was observed in the allelic frequency between the two groups. Analysis for the -31 T/C variant revealed no significant difference between patients and controls as far as the genotypic distribution and allelic frequencies are concerned. For CC vs TT,  $\chi^2 = 0.71$ ;  $p = 0.39$ , Odds ratio = 2.25 (95% CI = 0.35–14.6); for CC vs

TT+TC,  $\chi^2 = 0.01$ ;  $p = 0.91$ , Odds ratio = 0.94 (95% CI = 0.31–2.8) and for C vs T,  $\chi^2 = 0.2$ ;  $p = 0.7$ , Odds ratio = 1.2 (95% CI = 0.6–2.5) (Table V). Analysis for any possible haplotype revealed no specific haplotype block involving all the three SNPs to be associated with chronic periodontitis.

## Discussion

Periodontitis is a main cause of tooth loss in developed countries with increasing prevalence in the developing world [14]. Although microbial colonization is a well-established cause for the disease [15], recent studies emphasize the role of systemic diseases like diabetes and environmental factors (smoking, stress) in influencing the inflammatory and immune response towards the disease [16]. Additionally in the last few decades the role of host's genotype which influences the innate and acquired immune system has been well documented, suggesting a strong genetic component involved in the pathogenesis of the disease [17]. Studies involving human tissues and animal models strongly support the role of cytokines in influencing immune response in the periodontal disease [3].

Among several immune mediators, IL-1 is a major pro-inflammatory cytokine which is mainly produced by stimulated monocytes, macrophages, keratinocytes and endothelial cells [18]. Studies suggest that variation within IL-1 gene cluster influences the variation in synthesis of cytokines and certain haplotype groups resulting from different variants in IL-1 $\alpha$  and IL-1 $\beta$  gene increase the disease susceptibility [5]. Since there is genotypic and allelic variation for a gene in various ethnic populations globally and also in the Indian Population, exploring the prevalence of associated genes in a disease in various ethnic and racial groups is important for evaluating the use of genetic markers as diagnostic or prognostic tools. To the best of our knowledge this is the first study evaluating the association of three variants

Table II. Distribution of interleukin 1 $\beta$  polymorphisms (+3954, -511 and -31) genotype and allelic frequencies in periodontitis patients and controls.

Polymorphism	Genotypes			Total	Alleles		Total
	CC	CT	TT		C	T	
+3954	CC	CT	TT	29	37 (0.64)	21 (0.36)	58
	Periodontitis	11 (38%)	15 (51.7%)				
Control	CC	CT	TT	31	51 (0.82)	11 (0.18)	62
	Periodontitis	21 (67.8%)	9 (29%)				
-511	CC	CT	TT	29	24 (0.41)	34 (0.59)	58
	Periodontitis	9 (31%)	6 (20.7%)				
Control	CC	CT	TT	31	29 (0.47)	33 (0.53)	62
	Periodontitis	6 (19.4%)	17 (54.8%)				
-31	TT	TC	CC	29	22 (0.4)	36 (0.6)	58
	Periodontitis	2 (6.9%)	18 (62.1%)				
Control	TT	TC	CC	31	26 (0.4)	36 (0.6)	62
	Periodontitis	5 (16.1%)	16 (51.6%)				

Table III. Analysis of interleukin 1 $\beta$  (+3954) genotypes and alleles among periodontitis patients and controls.

Genotype	OR (95% CI)	$\chi^2$	<i>p</i> -value
TT vs CC	5.7 (0.53–61.7)	2.4	0.12
CT vs CC	3.2 (1.05–9.6)	4.3	0.03
TT vs CT	1.8 (0.2–20.0)	0.23	0.63
Dominant			
CC vs CT + TT	0.6 (0.24–1.5)	1.2	0.3
Co-dominant			
CT vs CC + TT	2.6 (0.90–7.6)	3.2	0.07
Recessive			
TT vs CC + CT alleles	3.5 (0.34–35.3)	1.2	0.27
C vs T	0.38 (0.2–0.9)	5.2	0.02
T vs C	2.7 (1.13–6.1)	5.2	0.02

OR, odds ratio; CI, confidence interval.

Table IV. Analysis of interleukin 1 $\beta$  (–511) genotypes and alleles among periodontitis patients and controls.

Genotype	OR (95% CI)	$\chi^2$	<i>p</i> -value
TT vs CC	1.2 (0.30–4.5)	0.05	0.82
CT vs CC	0.23 (0.06–0.94)	4.3	0.03
TT vs CT	4.9 (1.4–17.7)	6.3	0.01
Dominant			
CC vs CT + TT	1.9 (0.6–6.1)	1.1	0.30
Co-dominant			
CT vs CC + TT	0.21 (0.07–0.7)	7.3	0.007
Recessive			
TT vs CC + CT alleles	2.7 (0.90–7.9)	3.20	0.07
C vs T	0.80 (0.39–1.7)	0.35	0.55
T vs C	1.2 (0.60–6)	0.35	0.55

OR, odds ratio; CI, confidence interval.

Table V. Analysis of interleukin 1 $\beta$  (–31) genotypes and alleles among periodontitis patients and controls.

Genotype	OR (95% CI)	$\chi^2$	<i>p</i> -value
CC vs TT	2.25 (0.35–14.6)	0.71	0.39
TC vs CC	1.25 (0.40–3.84)	0.15	0.7
TC vs TT	2.81 (0.5–16.5)	1.34	0.24
Dominant			
TT vs TC + CC	0.4 (0.07–2.2)	1.22	0.27
Co-dominant			
TC vs TT + CC	1.53 (0.54–4.3)	0.7	0.41
Recessive			
CC vs TT + TC alleles	0.94 (0.31–2.8)	0.01	0.91
T vs C	0.84 (0.41–1.8)	0.2	0.7
C vs T	1.2 (0.6–2.5)	0.2	0.7

OR, odds ratio; CI, confidence interval.

(+3954C/T, –511C/T and –31T/C) of the IL-1 $\beta$  gene with chronic periodontitis.

The analysis of +3954 variant revealed the T-allele to be more frequent among the patients when compared with healthy controls and the CT genotype to be a significant risk factor for the development of the disease which is in accordance with studies from Brazil and South Indian states (Tamil Nadu, Maharashtra, Karnataka) [9,19–22]. This finding is in contrast to reports by Shete et al. [10] where the frequency of the CC genotype and C-allele has been reported to be associated with chronic periodontitis. On the other hand, a negative association has also been reported in populations from Northern Europe, Poland, Jordan and South Africa [11,23–25]. Further, a positive association has also been reported by two meta-analysis studies, one of which documents the CT genotype of chronic periodontitis patients to be a significant risk factor in Caucasians but not in Asians, and the other, which included 53 studies having 4178 cases and 4590 controls, revealed a strong association for the variant with disease susceptibility [26,27]. The evaluation of the second variant –511C/T revealed the TT genotype to be significantly higher among the patients and a significant association was found following the co-dominant genotypic model. A similar observation has been reported in studies involving patients from Macedonia, Brazilian blacks and mulattos [12,28]. However, meta-analysis by Nikolopoulou et al. [27] reports a weak positive association and another study reports a negative association for the variant [11]. Analysis for the –31 variant revealed no significant difference in the genotypic or allelic frequencies between patients and controls. Studies focussing on –31T/C variant were found to be almost nil, with only one report suggesting strong linkage disequilibrium of this variant with –511C/T among both patients and controls [10].

In conclusion, our study involving a North-Indian population suggests the –511 and +3954 variants to be risk factors for chronic periodontitis and that these two polymorphic sites encoded along IL-1 $\beta$  can be used as potential markers for chronic periodontitis, but it needs evaluation in a larger cohort of samples.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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