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Association of Interleukin-1β +3954 (Rs1143634) Gene Polymorphism in Periodontal Healthy and Chronic Periodontitis Patients

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Abstract:
Background: *Polymorphisms of various cytokine genes are known to influence the pathogenesis and severity of several inflammatory conditions. The association of these polymorphisms with chronic periodontitis(CP) susceptibility has been found to be diverse amongst different ethnic populations. This study was aimed to determine the distribution of IL-1β+3954 (rs1143634)gene polymorphismin healthy controls(HC) andCP and to explore their association with periodontal status.*
Methodology:*The above polymorphism was analysed amongst HC(n=56) and CP patients (n=79). Genotyping was performed using PCR-RFLPassay. Mann-Whitney U test and Kruskal Wallis ANOVA test were applied to find out the differences in genotypes of SNPs with clinical parameters and chronic periodontitis.*
Results:*No significant correlation was found between IL-1β+3954 (rs1143634)gene polymorphism and both groups though the frequencies of C/C genotype and allele T were higher in CP.*
Conclusion:*Lack of association ofIL-1β+3954 (rs1143634)gene polymorphismwasobserved with chronic periodontitis in the population of Northern Karnataka.*
Keywords: *Interleukin-1 beta, polymorphism, chronic periodontitis, gene, allele, genetics*

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I. Introduction

Periodontal disease is known to be a complex disease having multifactorialetiology.The interaction between the host and bacteria is important for periodontal disease pathogenesis.This involves cytokine mediatedcomplex interactions betweencells and the extracellular matrix .[1]Complex diseases are typically polygenic, inflammatory and immune responses in periodontitis can be influenced by genetic factors.The evidence of the genetic basis for periodontitishas been supported by familial aggregation,twin, segregation, linkage and association studies.[2,3]Reports on twin studies show the heritability of periodontal disease.[4,5]Also chronic periodontitis having familial aggregations was analysed with familial study designs.[6,7]

Role of genes and the polymorphisms in host responses leading to disease progression has been reported widely in the literature.[8]Genetic polymorphisms may be a risk factor for the disease susceptibility and may be protective for a disease.Genetic polymorphism in several aspects of immunity have been investigated as the host immune response has a major role in the pathogenesis of periodontitis.There are many candidate genes investigated in relation to chronic periodontitis.Cytokines have a significant role in inflammation and immunity.[9] IL-1 is a key cytokinein many chronic diseases such as rheumatoid arthritis[10],Alzheimer's disease[11]and periodontitis.[12]IL-1β is mainly produced by activated macrophagesand fibroblasts that release this extracellularprotein.The gene regulatingthe production of IL-1ß is located on the chromosome2q14.2.[14]IL-1β gene has three polymorphisms based on transitions between C and T at positions +3954/3953 (C→T, rs1143634), -511 (C→T, rs16944), and -31 (T→C, rs1143627) base pairs from the transcriptional site.[15,16]The host mediated interactions determining the disease phenotype can be understood with cytokine gene

polymorphisms studies.Several SNPs have been evaluated to determine the susceptibility of chronic periodontitis in various populations of the world with inconsistent results. In this study, we have analysed the association between SNP in IL1β (+ 3954) polymorphism in chronic periodontitis susceptibility in a hospital-based sample population from northern Karnataka,India.

II. Material & Methods

This case control study had 135 patients visiting the outpatient Department of Periodontics and Oral Implantology, ShriDharmasthalaManjunatheshwara College ofDental Sciences and Hospital (SDMCDSH) , Dharwad, India, betweenMarch 2016 and April 2017. An ethical clearance wasobtained from the institutional ethical committeeand informed written consent was obtained from allthe patients before their participation in the study. The study population was comprised of 63 males and 72 females.The inclusion criteria werepatients of 18 to 60 years old divided into two groups. Group 1 (Healthy controls) was comprised of 56 patients with no history of previous periodontal disease,no signs of gingival inflammation,absence of clinical attachment loss and no sites with probing depth > 3mm.Group 2 (Chronic Periodontitis) was comprised of 79 patients diagnosed with chronic periodontitis based on presence of inflammation, loss of clinical attachment, and loss of adjacent supporting bone as evidenced by radiographic examination.They had >30% of sites exhibiting a clinical attachment loss of ≥ 5mm and at least three teeth exhibiting sites of clinical attachment loss in at least two different quadrants. The exclusion criteria were tobacco smokers/chewers,immunosuppressed subjects,any patient who has undergoneperiodontal therapy in the last 3 months,any history of systemic disease,history of antibiotic intake in the past 3 months,pregnant and lactating women.The clinical parameters assessed were plaque index (PI)[17], gingival index (GI)[18], probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP).

Four mL of peripheral blood mononuclear cell (PBMC) samples was collected from all patients in EDTA coated vacutainers.The collection tubes were coded tomaintain confidentiality and transported to the laboratory in ice-cold condition (4°C).DNA was isolated using standard isolation kit (DNeasy Blood and Tissue Kit, Cat. No. 69506, QIAGEN, USA).DNA quantification and purity was assayed using UVspectrophotometer at 260nm and 280nm.

Table1: Primer sequences and reaction conditions

Primer Name	Sequence 5'-3'	Tm	Product Size	Enzyme used for RFLP	Genotype (digested fragment sizes in bp)	Genotype (digested fragment sizes in bp)	Genotype (digested fragment sizes in bp)	Reference
IL-1β, +3954C/T (rs1143634) Forward primer	GTTGTCATCAGACTTTGACC	47 °C	250bp	*Taq 1*	t/t (250)	c/t (250; 136; 114)	c/c (136; 114)	Meenakshi P et al.[19]
IL-1β, +3954C/T (rs1143634) Reverse primer	TTCAGTTCATATGGACCAGA							

The 25µl PCR reaction mixture was prepared that had 0.2 µM forward and reverse primer each; 200 M of dNTPs (NEB #NO447L); 1X Taqbuffer, 1unit Taq enzyme (NEB #MO273L); about 1000ng of DNA made up to volume with nuclease free water. Initial denaturation for 30 seconds at 95^0C followed by 35cycles of denaturation at 95^0C for 15 seconds and extension at 68^0C for 1 minute.After the PCR amplification, the amplicons were run through 4% agarose gel electrophoresis and the DNA bands were observed in gel documentation.All PCR products (10µl) were digested with 10units of restriction endonuclease enzyme.Restriction enzyme digestion was carried out by incubating at 37°C for 15 minutes(Table 1).

The restriction enzyme digestion products were subjected to agarose gel electrophoresis.4% agarose was prepared in 1X TAE buffer (300ml). To this 30µl of ethidium bromide was added.5µl of restriction enzyme digest product was mixed with 3µl of loading dye bromophenolblue.The electrophoresis was conducted at a constant voltage of 100V for 60minutes.The observed bands were analysed under the gel documentation chamber (Fig 1).

Statistical Analysis: The mean and standard deviation were calculated for all the continuous variables and frequency and percentage were calculated for all the categorical variables.Theassociationofallele and genotype frequencies for the SNPwith chronic periodontitis was assessed by Chi-square test.The odds ratio (OR) was determined to estimate the risk ofdeveloping severe periodontitis. The statistical analysis was carried out by

using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., Chicago, IL, USA). The statistical significance was set at 5% level of significance ($p<0.05$).

III. Results

The demographic characteristic of this case control study is summarized in Table 2.The mean plaque index for cases and controls was 3.15-11.06 and 0.66-0.45 respectively. The mean gingival index for cases and controls was 3.16-11.05 and 0.11-0.24 respectively. The probing pocket depth mean for cases and controls was 4.31-1-18 and 2.02-0.13 respectively. The mean of clinical attachment levels for the case and control groups was 5.92 -1.46and 1.91-0.29 respectively.The comparison of all these parameters in cases and controls was statistically significant.

Table2:- Clinical characteristics and parameters of the study population

	Chronic periodontitis	Control	P – value
Age (mean ± SD) (yrs)	40.42±8.72	32.64±5.94	0.0001*
Sex(M/F)	35/44	28/28	0.5131
PI (mean ± SD)	3.15±11.06	0.66±0.45	0.0001*
GI (mean ± SD)	3.16±11.05	0.11±0.24	0.0001*
PPD (mean ± SD) (mm)	4.31±1.18	2.02±0.13	0.0001*
CAL (mean ± SD) (mm)	5.92±1.46	1.91±0.29	0.0001*
BOP (mean ± SD) (mm)	1.00±0.00	2.00±0.00	0.0001*

No significant association between genotypes of IL-1β +3954 with clinical parameters was found (Table 3).Most of the cases (86%) had the severe type of disease (localized/ generalized) having ≥5 mm clinical attachment loss. The remaining (14%) had the moderate type of disease with 3–4 mm of clinical attachment loss; none had the mild type of disease.Also,no statistical significance was found when compared with severity of chronic periodontitis (Table 4).

Table 3: Comparison of clinical parameters with the genotypes

Genetic polymorphism	Clinical parameters	Genotypes			H-value	P- value
		c/c	**c/t**	**t/t**		P- value
IL-1β +3954	PI	1.39±0.88	1.35±0.77	1.36±0.99	0.0690	0.9660
	GI	1.18±1.05	1.19±1.02	0.85±0.83	0.5670	0.7530
	PPD	3.30±1.39	4.18±2.12	3.24±1.37	1.5370	0.4640
	CAL	4.18±2.22	5.32±3.01	4.19±2.26	1.3950	0.4980

Table 4:Association between severity of chronic periodontitis and genotypes

Genotypes	Severe CP	%	Moderate CP	%	Total	%	Chi-square	p-value
IL-1β +3954								**S**
c/c	25	35.71	45	64.29	70	88.61	2.2760	0.3200
c/t	4	66.67	2	33.33	6	7.59		
t/t	1	33.33	2	66.67	3	3.80		

The genotype C/C (88.61 % in cases and 91.07% in controls) was more prevalent than C/T(7.59 % in cases and 5.36% in controls) and T/T (3.80% in cases and 3.57%in controls) as well as allele C (94.94% in cases and 96.43% in controls)was more prevalent thanT allele (11.39% in cases and 8.93% in controls).However, there was no significant association of this genotype and alleles with the healthy controls and chronic periodontitis patients.The odds ratios forthe genotypes in cases and controls were OR:0.76 (95% CI:0.24-2.41) forIL-1β genotypes and for the alleles C and Twere OR:0.77 (95% CI:0.25-2.47) (Table 5).

Table 5: Genotype and allele frequencies of cytokines in cases and controls

Genotype	Cases	%	Controls	%	OR	95% CI		P value
	IL-1β+3954							
C/C	70	88.61	51	91.07	0.76	0.24	2.41	0.6440
C/T	6	7.59	3	5.36				0.608
T/T	3	3.8	2	3.57				0.945
	Alleles							
	Cases	%	Controls	%	OR	95% CI		P value
C	75	94.94	54	96.43	0.77	0.25	2.47	0.9924
T	9			8.93				0.6436

$p<0.05$

Agarose gel electrophoresis images representative of genotyping experiment results by PCR-RFLP are depicted in figure- 1.

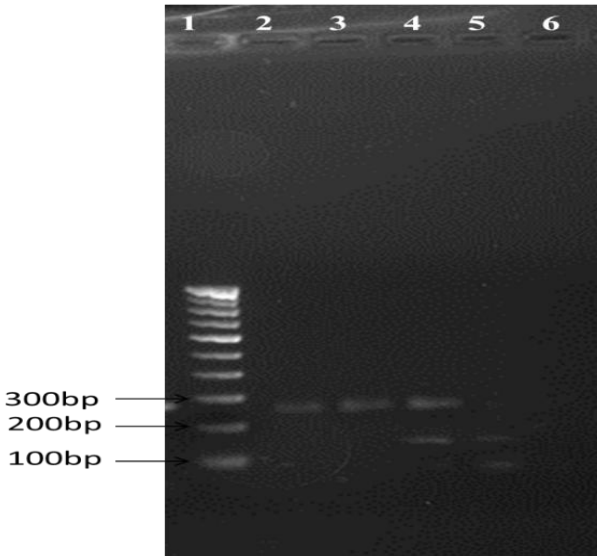

Fig 1: Agarose Gel (4%) Electrophoresis gel image of interleukin-1β +3954 (rs1143634) gene polymorphism

(Line 1: 100bp DNA Ladder (marker),Line 2:PCR product of 250bp of CP,Line 3:PCR product digested with *Taq 1* enzyme producing undigested fragment of 250bp (T/T genotype of CP),Line 4: PCR product digested with *Taq 1* enzyme producing digested fragments of 250bp; 136bp and 114bp (C/T genotype of CP),Line 5: PCR product digested with *Taq 1* enzyme producing digested fragments of 136bp and 114bp (C/C genotype of CP),Line 6: negative control).

IV. Discussion

Periodontal disease is a complex disease having the involvementof disease-modifying genes and their effect is modified by environmental factors.[20] These factors are bacterialplaque, smoking, coffee and alcohol consumption, stress and systemicfactors which may aggravate the pathology associatedwith the disease.[21] The initiationand progression of chronic inflammatorydiseases is regulated by cytokines. These are involved in the pathogenesis and clinical variability of chronic periodontitis. Their expression can be affected bythe presence of polymorphisms in cytokine genes thus they play an important role in resistance and susceptibility to periodontal disease.[21]Scientists stipulated that about 50% of the clinical differences of periodontal disease are evolved from gene polymorphisms.[4]IL-1β+3954polymorphismcan be associated with an increase in IL-1β production. IL-1β has an important role in the pathogenesis of periodontal disease. Pociot et al. found that monocytes from these patients produced more IL-1β thanpatients withoutIL1β+3954polymorphism.[22]Also, neutrophils from patients with periodontitis carrying IL-1 β+3954 had increased IL-1β levels compared topatients without thispolymorphism but these levels were not significant.[23]While allele 2 of the IL-1β polymorphism associated with decreased IL-1β secretion was found by Santtilaet al.[24]

The present analysis revealed that the C/C genotypeof IL-1β+3954 polymorphismwas more prevalent than C/T and T/T genotypes in CP than HC while the C allele waslightly more frequent among HC whencompared with CP while T allele was more frequent in CP.But this result did not reach the statistical significance. Allele T has been shown to be associated with severity of the periodontal disease.[20] Similar findingswere reported by Shete et al.[25] where thefrequency of the CC genotype but in contrast Callele was more frequent with chronic periodontitis.

A negative association was also documented in NorthernEurope, Poland, Jordan and South African populations.[26,27,28,29]The CT genotype was found to be a significant risk factor for the developmentof the disease in north Indian population and a positive association of this variant was observed in studies from Brazil and South Indian states.[30,31,32,33,34] A meta-analysis supporting a positive associationdocumenting the CT genotype of chronic periodontitis patients as a significant risk factor in Caucasians was observed but not in Asians[35]and the another included 53 studies with 4178 cases and 4590 controls reportingits strong association with disease susceptibility.[36]Lack of significant association ofIL-1β+3954polymorphism with theclinical parameters such as pocket probing depth, clinical attachment level, plaque and gingival indicesand also with the severity of chronic periodontitis was observed.

There is a large inconsistency of genetic polymorphisms in different studies suggesting the presence of several genetic profiles for the forms of periodontitis in different population or the diversity may be due to the detection methods and also the inter-individual variation in cytokine production.The protein product of the

genes may function differently though the SNPs do not change them. But SNPs have an effect on the gene product. This change contributes to a disease phenotype depending on the specific consequence ofthe particular genetic variant and on the type of disease along with environmental factors contributing to the disease risk.[37]Future strategies can be directed towards multi-centre research studies for cost- and time-effectiveness. Also,the microbiological application should be recommended due to the multifactorial characteristics of the periodontal disease.

V. Conclusions

Predominance of C/C genotype and T allele of IL-1β polymorphism appear to be associated with chronic periodontitis in Northern Karnataka population though it did not reach the statistical significance.

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