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Evaluation of genetic polymorphism of interleukin-1β +3954 (rs1143634) in chronic & aggressive periodontitis in Bengali population of West Bengal, India

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Abstract
Background & Objective: The genetic basis of periodontitis was demonstrated by formal genetic studies which were focused on a range of various candidate genes selected for their roles in the immune system like genes of the Interleukins (IL) which regulate the intensity of host immune-inflammatory response. This regulation of host response may be associated with the genetic polymorphisms, specifically single nucleotide polymorphisms of the genes of various Interleukins. Interleukin-1(IL-1) is a principal mediator of inflammatory responses acting on many cell types and is itself produced by many different cells, including macrophages, endothelial cells, B cells, fibroblasts, epithelial cells, astrocytes and osteoblasts in response to microorganisms, bacterial toxins and complement components. In the present study, attempt has been made to explore the role of IL-1β+3954 (rs1143634) gene polymorphism in Chronic as well as Aggressive Periodontitis in Bengali population of West Bengal, India.
Materials & Methods: Total 88 Bengali patients of both sex were recruited in this study and they were divided into 3 groups: Group A (Chronic Periodontitis group), Group B (Aggressive Periodontitis group) and Group C (Healthy control). The clinical parameters taken into consideration for the assessment of chronic and aggressive periodontitis were Plaque index, Calculus index, Gingival index, Probing pocket depth (PPD), Clinical attachment loss (CAL). 3 ml of peripheral venous blood was collected from each selected participants & transferred to a 3% EDTA containing serum vial and stored at -20°C for DNA extraction. DNA extraction was performed by phenol chloroform method & ethanol Precipitation. Genotyping of extracted DNA samples was carried out for locus IL-1β+3954 (rs1143634) by Real – time Polymerase Chain Reaction. Hardy–Weinberg equilibrium was tested for all the gene polymorphisms and association between genotypes and cases was examined by Odds ratio with 95% confidence interval (CI) and chi-square analysis using R statistical software. Allelic frequencies were calculate according to the number of different alleles observed and the total number of alleles examined. Statistical significance was defined as $p < 0.05$.
Results: Upon analysis of minor allele frequencies of total periodontitis cases and control, the results were found to be statistically insignificant with the p value of 0.7036.
Conclusions: The present study suggested a no association of single nucleotide polymorphism of IL-1β+3954 (rs1143634) with total periodontitis cases (both Chronic Periodontitis and Aggressive Periodontitis) in Bengali population of West Bengal, India.

Keywords: interleukin, real time polymerase chain reaction, minor allele frequency, genotyping, single nucleotide polymorphism

1. Introduction
In subjects susceptible to periodontitis, commonly an imbalance exists in between the host's immune system and the oral bacteria (Pihlstrom BL *et al* 2005 and Haffajee and Socransky 1995) [1, 2]. Cytokines are regulators of host responses to infection, immune responses, inflammation and trauma (Dinarello 2000) [3]. Prevalence of Chronic Periodontitis varies among various races. Demmer and Papapanou in 2010 [4] estimated the prevalence of severe periodontitis among participants aged approximately 40–50 years and the estimates were 21% in Germany and 16%, 28% and 32% in various populations from the United States. Jacob P. Shaju *et al* in 2011 estimated that in Indian population the prevalence rate of moderate Chronic Periodontitis is 17.5% in 35-44 years age group and 21.4% in 65-74 years of age group [5]. They also noticed that the prevalence of loss of attachment of > 3 mm in the 44 years age group is highest in Maharastra (78%) followed by Orissa (68%) and Delhi (46%). Genetic research can improve the understanding of the factors that mediate the immune response and explain why this response often greatly differs between individuals who have the same environmental context and comparable lifestyle habits. Kornman *et al* in 1997 first implicated the association of IL-1β+3954(rs1143634) gene polymorphism in Chronic Periodontitis [6]. Nikolopoulos *et al*. in 2008 performed a meta-analysis to investigate the association of the IL1α -889, IL1β +3954, IL1 β -511, TNF α -308 and IL6 -174 polymorphisms in aggressive periodontitis and chronic periodontitis [7]. They did not find any associations for aggressive periodontitis, but moderate and weak positive associations were found for the IL1 composite and IL1β -511 genotypes, respectively, in chronic periodontitis patients. In the present study, attempt has been made to explore the role of IL-1β+3954(rs1143634) gene polymorphisms in Chronic Periodontitis (CP) & Aggressive Periodontitis (AgP) in Bengali population of West Bengal, India.

2. Materials and Methods

Total 88 Bengali participants of both sex were selected selected from the OPD of department of Periodontics of Dr.R.Ahmed Dental College and Hospital, Kolkata and they were divided into 3 groups. Group A comprised of 49 CP patients; Group B comprised of 8 AgP patients and Group C comprised of 31 healthy volunteers. Diagnosis of CP and AgP were made as per AAP 1999 guidelines. Smokers, pregnant females, patients with any other systemic diseases, patients on Antibiotics or NSAIDs therapy or any periodontal therapy within past 6 months, were excluded from the study. The clinical parameters taken into consideration for the assessment of CP and AgP were Plaque index (Silness & Löe, 1964) [8], Calculus index (Calculus component of OHI-S, Green & vermillion 1964) [9], Gingival index (Löe & sillness, 1963) [10], Probing pocket depth (PPD),Clinical attachment loss (CAL). The study was carried out with written informed consent from all participants and was approved by the Ethics Committee of Dr R. Ahmed Dental College and Hospital (Chairman Prof. Dr. Haridas Adhikary) on 28th December 2016.

3. Sample collection

3 ml of peripheral venous blood was collected from each selected participants & transferred to a 3% EDTA containing serum vial and immediately transferred to Human Genetics Unit of Indian Statistical Institute, Kolkata to store at -20°C for DNA extraction.

4. DNA extraction from blood sample

DNA extraction was performed by phenol chloroform method & ethanol precipitation. 3 ml of whole blood was placed in a 15 ml Falcon tube.12 ml Reagent A (0.01M Tris-HCL, pH 7.4, 320 mM sucrose, 5 mM $MgCl_2$, 1% Triton X-100) was added in it for red cell lysis. The above mixture was then placed on a rotating blood mixer for 5 min at room temperature for twice. The whole mixture was centrifuged at 3000g for 5 min at room temperature for twice. The supernatant was discarded without disturbing cell pellet. Remaining moisture was removed by inverting the tube and blotting onto tissue paper. Then 1 ml reagent B (0.4m Tris-HCL, 150 mM NaCl, 0.06M EDTA, 1% sodium dodecyl sulphate, pH 8.0) was added for cell lysis. Then vortex briefly to suspend the cell pellet and added 250µL of 5M sodium perchlorate and mixed by inverting the tube several times. Then the tube was placed in water bath for 30 mins. Then allowed to cool at room temperature and 2ml ice cold chloroform was added and mixed on a rotating mixer for 30-60 mins. After that the tube was then centrifuged at 3000g for 5 mins. After centrifugation the upper phase of the tube was transferred into a clean Falcon tube using a sterile pipet. Then 2-3 ml ice cold ethanol was added and inverted the tube gently to allow DNA to precipitate. The DNA then spooled onto the hooked end by using a freshly prepared flamed Pasteur pipet. Then the DNA sample was transferred to a 1.5 ml Eppendrof tube and allowed to air dry. Finally, the sample was suspended in 200µl TE buffer.

5. Genotyping

Genotyping of extracted DNA samples was carried out for locus IL-1β+3954(rs1143634) using 7900HT (High Throughput) Fast Real – time PCR system instrument (Applied Biosystems, USA) by using allele specific Taqman MGB (Minor Groove Binder) probe labelled with fluorescent dyes FAM & VIC, according to manufacturer's protocols.

Hardy–Weinberg equilibrium was tested for all the three gene polymorphisms and association between genotypes and cases (CP & AgP) was examined by Odds ratio with 95% confidence interval (CI) and chi-square analysis using R statistical software. Allelic frequencies were calculate according to the number of different alleles observed and the total number of alleles examined. Statistical significance was defined as $p < 0.05$.

6. Results and Analysis

The study population of 88 participants comprised of 57 cases which include 26 males and 31 females; and among 31 controls (Group C) including 15 males and 16 females. Group A comprised of 20 males and 29 females with an average age of 41.43 years (range 24–55years) with the standard deviation of 8.06. Group B comprised of 6 males and 2 females with an average age of 29.44 years (range 18–50 years) with the standard deviation of 11.02. Group C comprised of 16 males and 15 females with an average age of 32.48 years (range 22–45years) with the standard deviation of 6.09. The distribution of sampled population according to the clinical parameters are shown in Table 1,2.

Table 1: Distribution of sampled population according to clinical parameters in Chronic Periodontitis case and control groups

	Plaque index (Mean±SD)	Gingival index (Mean±SD)	Calculus index (Mean±SD)	PPD (mm) (Mean±SD)	CAL (mm) (Mean±SD)
Group A	2.20±0.41	2.1±0.63	2.49±0.51	6.10±0.84	3.75±0.72
Group C	0.65±0.49	1.25±0.46	0.42±0.50	1.87±0.71	0.52±0.51

Table 2: Distribution of sampled population according to clinical parameters in Aggressive Periodontitis case and control groups

	Plaque index (Mean±SD)	Gingival index (Mean±SD)	Calculus index (Mean±SD)	PPD (mm) (Mean±SD)	CAL (mm) (Mean±SD)
Group B	1.5±0.53	1.75±0.63	1.63±0.52	6.38±1.06	6.88±1.13
Group C	0.65±0.49	1.25±0.46	0.42±0.50	1.87±0.71	0.52±0.51

- **Comparison of Allele Frequencies and Genotype Frequencies of IL1β+3954 [rs1143634] in Cases and Controls**

The analysis distributions of C and T alleles for IL-1β+3954 (rs11436934) showed more or less equal frequency of the C allele (54%) and T allele (46%) in patients with chronic periodontitis as well as in healthy control group[C allele (48%) and T allele (52%)], which achieved a statistically insignificant result with p value of 0.6843 [Table 3].

Table 3: Allele frequencies of IL-1β+3954 (rs1143634) single nucleotide polymorphism in chronic periodontitis patients versus healthy controls

Groups	Allele counts (frequency %) A G	Chi-square test (p value)
Chronic periodontitis (Group A)	53(54%) 45(46%)	$p = 0.2298^{*}$
Healthy controls (Group C)	30(48%) 32(52%)	

Higher frequency of CT heterozygous genotype (51%) for IL-1β+3954 (rs 1143634) has been found in Chronic Periodontitis cases (Group A) as compared to CC (31%) and TT (18%) genotypes. Also, in case of Control group (Group C) the frequency CT genotype was comparatively higher (77%) than CC (10%) and TT (13%) genotypes. The analysis of genotype frequencies of among Group A and Group C showed statistically significant difference with the p value of <0.0001 [Table 4]

Table 4: Genotype frequencies of IL-1β+3954 (rs1143634) single nucleotide polymorphisms in chronic periodontitis patients versus healthy controls

Groups	Genotype counts (frequency %) AA AG GG	Chi-square test (p value)
Chronic periodontitis (Group A)	15(31%) 25(51%) 9(18%)	$p < 0.0001^{*}$
Healthy control (Group c)	3(10%) 24(77%) 4(13%)	

*statistically insignificant p value

The analysis of distributions of C and T alleles for IL-1β+3954 (rs 11436934) showed more or less equal frequency of the C allele(43%) and T allele(57%) in patients with Aggressive Periodontitis(Group B) as well as in Healthy Control group[C allele (48%) and T allele (52%)], which achieved a statistically insignificant result with p value of 0.3169 [Table 5].

Table 5: Allele frequencies of IL-1β+3954(rs1143634) single nucleotide polymorphism in aggressive periodontitis patients versus healthy controls

Groups	Allele count (frequency%) A G	Chi-square test (p value)
Aggressive periodontitis (Group B)	7(43 %) 9(57%)	P=0.3169*
Healthy control (Group C)	30(48%) 32(52%)	

*statistically insignificant

Higher frequency of CT heterozygous genotype (87%) for IL-1β+3954 (rs1143634) has been found in Aggressive Periodontitis cases (Group B) as compared to TT (13%) homozygous genotypes. When compared with the genotype frequency of Healthy Control group (Group C), a statistically significant result was achieved with the p value of <0.0035 [Table 6]

Table 6: Genotype frequencies of IL-1β+3954 (rs1143634) single nucleotide polymorphism in aggressive periodontitis patients versus healthy controls

Groups	Genotype count (frequency%) AA AG GG	Chi-square test (p value)
Aggressive periodontitis (Group B)	- 7(87%) 1(13%)	0.0035
Healthy control (Group C)	3(10%) 24(77%) 4(13%)	

Upon analysis of minor allele frequencies of total periodontitis cases and control, the results were found to be statistically insignificant with the p value of 0.7036. Minor allele frequency of overall periodontitis cases and controls were 0.47368 and 0.5161 respectively; odds ratio was 1.185 and 95% confidence interval ranges from 0.49 – 2.84 [Table 7].

Table 7: Association of IL-1β+3954(rs 1143634) single nucleotide polymorphism with total periodontitis cases (chronic and aggressive periodontitis)

Gene	Periodontitis cases	Healthy control	Minor allele frequency (case)	Minor allele frequency (control)	Odds ratio	95% Confidence interval	P value
IL-1β+3954 (rs1143634)	57	31	0.47368	0.5161	1.185	0.49-2.84	0.7036*

*Statistically insignificant

4. Discussion

The association between the IL-1 genotype (IL-1β a+3954, rs1143634) and adult periodontitis was first established by Kornman *et al* (1997) [6], and after that many studies have been undertaken to explore this association. Convincing evidence has emerged that susceptibility to periodontal disease is partially based on a genetic predisposition to the disease. However, results of individual studies have been inconclusive. Regional and racial differences are likely to be the reasons for disparity in the results. However, the present study has certain limitations. The sample size was small and Bengali subjects were selected based on their names rather than through geneolgical study. Further well-designed study with larger sample size is necessary. Gore *et al* 1998 [11] showed significant association towards increased IL-lβ production by peripheral blood PMN cells in case of advanced periodontitis in American Caucasians who possessed the lL-1β+3954(rs1143634) allele 2. The increased frequency of the IL-1β+3954 1/2(CT) and 2/2(TT) genotypes (allele 1=C; allele 2=T) in patients with advanced periodontitis compared to those of the early and moderate stages of periodontitis suggests that allele 2, previously correlated with increased IL-lβ production, may predispose an individual to increased severity of periodontal disease.

Analysis of genotype frequency revealed higher frequency of CT (58%) genotype followed by CC (33%) and TT (9%). Similarly, in the present study has shown higher frequency of CT (51%) followed by CC (31%) and TT (18%) genotypes in Chronic Periodontitis group (Group A). However further analysis by using minor allele frequency showed statistically insignificant result with the p value of 0.7036. Lang *et al* 2000 [12] in a prospective longitudinal study on 323 swiss subjects attending the SPT (supportive periodontal therapy) programme at the prophylaxis clinic of the Department of Periodontology and Fixed Prosthodontics of the University of Berne, showed the composite genotype consisting of the less common allelic variants previously associated with severe periodontitis was detected in 35.3% of the Caucasian subjects, the vast majority of central European ancestry. The results indicated that genotype positive patients had a significantly elevated chance of presenting an increase in the BOP% over a 4-appointment recall period (p~0.03) after correcting for oral hygiene. In fact, patients who were genotype-negative had a 50% smaller chance of showing increases in BOP% during SPT.

Socransky *et al* 2000 [13] studied 108 subjects to compare microbiological parameters in IL-1 genotype negative and positive adult subjects with a range of periodontitis severities in an American population of Boston, USA. The data of this investigation indicated that subjects who exhibit a positive IL-1 genotype are more likely to be colonized by high levels of Red and Orange complex periodontal pathogens (*B. forsythus, Porphyromonas gingivalis, T. denticola*, the *F. nucleatum* subspecies, *F. periodonticum,Campylobacter rectus, C. showae, Eubacterium nodatum, S. constellatus, S. gordonii*, and *S.intermedius*).The increased levels of these organisms appear to occur primarily at depths of >6 mm pockets. It is speculated that over-expression of IL-1α and IL-1β in response to organisms in subgingival plaque may increase gingival inflammation, gingival crevicular fluid flow and subgingival species. These increases might place the genotype positive subject at greater risk for periodontal disease progression.

The results of most of the European and American studies in different Caucasian populations indicate the positive association between IL-1β+3954(rs1143634) and Chronic Periodontitis, but studies in other ethnic groups showed contradictory findings. GC Armitage *et al* in 2000 [14] showed only 7 out of 300 Chinese subjects (2.2%) carried IL-1 composite genotype consisting of allele 2 of both IL-1α+4845 and IL-1β+3954.

Some of the contradictory European studies showed lack of association between the IL-1 polymorphisms and generalised early onset periodontitis. Walker SJ *et al* 2000 [15] studied prevalence of IL-1α and IL-1β genotype polymorphism in an African-American population with Localised Juvenile Periodontitis(LJP) and they demonstrated the prevalence of the composite genotype with at least one allele 2 at each of the IL-1α+4845 and IL-1β+3954 loci was 14%(control group) and 8%(LJP group).Analysis of genotype frequency revealed higher frequency of CC (84%) genotype followed by CT (16%) and no TT genotype.

Where as in the present study data revealed higher frequency of CT genotype (87%) followed by TT genotype (13%) and no CC genotype in Aggressive Periodontitis patients (Group B). Similarly, in the control group (Group C) frequency of CT genotype was higher (77%) as compared to CC (10%) and TT (13%) genotypes. This difference is considered as statistically significant with the p value of 0.0035. However, further analysis with the minor allele frequency revealed statistically insignificant result with the p value of 0.3169. The frequency of minor allele (T) were found to be more or less similar in both groups (Aggressive Periodontitis group or Group B= 57%; control group or Group C=52%). Hodge PJ *et al* 2001 [16] examined IL-1α and IL-1β genetic polymorphisms in unrelated European white Caucasian patients with generalised early-onset periodontitis. There were no significant differences between experimental patients and controls for any of the genotype or allele frequencies investigated (p=1.0).

Similarly, Papapanou *et al* in 2001 [17] demonstrated no skewed distribution of the composite genotype between periodontitis cases and healthy controls (45.2% and 41.7% respectively).

Similarly, Anusaksathein *et al* 2003 [18] performed one study to determine the distribution of IL-1β+3954 and IL-1α-899 in a group of Thai subjects based on their periodontal status, including Chronic Periodontitis, Aggressive Periodontitis and healthy subjects. The CC genotype frequencies of aggressive periodontitis, chronic periodontitis and healthy controls were 92.3%,100% and 97.7 % respectively.

The first ever Indian study by Agarwal *et al* in 2006 [19] in a population of Maharastrian ethnicity demonstrated the distribution of IL-1β(rs1143634) allele 2 was comparatively higher in moderate and severe periodontitis group, and the distribution for the IL-1β(rs1143634) allele 1 was higher in control group. In comparison to this study, the present study showed statistically insignificant difference in the allele frequency in the study population. Nikolopoulos *et al*. in 2008 [7] explained in a meta-analysis that simultaneous carriage of the T allele at IL-1α -889 and at IL-1β +3953/4 loci seems to confer an additional risk compared with the separate effect of each SNP (single nucleotide polymorphism). Based on a smaller number of studies compared with the latter SNPs, the analysis provided an indication of a weak positive effect of the IL-1β -511C variant on chronic periodontitis. Study of Chilean population performed by Lo´pez *et al* 2009 [20] showed IL-1β +3954 TT genotype (Odd ratio =3.54; 95% Confidence interval =1.15 to 10.85), and IL-1β -511 CC genotype (Odd ratio = 2.10; 95% Confidence interval = 1.25 to 3.58) were significantly associated with periodontitis. On the contrary in the present study IL-1β+3954 CT genotype and IL-1β -511AG genotype were more frequent in both Chronic Periodontitis and control group.

Shete *et al* 2010 [21] demonstrated that in the Malayalam-speaking Dravidian population, allele C of IL-1β +3954(rs1143436) appeared to be an important risk factor for chronic periodontitis. IL-1β+3954 CC genotype was found to be more frequent in both chronic periodontitis and control group. Where as in case of IL-1β-511(rs16944), allele and genotype distributions showed no significant differences between periodontitis cases and control group. In the present study allele A of IL-1β-511(rs16944) and AG genotype was frequently found in both cases and control group. Allelic and genotypic distribution of IL-1β+3954 (rs1143436) showed no significant difference between periodontitis cases and control group.

Prakash *et al* in 2010 [22] showed that frequencies of IL-1β+3954(rs1143436) CT and TT genotypes may be related

to chronic periodontitis in Tamil speaking population from the State of Tamil Nadu in the Southern region of India. The chronic periodontitis group displayed a higher percentage of the T allele (28%) when compared to control (8.7%) groups. In contrast to this study, the present study showed no significant difference in T allele frequency between chronic periodontitis group and control group. Furthermore, CC and CT genotypes were more frequent as compared to TT genotype in chronic periodontitis cases.

The frequency of the C allele and TC genotype of IL-1β+3954, - 511 were found to be higher in chronic and aggressive periodontitis in Jordanian population according to Karasneh *et al* 2011 [23].

SS Masamatti *et al* 2012 [24] also showed the significantly higher prevalence of the TT genotype of the IL-1β (+3954) in the chronic periodontitis group (24%) than in both aggressive and control groups, 3.3% and 7.4%, respectively in individuals from the state of Karnataka in south Indian population. The homozygous genotype (CC) of IL-1β +3954 was the most frequent genotype in control subjects than in cases, whereas the heterozygous (CT) genotype dominated in cases than in controls.

Present study showed higher prevalence of the CT genotype of the IL-1β (+3954) in the Aggressive Periodontitis group (87%) than in both Chronic Periodontitis group (51%) and control group (77%) in Bengali population from Eastern India. Heterozygous (CT) genotype is more prevalent in all three study groups. There was no significant difference in the distribution of interleukin-1β+3954 (rs1143634) allele 2 homozygous genotype (TT) between overall periodontitis cases and healthy controls (18% vs 13%).

Contradictory results were also obtained by studies conducted by Gayathri *et al* 2011 [25] where they have found that CC genotype was the more frequently found genotype in periodontitis cases than in controls in a sample of Indian population of Karnataka state.

5. Conclusions

The present study revealed a no association of single nucleotide polymorphism of IL-1β+3954 (rs1143634) with total periodontitis cases (both CP and AgP) in our patient cohort. There was no significant difference in the distribution of interleukin-1β+3954 (rs1143634) allele 2 homozygous genotype (TT) between periodontitis cases and healthy controls (18% and 12% respectively). Further longitudinal study is essential to validate the biologic basis for genetic susceptibility testing, to establish the polymorphism of IL-1β+3954(rs1143634) as genetic marker for susceptibility to periodontitis. However, the ideal method of elucidating any association of genetic polymorphism with periodontitis would be to start before onset of the disease and following it up through the natural history. To accomplish this, the genotypes required has to be established first and then follow it up as the subject is exposed to various predisposing and risk factors.

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