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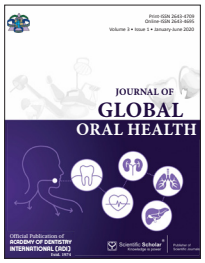

Research Article

Genetic polymorphism of interleukin-12β (rs7709212) in chronic and aggressive periodontitis in a group of the Bengali population of India

Avishek Das[1], Ashit Pal[1], Somen Bagchi[1]

[1]Department of Periodontics, Dr. R Ahmed Dental College and Hospital, Kolkata, West Bengal, India

***Corresponding author:**
Avishek Das,
Department of Periodontics
Dr. R Ahmed Dental College
and Hospital, Kolkata,
West Bengal, India

avidoc2010@gmail.com

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ABSTRACT

Objectives: 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). Bone proresorptive cytokines such as IL-1 and tumor necrosis factor-α are primarily regulated by a network of other T-cell derived cytokines, including IL-12 family. Specific objective of this study was to find out any association between IL-12β 3′ untranslated region (UTR) (rs7709212) gene polymorphism and chronic as well as aggressive periodontitis (AgP) in Bengali population of West Bengal, India.

Materials and Methods: A total of 88 Bengali participants of both sexes were recruited and were divided into three groups: Group A (chronic periodontitis [CP] group), Group B (AgP group), and Group C (healthy control). DNA extraction from collected peripheral venous blood was performed by phenol chloroform method and ethanol precipitation. Genotyping of extracted DNA samples was carried out for locus IL-12β 3′UTR (rs7709212) by real time-polymerase chain reaction. Association between genotypes and cases was examined by the odds ratio with 95% confidence interval and chi-square analysis using R statistical software. Statistical significance was defined as $P < 0.05$.

Results: On analysis of the minor allele frequencies of total periodontitis cases and control, the results were found to be statistically significant with the $P = 0.03045$.

Conclusion: The present study suggested a strong association of single nucleotide polymorphism of IL-12β (rs7709212) with total periodontitis cases (both CP and AgP) in the present study cohort.

Keywords: Interleukin, Real time polymerase chain reaction, Minor allele frequency, Genotyping, Single nucleotide polymorphism

INTRODUCTION

Periodontitis is a multifactorial chronic inflammatory disease involving the supporting tissues of the teeth and characterized by progressive attachment loss and alveolar bone loss. There are multiple risk factors for periodontitis such as local factors (dental plaque), behavioral factors such as smoking and alcohol, systemic factors like diabetes mellitus, and lastly the genetic factors. Although it is an infectious disease caused by microorganisms, host-susceptibility plays the important role in the development and progression of the disease. In subjects susceptible to periodontitis, commonly an imbalance exists in between the host's immune system and the oral bacteria.[1,2] Cytokines are regulators of host responses to infection, immune responses,

inflammation, and trauma.[3] The prevalence of chronic periodontitis (CP) varies among various races. The prevalence of severe periodontitis among participants aged approximately 40–50 years revealed estimates of 21% in Germany and 16%, 28%, and 32% in various populations from the United States.[4] In Indian population, the prevalence rate of moderate CP is 17.5% and 21.4% in 35–44 years and 65–74 years of age group respectively; the prevalence of loss of attachment of >3 mm in the 44 years age group is highest in the state of Maharashtra (78%) followed by Orissa (68%) and Delhi (46%).[5] 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. The association of Interleukin-1β+3954 gene polymorphism in CP had already been established.[6] Interleukin-12 (IL) is one of the potent regulator of bone pro-inflammatory cytokines like IL-1 and tumor necrosis factor-α.[7] However, any strong evidence regarding the association between genetic polymorphism of IL-12 and periodontitis has not been reported till now. One study showed insignificant association of polymorphisms of IL-12 (1188 A/C) and periodontitis.[8] In the present study, an attempt has been made to explore the role of single nucleotide polymorphism of IL-12β (rs7709212) gene in CP and aggressive periodontitis (AgP) in a group of the Bengali population of West Bengal, India.

MATERIALS AND METHODS

The case–control study was conducted between February 2017 to September 2018. A total of 88 Bengali participants (both male and female) with age group between 30 and 55 years, were selected from the Out Patient Department of the Department of Periodontics of Dr.R.Ahmed Dental College and Hospital, Kolkata and they were divided into three groups. Group A comprised 49 chronic periodontitis patients, Group B comprised 8 AgP patients, and Group C comprised 31 healthy volunteers. Diagnosis of CP and AgP was made as per AAP 1999 classification of periodontal diseases. Smokers, pregnant females, patients with some other systemic diseases, and patients on antibiotics or NSAIDs therapy or any periodontal therapy within past 6 months were excluded from the study. The clinical parameters that were considered in the assessment of CP and AgP are plaque index (Silness and Löe, 1964),[9] calculus index (Calculus component of Oral Hygiene Index-Simplified, Green and vermillion 1964),[10] gingival index (Löe and Sillness, 1963),[11] probing pocket depth (PPD), and clinical attachment loss (CAL). The inclusion criteria of all clinical parameters that had been used for assessing CP patients (Group A) were PPD (mm) ≥5 mm, CAL ≥3 mm, plaque index ≥2, gingival index ≥2, calculus index ≥2, and radiographic evidence of bone loss. Patients having rapid loss of attachment and tooth-supporting bone, otherwise healthy (i.e., not suffering from any systemic disease or condition that could be responsible for the present periodontitis), presence of familiar aggregation along with secondary features like inconsistent presence of etiologic factors and typical radiographic bone destruction pattern (arc-shaped) in molar-incisor group (AAP 1999 classification of periodontal diseases) considered as AgP case in Group B. Volunteers (age and sex matched) with healthy gingiva having PPD of 0–3 mm, no evidence of CAL, no evidence of radiographic bone loss, no signs of gingival inflammation, and not suffering from any systemic disease considered as healthy controls (Group C). The present study was conducted in accordance with the declaration of Helsinki and received approval from the Institutional Review Board of Dr R. Ahmed Dental College and Hospital (IRB No. DCH/73/8-19) and the written informed consent was obtained from all patients.

Sample collection

Three milliliter of peripheral venous blood was collected from each selected participant and 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.

DNA extraction from blood sample

DNA extraction was performed by the phenol chloroform method and ethanol precipitation. Three milliliter of whole blood was placed in a 15 ml Falcon tube. Twelve milliliters Reagent A (0.01M Tris-HCL, pH 7.4, 320 mm sucrose, 5 mm $MgCl_2$, 1% Triton X-100) were added into 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 3000 g 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 (0.4 m Tris-HCl, 150 mm NaCl, 0.06 M EDTA, and 1% sodium dodecyl sulphate, pH 8.0) were added to cell lysis. Then, vortex briefly to suspend the cell pellet and added 250 μL of 5 M sodium perchlorate and mixed by inverting the tube several times. Then, the tube was placed in a water bath for 30 min and then allowed to cool at room temperature and 2 ml ice cold chloroform was added and mixed in a rotating mixer for 30–60 min. After that, the tube was then centrifuged at 3000 g for 5 min. After centrifugation the upper phase of the tube was transferred into a clean Falcon tube using a sterile pipette. 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 pipette. Then, the DNA sample was transferred to a 1.5 ml Eppendorf tube and allowed to air dry. Finally, the sample was suspended in 200 μl TE buffer.

Genotyping

Genotyping of extracted DNA samples was carried out for locus IL-12β (rs7709212) using 7900HT (High Throughput) fast real-time PCR system instrument (Applied Biosystems, USA) using allele specific Taqman MGB (Minor Groove Binder) probe labeled with fluorescent dyes FAM (Fluorescein amidites) and VIC (Life technologies), according to manufacturer's protocols.

Hardy–Weinberg equilibrium was tested for the gene polymorphisms and association between genotypes and cases (Group A and B) was examined by Odds ratio with 95% confidence interval (CI) and Chi-square analysis using R programming software (R version 4.0.2). Allelic frequencies were calculated according to the number of different alleles observed and the total number of alleles examined. Statistical significance was defined as $P < 0.05$.

RESULTS AND ANALYSIS

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

Comparison of allele frequencies and genotype frequencies of IL12β [rs7709212] in cases and controls

Higher frequency of CC genotype (53.1%) for IL-12β (rs7709212) has been found in Group A as compared to CT (20.4%) and TT (26.5%) genotypes. The frequency of TT genotype was found to be higher in Group A (26.5%) than in Group C (3.2%). When compared with the genotype frequency of both Group A and Group C, a statistically significant result was achieved with $P < 0.0001$ [Table 3 and Figure 1].

The distributions of T and C alleles for IL-12β (rs7709212) between Group A and Group C showed a higher frequency (63%) of the C allele and comparatively lower frequency (37%) of T allele in patients with CP which achieved a statistically significant result ($P < 0.0001$). The frequency of minor allele T was found to be higher in Group A (37%) than in Group C (21%) [Table 4 and Figure 2].

Higher frequency of CT genotype (75%) for IL-12β (rs7709212) has been found in Group B as compared to CC (25%) genotypes. No TT genotype has been noticed in Group B. The frequency of CC genotype was found to be higher (61.3%) in Healthy Group C as compared to Group B (25%). When compared with the genotype frequency of both Group B and Group C, a statistically significant result was achieved with $P < 0.0001$ [Table 5 and Figure 3].

The distributions of T and C alleles for IL-12β (rs7709212) between the group with Group B and Group C showed a higher frequency of the C allele 62.5% and 79%, respectively.

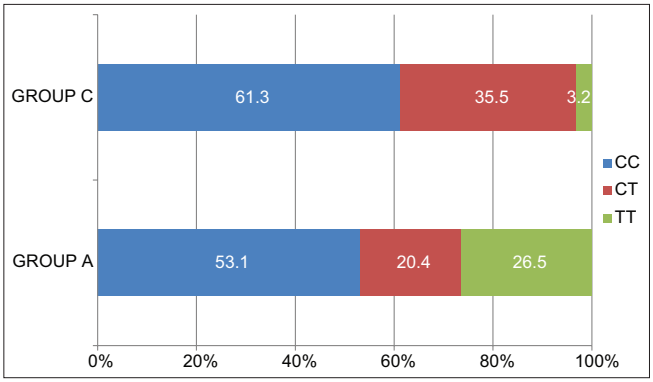

Figure 1: Genotype frequencies (%) of IL-12β (rs7709212) single nucleotide polymorphisms in chronic periodontitis patients versus healthy controls.

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

Table 3: Genotype frequencies of IL-12β (rs7709212) single nucleotide polymorphism in chronic periodontitis patients versus healthy controls.

Groups	Genotype counts (frequency %)			Chi-square test (*P*-value)
	CC	CT	TT	
Chronic periodontitis (Group A)	26 (53.1%)	10 (20.4%)	13 (26.5%)	<0.0001
Healthy control (Group C)	19 (61.3))	11 (35.5%	1 (3.2%)	

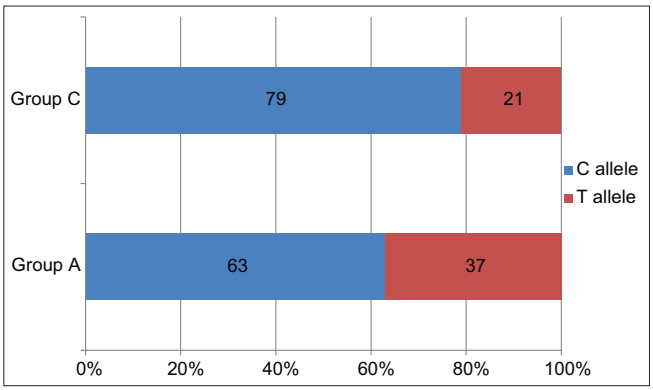

Figure 2: Allele frequencies of IL-12β (rs7709212) single nucleotide polymorphisms in chronic periodontitis patients (Group A) versus healthy controls (Group C).

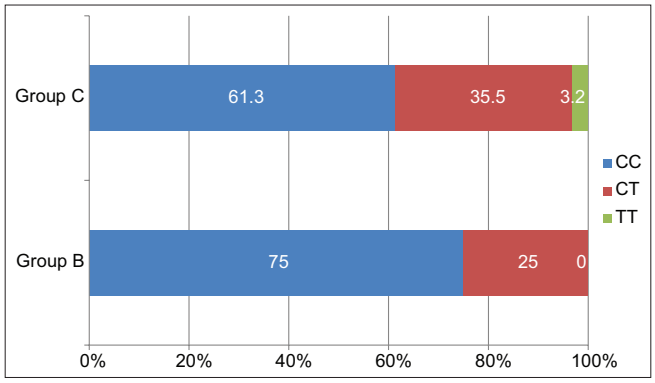

Figure 3: Genotype frequencies of IL-12β (rs7709212) single nucleotide polymorphism in aggressive periodontitis patients versus healthy controls.

The frequency minor allele T was found to be higher in Group B (37.5%) than in Group C (21%). The comparison of the distribution of C and T alleles among both Group B and Group C showed statistically significant result ($P < 0.0001$) [Table 6 and Figure 4].

As the sample size of Group B was too small, for the analysis of minor allele frequency, both the periodontitis cases (CP and AgP) were considered unified group of periodontitis. On analysis of the minor allele frequencies of total periodontitis cases and control, the results were found to be statistically significant with the $P = 0.03045$. Minor allele frequency of overall periodontitis cases and healthy controls were 0.36 and 0.2, respectively; odd ratio was 2.19 and 95% CI ranges from 1.07 to 4.51 [Table 7 and Figure 5].

Table 4: Allele frequencies of IL-12β (rs7709212) single nucleotide polymorphisms in chronic periodontitis patients versus healthy controls.

Groups	Allele counts (frequency %)		Chi-square test (*P*-value)
	C	T	
Chronic periodontitis (Group A)	62 (63%)	36 (37%)	<0.0001
Healthy controls (Group C)	49 (79%)	13 (21%)	

Table 5: Genotype frequencies of IL-12β (rs7709212) single nucleotide polymorphism in aggressive periodontitis patients versus healthy controls.

Groups	Genotype counts (frequency %)			Chi-square test *P*-value
	CC	CT	TT	
Aggressive periodontitis (Group B)	2 (25%)	6 (75%)	-	<0.0001
Healthy controls (Group C)	19 (61.3)	11 (35.5%)	1 (3.2%)	

Table 6: Allele frequencies of IL-12β (rs7709212) single nucleotide polymorphism in aggressive periodontitis patients versus healthy controls.

Groups	Allele counts (frequency %)		Chi-square test (*P*-value)
	C	T	
Aggressive periodontitis (Group B)	10 (62.5%)	6 (37.5%)	<0.0001
Healthy controls (Group C)	49 (79%)	13 (21%)	

DISCUSSION

Genetic basis of the susceptibility to periodontal disease has been supported by several convincing evidence. However,

Table 7: Association of IL-12β (rs 7709212) single nucleotide polymorphism with total periodontitis cases (chronic and aggressive periodontitis).

Gene	Periodontitis cases (Group A+Group B)	Healthy control	Minor allele frequency (case)	Minor allele frequency (control)	Odds ratio	95% confidence interval	*P*-value
IL-12β (rs7709212)	57	31	0.36	0.2	2.19	1.07–4.51	0.03045*

*Statistically significant

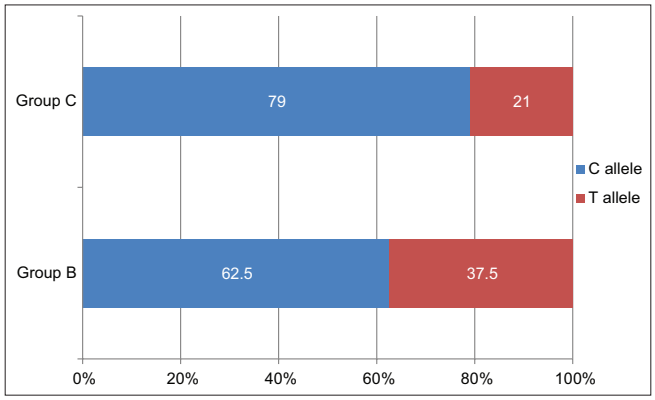

Figure 4: Allele frequencies of IL-12β (rs7709212) single nucleotide polymorphism in aggressive periodontitis patients versus healthy controls

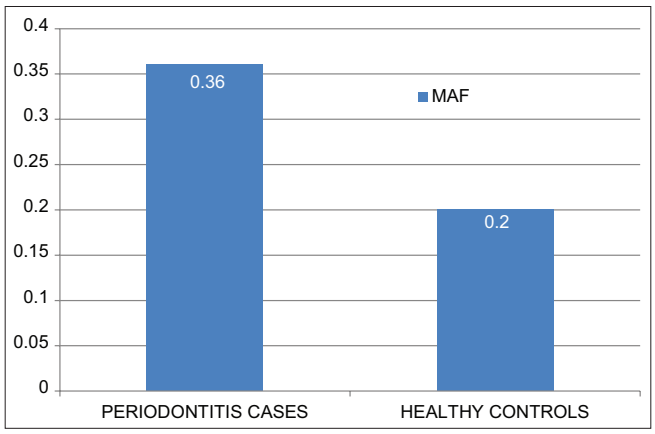

Figure 5: Comparison of minor allele frequency between periodontitis cases and controls. MAF=Minor allele (T allele) frequency.

findings of individual research have been inconclusive. Regional and racial differences are likely to be the reasons for disparity in the results. A strong association between the IL-1β C-511T (rs16944) polymorphism and periodontitis had been established in Afro-Americans (blacks) and mulattos[12] and Chinese population.[13] However, an updated meta-analysis suggested a non-significant association between IL-1β C-511T polymorphism and CP.[14] Nikolopoulos *et al.*, in 2008, observed weak positive association for IL1β -511 genotypes in CP patients.[15]

Apart from IL-1β, the role of IL-12 in periodontal bone resorption has been demonstrated in IL-12-deficient mice, in which periodontitis was induced with *P. gingivalis*.[16] The underlying mechanism behind IL-12-mediated alveolar bone destruction through the induction of RANKL expression by human PDL cells and upregulation of the RANKL/OPG ratio was demonstrated by Ayuthaya *et al.* in 2017.[17] Polymorphisms in genes for IL-12 were found to be functionally relevant with various chronic inflammatory diseases like psoriasis,[18] refractory ulcerative colitis.[19] Periodontitis is also a complex multifactorial chronic inflammatory disease. Hence, the polymorphisms in genes for IL-12 are likely to be functionally relevant with periodontitis. However, the survey of the literature indicates no strong evidence of association of IL-12β polymorphism with any form of periodontitis. Reichert *et al.*, in 2008, studied the possible association of polymorphisms of IL-12 (1188 A/C) and periodontal conditions.[20] The results showed the allele and genotype frequencies of both investigated polymorphisms were not significantly different between subjects with periodontitis and periodontitis-free controls. However, in the total study group, IL-12 AA–positive subjects had a significantly higher bleeding index than individuals who expressed IL-12 CC (68.2% versus 50.0%, $P = 0.025$). They concluded that IL-12 polymorphisms are not suitable diagnostic features for aggressive and CP.

The present study showed higher frequency of TT genotype (26.5%) in Group A than in Group C (3.2%). The frequency of minor allele T was found to be higher in Group A (37%) than in Group C (21%). No TT genotype has been noticed in Group B. However, the frequency of minor allele T was found to be higher in Group B (37.5%) than in Group C (21%). On analysis of the minor allele frequencies of overall periodontitis cases and control group, the results were found to be statistically significant with $P = 0.03045$. However, the present study has certain limitations. The sample size was small and Bengali subjects were selected based on their names rather than through genealogical study. Further, well designed study with larger sample size is necessary.

CONCLUSION

The present study revealed a strong association of single nucleotide polymorphism of IL-12β (rs7709212) with total periodontitis cases (both CP and AgP) in our patient cohort.

To the best of our knowledge perhaps, this was the first study of its kind performed in Eastern India and for the 1st time the association of IL-12β (rs7709212) with periodontitis has been explored. Further, longitudinal study is essential to validate the biologic basis for genetic susceptibility testing, to establish the polymorphism of IL-12β (rs7709212) as a genetic marker for susceptibility to periodontitis. However, the ideal method of elucidating any association of genetic polymorphism with periodontitis would be to start before the 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.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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