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Genetic Polymorphism of Prostaglandin E2 EP4 Receptors in Chronic and Aggressive Periodontitis Patients

Dental Science

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ABSTRACT

Aim: PGE2 has been reported to be associated with periodontal attachment loss and alveolar bone resorption, which suggests that PGE2 is involved in the pathogenesis of periodontal disease. Among the receptors of PGE2, EP4 receptor regulate inflammatory and immunological responses in periodontal lesions. The present study was aimed at evaluating a possible association of single nucleotide polymorphism of PGE2 EP4 receptor gene with susceptibility and severity of chronic and aggressive periodontitis. **Materials and Methods:** A total of 105 subjects were divided into 3 groups of 35 patients each and grouped as Group I- 35 Healthy controls, Group II- 35 Chronic periodontitis patients, Group III- 35 Aggressive periodontitis patients. 2ml of blood sample was collected from all the subjects and subjected to assessment of polymorphism of PGE2 EP4 receptor using polymerase chain reaction technique.The analysis of variance (ANOVA) and chi-square test was used to analyze the genotype distribution among chronic, aggressive periodontitis patients and healthy controls. **Results:** Significant differences were found between the frequency of PGE2 EP4 receptor genotypes in periodontitis group and control group. CC and GC genotype were more prevalent in aggressive, chronic periodontitis patients and GG genotype was prevalent in healthy controls. **Conclusion:** The GC and CC genotype of PGE2 EP4 receptor genetic polymorphism may increases the susceptibility to chronic and aggressive periodontitis respectively and that the GG genotype found in healthy controls may protect against periodontitis.

KEYWORDS:

Prostaglandin E2 EP4 receptors; Chronic periodontitis; Aggressive periodontitis; Genetic polymorphism

INTRODUCTION

Prostaglandins (PGs) play important roles in regulating diverse cellular functions under physiological and pathological conditions.[1] In response to stimuli, arachidonic acid released from membrane phospholipid is metabolized to several types of PGs including PGE2 by cyclooxygenase (COX).[2] PGE2 is increased in periodontal site demonstrating inflammation, attachment loss, osteoclastic bone resorption, collagen destruction and induction of matrix metallo proteinases. Primary cells responsible for PGE2 production in the periodontiumare macrophages and fibroblasts.[3,4] Numerous studies have indicated that prostaglandin E2 are involved in the pathogenesis of periodontal disease. Elevated prostaglandin E2 levels are detected in the gingiva and gingival crevicular fluid of patients with periodontal diseases, compared to periodontally healthy subjects.[5,6] Genetic factors are important determinants of periodontitis susceptibility and progression. Studies on humans and animals indicate that genetic factors which impair inflammatory and immune responses in general, affect periodontitis experience specifically.[7] Initial studies indicate that the most common source of human genetic variation is found in single nucleotide polymorphisms.[8] Single nucleotide polymorphisms can affect gene function. Thus, it may make considerable sense to use single nucleotide polymorphisms for more accurate diagnosis of diseases as well as improving prognostic processes and even developing novel therapies.[9]

PGE2 exerts its biological actions via specific PGE receptors on target cells, and 4 distinct subtypes of PGE receptors-designated EP1, EP2, EP3, and EP4—have been identified and cloned, each with unique signal transduction mechanisms as a result of coupling to different G proteins.[10,11] EP4 receptor cDNA encodes a 488-amino acid polypeptide with a predicted molecular mass of ~53 kDa. The EP4 receptor couples to a Gs-type G protein leading to stimulation of adenylatecyclase and increased intracellular cyclic AMP levels.[12]

Ep4 (PTGER4) mediates inhibition of IL-12 production by PGE2 in human monocytes stimulated with polysaccharides from *Actinobacil lusactinomycetemcomitans* and IFN-γ.[13] It has been demonstrated that EP4 receptors are involved in the regulation of TNFα production in monocytes and intercellular adhesion molecule-1 expression in human gingival fibroblasts.[14,15] Activation of EP4 receptors may regulate inflammatory and immunological responses in periodontal lesions.[13] EP4 receptors mediates downregulation of IL-1α induced IL-6 production by PGE2 in human periodontal ligament cells and downregulates IL-1β-elicited IL-6 synthesis through cAMP-dependent pathways in human gingival fibroblasts.[16,17] EP4 receptors have shown to activate osteoblast directly and osteoclast indirectly via osteoblast activation in human osteoblast cell lines.[18] EP4 receptors mediates suppression of both Th1 (T helper type 1) - and Th2-polarized antigen-specific human T-cell responses and EP4 receptor enhances BCR (B cell receptor)-induced apoptosis of immature B cells.[19,20]

Recent studies have shown the association of PGE2 EP4 single nucleotide polymorphism in many diseases like aspirin-intolerant asthma.[21] Expression and function of EP4 receptors on target cells by the action of PGE2 may play critical role in controlling inflammatory periodontal conditions. In the present study, we evaluated the possible association of single nucleotide polymorphism of PGE2 EP4 receptor gene with susceptibility and severity of chronic and aggressive periodontitis.

MATERIALS AND METHODS

Subject population

The present study employed a case-control design involving 105 subjects visiting the outpatient section of the Department of Periodontics, Rajarajeswari Dental College and Hospital, Bangalore, India. The total of 105 subjects were divided into 3 groups, composed of 35 subjects each were grouped as follows: Group I- 35 Healthy controls, Group II- 35 Chronic periodontitis patients, Group III- 35 Generalized aggressive periodontitis patients.

A clinical diagnosis of chronic periodontitis and generalized aggressive periodontitis was based on criteria established in 1999 at the international workshop for a classification of periodontal diseases and conditions. Chronic periodontitis patients were defined as those with periodontal pockets greater than 5 mm and clinical attachment loss greater than 3mm with moderate to severe bone loss and generalized aggressive periodontitis patients with periodontal pockets greater than 5 mm and generalized proximal attachment loss affecting at least three teeth other than first molars and incisors. All patients were systemically healthy and had not received periodontal treatment or antibiotics for at least 6 months before the clinical examination and sampling. Other exclusion criteria were smokers, patients with history

of systemic diseases, chronic use of anti-inflammatory drugs, bleeding disorders or patients on immunosuppressive agents, pregnancy and lactation. Clinical parameters included were gingival index (GI), pocket probing depth (PPD) and clinical attachment loss (CAL).

The research protocol was approved by the ethical review board of the Rajarajeswari Dental College and Hospital, Bangalore, India, and the subjects who satisfied the inclusion criteria of the study were selected. An informed consent was signed from each subject after explaining the study design.

Preparation of Template DNA for Polymerase Chain Reaction (PCR)

A total of 2ml venous blood was collected from each subject by venipuncture from the antecubital fossa and transferred to aliquots containing EDTA and sent to a laboratory for PCR analysis of single nucleotide polymorphism of prostaglandin E2 EP4 receptor gene.

Genomic DNA was extracted from whole blood in the following stages: At first approximately 100 µl of the blood sample was mixed with Tris-EDTA (TE) buffer incubated for 5 min and then washed repeatedly with the same buffer to obtain cell sediment. Then, 400 µl of lysis buffer I (4 M guanidiniumthiocyanate, 0.5% N-lauroylsarcosine, 1 mMdithiothreitol, 25 mM sodium citrate, and 40 µg of glycogen/tube) was added, incubated for 5 minutes, and centrifuged again. The supernatant was discarded and lysis buffer II (Tris-HCL, Nonidet-P40, Tween 20, and freshly prepared proteinase K 100 µg/ml) was added to the sediment. The tubes were kept at 65°C in a water bath for 2 h, followed by boiling for 10 min. The tubes were cold-stored at -20°C until processed by the PCR technique. DNA amplification was done using PCR.

PCR Amplification

PCR was performed with following primer sequences. Forward primer:

5'-CCTCCTGAGAAAGACAGTGCT-3', Reverse primer, 5'-AAGACACTCTCTGAGTCCT-3'. PCR reaction was performed in 25 mM of each primer, 2.5mM of each dNTP and 2.5 units of Taq DNA polymerase in an automated DNA thermal cycler. The PCR amplification was performed with a Perkin-Elmer Gene Amp PCR system 9600, which consisted of 35 cycles of denaturation at 97°C for 1 min, annealing at 60°C for 1min and extension at 72°C for 3min.

Confirmation of PCR products with restriction enzyme

The PCR products were digested with restriction enzyme Ava II. To the 10 µl of PCR reaction mixture, 18 µl of nuclease-free water, 2 µl of buffer R, and 1 µl of enzyme Ava IIwas added. Then the mixture was gently mixed and spun down for a few seconds. The mixture was then incubated at 37°C for 16 hours and thermal inactivation was performed. Ava IIenzyme was deactivated by heating at 65°C for 10min. Enzyme cuts between GG sequences. After this the products were resolved by electrophoresis on 2% agarose gel and stained with ethidium bromide and was visualized in an ultraviolet transilluminator.

Statistical analysis

The analysis of variance (ANOVA) and chi-square test was used to analyze the genotype distribution among chronic, aggressive periodontitis patients and healthy controls. Odds ratios were used to measure the strength of the associations. A p value of <0.001 was considered significant. The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate tables.

RESULTS

This present case-control study was carried out on randomly selected 105 subjects, divided into 3 groups. Group I included 35 subjects with healthy periodontium (mean age 24.60±3.07, 19 males and 16 females), Group II consisted of 35 chronic periodontitis patients (mean age 39.57±9.72, 19 males and 16 females) and Group III consisted of 35 aggressive periodontitis patients (mean age 29.89±4.59, 24 males and 11 females) in the age range of 19-60 years. The prevalence of the single nucleotide polymorphism of prostaglandin E2 EP4 receptor gene was compared.

The subjects with healthy periodontium genotype distribution were as follows (Table 1): GG in 9 (25.7%), CC in 8 (22.9%) and GC in 18 (51.4%) subjects. The genotypes in the chronic periodontitis patients were as follows: GG in 2 (5.7%), CC in 12 (34.3%) and GC in 21 (60%) patients. The genotypes in the aggressive periodontitis patients were as follows: GG in 5 (14.3%), CC in 24 (68.6%) and GC in 6 (17.1%) patients. The differences in the distribution of the PGE2 EP4 receptor genotypes between healthy controls and periodontitis patients was statistically significant (p=0.001).

Table 1 Distribution of Genotypes in three groups of study subjects

Genotype	Group I	Group II	Group III	Significance		
				Group I-II	Group I-III	Group II-III
GG	9(25.7%)	2(5.7%)	5(14.3%)	χ^2=5.490 P=0.064+	χ^2=15.100 P=0.001**	χ^2=13.600 P=0.001**
CC	8(22.9%)	12(34.3%)	24(68.6%)			
GC	18(51.4%)	21(60%)	6(17.1%)			
Total	35(100%)	35(100%)	35(100%)			

Chi-square test

The genotypes distribution by gender is listed in Table 2. Out of 62 men, 10 (16.1 %) had GG genotype, 28 (45.2 %) had CC, and 24 (38.7%) had GC. Among the 43 women, 6 (13.9%) had the GG genotype, 16 (37.2 %) had CC, and 21(48.8 %) had GC. There was no statistically significant difference between gender (p=0.586). The correlation between the individual genotype (GG, CC, GC) and clinical parameters (GI, PPD, CAL) among three study groups were as follows (Table 3) and the difference among the three groups was statistically significant (p<0.001).

Table 2 Distribution of study subjects according to genotypes and gender

Genotype	Total		Male		Female	
	No	%	No	%	No	%
GG	16	15.2	10	16.1	6	13.9
CC	44	41.9	28	45.2	16	37.2
GC	45	42.8	24	38.7	21	48.8
Total	105	100.0	62	100.0	43	100.0
Inference	χ^2=1.07; P=0.586					

Table 3 Correlation of Genotypes with respect to clinical parameters (GI, PPD and CAL) in three groups studied

	Group I	Group II	Group III	P value
Gingival Index				
• GG	0.50±0.17	2.11±0.43	1.70±0.34	<0.001**
• CC	0.46±0.21	2.06±0.12	1.78±0.32	<0.001**
• GC	0.45±0.19	2.19±0.31	1.88±0.29	<0.001**
• P value	0.814	0.411	0.657	-
Pocket probing depth				
• GG	1.33±0.50	6.00±0.00	8.40±1.34	<0.001**
• CC	1.25±0.46	7.25±1.36	8.88±1.26	<0.001**
• GC	1.00±0.34	7.71±1.31	8.83±1.60	<0.001**
• P value	0.118	0.181	0.768	-
Clinical attachment loss				
• GG	0.0	6.00±1.41	8.20±2.05	<0.001**
• CC	0.0	7.17±1.75	7.92±1.21	<0.001**
• GC	0.0	6.81±1.44	8.33±1.86	<0.001**
• P value	NS	0.583	0.791	-

ANOVA test

DISCUSSION

In recent years, Interleukin-1, IL-4, IL-6 and immunoglobulin G Fc receptor gene polymorphism have been associated with increased risk of chronic and aggressive periodontitis.[22-25] There are currently no data to indicate that variations in the genes for PGE2 or its receptors

influence the severity of periodontitis.[26] In this regard, this is the first study being conducted to assess the prevalence of single nucleotide polymorphism of prostaglandin E2 EP4 receptor gene in aggressive and chronic periodontitis patients to the best of our knowledge.

In our study, the overall genotype distribution was significantly different between groups. The results showed that the CC genotype was more frequent in the periodontitis group and that the GG genotype was more frequent in controls. Patients with chronic periodontitis had higher prevalence of GC (60%) than did controls (51.4%) and aggressive periodontitis patients had higher prevalence of CC (68.6%) homozygosity than chronic periodontitis (34.3%) patients, suggesting that the GC genotype plays a role in the susceptibility to chronic periodontitis and CC genotype plays a role in the susceptibility to aggressive periodontitis. In the present study, the GG genotype was more prevalent in healthy subjects (25.7%) than in chronic (5.7%)) and aggressive periodontitis patients (14.3%)), indicating that this genotype might reduce susceptibility to periodontitis, which explains its protective function. It was observed that GC genotype distriution was marginally higher in chronic periodontitis group than in controls; hence it can be contemplated that PGE2 EP4 receptor genotype may predispose to periodontal disease through stimulation of an excessive inflammatory cascade, which increase the risk for tissue destruction. This result may suggest development of periodontitis in an individual depends on the collective presence of number of environmental risk factors in conjunction with genetic risk factors at a given time point.

In the present study, significant results were observed for aggressive periodontitis group with the distribution of CC genotype (68%); hence it can be contemplated that EP4 receptor CC genotype may predispose to aggressive periodontitis through increased production of prostaglandin E2. It can be assumed that PGE2 EP4 receptor polymorphism can lead to dysregulation of action of EP4 receptor which may in turn result in dysregulation of action of PGE2 leading to periodontal destruction. PGE2 release from monocytes from patients with severe or aggressive periodontitis is greater than that from patients with little to no periodontal destruction. It has been postulated that high-risk periodontal patients have a "monocyte hypersecretory trait" that results in an exaggerated response both locally and systemically to bacterial lipopolysaccharide (LPS).The presence of elevated levels of A. actinomycetemcomitans, evidence of phagocyte abnormalities and hyper-responsive monocytes/macrophages leading to elevations in PGE2 and IL-1β in response to LPS. This hyper-responsive phenotype could be a result of genotype polymorphism that can increase connective tissue or bone loss due to excessive production of these catabolic factors.[27] Kim et al reported the potential association between prostanoid receptor gene polymorphisms and the aspirin-intolerant asthma phenotype.[21]

The correlation between the individual genotype (GG, CC, GC) and gingival index (GI), pocket probing depth (PPD) and clinical attachment loss (CAL) among three study groups was done using ANOVA test which showed statistically significant difference ($p<0.001$). The overall result of the study suggest that subjects with PGE2 EP4 receptor genotype CC and GC (homozygous/ heteroz ygous) have more severe destruction and are more susceptible to periodontitis as compared to subjects having GG genotype.

Although reports on the genetic polymorphisms associated with periodontal disease are increasing, one can summarize that there is a large inconsistency on particular polymorphisms in different studies. If the prevalence of this polymorphism varies in different populations, the association with susceptibility to periodontitis might also vary. This would lead to discrepancies in genetic polymorphism studies conducted on periodontitis patients of different ethnicities and diverse genetic backgrounds. Apart from racial differences, the differences might occur due to a number of confounding factors, including clinical diagnosis, environmental variables, biologic plausibility, penetrance, and logic of association studies.[28]

CONCLUSION

The present study results concluded that the PGE2 EP4 receptor GC and CC genotype is more prevalent in chronic and aggressive periodontitis patients respectively than in healthy controls and that the reverse is true for the GG genotype. This suggests that the GC and CC genotype of PGE2 EP4 receptor genetic polymorphism increases the susceptibility to chronic and aggressive periodontitis respectively and that the GG genotype protects against the susceptibility to periodontitis. The study of pro-inflammatory mediator PGE2 EP4 receptor gene polymorphisms may contribute to an understanding of the host mediated interactions that determine the disease phenotype. Future studies conducted on large sample size involving the functional polymorphism of PGE2 receptors would enlighten us more regarding the role of PGE2 in susceptibility to periodontitis.

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Conflicts of interest

There are no conflicts of interest

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