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Original Article

Plasminogen activator inhibitor-1 polymorphisms as a risk factor for chronic periodontitis in North Indian population

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ABSTRACT

Objectives: Impaired plasminogen activator inhibitor-1 (PAI-1), controlling coagulation and the fibrinolytic system is supposed to be involved in the pathogenesis of periodontitis. This study was performed to examine the association of PAI-1 gene polymorphisms with Chronic Periodontitis (CP) and alveolar bone loss severity involved with the disease and for understanding the role of genetic contributions in disease progression.

Methods: 87 volunteers were included in the study. Genomic DNA was isolated from peripheral blood, subsequently, DNA samples were subjected to polymerase chain reaction and endonuclease digestion. Direct gene sequencing were performed for all the samples to identify genotype polymorphisms (rs 11560324) in the 3' untranslated region of PAI-1 gene. For bone loss assessment full mouth IOPA was taken.

Results: Statistical analysis showed that for SNP PAI-I in 3' UTR, genotype CC (homozygous mutant) and allele C (mutant) has a risk associated with CP, although statistically significant association was not found. An increased risk of association of disease severity with CG (heterozygous mutant) and CC (homozygous mutant) genotypes, i.e., an increased carriage rate of genotype CG and CC (homozygous mutant) was evident with the increase in the severity of CP, highlighting an increased susceptibility to CP due to this gene polymorphisms.

Conclusion: PAI-1 genotype has a risk association with CP and alveolar bone loss severity in North-Indian population.

1. Introduction

Chronic periodontitis is a complex multifactorial disease which affects the periodontium and results in its destruction if timely intervention is not done. Though periodontal pathogenic bacteria are an integral part of the pathogenesis, the knowledge of involved host-bacterial interaction is equally important. It is now understood that genetic variation, along with other environmental factors (stress, smoking)[1] are involved in the aetiology and progression of the disease.

When the association between periodontal and systemic disease is considered, PAI-1 (Plasminogen Activator Inhibitor- 1) gene polymorphisms can further provide the necessary evidence. It is reported that Plasminogen Activators (PA) involved in plasmin activation which plays a vital role in the vascular events and tissue remodeling are also associated with the pathogenesis of the periodontal disease.[2–4] Any alteration in the coagulation and fibrinolytic system also seems to be involved in the progression of the disease (e.g. Cardiovascular disease).[5] Increased plasmin and PA activity have been observed in inflamed gingival and periodontal tissues.[6,7] Presence of PAI-1 m-RNA in blood vessels of CT of pathogenic periodontium indicates its role in vascular remodeling in the disease progression.[8] A Hind-III restriction fragment length polymorphism (RFLP) in the 3' end of PAI-1 gene is reported to be associated with the disease with vascular component.[9–13] Studies have reported a 4 G/5G promoter polymorphism of PAI-1 gene to be linked with a disease like Chronic Heart Disease,[14–16] meningococcal septic shock[17] and periodontal disease.[18]

The study was performed to explore the relation between Single Nucleotide Polymorphisms (SNPs) of PAI-1 gene (rs 11560324) with chronic periodontitis and alveolar bone loss severity involved in chronic periodontitis in North Indian population.

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2. Material and methods

2.1. Volunteer selection and study design

A total of 87 volunteers were recruited for this case-control study from the outpatient wing of Department of Periodontics at Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow, Uttar Pradesh, India. Candidates over the age of 35 years were included in the present study who were able to donate blood as it is seen that genetic predisposition of adults can be better evaluated in older patients. Moreover, it is reported that chronic periodontitis is the most commonly occurring form of periodontitis affecting adults,[19] which becomes clinically more significant after the age of 30.[20] The study protocol included a customized proforma for systematic recording of the observations and information. It included a detailed case history record for clinical examination and periodontal charting and a consent form. Volunteers with any history of systemic disease, on the antibiotic regime in the last 3 months before the study, pregnant and lactating females, females on hormone replacement therapy or post-menopausal females, tobacco chewers and smokers, and volunteers with T score < -1.0 in Bone Mass Index were excepted from this study. The periodontal examination was done via single examiner with the help of UNC-15 probe. Gingival index,[21] plaque index,[22] clinical attachment level, and probing pocket depth were measured for the record of clinical periodontal parameters. The volunteers diagnosis for examine clinical criteria was based on 1999 International Workshop for Classification of Periodontal Diseases and Conditions.[19]

The volunteers were categorized into two groups. Control Group (Group I) that included 45 periodontally healthy volunteers (34 Male and 11 Female) with at least 20 natural teeth present without periodontal disease, and with no evidence of radio-graphical bone loss (relative to the distance from the apex to a point 2 m m apical to the CEJ). However, test Group (Group II) which included 42 volunteers (31 male and 11 female) suffering from chronic periodontitis with at least 20 natural teeth present with periodontal disease. They were further divided into 3 sub-groups. Group IIA: Mild bone loss i.e., one or more sites with < 25% crestal bone loss, Group IIB: Moderate i.e, at least one site demonstrated 25–50% crestal bone loss, with no teeth with severe bone loss and Group IIC: Severe bone loss i.e, at least one site with > 50% crestal bone loss.

2.2. Determination of alveolar bone loss

Alveolar bone loss (ABL) was confirmed from full mouth IOPA radiographs. Radiographs were obtained by paralleling radiographics technique (long-cone technique/right-angle technique) as described previously[23] using commercially available film holder. Intraoral dental films size two (E speed, Eastman Kodak Co., Rochester, NY, USA) were exposed to an X-ray source (Satellec X-Minf AC, 70 kV, 8 mA, Birmingham, UK) for 0.5 s. All the study radiographs were digitalized. X-rays films were scanned using Scanner HP Scanjet 2400 series; the scanned images were then analyzed AutoCAD-2007 software (Fig. 1).

2.3. Sample collection and selection of SNP

Blood samples were collected from Babu Banarasi Das College of Dental Sciences. Samples were collected in EDTA vials and immediately stored in 40 C until processed for DNA isolation. The SNP's selected for the present study is PAI-I (rs11560324) was based on their role in susceptibility to chronic periodontitis.[24,25]

2.4. Genotype identification

Genomic DNA was isolated from blood samples using DNA isolation kit (Qiagen, cat no 51106) according to the manufacturer's protocol. Isolated DNA was undergone for PCR amplification with 10 pmol each

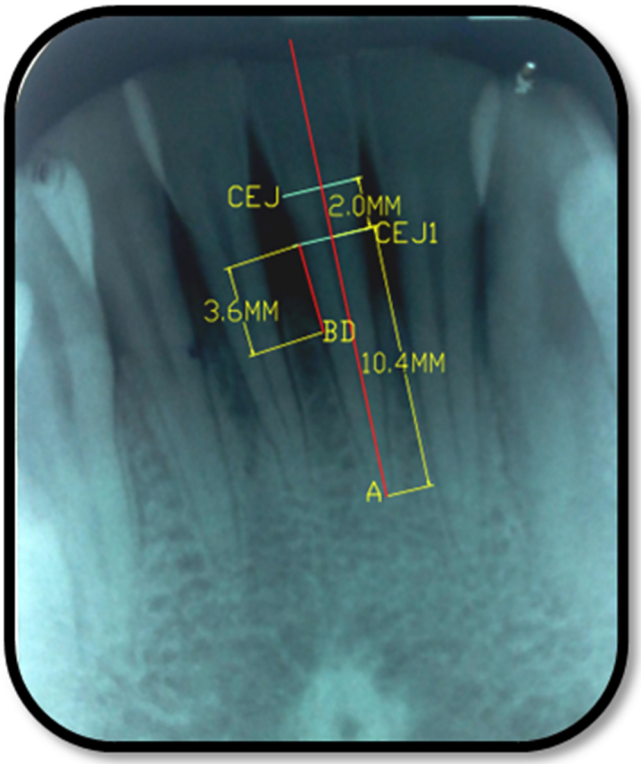

Fig. 1. Measurement of bone loss with AutoCAD software.
Note: CEJ: Cemento Enamel Junction; CEJ_1: 2 mm apical to the CEJ; BD: most coronal point where the periodontal ligament space showed a continuous width; A: apex of the tooth.

Table 1
The Primer Sequences used to amplify each marker in Polymerase chain reaction.

Single nucleotide polymorphism	PRIMER SEQUENCE	SIZE OF PCR PRODUCT
PAI-I near 3′UTR (C-G)	FP:5'GCCTCCAGCTACCGTTATTGTACA3' RP:5'CAGCCTAAACAACAGAGACCCCC3′.	755

FP: Forward primer, RP: Reverse primer, bp: Base Pair.

of forward and reverse primer of PAI-1 (Hind-III) polymorphism (Table 1). For PCR, thermal cycler (MJ Research PTC-100 Thermal Cycler, Watertown, MA, USA) was used; Taq DNA & 10X buffer were purchased from Sigma Aldrich, dNTP from Fermentas life sciences and Primers were procured from Integrated DNA Technologies. PCR products were digested with restriction endonucleases Hind III and the genotypes were determined from ethidium bromide-stained gels under ultraviolet light (Table 2). Direct DNA sequencing of the PCR products was performed using PCR clean up Gel extraction kit (Qiagen) based on silica membrane technology and ABI3500 automated DNA Sequencer (Applied Biosystems, Foster City, CA) to obtain sequence-specific chromatograms. Furthermore, DNA sequencing was also carried out for the detection of novel SNPs and validation of the results obtained through restriction fragment length polymorphism analysis.

2.5. Statistical analysis

The frequency (count) data of genotypes and alleles among groups (periodontal volunteers and controls) were analyzed for SNP's of PAI-I near 3′ UTR. Analysis and graphs were done on Graph Pad Prism (version 3.0). Data were analyzed as Mean ± SD and percentage. The Chi-square test was used to compare categorical variables. The

Table 2
Details of Restriction enzyme used and length of fragments generated upon Restriction digestion.

Single nucleotide polymorphism	Restriction Enzyme	Restriction fragment lengths of genotypes		
		Homozygous Wild type	Heterozygous Mutant	Homozygous Mutant
PAI-I near 3′ UTR (C-G)	Hind III	GG 755 bp	CG 755 + 567 + 188 bp	CC 567 + 188 bp

Table 3
Age distribution of (n = 87) Group I and II.

Subjects	N	Minimum	Maximum	Mean	Std. Deviation	P Value
Group I	45	35	50	43.95	4.43	> 0.05*
Group II	42	37	50	44.85	4.47	

variables between Group I and Group II were compared by Mann-Whitney U test and Student t-test was used. The p-value less than 0.05 were considered as significant. All the analysis were carried using SPSS (19.0 version).

3. Results

The mean age for chronic periodontitis volunteers was (± SD) 44.85 (± 4.47) and 43.95 (± 4.43) years for control volunteers. No statistically significant differences were found in age distribution between the study groups (Table 3). Genotype and allelic distribution of PAI-1 11560324 polymorphisms in the control and chronic periodontitis groups (Table 4 and 5). The odds ratio for Heterozygous (CG) and Homozygous Genotype (CC) increases over wild-type (GG) with the introduction of the mutant allele (C) (0.95 and 2 respectively). In case of homozygous genotype, the odds ratio is 2 and for the combined mutant group, i.e., Heterozygous and Homozygous Genotype it is 1.91, which shows clearly that Homozygous and Combined Mutant genotype has a risk associated with chronic periodontitis. Also, mutant allele has higher odds ratio over wild allele (1.87), and the association is significant with chronic periodontitis (X2 = 4.1).

In the test group, 23.8% had a mild bone loss (Group IIA), 28.6% with moderate (Group IIB) and 47.6% with the severe bone loss (Group IIC). The overall frequency distribution of genotypes and alleles among Group II-A, IIB and IIC is summarized in Table 6. The odds ratio for having severe periodontal disease increases within each genotype group. For Wild-type group, it is 1.8 over the mild group. For the Heterozygous group, it increases from 2.42 to 6.92 which is significant. For the Homozygous group, it increases from 3.33 to 6.0. When all mutant groups are taken as a whole, it increases from 2.77 to 6.52 which is significant. It shows that the increase in the severity of chronic periodontitis is significantly associated with Homozygous and Heterozygous genotype. However, on comparing the genotype frequency showed no significant difference (p > 0.05).

Table 5
Allelic Distribution in Group I and II.

Allele Type	Allele	Group I	Group II	Odds Ratio*	X^2 Value	p Value
Wild Type	G	50 (55.5%)	32 (39%)			
Mutant	C	40 (44.4%)	48(58.5%)	**1.87 (1.01- 3.45)	4.1	***0.04

Table 6
Tabulation of Group IIA, IIB and IIC in each genotype group.

Types of Genotype	Genotype	Test (n = 42)	Odds Ratio	
Wild Type	GG (n = 8)	Group IIA	5 (62.5%)	–
		Group IIB	1 (12.5%)	–
		Group IIC	2 (25%)	–
Heterozygous Mutant	CG (n = 20)	Group IIA	3 (15%)	**
		Group IIB	6 (30%)	2.42 (0.51 -11.51)
		Group IIC	11 (55%)	*6.92 (1.5-31.3)
Homozygous Mutant	CC (n = 14)	Group IIA	2 (14.2%)	**
		Group IIB	5 (35.7%)	3.3 (0.52-21.2)
		Group IIC	7 (50%)	6 (0.96-37.2)
Combined Mutants	CC + CG (n = 34)	Group IIA	5 (14.7%)	**
		Group IIB	11 (32.3%)	2.77 (0.84-9.12)
		Group IIC	18 (52.9%)	*6.52 (2.03-20.89)

4. Discussion

In the PAI-1 gene (chromosome 7 at 7q21.3-q22; gene accession no AC004876; gene ID 5054), polymorphism present either single or both the alleles which lies outside of the protein coding region (bases 23 159–34 338) within the 3' untranslated region (UTR) (bases 34 339–126 462), and identified as the presence of Hind III restriction site.[26] All the volunteers included in the study were strictly from North Indian subpopulation to maintain homogeneity of the ethnic group.[27]. In the present study, although no statistically significant association was found between PAI-1 11560324 G > C and chronic periodontitis (Table 3), however, the trend is noteworthy. In a similar study, a significant association between polymorphisms in PAI-1 gene and chronic periodontitis in Czech population was reported by Holla et al[18] whereas Gurkan et al. found no significant association of PAI-1 genotype and chronic periodontitis in Turkish population.[4] Here, we observed the presence of mutant (C) allele increased the risk, and severity of periodontitis. A similar result was reported by DeCarlo et al. in 2007.[26]

At present, the exact mechanism of action of this polymorphism cannot be explained as it is present in 3'UTR. However, it is believed that within the coding region of PAI-1 gene, several non-synonymous coding SNPs are closely associated to Hind III in 3'UTR, which may be

Table 4
Genotypic Distribution in Group I and II.

Type of Genotype	Genotype	Group I (n = 45)	Group II (n = 42)	Odds Ratio*	X^2 Value	p Value
Wild Type	GG	14(31.1%)	8(19.1%)	0.521	1.67	0.19
Heterozygous Mutant	CG	22(48.8%)	20(47.6%)	0.95(0.40- 2.2)	0.01	0.92
Homozygous Mutant	CC	9 (20%)	14 (33.3%)	2(0.75-5.2)	1.99	0.15
Combined Mutants	CG + CC	31(68.9%)	34 (80.1%)	1.91 (0.70 - 5.1)	1.67	0.19

linked with primary sequence changes. This can be a probable reason for the clinical relationship with the polymorphisms. It is reported that activation of coagulation and inflammation are related and there is a single pathway for both coagulation and inflammation in primitive species.[28]

Micro-vascular age changes in gingiva present as intimal thickening of arteries and arterioles and accumulation of peri-luminal extracellular matrix accumulation (ECM) to periodontal disease.[29–32] To attain homeostasis, the balance between mechanism which promotes and inhibits ECM degradation is necessary, which is further dependent on PA and PAI for its activation.[2,3] Hence, it can be said that PA and PAI are as important for the pathogenesis of periodontal disease as they are essential for other vascular component related diseases like coronary artery disease and diabetes.[26] It has been reported that growth factors and pro-inflammatory cytokines (e.g. IL-1, TNF, TGF, TLR4 gene)[33,34] and other pluripotent factors are involved in inflammatory reactions (Endothelium ACE).[35] The PAI-1 genotype identified here may have a role in biological changes that can lead to severe chronic periodontitis.

Identification of specific genetic markers will help in early detection of highly susceptible individuals to periodontal disease and may help in the development of pathology based treatment. To the best of our knowledge, this is the first report on the association of SNP of PAI-1 gene with chronic periodontitis and severity of alveolar bone loss associated with the disease in North Indian population. However, further studies with large sample size are required to understand the role of PAI-1 gene polymorphisms in periodontal disease in the different population.

Ethical approval

The study conformed to the ethical guidelines of the Helsinki Declaration and was evaluated and approved by the Institutional Ethical Committee. A written informed consent was obtained from all participants of the study.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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