Original Article

Association of SRXN1 Receptor Gene Polymorphism with Susceptibility to Periodontitis

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Abstract

Background: Emerging evidence suggests that oxidative stress forms a key component in the etiopathogenesis of periodontitis. Literature evidence have shown potential antioxidants responsible for combating the pro-oxidants which stress the periodontium, but the peroxiredoxin-sulfiredoxin system is explored very minimally in periodontal disease. Thus, the present study was aimed to evaluate the genetic association of SRXN1 receptor gene polymorphism (rs6053666). **Materials and Methods:** A total of 100 subjects were recruited for this study, which included 50 Periodontitis patients (Stage II and above based on the criteria of American Association of Periodontology-2018) and 50 periodontally healthy or mild gingivitis. Genomic DNA was extracted from the whole blood collected from the subjects. DNA was amplified using specific primers flanking the BtgI region of the SRXN1 receptor gene. The amplicon was further subjected to genotyping using restriction fragment length using BtgI enzyme. The genotype obtained based on the restriction fragment length polymorphism pattern was recorded and used for statistical analysis. The distribution of genotypes and allele frequencies in the periodontitis and control groups were compared using the Chi-square test. The risk associated with individual alleles or genotypes was calculated as the odds ratio with 95% confidence intervals. Statistical significance in all tests was determined at $P < 0.05$. **Results:** The genotype frequency and distributions of SRXN1 receptor BtgI polymorphism did not differ significantly at $\chi 2df$ ($P = 0.557$). Our study results showed that homozygous and heterozygous mutant genotypes had no significant difference (CC vs. CT + TT) between the periodontitis patients and control group with a $P = 0.4266$. The detected frequency of CT (38% vs. 34%) and TT (42% vs. 52%) genotype showed no significant difference between control and test group. There was no significant difference in C allele (39% vs. 31%) and T allele (61% vs. 69%) between the test and control group. **Conclusion:** The present study denotes that SRXN1 receptor gene polymorphism is not associated with periodontitis in the study group analyzed.

Keywords: *Alleles, periodontitis, polymorphism, SRXN1 receptor*

Introduction

Periodontitis is a chronic inflammatory disease, influenced by multiple factors that caused destruction of the supporting tissues and resulted in tooth loss.[1-4] There are various risk factors of periodontitis like diabetes mellitus, smoking which are modifiable risk factors. Whereas Genetics is a nonmodifiable risk factor risk factor which plays a role in determining the host susceptibility to periodontal destruction.[5] A number of single-nucleotide polymorphisms (SNP) such as interleukin (IL) receptors, Vitamin D receptor, matrix metalloproteinase receptors are determinants in disease susceptibility of genetically complex disease such as chronic periodontitis.[6-9]

Oxidative stress is an important cause of cell damage associated with initiation and progression of many chronic diseases.[10-12] Oxidative stress is the distribution in the pro-oxidant and antioxidant balance, in favor of the former resulting in potential tissue damage. All the cells have intrinsic storage of antioxidant molecules in order to overcome the oxidative stress.[13] Oxidation-reduction reactions of molecular oxygen by various enzymes results in the production of molecules such as superoxide anion, hydrogen peroxide, hydroxyl radicals, singlet oxygen, nitric oxide, hypochlorous acid which together are termed as "reactive oxygen species"(ROS).[13] In the human body all the cells are capable of generating ROS, of which polymorphonuclear neutrophils are of prime importance in relation to periodontitis.[14] Neutrophils are the first line of defense and are located

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at the sites of microbial invasion which are activated by inflammatory mediators and can generate increased levels of ROS, which not only attack the periodontopathogenic but also the surrounding tissues.[15] ROS is usually generated by nicotinamide adenine dinucleotide phosphate oxidase (Nox) system present in neutrophils which catalyzes the reduction of molecular oxygen to superoxide anion.[16] Many studies have shown that ROS regulates the formation and function of osteoclasts i.e., the activation and bone resorption ability.[17-19] Bone resorption which results in alveolar bone loss and ultimately tooth loss is the hallmark of periodontal disease.

Hydrogen peroxide is one among the main ROS present in the body. It takes part as a signaling molecule in the regulation of a variety of biological processes. It also acts as a double-edged sword due to its accumulation which can result in cell apoptosis and tissue damage. A cell culture study where human periodontal periodontal ligament cells (PDLC's) were procured from extracted premolars was exposed to hydrogen peroxide in a dose dependent manner and it showed hydrogen peroxide induced apoptosis of PDLC's in a dose and time dependent manner.[20] The antioxidants in action against hydrogen peroxide are catalase, peroxiredoxin, thioredoxin, glutathione peroxidase and they have grouped as "peroxidases."[21,22]

Scavenging of hydrogen peroxide is largely mediated by the peroxiredoxin-sulfiredoxin system of antioxidants.[23] The activation of peroxiredoxins is dependent on sulfiredoxin. In a cross sectional study, the expression of peroxiredoxins I, II was found to be up-regulated in the gingival biopsies of Type-2 diabetic patients.[24] Still dated there is a controversy regarding the expression/amount of antioxidants and the periodontal status. There has been contradicting evidence where some studies suggest that periodontitis is related to a decreased expression of antioxidant defenses,[25,26] whilst few other studies have suggested that the antioxidant defences are upregulated in periodontal disease.[27,28]

Expression of sulfiredoxin has been observed in macrophages, neurons, hepatocytes, alveolar cells and in tumor cells.[29-33] It has been implicated in numerous oxidative stress induced conditions such as atherosclerosis,[34] chronic obstructive pulmonary disease,[31] and in a wide range of carcinomas involving the various organs of the body.[35-37] In the various studies, sulfiredoxin has been projected from being an antioxidant to a prognostic marker and a potential chemotherapeutic agent.[38] A recent cross-sectional study has shown that sulfiredoxin levels are highly expressed in the gingival tissues of chronic periodontitis.[39] Thus the present study was first of its type to evaluate the association of SRXN1 receptor gene SNP rs6053666 in periodontitis patients.

Materials and Methods

The study complies with Declaration of Helsinki. A total of 100 individuals who reported to the Department of Periodontics were included in this cross sectional study. The sample size was calculated based on the previous study by Kaarthikeyan *et al*.[40] based on which sample size of 100 was derived keeping the power of the study as 80%. The subjects were divided into a Periodontitis group and a control group based on the clinical examination of probing pocket depth, clinical attachment loss and bleeding on probing. The periodontitis group contained 50 patients (male-26; female-24) and the control group had 50 subjects (male-26; female-24). The Periodontitis patients were diagnosed based on the criteria of American Association of Periodontology (AAP)-2018.[41]

Inclusion criteria

Control group

Patients who are systemically and periodontally healthy or mild gingivitis (gingival index <1).

Test group

Patients who are systemically healthy, diagnosed as Stage II periodontitis or above based on the criteria of American AAP-2018.

Exclusion criteria

Smokers, pregnant or lactating mothers, immunocompromised individuals, subjects who underwent periodontal therapy within the past 6 months were excluded from this study.

The ethical clearance was obtained from the Institutional Review Board of Saveetha Dental College and Hospitals, Saveetha University IHEC/SDC/PERIO-1801/21/63 and written informed consent was obtained from all the patients who participated in the study.

Sample collection

A volume of 2 ml of venous blood was collected from antecubital fossa and dispersed into a sterile tube containing a pinch of ethylene diamine tetra acetic acid. It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified Miller *et al*. 1988 protocol.[42]

Polymerase chain reaction and restriction endonuclease digestion

SRXN1 receptor gene (*BtgI*) polymorphism (*rs6053666*) was assessed by polymerase chain reaction (PCR) amplification and digestion. The following primers, forward primer: 5'-GGATACAGCAGCCATCTTGG-3' and reverse primer: 5'-CTTGAAGAGCCACGTGCTG-3' were used for amplification of DNA spanning the *BtgI* polymorphic site, of the SRXN1 receptor gene. The amplification of DNA was performed in 20 µl volumes using 10 ng of

genomic DNA, 5 pmol/µl each of forward and reverse primers along with PCR master mix (Takara, Japan). The cycling conditions were as follows: Initial denaturation at 94°C for 5 min, denaturation at 94°C for 35 s, annealing at 60°C for 35 s, extension at 72°C for 35 s, and a final extension at 72°C for 5 min. 5 µl of PCR product was checked on a 2% agarose gel [Figure 1]. Fifteen microliter of PCR product was digested using *BtgI* restriction enzyme procured from New England Biolabs, England. Digestion was carried out at 37°C for 2 h. The digested product was visualized on a 2.5% agarose gel and the results were documented [Figure 2].

Statistical analysis

All statistical analyses were performed using the Statistical package for the Social Sciences Version 23.0 for Windows (SPSS Inc., Chicago, IL, USA). The distribution of genotypes and allele frequencies in the periodontitis and control groups were compared using the Chi-square test. The risk associated with individual alleles or genotypes was calculated as the odds ratio with 95% confidence intervals. Statistical significance in all tests was determined at $P < 0.05$.

Results

The periodontitis group contained 50 patients with the mean age of 39.02 ± 8.22 years. The control group contained 50 periodontally healthy or mild gingivitis subjects with mean age of 41.34 ± 7.49. The clinical characteristics of the subjects in periodontitis and control groups are shown in Table 1. The genotype and allele frequencies of the group are shown in Tables 2 and 3. The genotype frequency and distributions of SRXN1 receptor BtgI polymorphism did not differ significantly at χ2df ($P = 0.557$). Our study results showed that homozygous and heterozygous mutant genotypes had no significant difference (CC vs. CT + TT) between the periodontitis and control group with a $P = 0.4266$. The detected frequency of CT (38% vs. 34%) and TT (42% vs. 52%) genotype showed no significant difference between control and periodontitis group. There was no significant difference in C allele (39% vs. 31%) and T allele (61% vs. 69%) between the periodontitis and control group.

Table 1: Demographic data of periodontitis and control groups

Clinical characters	Periodontitis group	Control group
Number of subjects		
Male	26	26
Female	24	24
Total	50	50
Mean age	39.02±8.22	41.34±7.488
Clinical attachment loss	6.13±1.29	-
Probing pocket depth	5.48±1.15	1.60±0.57
Gingival index	1.74±0.22	0.76±0.16

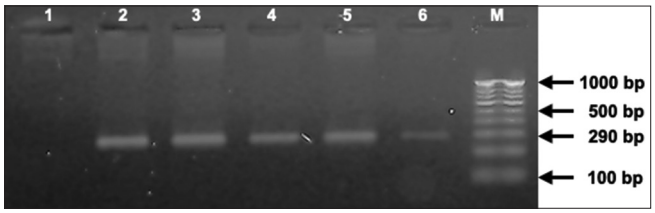

Figure 1: Agarose gel electrophoretogram of T/C polymorphism (rs6053666) of SRXN1 gene showing 290 bp amplicon in lanes 2–6 (Lanes 7 [M]: 100 bp DNA ladder), Lane 1: Negative control

Discussion

The genetic polymorphism influences susceptibility of periodontitis and there are various gene polymorphisms which are shown to play a role in periodontitis.[6-9] Experimental evidence gathered from various studies have identified genes encoding immune-regulatory and immune-modulatory molecules such as chemokines (CXCR2), cytokines (IL), surface receptors (Vitamin D receptors), antigen recognition proteins (FC gamma) etc.,[43]

Our study results showed that the genotype frequency and distributions of SRXN1 receptor BtgI polymorphism did not differ significantly at χ2df ($P = 0.557$). Our study results showed that homozygous and heterozygous mutant genotypes had no significant difference (CC vs. CT + TT) between the periodontitis and control group with a $P = 0.4266$. The detected frequency of CT (38% vs. 34%) and TT (42% vs. 52%) genotype showed no significant difference between healthy control and periodontitis group. There was no significant difference in C allele (39% vs. 31%) and T allele (61% vs. 69%) between the periodontitis and control group.

Sulfiredoxin is primarily located in the cytosol and in case of severe oxidative stress, it translocates into the mitochondria.[44] Human sulfiredoxin gene is located on chromosome 20 in the region of short arm p13 where the gene is 6632 bp in length, composed of 2 exons. The size of Srx mRNA is 2580 bp.[45] Sulfiredoxin transcript contains two exons and the catalytic domain of sulfiredoxin reducing enzyme activity is localized in the exon.[46]

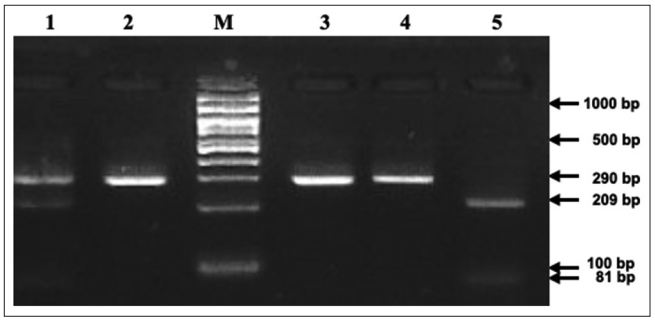

Figure 2: BtgI digestion of polymerase chain reaction amplified product (Lane 1: CT heterozygous, 2-4: TT homozygous and 5: CC-homozygous variant)

Table 2: Genotype frequencies of SRXN1 gene polymorphism (rs6053666) among the cases and controls

Groups	CC	CT	TT	C	T	HWE (*P*)*
Case (*n*=50)	7	17	26	0.31	0.69	0.146
Control (*n*=50)	10	19	21	0.39	0.61	0.154

*For departure from HWE, Chi-square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly χ^2 df (*P*=0.557). HWE: Hardy-Weinberg equilibrium

Table 3: Overall genotype distribution of the SRXN1 gene polymorphism (rs6053666) gene polymorphism in cases and controls

Genotypes	Case	Control	Unadjusted OR (95% CI)	*P*
Dominant				
CC	7	10	0.65 (0.2262-1.8748)	0.4266
CT+TT	43	40		
Recessive				
TT	26	21	1.49 (0.6793-3.2945)	0.3173
CT+CC	24	29		
Allele				
C	31	39	0.7027 (0.3919-1.2601)	0.2364
T	69	61		

OR: Odds ration; CI: Confidence interval

Previous study by Kunnas *et al*. showed that rs6053666 of SRXN1 was associated with cerebrovascular disorder.[47] Also studies have shown that the C allele of rs6053666 of SRXN1 gene was associated with a decrease in breast carcinoma.[48] Previous study done by Ramesh *et al*. has shown that sulfiredoxin were significantly increased in the gingival tissue samples of chronic periodontitis patients and this paved the way for conducting the present study to analyse the association between SRXN1 gene polymorphism and periodontitis. But the results of the present study showed no significant relation between SRXN1 gene polymorphism and periodontitis. This suggests that the increased sulfiredoxin in chronic periodontitis observed in the previous study could be due to the local oxidative imbalance due to the presence of microorganisms. It has been reported that bacterial lipopolysaccharide at the local site of inflammation induces the sulfiredoxin expression.[28,29,39] However in the present study we have evaluated only the SRXN1 receptor gene polymorphism and further genetic studies are warranted to rule out any other genetic predisposition for periodontitis with respect to sulfiredoxin gene.

Hence, studies in a large population size including various ethnic groups at multicenter are required to arrive at a statistically significant observation.

The present study was limited to genetic polymorphism in periodontitis (Stage II and III and Grade B) patients; future studies need to be done as a multicentered study, different ethnic groups in a larger study sample.

Conclusion

The present study denotes that SRXN1 gene polymorphism is not associated with periodontitis (Stage II and III and Grade B) in the study group analyzed. Further studies are required to explore the interaction of BtgI-SRXN1 receptor gene with microbial and environmental factors in the etiopathogenesis of Periodontitis and link between SRXN1 receptor gene in periodontitis patients with systemic diseases.

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Consent for publication

The patient has given valid and informed consent for publication.

Declaration of patient consent

The authors certify that we have obtained all appropriate patient consent forms. In the form the patient has given his consent for his image and other clinical information to be reported in the journal. The patient understands the name and initials will not be published and due efforts will be made to conceal the identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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