Association of Cathepsin G gene polymorphism in chronic periodontitis.

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Abstract

Periodontitis is an inflammatory disease with the underlying etiology that is unknown. Cathepsin is well known genes that play important roles in periodontitis by interacting with autophagy-associated molecules in pathogenesis. Several studies have established that the role of Cathepsin G (CTSG) gene is a bacterial serine protease stored in the neutrophil azurophilic granules and the mutation of this gene established correlation between many other diseases like osteomyelitis and alzheimer's diseases. Hence, the aim of the current study was to assess the association between Chronic Periodontitis (CP) and polymorphism of CTSG. Gingival tissue and blood samples are collected from both the control and case group and DNA isolation was done. CTSG N125S polymorphism was genotyped in 30 patients with CP and 30 healthy controls. DNA was obtained from patients with CP and controls. PCR amplification and Sequencing of Cathepsin G gene were completed and the data was analysed. The CTSG N125S genotype frequency was calculated. The percentage of occurrence of heterogeneous diseased mutation genes were found to be 1.67% and 5% respectively. The genotype frequency was found to be 0.77 in CP patients and 0.93 in controls. There was no statistically difference between CP patients and healthy controls. The study concludes that CTSG N125S polymorphism was not associated with CP. However, further studies have to be conducted with more samples to identify the correlation between chronic periodontitis and polymorphism of Cathepsin G gene.

Keywords: Chronic periodontitis, Cathepsin G gene mutation, Single nucleotide polymorphism, Genotype frequency, Inevative study.

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Introduction

Periodontitis is a most prevalent inflammatory disease that involves the periradicular tissues that can even lead to loss of ligamentous support of the tooth to the alveolar bone leading to mobile teeth and finally loss [1]. It has been reported that over 10% of the adult population are diagnosed with periodontitis while 30% of individuals above the age of 50 years are found to have severe form of periodontitis. It is a condition of periodontal tissues with swelling of gingiva, alveolar bone loss, along with tooth movement. The etiology of periodontitis is established in association with certain genetic and environmental factors [2]. Host immune response and presence of oral microorganism contributes to the onset and progression of periodontitis [3].

The pathogenesis of periodontitis is still a debate between the interplay of host gene variants and bacterial infections. Periodontitis that is caused due to bacterial infection induces the host immune response and involves the inflammatory process. The presence of bacteria can cause initiation of periodontitis, but the progression and severity of the disease are not in concordance with the type and amount of microbial species [4]. As concerned with the bacterial invasion, the production of pro-inflammatory factors like TNF-α, IL-6 and prostaglandin E2 are found to be left into the circulation and cause systemic or distant effects of the bacteria. This indeed produces the systemic cellular and molecular markers of inflammation in periodontitis. The role of gene and gene polymorphism or change in the expression of gene or in the encoded proteins ultimately results in alterations in innate and adaptive immunity that leads to the outcome of disease [5]. The Cathepsin family plays a significant role in periodontitis by interacting with autophagy–associated molecules in pathogenesis [6]. Previous research has established that increased mRNA and protein levels of Autophagy-Related Genes (ATGs) are found in peripheral blood mononuclear cells from periodontally compromised patients [7].

Cathepsin, a term derived from the Greek word 'kathepsein', that has a meaning of digest, is a protease that is functionally active in a slightly acidic environment. There are 11 human cysteine Cathepsin isoforms, referred to as B, C, F, H, K, L, O, S, V, X, and W. Cathepsins are primarily intracellular enzymes responsible for nonspecific bulk proteolysis in the endosomal/lysosomal system, which degrades both intracellular and extracellular proteins [8].

However, Cathepsins are involved in producing immune modulators by the limited proteolysis processing. Mutation of the Cathepsin gene causes various kinds of syndromes namely papillon–lefevre syndrome, chediak-higashi syndrome, down syndrome and so on.

In papillon-lefevre syndrome, Cathepsin S gene (CTSC) might influence periodontitis progression through its role in epithelial differentiation or desquamation. This syndrome is seen in association with palmoplantar keratosis, which is characterized by thickening of skin in palms of hand and soles of feet [9]. The three serine proteases of the chemotrypsin family are

Human Neutrophil Elastase (HNE), Cathepsin G and proteinase 3 are stowed in the primary granulae (azurophil) of Polymorphonuclear Neutrophils (PMNs). The activities of these three serine protease are based on a catalytic triad of certain three proteins that are parted in their primary structure but brought together in tertiary structure. They are initially identified as degenerative enzymes that are accountable for eliminating intracellular pathogens and breaking down of tissues at certain inflammatory sites and later, they are acclaimed as possible molecular targets for anti-inflammatory agents. Cathepsin G gene establishes role in development of inflammation by causing migration of neutrophils, antigen-presenting cells and monocytes by changing chemokine ligand 5 and chemokine ligand 15 into more potent chemotactic factors by proteolytic processing of CTSG and converting prochemerin into chemerin, which is a novel chemoattractant factor that specifically attracts Antigen-Presenting Cells (APC) through its receptor ChemR [10].

Cathepsin G gene is able to directly activate Protease-Activated Receptors 4 (PAR4) at the surface of platelets, which may lead to platelet secretion and aggregation, and the interaction between neutrophils and platelets at the sites of inflammation or vascular injury [11]. The adverse effects of Cathepsin G gene is that it causes breakdown of protein matrix and activates macrophages thereby stimulating the infiltration of inflammatory cells. Thus, Cathepsin G gene plays a vital role in self-propagating, chronic inflammation. This gene increases the production of antigen-specific antibodies by activating T cells in BALB/C mice. The gene holds together to lymphocytes, including CD4+, CD8+, natural killer and B cells with a thrombin-like receptor, hat augments the cytotoxicity of natural killer cells, activates reactive T cells and rise the cytokines and antigen-specific antibody production [12]. The polymorphism of Cathepsin G gene in the position of N125S delineates that the levels of CTSG is higher in osteomyelitis individuals who have CTSG gene allele when compared with AA genotype [13]. Previous articles have stated the correlation between the polymorphism of CTSG in association with the risk of alzeimer's disease. It is found that there is no association between the polymorphism of this gene with alzeimer's disease [14].

A case control study that was conducted in 2001 affirmed that Ser125 allele is significantly associated with rise in elevated plasma fibrinogen levels that are predominantly seen in allele carriers [15]. It is substantiated that ulcerative colitis, Crohn's disease and primary sclerosing cholangitis are frequently seen to be confederated with autoantibodies directed against polymorphonuclear neutrophils of Cathepsin G gene [16]. Even though Cathepsin G gene is found to have a role in inflammatory diseases, the association between chronic periodontitis and the inflammatory pathway of Cathepsin G gene is not yet evaluated. Our team has extensive knowledge and research experience that has translate into high quality publications [17-36]. Hence, the current study aims to assess the association between chronic periodontitis and polymorphism of Cathepsin G gene.

Materials and Methods

The study population is divided into two groups namely the case (CP) and control group and each group comprises 30 participants presenting for treatment at the Department of Periodontitis at Saveetha dental college and hospitals. The age group of the study population is 20-70 years old patients with chronic periodontitis in the case sample. Gingival tissue and blood samples are collected from both the case and control group for DNA isolation. DNA was obtained from patients with CP and controls tissues using the QIAamp kit. CTSG N125S polymorphism was assessed by Polymerase Chain Reaction (PCR) amplification. A 263-bp product of CTSG N125S polymorphism was amplified using the primers 5'- GCTGAGCGGGAACGCCTACA-3' and 5'-CCGGTCCCCCACACAAATCT-3'. PCR products were electrophoresed on a 2% agarose gel. PCR products were purified and directly sequenced using BigDye terminator cycle sequencing kit and 3730XL Genetic Analyzer. Data were analyzed using SPSS version 23.0 for Windows (SPSS, Chicago, IL, USA) and a p value less than 0.05 was considered significant. The odds ratio and the 95% Confidence Interval (CI) were calculated. The ethical approval of the current study was obtained from the institutional ethical board (Ethical approval number: IHEC/SDC/UG-1871/21/175).

Results

The genotypic frequencies of Cathepsin G gene polymorphism among the control and the case group are indicated in Table 1 where among 60 participants, 30 participants belong to the control group and 30 participants belong to the case group. The case group had 27 participants with homogeneous normal genotype (AA) and heterogeneous genotype (AG) was observed in 3 participants. The control group had 29 participants with homogeneous normal genotype (AA) while 1 participant had heterogeneous genotype (AG) among the study population. Figure 1 shows the percentage of mutation seen in the case group in association with the types of genomic mutation. 90% of the case group reported to have homogeneous genotype percentage while 10% reported to have heterogeneous diseased mutation genotype percentage among the 30 participants in the case group. Figure 2 represents the percentage of mutation observed in the control group along with their genomic mutation. 96.6%reported to have homogeneous normal genotype percentage while 3.3% reported to have heterogeneous diseased mutation genotype percentage among 30 participants of the control population. Figure 3 signifies the correlation between the percentage of mutation observed in both case and control group along the type of genomic mutation. 48.3% and 45% of the study population reported to have homogeneous normal genotype in the control and case group while heterogeneous diseased mutation genotype was reported in 1.6% and 5% of the control and case group among the study population. The genotype frequency of CTSG N125S polymorphism did not differ significantly at χ2df (P=0.300). Our study results showed that the prevalence of homozygous and heterozygous mutant genotypes had no significant difference between the CP and healthy control group (Table 1).

Groups	AA	AG	GG	A	G	HWE (p vale)*
Case (N=30)	27	3	0	0.95	0.05	0.77
Control (N=30)	29	1	0	0.98	0.02	0.93

Table 1. *Represents the genotype frequencies of Cathepsin G gene polymorphism among the control and case sample population. AG, AA and GG indicate the homogeneous normal genotype, heterogeneous genotype and homogeneous diseased genotype. Hardy-Weinberg Equilibrium (HWE) represented the p value of the control group and case study population. Among 60 participants, homogenous normal genotype was seen in 27 people in the case group and 29 people in the control group. Heterogeneous genotype found in 1 participant in the control group whereas 3 participants had heterogenous genotype in the case group. Homogeneous diseased genotype was not reported in both the samples of control and case groups. Hardy-Weinberg Equilibrium (HWE) represented the p value of the control group as 0.77 and 0.93 for the case group respectively which is not statistically significant.*

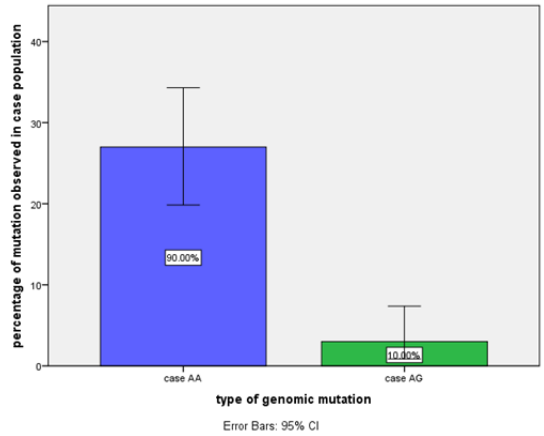

Figure 1. *Represents the percentage of mutation observed in case population. The X-axis represents the types of genomic mutation seen in chronic periodontitis patients and Y-axis represents the percentage of genomic mutation. Blue colour indicates the periodontitis patients who have reported to have homogenous normal genotype among the population which was 90% and yellow colour indicates the percentage of population with heterogeneous genotype that accounts for 10%. Homogeneous mutation genotype was not recorded among the study population. The p value was 0.77 (>0.05) which is statistically not significant.*

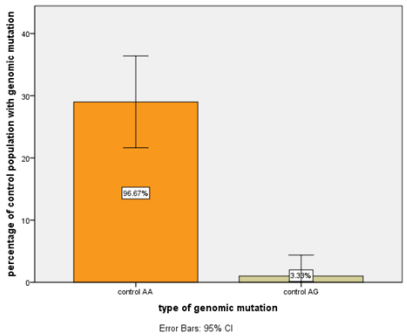

Figure 2. *Represents the percentage of the control population with genomic mutation. The X-axis represents the type of genomic mutation observed in the control group and Y-axis represents the percentage of mutation in the control population. 96.67% of the control group had a homogeneous normal genotype that is indicated by orange colour while 3.33% had heterozygous genotype that is represented by yellow colour. Homogenous diseased mutation genotype was not recorded in the control population. The p value was 0.93 (>0.05) which was statistically not significant.*

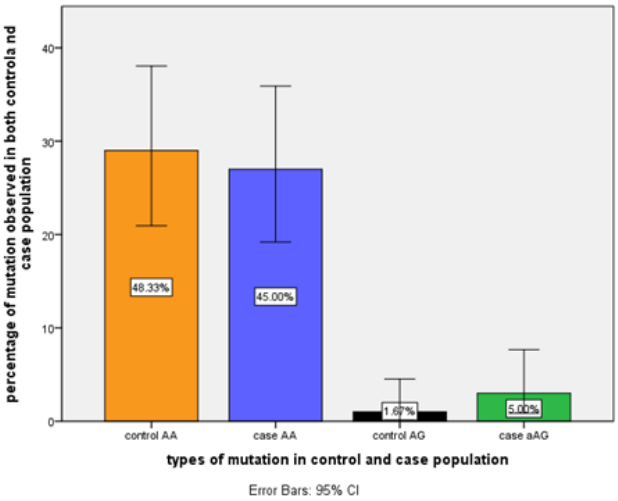

Figure 3. *Represents the percentage of mutation that is observed in both control and case population in association with their type of mutation. The X-axis represents the types of mutation while Y-axis represents the percentage of mutation in both control and case population. 48.33% reported to have homogeneous normal genotype that was indicated by orange colour while 1.67% reported to have heterogeneous genotype that was represented by black colour among the control population. 45% reported to have homogeneous normal genotype that is indicated by blue colour while 5% reported to have heterogeneous genotype that is represented by green colour among the case population.*

Discussion

The association between cardiovascular diseases to that of mutation in Cathepsin G gene is stated in an article of 2001.

The study results indicated that the CTSG Ser125 allele is associated with plasma fibrinogen levels in Myocardial Infarction (MI) patients from the Etude Cas-Temoin de I'Infarctus du Myocarde (ECTIM) study and with Brain Infarction (BI) in the GENIC study in patients without a previous history of cardiovascular or cerebrovascular diseases.

The study indicated that the activation of neutrophils causes release of Cathepsin G release from their granules thereby producing platelet activation along with the release of platelet thrombogenic products which could cause a rise in intravascular thrombosis [37].

The association between the mutations of Cathepsin G gene with osteomyelitis was conducted in 2019 among the Spanish population.

The result manifested significant association between the occurrence of osteomyelitis and mutation of Cathepsin G gene [38].

Previous certain studies stated that this gene can be used as a potential marker for prevention of post-surgical pain [39].

CTSG contributed to the development of pain hypersensitivity owing to its chemotactic properties, leading to an increase in neutrophils and release of inflammatory mediators in the spinal cord. CTSG also promoted astrocyte activation upon peripheral inflammation [40].

Previous studies have stated that variations in the CTSG gene were associated with minimized risks of chronic postsurgical pain. CTSG represents a potential target for chronic pain intervention [41].

Furthermore, preoperative determination of CTSG gene polymorphisms may facilitate perioperative physicians to formulate an appropriate plan to prevent chronic postsurgical pain [42].

A study reported the association between polymorphism of the Cathepsin G gene with the risk of alzheimer's disease.

The results showed that there is no significant correlation between the disease and the polymorphism of genes of interest [43].

All the above-mentioned systemic diseases have inflammatory pathways that are associated with mutation of CTSG in Ser125 allele. The current study adopted the Single Nucleotide Polymorphism (SNP) of Ser125 in Cathepsin G gene due to its vital role in inflammation.

Since periodontitis is also an inflammatory disease, the Cathepsin G gene mutation in Ser125 is specified. The present study states that the N125S polymorphism of CTSG was not associated with periodontitis.

Conclusion

Cathepsin G gene plays an important role in the pathway of inflammation and hence its association between chronic periodontitis is explored in the current study.

The N125S polymorphism of Cathepsin G gene has no association with chronic periodontitis. Hence, further studies have to be conducted with increased sample size to obtain the correlation of Cathepsin G gene mutation with chronic periodontitis.

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Conflict of Interest

The authors declare no conflict of interest.

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