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Genetic association of GPX-1 gene polymorphism (rs1050450) with susceptibility to Chronic periodontitis– a case control study in south Indian population.

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ABSTRACT:

AIM: The purpose of this pilot study was to determine the association between the GPX-1 gene polymorphism (rs1050450) with susceptibility to Chronic Perodontitis in the south indian population.
INTRODUCTION: Periodontal disease is a widespread, chronic multimicrobial immuno-inflammatory illness. An essential enzymatic antioxidant, glutathione peroxidase (GPX-1), guards periodontal tissues against oxidative stress. Since members of the glutathione peroxidase family catalyse the reduction of organic hydroperoxides and hydrogen peroxide (H202) by glutathione and so protect cells from oxidative damage, the protein produced by this gene is a member of that family. Therefore, the disease genes in complex disorders are viewed as moderating disease genes.
MATERIALS AND METHODS: The study was conducted in saveetha dental college, chennai.
50 wet samples were collected after receiving ethical clearance, with N=25 case and N=25 control groups. After genomic extraction of the samples, PCR-RFLP based genotyping assays were performed using the specific forward and reverse primers flanking the locus at (rs1050450). The genotype distribution and allele frequencies between the study groups were determined using chi-square test.
RESULTS: For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. The genotype frequency of cases and controls do not

differ significantly χ 2df (P =0.5688). No significant difference was observed between the case and control groups as (P<0.05).
CONCLUSION: From the study, it has been concluded that there is no significant association of GPX-1 gene polymorphism rs1050450 with susceptibility to chronic periodontitis. Since it's a pilot study the sample size was kept as N=25 in the case group and control group. Sample size should be increased to determine the actual association between the genetic marker(rs1050450) and the disease phenotype (CP).

KEYWORDS: Chronic periodontitis, Polymorphism, Glutathione peroxidase, Ancestral allele, Genotype, Oxidative stress.

INTRODUCTION:

Damage to the periodontal tissues is a symptom of periodontitis, a chronic inflammatory illness mediated by host and bacterial interactions that may lead to tooth loss(Huang and Jia, 2022). Periodontal disease is a widespread, chronic multimicrobial immuno-inflammatory illness(Musurlieva, 2015). Inflammatory mediators activate polymorphonuclear leukocytes at the sites of microbial invasion, resulting in higher levels of reactive oxygen species (ROS), which not only destroy the pathogens but also the host surrounding tissues(Kõll, 2006). The action of a leukocytes membrane-bound enzyme termed reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which catalyses the reduction of molecular oxygen to superoxide anion, starts the cascade of ROS generation(Kõll, 2006; Li *et al.*, 2022). One of the key enzymes in the host's cytosolic defence against oxidative stress is glutathione peroxidase(Tang *et al.*, 2022). Glutathione peroxidase prevents oxidative damage, protein deterioration, and lipid peroxidation brought on by cytotoxic peroxide.
Monogenic mutations called polymorphisms arise when a nitrogenous base is changed to a different base pair(de Castro *et al.*, 2022). They are regarded as typical differences within the population and might or might not affect the phenotype(Ruehr, 1992; de Castro *et al.*, 2022). Different polymorphic genes have been linked to a higher risk of developing chronic periodontitis, according to several studies(Kimura, 1982).
A polymorphic variant of a gene can result in the expression or manufacture of a protein with aberrant properties, which may either cause or be linked to disease(Xu *et al.*, 2022). For instance, a polymorphic version of the CYP4A11 enzyme gene in which thymidine replaces cytosine at the nucleotide 8590 position of the gene generates a CYP4A11 protein that substitutes serine for phenylalanine at the amino acid position of the protein(Ghoreshi *et al.*, 2022). The blood pressure-regulating eicosanoid 20-hydroxyeicosatetraenoic acid cannot be produced from arachidonic acid by this mutant protein(Lange *et al.*, 2022).
The majority of genetic studies on periodontitis have concentrated on gene variants that affect the immune system, processes that destroy tissue, or metabolic systems(Demkovych *et al.*, 2022). The genetic polymorphisms might, in some circumstances, influence how a protein is expressed or how innate and adaptive immunity function, which could change the course of the disease. A disease may also be protected from by genetic polymorphisms. As with other complicated diseases, the pathophysiology of periodontitis is characterised by several biological pathways that result in the same clinical manifestations(Dumitrescu and Davison, 2019; Demkovych *et al.*, 2022). There may be a little overall contribution and relative risk from different genes and their polymorphisms to the susceptibility and severity of disease(Chakraborti *et al.*, 2019). Polygenic disorders are often complex diseases. Therefore, the disease genes in complex disorders are viewed as moderating disease genes(Ji and Choi, 2013).
It is crucial to understand that various ethnic populations may not share the same quantity and kind of disease-modifying genes for the same disease.
The aim of the study is to analyse if GPX-1 gene can be a reliable biomarker in biological fluids to evaluate periodontal status and results of periodontal treatment.

MATERIALS AND METHODS:

The present study was carried out in saveetha dental college, Chennai, India. This study included a total of 50 samples. The study protocol was approved by the ethical committee and informed con- sent was obtained from all the participants. The samples were divided in two groups, each comprising 25 chronic periodontitis samples (case group) and 25 normal healthy samples (control group). The samples were collected in an EDTA tube to prevent coagulation.

Diagnosis of the periodontal disease: The patients' diagnoses were made in accordance with the standards established by the International Workshop for a Classification of Periodontal Diseases and Conditions in 1999 (IWC 1999). To determine the severity and extent of the condition, clinical and physical measurements were taken, including assessments of probing depth, bleeding on probing, tooth movement, presence of teeth, accumulation of calcified plaque, and symptoms of inflammation.
DNA extraction: Each participant provided a 3 mL sample of peripheral blood, which was drawn into an EDTA tube with heparin and kept cold until processed. The Quick-gDNA MiniPrep Kit was utilised for the DNA extraction process. The products were kept at 15 C until the amplification and were confirmed by horizontal electrophoresis in 1% agarose.
Genotyping of (rs1050450) Polymorphisms among the cases and controls: Genotyping of the (rs1050450) region was performed with polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)
GPx1(Forward) -5'- TCCAGACCATTGACATCGAG-3′
GPx1(Reverse) -5'- ACTGGGATCAACAGGACCAG-3′
Cycling was carried out as follows: initial denaturation at 94C for 3 min, 32 cycles each at 94C for 30 s, 55C for 30 s, 72C for 45 s, and one cycle at 72C for 5 min. Digestion of PCR products with Apal, restriction endonuclease enzymes yielded Homozygous: CC - 170+52 bp; Heterozygous: CT - 222+170+52 bp; Homozygous: TT - 222 bp.
Statistical analysis: Due to the nature of the variables, the analysis was performed in different proportions to distinguish between the frequencies of each polymorphism. Statistical analysis was carried out using the 20 version of SPSS, chi-square test.
programme (SPSS 20.0; SPSS Inc., Chicago, IL, USA).

RESULTS:

Genotype frequencies and overall genotype distribution of *GPX-1* gene polymorphism (*rs1050450*) among the chronic periodontitis patients and normal healthy subjects are represented in Table 1 and Table 2 respectively. Agarose gel electrophoretogram showing polymorphism of *GPX-1* spanning the (*rs1050450*) polymorphic site and Agarose gel electrophoretogram showing *Apal* digested amplicon of *GPX-1* gene (*rs1050450*) at the polymorphic site are presented in Figure-1 and 2 respectively. The allele frequency of *GPX-1* gene polymorphism (*rs1050450*) in different populations acquired from the Ensembl database is depicted in Graph 1. The genotype frequency of cases and controls show no significant difference (P =0.5688) i.e P<0.05. The present study showed that the prevalence of homozygous and heterozygous mutant genotypes had no significant differences (CC vs CT+TT) between the chronic periodontitis and control group with a p-value of 0.5693 and (CT+CC vs TT) showed statistically insignificant results with a p-value of 1.00 Comparison of alleles C and T also produced a insignificant p value of 0.6296, indicating there is no risk genotype associated with *GPX-1* gene polymorphism (*rs1050450*) with susceptibility to Chronic periodontitis.

Groups	CC	CT	TT	C	T	HWE (p value)*
Case (N=25)	13	12	0	0.76	0.24	0.305
Control (N=25)	15	10	0	0.80	0.20	0.211

Table 1: Genotype frequencies of GPX-1 gene polymorphism (rs1050450) among the cases and controls.

For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly χ 2df
(P =0.5688). No significant difference was observed between the case and control groups as (P<0.05).

Dominant				
Genotypes	**Case**	**Control**	**Unadjusted OR [95% CI]**	**P value**
CC	13	15	0.7222 [0.2355 - 2.2150]	0.5693
CT + TT	12	10		
Recessive				
CT + CC	25	25	1.0000 [0.0191 - 52.3653	1.0000
TT	0	0		
Allele				
C	38	40	0.7917 [0.3063 - 2.0459]	0.6296
T	12	10		

Table 2: Overall genotype distribution of the GPX-1 gene polymorphism (rs1050450) in cases and controls

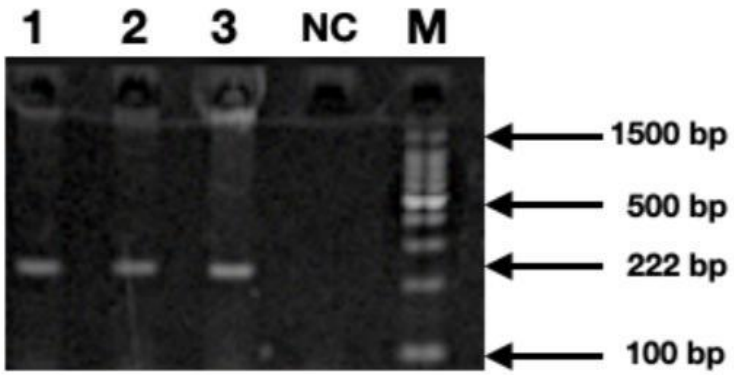


Figure 1: Agarose gel electrophoretogram showing partial amplification of GPX-1 gene spanning polymorphic site (rs1050450) run along with standard DNA ladder [Lane M = 100 bp DNA marker]

Using the standard DNA ladder the amplicon size was found to be 222 base pairs which is depicted in figure 2.
And the allele frequencies of the South Asian population and that of the present study was found to be the same as depicted in graph 1.

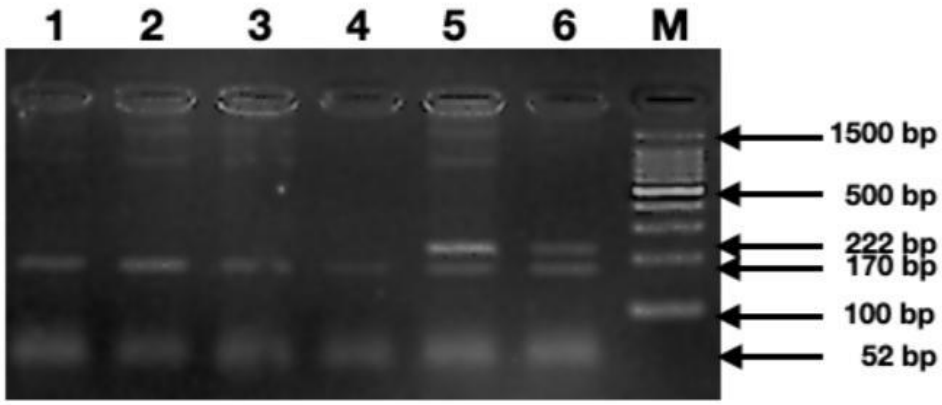
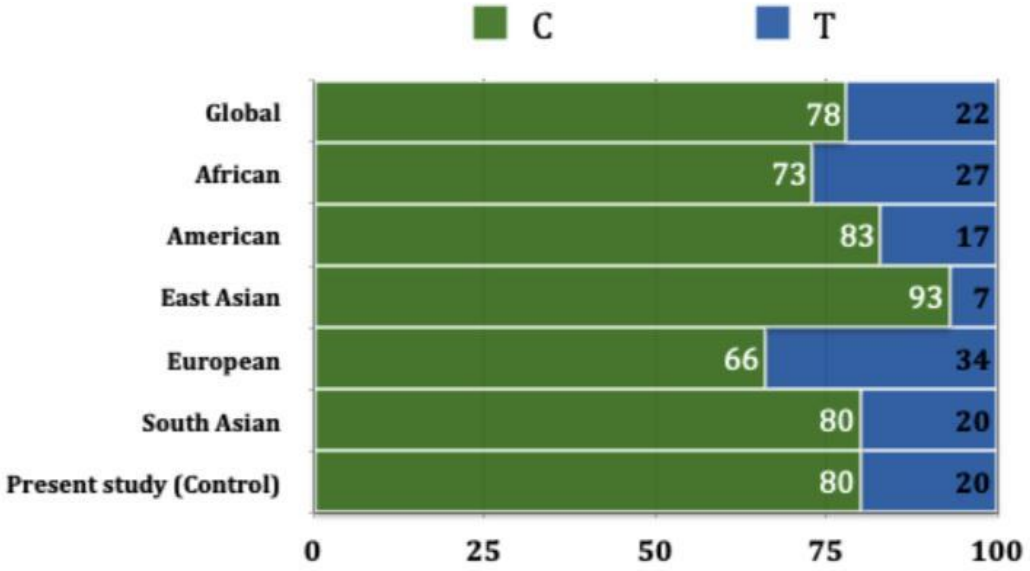


Figure 2: Agarose gel electrophoretogram showing ApaI digested amplicon of GPX-1 gene at (rs1050450) (Homozygous: CC - 170+52 bp; Heterozygous: CT - 222+170+52 bp; Homozygous: TT - 222 bp) [Lane M = 100 bp DNA marker]



Graph 1: The graph depicts the allele frequency of GPX-1 polymorphism in different population [Data acquired from Ensembl database]

The findings indicated that there is no significant association with the GPX-1 gene polymorphism (rs1050450) with that of chronic periodontitis.

DISCUSSION:

It is well known that all illnesses, whether brought on by infectious agents or not, have a hereditary and environmental component(Dai and Zhu, 2022). Over the years, periodontal research has primarily concentrated on a small number of antioxidant systems, including glutathione, catalase, and superoxide dismutase(Araújo *et al.*, 2019). Periodontitis and diabetes mellitus are two such complicated disorders whose etiopathogenesis is influenced by a variety of environmental and genetic variables(Araújo *et al.*, 2019; *Glutathione System and Oxidative Stress in Health and Disease*, 2020). This gene produces a protein that is a member of the glutathione peroxidase family(Morales-Gonzalez, 2013). This family's enzymes catalyse the reduction of organic hydroperoxides and hydrogen peroxide (H2O2) by glutathione, preventing oxidative damage to cells(Morales-Gonzalez, 2013; Dumitrescu and Davison, 2019). According to additional research, H2O2 is also necessary for the mitochondria to operate, the maintenance of thiol redox balance, and signal transmission mediated by growth factors. As a result, glutathione peroxidases are also engaged in controlling these activities by limiting H2O2 accumulation(Hovav, Wilensky and Allam, 2021). In vertebrates, this gene family has a number of isozymes that differ in their cellular localization and substrate specificity.

Several associations of the *GPX-1* gene with various other diseases and systemic illness have been recorded in the past studies conducted, including diabetes mellitus, breast cancer, in-stent restenosis, CVD etc.
In a study conducted the Polymorphisms in the *GPX-1* and *MnSOD* genes showed an increased risk of breast cancer, with an odd's ratio of 1.87 and that individuals who carried both the *Ala16Ala* genotype of *MnSOD* and the Leu198Leu genotype of *GPX-1* showed an increased risk of breast cancer. While neither allele alone showed any change in breast cancer risk(Cox, Tamimi and Hunter, 2006; Hovav, Wilensky and Allam, 2021).
A study conducted in patients with type 2 diabetes concluded that *GPx-1* variants might contribute to the development of diabetes and both *GPx-1* and *NQO1* variants confirmed the association of CAD in people with T2DM of South Indian population(Dworzański *et al.*, 2020).
A study revealed that minor alleles of the *eNOS* gene polymorphism *298G/T* and the *GPx-1* gene polymorphism *599C/T* are linked to a higher risk of in-stent restenosis. The minor allele of the *599C/T* polymorphism in the *GPx-1* gene causes an increase in free-radical processes while decreasing *GPx* activity(Shuvalova *et al.*, 2012).
According to a study that looked at the genetic relationship between the *GPX-1* gene polymorphism (*rs1050450*) and CVD susceptibility, the Pro198Leu and Pro197Leu polymorphisms in the *GPX-1* gene significantly enhanced the risk of CVD in the East Asian population(Zhang *et al.*, 2014).

In the present study, the (*rs1050450*) polymorphism of the *GPX-1* gene was investigated by the PCR RFLP method. The genotype and allele frequencies of the study population were analysed and compared. And observed no significant association (P <0.5688) between the polymorphism*(rs1050450)* of the *GPX-1* gene and the occurrence of chronic periodontitis.
In a review by Zhang et al, the C allele is linked to periodontal disease progression(Jiang *et al.*, 2014; Zhang *et al.*, 2014). Compared to Japanese and Chinese populations, the Caucasian population has a significantly higher frequency of the C allele(Jiang *et al.*, 2014). According to Brett et al, the GC genotype is carried by people with chronic diseases and is overexpressed in CP genotypic patients(Sternes *et al.*, 2022). According to Babel et al, patients with periodontitis were more likely to have the *IL-6 - 174 CC* genotype, which codes for low phenotypic production of IL-6(Babel *et al.*, 2006).
The study was limited only to a small sample size, that is only within the study population (N=25 in the case group and control group).
Further studies should encompass larger sample sizes for more accurate results and to determine the actual association between the genetic marker(rs1050450) and the disease phenotype (CP).

CONCLUSION:

Complex diseases with strong environmental, immunological, and genetic etiologies include Chronic periodontitis and Diabetes mellitus. Through this study we can conclude that there was no significant association between the *GPX-1* gene polymorphism (rs1050450) and Chronic periodontitis.
More extensive genetic research with higher sample numbers and haplotype analysis, followed by confirmatory clinical trials, will further reveal the susceptibility or resistance allele that contributes to the disease. The pathophysiology of chronic periodontitis will be better understood through studies of other functional variants in the *GPX-1* gene, providing knowledge that may one day be useful for diagnostic and therapeutic purposes.

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