GENETIC ASSOCIATION OF CATALASE 21 GENE POLYMORPHISM (RS7943316) WITH SUSCEPTIBILITY TO CHRONIC PERIODONTITIS IN SOUTH INDIAN POPULATION

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Abstract

Background: An inflammatory disease called periodontitis can eventually result in tooth loss and other serious systemic diseases by affecting the tissues that support the teeth.The catalase enzyme is produced by the CAT gene, which is observed in the 13th position on the short arm of chromosome 11.The CAT-21A/T polymorphism may enhance a person's susceptibility to a multitude of systemic diseases. Therefore, in this study, we investigated its contribution to the likelihood of developing chronic periodontitis.

Aim: The purpose of the study is to investigate the connection between the risk of developing chronic periodontitis and the CAT 21 gene polymorphism (rs7943316).

Background: This study included a total of 50 participants. Blood was drawn from each subject in a control group A (N = 25) and a group B (N = 25) in order to extract their genomic DNA. DNA was amplified by using specific primers that focused on the polymorphism region of the Catalase 21 gene (rs7943316). Next, genotyping of the amplicon was performed using the Pvu II enzyme and (RFLP)restriction fragment length polymorphism. The genotype identified by the RFLP pattern was noted and statistically evaluated.

Results: The genotype frequency of the catalase 21 polymorphism did not vary significantly between the cases and controls 2df (p = 0.819).With a p-value of 07654, the present research showed that there was no significant difference in the prevalence of homozygous and heterozygous mutant genotypes between the control and case groups (TT vs AT+AA). With a p-value of 0.3059, (AT+TT vs AA) also showed statistically insignificant results.

Conclusion: From the study, it has been concluded that there is no significant association between CAT 21 gene polymorphism with susceptibility to periodontitis among South Indian population.

Keywords:Alleles, Chronic Periodontitis, Polymorphism, Catalase 21

1. Introduction

Cellular components such as nucleic acids, proteins, and lipids can chemically react with reactive species released in the cell during normal cellular metabolism leading to their

oxidative modification which can change their composition and potentially harm their cellular activities. To lessen or combat the negative effects of reactive species and/or their byproducts, cells have fortunately evolved with a number of antioxidant defence mechanisms.An imbalance in the homeostasis between the levels of antioxidants and reactive species would cause oxidative stress.[1]

The two types of antioxidants used to remove reactive oxygen species from the environment are scavenging antioxidants and preventive antioxidants. An example of a preventive antioxidant is catalase (CAT). Hydrogen peroxide is broken down into water and oxygen by catalase (CAT), one of the vital natural antioxidant enzymes.[13]

Periodontitis on the other hand is an inflammatory and complex disease initiated by the subgingival biofilm and modulated by the hosts dysfunctional inflammatory/immune response.In gingiva and periodontal tissues, polymorphonuclear leukocytes (PMNL) are the principal inflammatory cells. It is believed that PMNLs, which are inherently capable of creating ROS, become functionally hyperactive in periodontitis and produce more ROS as a consequence disrupting the homeostasis.[14]

Free radicals have a negative impact on the structural and tissue integrity of periodontal tissues, whereas antioxidants counteract this destruction. Reduced enzymatic activity and increased ROS sensitivity may be the results of allelic variants in the antioxidant genes that code for CAT enzymes. Different clinical forms of periodontitis have been linked with genetic polymorphisms. [14]Numerous epidemiologic studies have shown that a single nucleotide polymorphism in the CAT gene was connected to a number of systemic diseases, Previous studies have assessed for the CAT gene polymorphisms in the coding and non coding regions but very few studies have assessed for the polymorphism in the promoter region.[7] This is the first study to examine how the catalase 21 gene polymorphism (Rs7943316) in the promoter region relates to a population's susceptibility to chronic periodontitis.

2. Materials And Methods:-

This study included participants from Chennai, Tamil Nadu, India. This study includes 50 patients from Chennai's Saveetha Dental College. The subjects were divided into case group A and control group B with (N = 25) based on a clinical examination. The Case group included 25 patients ranging in age from 40 to 60. (17 men and 8 women). The control group included 25 healthy subjects aged 40 to 60. (17 men and 8 women).

The participants were questioned about their comprehensive dental history, any family members who had suffered from chronic, severe periodontitis, their smoking habits, and their overall health issues. Despite having chronic periodontitis, the participants in the study had good overall health. Immunocompromised individuals, breastfeeding or pregnant women, and individuals who had recently undergone surgery were all barred from participating in this study. The Institutional ethical committee has approved the project.

Collection of Sample and DNA extraction.

The antecubital fossa was used to obtain 5 ml of venous blood, which was then dispersed in a sterile tube with a small percentage of ethylenediaminetetraacetic acid.To avoid the formation of a clot, it was thoroughly mixed. In accordance with a modified Miller et al. 1998 procedure, DNA isolation was performed .[16]

PCR and restriction endonuclease digestion

The catalase 2 polymorphisms were evaluated using restriction digestion and PCR polymerase chain reaction. The primers forward 5′ TAAGCCAAGGCAAAATTGAG and reverse 5′ CTTCAAAATTTATGTTCCTCTGC were used to encode the DNA with catalase 21 polymorphism. For amplification of DNA in 20 Lvolumes 10 ng of genomic DNA was mixed with 5 pmol/L of each of the forward and reverse primers, and PCR Master Mix.

The cycle started with Initial denaturation of 5 minutes at 94°C ,initial annealing of 35 seconds at 60 °C followed by Initial extension of 72°C for 35 seconds and final extension at 72°C for 5 minutes.5L volume of the PCR was examined on 1% agarose gel and 15L of the PCR product was digested with a Catalase 21 restriction enzyme.The digestion took place for two hours at 37°Cb and the digested product was analysed.

Statistical analysis

SPSS version 23.0 for Windows was used to undergo statistical analyses (SPSS, Chicago, IL, USA). The genotype and allele frequency distributions in the OSCC and control groups were compared using the independent t test. Using the odds ratio (OR) and 95% confidence intervals, the risk connected to particular alleles or genotypes was calculated. P.05 was chosen as the statistical threshold of significance for all tests.[6]

3. Result

Table 1 shows the participants in the Chronic Periodontitis and Control groups' demographic characteristics as well as their clinical characteristics. For patients and controls, respectively, Tables 2 and 3 display the genotype frequencies of the gene polymorphism (rs7943316) and the overall genotype distribution. The Catalase 21 gene's PvuII-digested amplicon is displayed in Figures 1 and 2 at (rs7943316). Using information from the Ensembl database, Graph 1 shows the allele frequency of the Catalase21 (rs7943316) polymorphism in various populations.[7] The genotype frequency of the ESR1 polymorphism did not significantly differ between patients and controls (p = 0.819, 2df). The results of this study showed no significant differences in the prevalence of homozygous mutant genotypes and heterozygous mutant genotypes (TT). With a p-value of 0.7654, the current study found no discernible difference in the prevalence of homozygous and heterozygous mutant genotypes between the chronic periodontitis group and the control group had no discerResults between AT+AA and AA were statistically unremarkable, with a p-value of 0.3959.[8]

Table 1: Genotype frequencies of Catalase 21 A/T gene polymorphism (rs7943316) among the cases and controls

Groups	TT	AT	AA	T	A	HWE (p value)*
Case (N=25)	8	11	6	0.54	0.46	0.567
Control (N=25)	9	13	3	0.62	0.38	0.604

Table 2: Overall genotype distribution of the Catalase 21 gene polymorphism (rs7943316) in cases and control

Dominant				
Genotypes	**Case**	**Control**	**Unadjusted OR [95% CI]**	**P value**
TT	8	9	0.8366 [0.2592 - 2.7004]	0.7654
AT + AA	17	16		
Recessive				
AT + TT	19	21	0.4524 [0.0991 - 2.0653]	0.3059
AA	6	3		
Allele				
T	27	31	0.7195 [0.3242 - 1.5967]	0.4183
A	23	19		

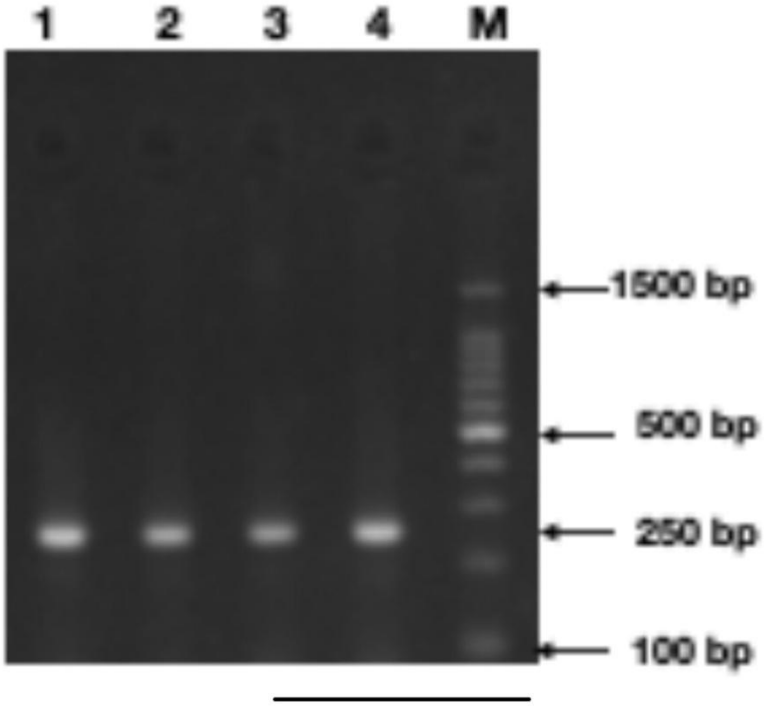

Figure 1: Agarose gel electrophoretogram showing T/A polymorphism of Catalase 21 gene spanning the (rs7943316) polymorphic site [Lane M = 100 bp DNA marker]

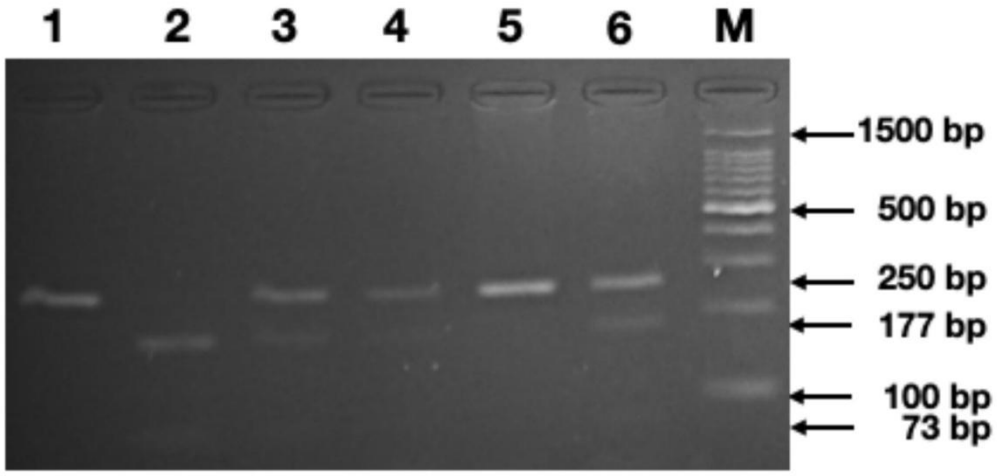

Figure 2: Agarose gel electrophoretogram showing HinfI digested amplicon of Catalase 21 gene (rs7943316) at the polymorphic site (Homozygous TT - 177+73 bp; Heterozygous AT-73+177+250 bp; Homozygous AA - 250 bp) [Lane M = 100 bp DNA marker]

Primer information:
Catalase 21: Forward: - 5‘-AATCAGAAGGCAGTCCTCCC-3 ‘Catalase 21: Reverse: - 5‘-TCGGGGAGCACAGAGTGTAC-3‘
Amplicon size: 250 bp, Annealing temperature: 66 degree C for 30 second

4. Discussion

An essential endogenous antioxidant enzyme called catalase21 detoxifies H2O2 into oxygen and water, thereby minimizing the harmful effects of reactive oxygen species. The endogenous antioxidant enzyme catalase21 is in charge of regulating the concentrations of reactive species. SNPs in the catalase gene may be connected to a variety of systemic diseases, according to several epidemiologic studies.[20] Four polypeptide chains, each longer than 500 amino acids, make up the tetramer catalase21. Its four heme groups, which contain iron, enable the enzyme to interact with hydrogen peroxide.

In recent years, periodontitis has been seen as a complex disease. Similar to other complex diseases like diabetes, Alzheimer's, rheumatoid arthritis, and others, periodontitis is linked to variations in a number of genes, each of which contributes to a modest proportion of the disease risk.[17]

The practical application of genetics in periodontitis is inherently limited. Careful diagnosis, prompt treatment, and prevention may be offered if the susceptible patients could be identified and analysed. Hence the present study was done to analyse the role of catalase 21 gene polymorphisms in the susceptibility of chronic periodontitis.[19]

Catalase was discovered to be crucial in the adaptive response to oxidative stress, even though it was not necessary for some cell types under normal conditions. However, it has been noted that reactive oxygen species inactivation may not be obtained with just an adaptive increase in catalase activity. Further lowering catalase enzyme activity may result from reduced catalase gene expression brought on by the presence of a mutant allele.

The present study is the first to examine the polymorphism region in the promoter region of the Catalase 21 gene in people with chronic periodontitis. According to a study[7] on healthy adults, the CAT-21A/T (rs7943316) gene promoter polymorphism was present in 18.26% of individuals with the wild type genotype (AA) and 81.74% of cases with the altered genotype (AT/TT). The study, however, was unable to be compared with our study because it only examined the prevalence of the catalase gene polymorphism in healthy persons, not those suffering from any complex disorders.

They investigated whether the catalase gene polymorphism (Catalase, C-262 T, rs1001179) affected how people responded to root planing treatment, and they found that the results were statistically significant and that people who had this polymorphism were more responsive to treatment. [15]The limitations of the study includes the small sample size derived from low allelic frequency(catalase 21AA genotype).

5. Conclusion

The Present research concluded that there is no association between the CAT 21 gene polymorphism and periodontitis susceptibility in the South Indian population.

Our team has a lot of experience and research knowledge, which has resulted in publications of the highest caliber.

References

[1] Ankita Nandi, Liang-Jun Yan, Chandan Kumar Jana, Nilanjana Das, "Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases", Oxidative Medicine and Cellular Longevity, vol. 2019, Article ID 9613090, 19 pages, 2019. https://doi.org/10.1155/2019/9613090

[2] Chaitanya, N.C. *et al.* (2017) 'Role of Vitamin E and Vitamin A in Oral Mucositis Induced by Cancer Chemo/Radiotherapy- A Meta-analysis', *Journal of clinical and diagnostic research: JCDR*, 11(5), pp. ZE06–ZE09.

[3] DeSouza, S.I. *et al.* (2014) 'Mobile phones: the next step towards healthcare delivery in rural India?', *PloS one*, 9(8), p. e104895.

[4] Jain, A.R. (2017) 'Clinical and Functional Outcomes of Implant Prostheses in Fibula Free Flaps', *World journal of dentistry*, 8(3), pp. 171–176.

[5] Jayaraj, G. *et al.* (2015) 'Stromal myofibroblasts in oral squamous cell carcinoma and potentially malignant disorders', *Indian journal of cancer*, 52(1), pp. 87–92.

[6] Khalid, W. *et al.* (2016) 'Role of endothelin-1 in periodontal diseases: A structured review', *Indian journal of dental research: official publication of Indian Society for Dental Research*, 27(3), pp. 323–333.

[7] Khalid, W. *et al.* (2017) 'Comparison of Serum Levels of Endothelin-1 in Chronic Periodontitis Patients Before and After Treatment', *Journal of clinical and diagnostic research: JCDR*, 11(4), pp. ZC78–ZC81.

[8] Putchala, M.C. *et al.* (2013) 'Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity -- a systematic review', *Archives of oral biology*, 58(6), pp. 563–574.

[9] Rao, N.G. *et al.* (1989) 'Mandibular canine index--a clue for establishing sex identity', *Forensic science international*, 42(3), pp. 249–254.

[10] Sekhar, C.H., Narayanan, V. and Baig, M.F. (2001) 'Role of antimicrobials in third molar surgery: prospective, double blind,randomized, placebo-controlled clinical study', *The British journal of oral & maxillofacial surgery*, 39(2), pp. 134–137.

[11] Sitharthan, R. *et al.* (2019) 'Automated power management strategy for wind power generation system using pitch angle controller', *Measurement and Control*, 52(3-4), pp. 169–182.

[12] T, P. *et al.* (2020) 'Prevalence of cleft lip: A retrospective hospital based study', *International Journal of Research in Pharmaceutical Sciences*, pp. 1911–1917. Available at: https://doi.org/10.26452/ijrps.v11ispl3.3643.

[13] Wang, Y., Andrukhov, O., & Rausch-Fan, X. (2017). Oxidative Stress and Antioxidant System in Periodontitis. Frontiers in Physiology, 8.doi:10.3389/fphys.2017.00910

[14] Trivedi S, Lal N. Antioxidant enzymes in periodontitis. J Oral Biol Craniofac Res. 2017 Jan-Apr;7(1):54-57. doi: 10.1016/j.jobcr.2016.08.001. Epub 2016 Aug 25. PMID: 28316923; PMCID: PMC5343161.

[15] Lee CY, Chang CH, Teng NC, Chang HM, Huang WT, Huang YK. Associations between the phenotype and genotype of MnSOD and catalase in periodontal disease. BMC Oral Health. 2019 Aug 30;19(1):201. doi: 10.1186/s12903-019-0877-3. PMID: 31470840; PMCID: PMC6717336.

[16] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988 Feb 11;16(3):1215. doi: 10.1093/nar/16.3.1215. PMID: 3344216; PMCID: PMC334765.

[17] Laine, M.L., Crielaard, W. and Loos, B.G. (2012), Genetic susceptibility to periodontitis. Periodontology 2000, 58: 37-68. https://doi.org/10.1111/j.1600-0757.2011.00415.x

[18] Nawab SN, Zehra S, Fawwad A, Azhar A. A Study on *Catalase* Gene Promoter Polymorphism *-21 A/T (rs7943316)* in Healthy Pakistani population. Pak J Med Sci. 2017 Nov-Dec;33(6):1521-1524. doi: 10.12669/pjms.336.13188. PMID: 29492090; PMCID: PMC5768856.

[19] Tarannum F, Faizuddin M. Effect of gene polymorphisms on periodontal diseases. Indian J Hum Genet. 2012 Jan;18(1):9-19. doi: 10.4103/0971-6866.96638. PMID: 22754216; PMCID: PMC3385187.

[20] Vijayalakshmi R, Geetha A, Ramakrishnan T, Emmadi P. Genetic polymorphisms in periodontal diseases: An overview. Indian J Dent Res [serial online] 2010 [cited 2023 Feb 4];21:568-74.

[21] Stephen M. Sudi, Salma Kabbashi, Imaan A. Roomaney, Marwa Aborass, Manogari Chetty. (2022) The genetic determinants of oral diseases in Africa: The gaps should be filled. *Frontiers in Oral Health*3.

[22] Kinane DF, Hart TC. Genes and Gene Polymorphisms Associated with Periodontal Disease. *Critical Reviews in Oral Biology & Medicine*. 2003;14(6):430-449. doi:10.1177/154411130301400605