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Incidence of multidrug resistance gene 1 3435C/T single nucleotide polymorphism in patients with amlodipine induced gingival enlargement – A case control study

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ABSTRACT

Background: Periodontal diseases are multifactorial in nature with genetic cause as one of the risk factors. In current era, periodontal researches have widely expanded to explore the genetic background of periodontal diseases. Also, genetic predisposition remains as one of the causes for Drug-induced Gingival enlargement.

Objective: To identify MultiDrug Resistance gene (MDR) 1 C3435T single nucleotide polymorphism (SNP) in patients under amlodipine therapy with and without gingival enlargement.

Methods: About 50 hypertensive patients were selected. They were divided into two groups; in that, 25 hypertensive subjects under amlodipine therapy with drug-induced gingival enlargement and 25 hypertensive subjects under amlodipine therapy with healthy gingiva were included as study and control group respectively. The blood Samples were collected from both groups for analyzing genotype distribution and finding the association of C3435T SNP with drug-induced gingival enlargement by PCR method.

Results: Our present study demonstrated no significant difference between genotypes and severity of gingival enlargement as the *p* value was less than significance value of 0.05. Therefore, the study concludes there is no association of gingival enlargement with specific genotype.

Conclusion: Within the limits of the present study, it can be concluded that the susceptibility for drug-induced gingival overgrowth is not influenced by the MDR 1 C3435T single nucleotide polymorphism. Since the study was undertaken in a small sample size, definite conclusion cannot be drawn. Hence, further genetic studies in MDR 1 gene in a large population are necessary for justifying the genetic predisposition to drug-induced gingival overgrowth.

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1. Introduction

Periodontal diseases are multifactorial in nature; for which, several risk and susceptibility factors are proposed in the natural history of periodontitis.[1] Susceptibility factors are non-modifiable determinants such as age, gender and genotype (genetic make-up). Drug-induced gingival overgrowth (DIGO) is one of the common oral conditions seen among patients seeking dental care, and also occurs as a side effect following the administration of three main groups of drugs namely antiepileptics, calcium channel blockers and immunosuppressants.[2]

Among the various risk factors responsible for drug-induced gingival enlargement, evidence suggests that the genetic factors in the form of mutation or gene polymorphism attributed to inter-individual variability in susceptibility to gingival enlargement.[3] Genetic markers that have been investigated in relation to drug-induced gingival overgrowth include cytochrome P450genes, human lymphocyte antigen expression, integrin genes and MultiDrug Resistance (MDR) 1 gene localized in a position C3435T of exon 26.[4]

MDR 1 gene encodes a drug transporter called P-glycoprotein (P-gp) that limits a wide variety of drugs from penetrating cells and depositing them into the extracellular space.[5] The multidrug resistance 1 (MDR1) gene, also known as ABC subfamily B member 1 transporter (ABCB1) gene is highly polymorphic. In recent years, according to the single nucleotide polymorphism (SNP) database maintained by the National Center for Biotechnology Information (NCBI), ~66 coding single-nucleotide polymorphisms (SNPs) have been identified that may alter the expression of P-gp or its protective physiological function.[6] Recent evidence suggests that amlodipine acts as a substrate of P-gp.[7] Thus MDR1 polymorphisms may alter P-gp expression which in turn affects amlodipine plasma levels and was analyzed as a risk factor for gingival overgrowth induced by calcium channel blockers.[8] The present study is a pioneer study in Chennai population under taken to identify MDR 1 gene polymorphism in subjects under amlodipine therapy.

2. Methods

A case–control study was performed in 50 hypertensive patients undergoing therapy with amlodipine. The patients were dichotomized into two groups. The control group (Group A) included 25 hypertensive patients with healthy gingiva constituting 13 males and 12 females, aged 33–78 years and the study group (Group B) included 25 hypertensive patients presented with gingival enlargement constituting 8 males and 17 females, aged 34–66 years. The study protocol was approved by Institutional Ethical Committee (IEC), Tamil Nadu Government Dental College and Hospital, Chennai, India. The study and control groups were recruited from the Tamil Nadu Government Dental College and Hospital, Chennai and Rajiv Gandhi Government general hospital, Chennai respectively. The written, informed consent was obtained from each subject before enrolment in the study.

Demographic and medical data and smoking history were recorded from all patients at the time of first examination. The patients who were diagnosed as a case of drug-induced gingival enlargement and taking amlodipine as the sole antihypertensive drug with a dosage of 2.5–10 mg were considered among the inclusion criteria. At the beginning of the study, the severity of gingival enlargement was assessed by Gingival enlargement index. They were then subjected to periodontal debridement and nonsurgical periodontal therapy, consisting of supra gingival and subgingival scaling with both ultrasonic and hand instruments. The whole oral cavity was examined radiologically as part of the periodontal screening process to exclude other possible maxillofacial diseases.

3. Genomic DNA extraction

1 ml of venous blood was taken from the patients with ethylene diamine tetra-acetic acid as an anticoagulant. The blood samples were thawed in batches of eight. Genomic DNA was extracted from leukocytes by column-based protocol. The MDR1 polymorphisms were determined by polymerase chain reaction. The PCR amplification consisted of an initial denaturation for 2 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s. PCR uses two primers, each complementary to opposite strands of the DNA of interest, which has been denatured by heating. One primer directs the synthesis of a strand of DNA, which can then be primed by the second complementary primer. The PCR products can be identified by its size using agarose gel electrophoresis.

3.1. *Gel electrophoresis*

5 μl aliquots of amplified PCR products were analyzed by running them in a 1.5% agarose gel at 100 V for 30 min with 1X TAE (Tris Acetate EDTA) buffer. The DNA bands were visualized by staining the gel with ethidium bromide (a DNA intercalating agent that fluoresces when excited by UV in the range of 302 nm to 364 nm), and images were captured with gel documentation unit (Fig. 1).

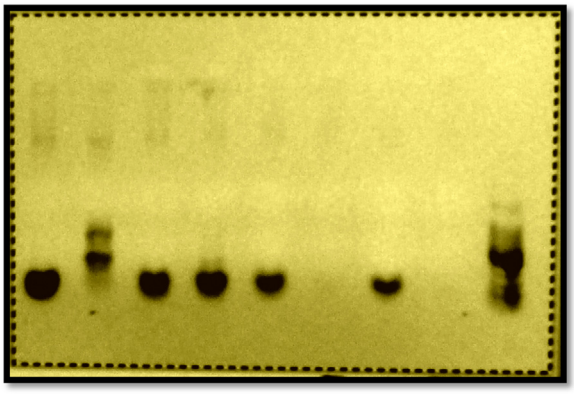


Fig. 1 – Amplified PCR products image captured with gel documentation unit.

Table 1 – Chi-square analysis test for genotype distribution.

SNP	Amlodipine – normal ($n = 25$)	%	Amlodipine induced GH ($n = 25$)	%	Total	%	p Value	
CC	10	40	9	36	19	38		
TT	6	24	6	24	12	24		
CT	9	36	10	40	19	38		
Total	25	100	25	100	50	100		
	(χ^2)						$p = 0.11$ (>0.05)	NS

The table shows the percentage of different genotypes among total sample size.

- Percentage of CC genotypes among total sample size is 38%.
- Percentage of CT genotypes among total sample size is 38%.
- Percentage of TT genotypes among total sample size is 24%.

The chi-square analysis test for genotype distribution among control and study group revealed the p value as 0.11 which is not statistically significant (NS, not significant).

4. Results

25 hypertensive subjects with healthy gingiva and 25 hypertensive subjects with enlarged gingivae required treated with amlodipine were included in the study. The percentage of control and study population among total sample size was 50% and 50%, respectively. The criteria for p value were set as significance level of 0.05. The results demonstrating the value less than 0.05 considered statistically significant.

The respective frequency of MDR1 genotypes found in control group could be 40% in homozygous CC genotype, 36% in heterozygous CT genotype and 24% in mutant TT genotype, whereas, in the study population, the genotypes were 36% in homozygous CC genotype, 40% in heterozygous CT genotype, and 24% in mutant TT genotype (Table 1). The percentage of CT genotype exhibiting gingival enlargement was slightly higher when compared to other genotypes. There were no significant differences in the frequency of 3435CC, 3435CT and 3435TT genotypes between patients with and without gingival overgrowth ($p = 0.11$). These results are consistent with De Iudicibus et al.'s study[10] who demonstrated CT genotype exhibiting severe gingival overgrowth. However, the genotype distribution is not statistically significant between the control and study groups. This is consistent with Drozdzik et al.'s study[11] who demonstrated similar results for the genotype distribution between the control and study groups.

Likewise to genotypes, distribution of alleles was similar in patients with gingival overgrowth and healthy gingiva ($p = 0.02$) (Table 2). Also, the severity of gingival enlargement is not associated with the specific genotype (p value = 0.276) (Table 3).

Table 2 – Chi-square test for allele frequency.

	C	%	T	%
Amlodipine – normal ($n = 25$)	19	76	15	60
Amlodipine induced GH ($n = 25$)	19	76	16	64
Total = 50	38	100	31	100
%	76		62	
(χ^2)	0.02	$p > 0.05$	NS	

From this table we infer that,

- Percentage of C allele in control group: –76
- Percentage of C allele in study group: –76
- Percentage of T allele in control group: –60
- Percentage of T allele in study group: –64

p Value = 0.02 which is not statistically significant (NS, not significant).

5. Discussion

Much effort has been done in recent years to identify the allelic variants of genes that can be used to assess disease risk for periodontal diseases. Drug-induced gingival overgrowth or enlargement is one of the periodontal diseases that manifests as abnormal growth of the gingiva due to side effects in patients treated with anti-convulsants, immunosuppressants and calcium channel blockers. An appraisal of the various investigations into the pathogenesis of DIGO supports the hypothesis that it is multifactorial with genetic factors being one of the risk factors for gingival overgrowth. Genetic factor that has been investigated in relation with drug-induced gingival enlargement comprises Fibroblast

Table 3 – Correlation of genotypes with severity of gingival enlargement. Independent-samples Kruskal–Wallis test.

Hypothesis test summary				
	Null hypothesis	Test	Sig.	Decision
1	The distribution of SEVERITY is the same across categories of GENEOTYPE	Independent-Samples Kruskal–Wallis Test	0.276	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is 0.05. Statistically no significant difference between genotypes with respect to severity.

heterogenecity, Cytochrome P450 gene polymorphism, Human lymphocyte antigen expression (HLA), Multidrug resistance gene MDR 1 C3435T polymorphism and MDR1 G2677T/A polymorphism.[9]

Our present study focused on MDR 1 C3435T genotype association with amlodipine induced gingival enlargement since only very few studies have been conducted worldwide to correlate the association of MDR 1 C3435T with DIGO. Among these, a polymorphism in exon 26 (C3435T) and a polymorphism in exon 21 (G2677T/A) have been correlated with the expression and activity of P-glycoprotein.[4] P-glycoprotein is expressed in the endothelial layers of blood vessels in gingival tissue,[8] the C3435T mutated genotype, which is associated with a lower expression and activity of P-glycoprotein, could result in an increased concentration of the drug in this tissue, and hence in the increased hypertrophy.

When genetic studies in India were explored, until now, only two case reports have been published in India to find the association of MDR1 3435C/T polymorphism with gingival enlargement. A pharmacogenetics analysis by NSC Charles et al. in 2012[12] and by Naik et al. in 2015[13] demonstrated the association of MDR1 3435C/T polymorphism with gingival enlargement. The improved knowledge on genetic predisposition to gingival overgrowth will provide a way for analysis of a new genetic marker for medical testing and a safer individualized medication to treat the most common disorders.

6. Conclusion

Identification of variations in genes and analysis of their effect may lead to a better understanding of their impact on gene therapy. On the basis of the results of the study, no significant incidence of MDR 1 gene polymorphism in patients with gingival overgrowth was demonstrated. Also, the association between the MDR1 gene polymorphism and severity of gingival overgrowth in hypertensive patients was statistically insignificant. The study will remain as a basic tool for future researchers in the field of genetics.

Conflicts of interest

The authors have none to declare.

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