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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).

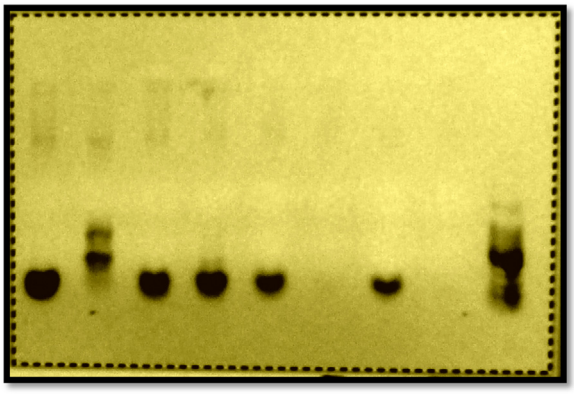

Fig. 1 – Amplified PCR products image captured with gel documentation unit.

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