Research Letter

Drug-Induced Gingival Overgrowth: The Genetic Dimension

Noronha Shyam Curtis Charles[1], Rahul Chavan[2], Ninad Joshirao Moon[3], Srinivas Nalla[4], Jaydeepchandra Mali[5], Anchal Prajapati[6]

[1]Department of Oral Pathology and Microbiology, Mahatma Gandhi Postgraduate Institute of Dental Sciences, Puducherry, [2]Department of Periodontics, Shri Guru Gobind Singh Educational and Welfare Society, Burhanpaur, Madhya Pradesh, [3]Department of Periodontics, RKDF Dental College and Research Centre, Bhopal, [4]Department of Orthodontics and Dentofacial Orthopedics, Al Badar Rural Dental College and Hospital, Daryapur, Gulbarga, [5]Department of Periodontics, Vaidik Dental College and Research Centre, Daman, [6]Department of Dental and Implant Surgery, Pramukh Swami Medical College, Karamsad, Anand, India

Abstract

Background: Currently, the etiology of drug-induced gingival overgrowth is not entirely understood but is clearly multifactorial. Phenytoin, one of the common drugs implicated in gingival enlargement, is metabolized mainly by cytochrome P450 (CYP)2C9 and partly by CYP2C19. The CYP2C9 and CYP2C19 genes are polymorphically expressed and most of the variants result in decreased metabolism of the respective substrates. **Aims:** The present study was undertaken to investigate the influence of the CYP2C9*2 and *3 variant genotypes on phenytoin hydroxylation in subjects diagnosed with epilepsy from South India, thus establishing the genetic polymorphisms leading to its defective hydroxylation process. **Materials and Methods:** Fifteen epileptic subjects, age 9 to 60 years were included in the study. Among the study subjects, 8 were males and 7 were females. Genomic DNA was extracted from patients' blood using Phenol-chloroform method and genotyping was done for CYP2C9 using customized TaqMan genotyping assays on a real time thermocycler, by allelic discrimination method. The genetic polymorphisms *1, *2 and *3 on CYP2C9 were selected based on their function and respective allele frequencies in Asian subcontinent among the Asian populations. **Results:** CYP2C9*1*2 and CYP2C9*3/*3 were identified with equal frequency in the study population. There were seven subjects with CYP2C9*1/*2 genotype (heterozygous mutant), one subject with CYP2C9*1/*1 (wild type) and seven study subjects with CYP2C9*3/*3 (homozygous mutant). **Conclusion:** The results obtained in the present study will be helpful in the medical prescription purposes of phenytoin, and a more personalized patient approach with its administration can be advocated.

Keywords: CYP2C9, Gene polymorphism, Gingival enlargement, Phenytoin, South Indians

Address for correspondence: Dr. NSC Charles, Department of Oral Pathology and Microbiology, Mahatma Gandhi Postgraduate Institute of Dental Sciences, Puducherry - 605 006, India. E-mail: charlesnsc36@gmail.com

Introduction

In today's day and age of personalized lifestyles and choices, the foray of personalization into the field of medicine and custom-made drug administration regimes is inevitable. The pathogenesis of drug-induced gingival overgrowth is baffling and there appears to be no unifying hypothesis that links together the three commonly implicated drugs, i.e., anticonvulsants, calcium channel blockers and immunosuppressants.[1] The pharmacologic effect of each of these drugs is different but all of them seem to act similarly on secondary target tissue, i.e., the gingival connective tissue, causing common clinical histopathological findings.[2]

Phenytoin, a widely used anti-epileptic drug, has been shown to induce gingival overgrowth by its interaction with epithelial keratinocytes, a subpopulation of "sensitive" fibroblasts, and collagen.[3] Phenytoin is metabolized almost extensively by the cytochrome P450

Access this article online

Quick Response Code:

Website: www.najms.org

DOI: 10.4103/1947-2714.141651

(CYP)2C9 enzyme and to a small extent by CYP2C19 to its major metabolite 5-(para-hydroxyphenyl)-phenylhydantoin (p-HPPH).[4] CYP2C9, the gene encoding this enzyme, is polymorphically expressed with about 20 variant alleles.[5] Of these, CYP2C9*2 and CYP2C9*3, are the most common variants.[3] In vitro studies have suggested a significant reduction in CYP2C9 activity with the *3 allele, but only a slight reduction with *2.[6]

With this background, the present study was undertaken to investigate the influence of the CYP2C9*2 and *3 variant genotypes on phenytoin hydroxylation in subjects diagnosed with epilepsy from South India, thus establishing the genetic polymorphisms leading to its defective hydroxylation process.

Materials and Methods

A total of 15 patients diagnosed with epilepsy with age ranging from 9 years to 60 years were recruited to the study after the ethical committee (IRB) approval. Of these, 8 patients were males and 7 patients were females. Five milliliters of blood was extracted from each subject's antecubital vein in a polypropylene tube containing 100 µl of anticoagulant (10% ethylene diamine tetra-acetic acid). Phenol-chloroform method was used to extract the genomic DNA. The extracted DNA was diluted to 50 ng/µl concentration and was stored at minus 20°C. The samples of each of the study groups were genotyped for CYP2C9 using customized TaqMan genotyping assays on a real time thermocycler, by allelic discrimination method (Applied Biosystems real time thermocycler 7300, Foster City, CA) and the same methodology was validated by performing direct gene sequencing. The genetic polymorphisms *1, *2 and *3 on CYP2C9 were selected based on their function and respective allele frequencies in Asian subcontinent among the Asian populations. Primers used in the study were 5-CACTGGCTGAAAGAGCTAACAGAG-3 forward primer, 5-GTGATATGGAGTAGGGTCACCCAC-3 reverse primer.

Results and Discussion

The results of the present study revealed that CYP2C9*1*2 and CYP2C9*3/*3 were identified with equal frequency in the study population. There were seven subjects with CYP2C9*1*2 genotype (heterozygous mutant), one subject with CYP2C9*1*1 (wild type) and seven study subjects with CYP2C9*3/*3 (homozygous mutant). The results for all the 15 subjects included in the study are depicted in the gel electrophoresis picture [Figure 1].

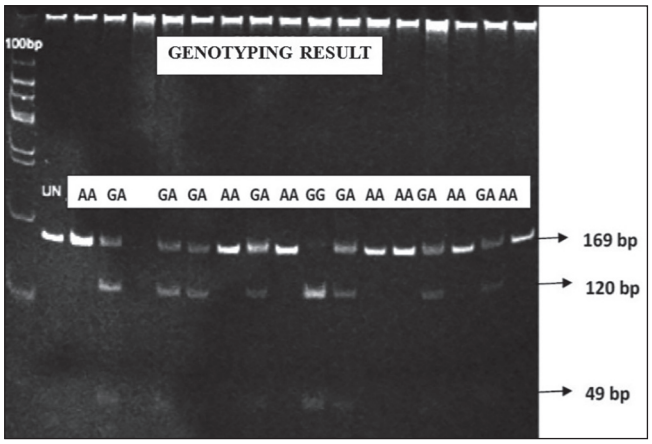

Figure 1: Gel electrophoresis photograph depicting seven subjects identified with heterozygous mutant (GA) genotype, seven subjects identified with homozygous mutant (AA) genotype, and one subject identified with wild type normal (GG) genotype

The availability of diagnostic aids has seen a quantum jump during the last decade. In the forefront today, in fact leading the way is genetics and genetic studies. Genetic studies have enabled the identification of gene mutations, which make patients susceptible to gingival overgrowth, secondary to drug use. The identification of patients at risk is of paramount necessity. Gingival overgrowth still presents an enigma to modern day science as its etiology still hasn't completely been understood. This stems from the fact that many factors may be responsible either alone or as a combination of agents responsible for causing it. In addition it is a well-known fact that several risk factors exist in the oral cavity that may precipitate the condition, again either alone or as a combination of factors. Some of the known factors are poor oral hygiene, gingival inflammation either acute or chronic, periodontal inflammation, presence of periodontal pockets, duration and dosage of the drug therapeutics. Fibroblasts have also been shown to be vulnerable to such drugs producing a so-called fibrogenic response. Various experiments have been conducted that have proven that fibroblasts from drug-induced gingival overgrowth patients showed an elevated level of collagen synthesis.[2,7,8] An up-regulation of collagen synthesis has also been observed in various studies when fibroblasts have been exposed to pro-inflammatory cytokines, coupled with the administration of the drug nifedipine, coming to the conclusion that gingival overgrowth occurs not due to one but a variety of factors. Lastly the role of MMPs, which have shown interference in the synthesis and functional capability of collagenases cannot be ruled out as also one of the variety of factors, which predispose a patient to gingival overgrowth.[2,8]

The main route of elimination of phenytoin is through hepatic oxidation under CYP2C9 (90%) and CYP2C19 (10%) metabolism. Therefore, the genetic

polymorphisms on CYP2C9 but naturally were selected. Phenytoin metabolism rate can be reduced by 25% to 50% depending on the individual genetic polymorphism and drug interaction through the same pathway.[9,10] The genetic polymorphisms on CYP2C19 have been selected and researched on by other studies but due to its relatively low frequency of only 10%, this study concentrated on polymorphism related to CYP2C9. Polymorphisms were analyzed by using Real Time Polymerase Chain Reaction (RT-PCR) and Polymerase Chain Reaction -Restriction Fragment Length Polymorphism (PCR-RFLP) methods.

The results of the present study revealed that CYP2C9*1*2 (heterozygous mutant) and CYP2C9*3/*3 (homozygous mutant) were identified with equal frequency in the study population. These findings point to an equal role of the heterozygous mutant and homozygous mutant alleles in altering the phenytoin metabolism in epileptic patients. The findings of the present study are in contrast to the findings of the previous in vitro studies, which suggested that a significant reduction was observed in the activity of CYP2C9 with the *3 allele as compared to *2 allele.[4] On the contrary, the findings of our study in the form of more frequent homozygous and heterozygous mutant alleles as compared to the wild type allele are in support of the findings of the study conducted by Aynacioglu *et al* in the Turkish population, which showed significantly increased phenytoin levels and significantly reduced p-HPPH/phenytoin ratios in individuals with CYP2C9*1/*2, *2/*2 and *1/*3 compared to *1/*1 genotypes.[11] The observations of this study do not pinpoint on any single allele to be responsible for altered phenytoin metabolism in epileptic patients and hence lend support to the existing non-unifying hypothesis behind gingival overgrowth observed in epileptic patients consuming phenytoin.

In the light of the findings of the present study it can be concluded that phenytoin-induced gingival overgrowth is an outcome of various factors, but the common factor in each of these cases seems to be the usage of drugs for the treatment of epilepsy. The CYP2C9 polymorphism is responsible for modification of the inflammatory response to phenytoin. The definitive treatment to such cases is drug substitution, which seems to be the easiest and safest norm, but having said that science today has advanced leaps and bounds and it is possible in today's day and age to determine individual single nucleotide polymorphism (SNP) profiles to identify differences in our DNA. The future holds great promise and in the coming years, one can expect breakthrough cutting-edge research in pharmacogenetics, using SNP profiles and other types of DNA analysis, to provide safer and more effective tailor-made individually exclusive drug therapies for each and every individual based on one's unique genetic profiling/fingerprint. The future is now, it is for the agencies responsible to identify and diversify into research methodologies that benefit the future of mankind making drugs safer and easier to use and not abuse.

References

1. Joshipura V. Sodium valproate induced gingival enlargement with pre-existing chronic periodontitis. J Indian Soc Periodontol 2012;16:278-81.
2. Charles N, Ramesh V, Babu KS, Premalatha B. Gene polymorphism in amlodipine induced gingival hyperplasia: A case report. J Young Pharm 2012;4:287-9.
3. Rosemary J, Surendiran A, Rajan S, Shashindran CH, Adithan C. Influence of the CYP2C9 & CYP2C19 polymorphisms on phenytoin hydroxylation in healthy individuals from south India. Indian J Med Res 2006;123:665-70.
4. Giancarlo GM, Venkatakrishnan K, Granda BW, von Moltke LL, Greenblatt DJ. Relative contributions of CYP2C9 and 2C19 to phenytoin 4-hydroxylation *in vitro*: Inhibition by sulfaphenazole, omeprazole, and ticlopidine. Eur J Clin Pharmacol 2001;57:31-6.
5. Human Cytochrome P450 (CYP) Allele Nomenclature Committee. (Accessed November 6, 2005, at http://www.imm.ki.se/cypalleles/cyp2c9.htm).
6. Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: A comprehensive review of the *in vitro* and human data. Pharmacogenetics 2002;12:251-63.
7. Johnson RB, Zebrowski EJ, Dai X. Synergistic enhancement of collegenous protein synthesis by human gingival fibroblasts exposed to nifedipine and interleukin-1-beta *in vitro*. J Oral Pathol Med 2000;29:8-12.
8. Correa JD, Queiroz-Junior CM, Costa JE, Teixeira AL, Silva TA. Phenytoin-induced gingival overgrowth: A Review of the molecular, immune, and inflammatory features. ISRN Dent 2011;2011:497850.
9. Twardowschy CA, Werneck LC, Scola RH, Borgio JG, De Paola L, Silvado C. The role of CYP2C9 polymorphisms in phenytoin-related cerebellar atrophy. Seizure 2013;22:194-7.
10. Twardowschy CA, Werneck LC, Scola RH, De Paola L, Silvado C. CYP2C9 polymorphism in patients with epilepsy: Genotypic frequency analyzes and phenytoin adverse reactions correlation. Arq Neuropsiquiatr 2011;69:153-8.
11. Aynacioglu AS, Brockmoller J, Bauer S, Sachse C, Guzelbey P, Ongen Z, *et al*. Frequency of cytochrome P450 CYP2C9 variants in a Turkish population and functional relevance for phenytoin. Br J Clin Pharmacol 1999;48:409-15.

How to cite this article: Charles NC, Chavan R, Moon NJ, Nalla S, Mali J, Prajapati A. Drug-Induced gingival overgrowth: The genetic dimension. North Am J Med Sci 2014;6:478-80.

Source of Support: Nil. **Conflict of Interest:** None declared.