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VKORC1 GENE POLYMORPHISM STUDY USING PCR-RFLP OF C.1639G>A LOCUS IN RURAL MAHARASHTRA POPULATION

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ABSTRACT

Introduction: The dose requirements for oral anticoagulants in thromboembolic events are influenced by promoter polymorphism in the VKORC1 gene. However, limited data are available on the influence of the polymorphism in various Indian populations. **Objectives:** The present study aimed to detect the VKORC1 polymorphism & its genotype distribution in study population. **Materials and Methods:** Fifty healthy subjects from the west rural Maharashtra region were genotyped for VKORC1-1639 G>A by polymerase chain reaction and restriction fragment length polymorphism. **Results:** The VKORC1 - 1639G>A allele frequency in the study population (n = 50) was observed. GA, GG, AA were found in range of 12 %, 6%, 82%. These findings are in concurrence with the effect of the polymorphism present at promoter region on vitamin K epoxide reductase activity. **Conclusion**: The wild, Heterozygous & variant homozygous genotypes of VKORC1 distribution in Maharashtra population is different from other known racial groups. This VKORC1-1639 G>A status can be indicative of establishing the therapeutic dose of oral anticoagulants in west rural Maharashtra population in India

KEYWORDS: Allele frequency, drug dose, genotype, polymorphism, VKORC1.

INTRODUCTION

Coumarin derivative warfarin is widely used in prophylaxis for thromboembolic events. Warfarin inhibits an enzyme vitamin K epoxide reductase, specifically the VKORC1 subunit, [1] we carried out this study in west Maharashtra population to find out the polymorphism of

VKORC1. Warfarin with narrow therapeutic index associated with pharmacokinetic & pharmacodynamic variations thus requiring therapeutic monitoring. [2] However, large inter-individual and intra-individual variation in responsiveness to warfarin indicates that subsets of patients are not attaining optimal therapy, this makes dosing problematic. Under-anticoagulation can result in thrombosis but over-anticoagulation can result in dangerous bleeding episodes. [3] These variations are also contributed by genetic constitution of a patient. Warfarin efficacy and safety critically dependent on maintaining the INR within the therapeutic range. [4] Recently, single nucleotide polymorphisms (SNPs) have been linked to the variations observed in efficacy and toxicity for warfarin. Common genetic variations in vitamin K epoxide reductase (VKOR) have been discovered to significantly influence the oral anticoagulant maintenance dose requirements, [5] Twenty-eight polymorphisms have been described in this gene among these VKORC1-1639 G>A is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. [6-7]

MATERIALS AND METHODS

Study conduct: The present study was carried out on healthy individuals. The recruitment of 50 subjects depends on eligibility of age 18 years to 65 years of both genders, willing and able to give written informed consent. Subjects not related to west rural Maharashtra were excluded.

Patients-Sample collection & DNA isolation

The Maharashtra ethnicity was based on place of residence in the last three generations, food habits and the mother tongue (Marathi). Blood samples were collected in ethylenediaminetetraacetic acid and genomic DNA was extracted from peripheral venous blood by using the standard Non enzymatic salting-out method. [8] The quality and quantity of DNA was checked by gel electrophoresis and spectrophotometer**.** The ratio of absorbance at 260 and 280 nm of DNA were between 1.6 and 1.9. The isolated DNA was stored at −80ºC.

Genotyping of VKORC1 (-1639G>A, rs9923231) allele by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method: The c.1639G > A polymorphism marks the VKORC1*2 haplotype, [9] and its association with increased response to acenocoumarol has been shown in a group of healthy subjects [10] and a single VKORC1 (-1639G>A) snp shown strongest influence on predictive dose of warfarin among 3 racial groups. [11]

Polymerase chain reaction

A fragment of size of 290 bp was amplified using forward and reverse primers. [12]

Amplification was carried out using a GeneAmp PCR System (Applied Biosystems, USA). The amplified DNA fragments were resolved on a 2% agarose gel spiked with Ethidium bromide. The gel was visualised under UV transilluminator and photographed using Gel documentation system (Kodak R-2000).

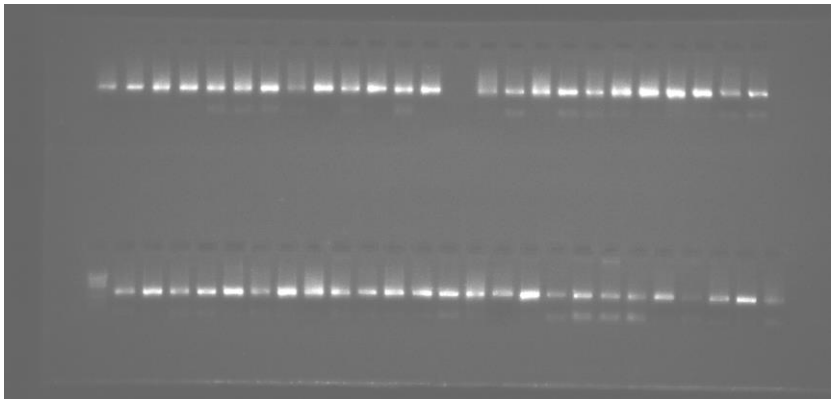

Fig.1: VKORC1 PCR Product; *Lane1:s1-s25, Lane2:well26 ladder,s27-s50*

RFLP Method

The RFLP Reaction using *MspI* restriction Enzyme from New England Biolabs. The digested PCR product was loaded on 3% agarose gel. The MspI enzyme digestion of this product produced the following profiles.

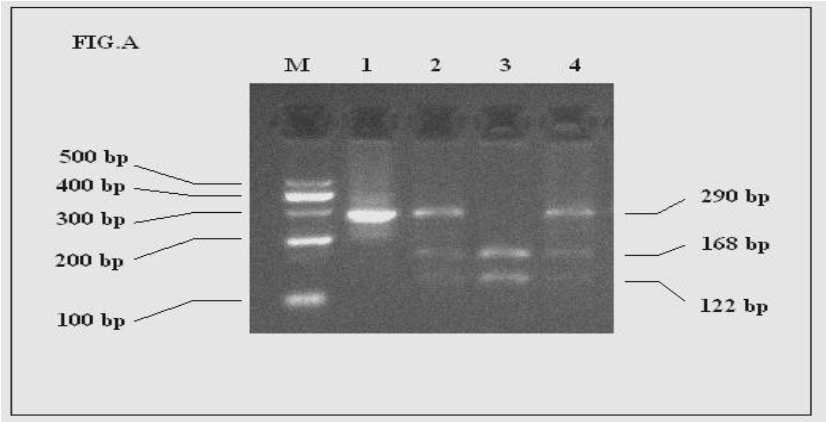

Fig.2: VKORC1 PCR RFLP

Lane M: 100-500 bp DNA ladder; Lane 1: Undigested PCR product; Lane 2, 4: MspI digested PCR products showing heterozygous mutant profile; Lane 3: MspI digested PCR product showing homozygous mutant profile.

RESULTS

Table.1: MspI Enzyme digestion profile

Genotype	Band pattern	Mutation
GG	290	Wild type
GA	290+168+122	Hetero mutant
AA	168+122	Homo mutant

VKORC1 1639-GA was the most abundant genotype of VKORC1 1639 allele. The two homozygote -GG and –AA genotypes had frequencies of 6% and 82% & GA heterozygous genotype had frequency of 12% of distribution in the study population.

Table.2: Frequency of VKORC1-1639 Allele

Genotype	Mutation	Total=50	Genotype distribution	Allele frequencies
GG	Wild type	3	(6%)	G = 12
GA	Heterozygous mutant	6	(12%)	
AA	Homozygous mutant	41	(82%)	A = 88

DISCUSSION

The required dose of warfarin to achieve the target level of anticoagulation is variable in individual to individual and depends on environmental factors & variations in pharmacokinetics as well as interfering pharmacotherapy. [13] Each population has its unique pattern of polymorphism in genes, playing a significant role in drug efficacy & safety. [14] As demonstrated in Table 1, the results of the present study on west Maharashtra population represented that patients with one mutated allele VKORC1 G1639A is presented. In this study, the frequency of VKORC1-1639AA is 82%. The frequency of VKORC1-1639 AA in Asians 90%, Caucasians 40-50%.[15] African-Americans 0% and Europeans 23%, respectively. [16]

Clinicians should not ignore the large amount of new information pertaining to pharmacogenetic testing and should apply it in the already complex process of individual's therapeutic decision-making particularly when the individuals' safety is a matter of concern. Use of genetic polymorphisms for CYP2C9 and VKORC1, environmental and clinical factors to estimate the warfarin dosage could potentially reduce the risk of overdose during warfarin therapy. Testing for VKORC1 polymorphism complemented with testing for CYP2C9 polymorphisms, may become one of the necessary standards prior to starting warfarin therapy.

CONCLUSION

Individualized medicine is becoming a reality in future medicine. The frequency of VKORC1-1639AA is 82% near to frequency of VKORC1-1639 AA 92% in Asians and differs with African-Americans and Europeans. Identifying these polymorphisms in patients prior to starting the therapy may help clinicians to choose the appropriate strategy to reduce adverse drug reactions such as bleeding, as well as for better therapeutic implication.

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