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The Study on Demographic factors and VKORC1- 1630 G>A gene Polymorphism by using PCR-RFLP in the South Indian Population

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ABSTRACT: Deep vein thrombosis, a disease with a high mortality risk. Thomboembolic conditions require oral anticoagulants. Warfarin is used to treat DVT, everybody has a distinct range of dose requirements, and dose variation is influenced by genetic factors. Variations in the dose requirements for warfarin are primarily caused by polymorphism of the gene for vitamin K epoxide reductase complex 1 (VKORC1). The data on promoter polymorphism is limited at present Indian population. The given study is aimed to detect VKORC1 polymorphism distribution in South Indian Population. Ninety six healthy individuals are taken from south Indian population and genotyped for VKORC1-1639 G>A by PCR-RFLP Method.

The VKORC1-1639G> A in the research group, a gene frequency was found. GG, GA, and AA were discovered in the range of 63.5%, 22.9%, and 13.5%. These results are consistent with how the mutation in the promoter region affects the function of vitamin K epoxide reductase. The prevalence of the wild, heterozygous, and variant homozygous alleles of VKORC1 in the South Indian community differs from that of other known ethnic groups. The therapeutic dosage of oral anticoagulants in the people of South India can be determined using this VKORC1-1639 G>A condition.

Keywords: Warfarin, Polymerase chain reaction, VKORC1 gene, polymorphism.

INTRODUCTION

The focus of pharmacogenomics and pharmacogenetics is on the interactions between genes and drugs. The most significant factor influencing variance in drug response is variation in pharmacogenetically relevant genes (Bala *et al.,* 2021). Warfarin is anticoagulant and therapeutic window is quite limited, and its effectiveness varies greatly between various people (Li *et al.,* 2022). Warfarin, a coumarin product, is frequently used in deep vein thrombosis, atrial fibrillation, atrial cardiac arrhythmias, heart valve disease, replacement of artificial heart valves, and orthopedic patients to prevent thromboembolism (Rad *et al.,* 2019; Sconce *et al.*, 2005). Serious side effects like haemorrhage or thromboembolism have been linked to either over- or under-dosing on warfarin, respectively (Yilmaz, 2019; Berling *et al.*, 2017; Sahin *et al.*, 2019). The blood clotting process depends on the gamma-carboxylation of vitamin K-dependent clotting factors such as FII- Prothrombin, F VII- stable factor, F IX- plasma thromboplastin component, and F X- Stuart Prower factor (Rad *et al.,* 2019). The alleles for the cytochrome P450 (CYP) 2C9 and VKORC1 have an impact on the pharmacodynamics and pharmacokinetics of warfarin, respectively (Colet *et al.,* 2021). The VKORC1 subunit of the enzyme vitamin K epoxide reductase is particularly inhibited by warfarin (Sajja *et al.*, 2014). This research was conducted to determine the polymorphism among south Indian population. Since warfarin has a limited therapeutic index and is accompanied by pharmacokinetic and pharmacodynamic variations, therapeutic monitoring is necessary (Ma and Lu 2011). The anticoagulant effect of warfarin, an oral anticoagulant is due to the inhibition of carboxylation of vit k dependent proteins (Hirsh and Dalen 1998). Over-anticoagulation can cause bleeding events while under-anticoagulation can cause thrombosis (Gullov *et al.*, 1994). About 90% of human variations are founded on single nucleotide polymorphism, which affects both its therapeutic efficacy and toxicity (Bala *et al.,* 2021). In Asian groups, polymorphism of the vitamin K epoxide reductase complex subunit 1 (VKORC1) gene promoter is linked to a reduction in warfarin dosage (Takahashi *et al.*, 2006). The body weight, age, food, drug, vitamin K intake, and social habits like drinking or smoking have an impact on the amount of warfarin that needs to be taken (Whitley *et al.*, 2007; Dang *et al.,* 2005; Walenga and Adiguzel 2010). The therapeutic efficacy and toxicity vary from individual to individual, and these human variations are due to single nucleotide polymorphisms (Marsh and McLeod 2006). Studies

have been conducted on genetic variants in the VKORC1 gene that are known to affect how sensitively warfarin therapy works (Shukla *et al.*, 2013; Rost *et al.*, 2004). Warfarin's target enzyme, vitamin K epoxide reductase, is encoded by the VKORC1 gene. Patients who have the polymorphism -1639G>A in the VKORC1 gene promoter are more responsive to warfarin and need fewer doses (Dean *et al.*, 2018). It is believed that the polymorphism, which affects a transcription factor binding site and lowers protein expression, appears in the promoter region of VKORC1. Because of this, patients beginning warfarin therapy who are 1639A carriers need lower starting and ongoing doses of the medication than 1639G carriers (Pharm, 2012). There have been 28 polymorphisms identified in this gene, and VKORC1-1639 G>A is one of them. This polymorphism is linked to inter-individual variation in the dose of warfarin (Yuan *et al.*, 2005; D'Andrea *et al.,* 2005). The VKORC1 gene has single nucleotide polymorphisms (SNPs) that correspond to three distinct haplotypes: haplotypes 2, 3, and 4. The most significant haplotype is VKORC1 *2, which is linked to inter-individual variation in the dose of warfarin's anticoagulant effect and a reduced dose of oral anticoagulant requirement (Vesa *et al*., 2016). The gene VKORC1 has been found to be the most significant predictor of the need for warfarin doses in a number of trials (Gage *et al.*, 2008).

MATERIAL AND METHODS

In the current research, 96 healthy individuals between the ages of 18 and 65 were included. There were 51 men and 45 women (Fig. 1). All participants in the research had provided Informed consent. This research was conducted in the year 2020 at the Gayatri Vidya Parishad Institute of Health Care and Medical Technology in Visakhapatnam, Andhra Pradesh, after the approval of Institutional Ethics Committee.

An expert conducted an initial discussion with each patient to gather clinical and demographic data such as age, gender, dietary habits (including intake of nutritional supplements, alcohol, and coffee), and weight. Age and weight were represented as a group and were also calculated as Mean (Table 1). Individuals who failed to give their informed consent form had risk factors like hypertension, diabetes mellitus, or liver conditions, or were taking CYP2C9 inducers or inhibitors were not included.

Sample gathering from patients and DNA isolation. 5mL of peripheral blood was drawn and placed in EDTA and sodium citrate for coagulation and molecular investigations.

We used the salting out method to separate the genomic DNA from white blood cells and for quantity and purity of the extracted DNA, agarose gel electrophoresis and spectrophotometer was followed. The pure DNA was stored at a temperature of -80 °C.

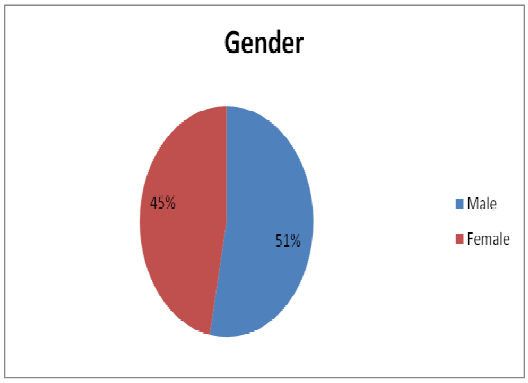

Fig. 1. Distribution of gender.

Table 1: Demographic details of the participants.

Patient Details	Test Group
Age In Groups	
20-30	32.3%
31-40	33.3%
41-50	21.9%
51-60	12.5%
61-70	0%
Mean	36.9
Weight In Groups	
30-45	10.4%
46-60	38.5%
61-75	51.0%
Mean	59.5

Polymerase chain reaction (PCR): The VKORC1 (-1639G>A, rs9923231) was genotyped using PCR and restriction fragment length polymorphism (RFLP). An amplified fragment of 290 nucleotides was created using forward and reverse primers. The primers used were: Premier v.5programs, the human-specific primers VKORC1-F, 5'-GCCAGCAGGAGGGAAATA-3', and VKORC1-R, 5'-AGTTTGGACTACAGGTGCCT- 3' were used. The following steps were used during the 35 cycles of the PCR process: denaturation for 5 min at 95°C followed by annealing for 45 s at 59°C and extension for 45 s at 72°C. PCR products were visualized on a 2% agarose gel stained with ethidium bromide (Fig. 2).

RFLP Method: MspI restriction Enzyme (Takara Bio Inc., India) was used for restriction fragment length polymorphism reaction. A 2% quantity of agarose gel was utilised for to visualize digested products. A 290 bp DNA segment for VKORC1 was amplified by PCR (Table 2).

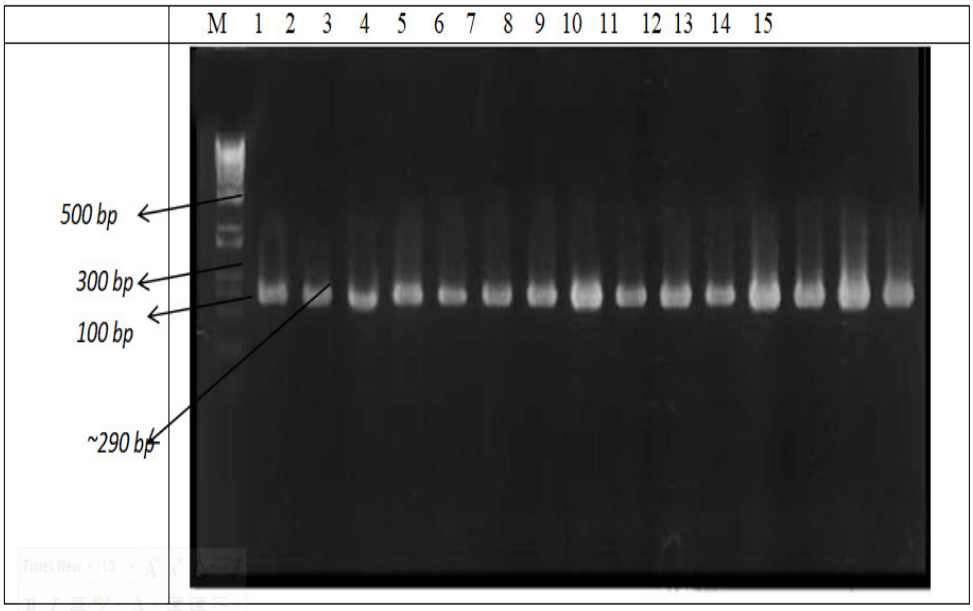

Fig. 2. VKORC1- PCR amplified bands on agarose gel.

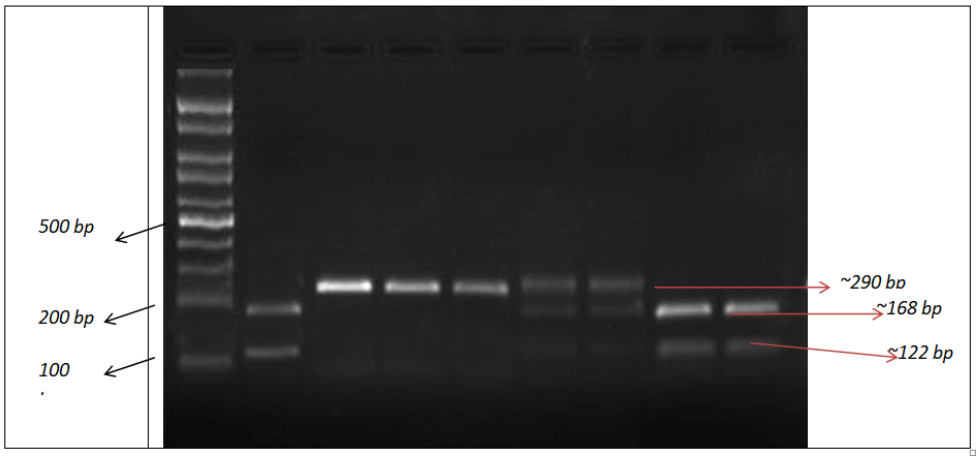

Fig. 3. Restriction digested banding patters of VKORC1 gene. Lane M: 100-500 bp DNA ladder; Lanes 1, 7& 8: showing homozygous mutant genotype; Lanes 2, 3, 4 showing undigested PCR products of homozygous genotype; and Lanes 5 and 6 showing heterozygous genotype.

RESULTS AND DISCUSSION

In the study population, the two homozygote genotypes GG and AA had frequencies of 63.5% and 13.5%, respectively, and the GA heterozygote genotype had a frequency of 22.9%.

The genotype GG, GA, and AA are homozygous wild, heterozygous, and homozygous mutant respectively. The allelic frequency G is 143 (75%), and A is 49 (25%) by Hardy weinberg equilibrium (HWE) (Table 3).

Table 2: Band pattern of VKORC1 by RFLP.

Genotype	Band Pattern	Mutation
GG	290	Wild Type
GA	290+122+168	Heterozygous mutant
AA	122+168	Homozygous mutant

Table 3: Genotype frequency of VKORC1.

Genotype	Mutation	Genotype frequency (%)	Allele Frequencies (%)
GG	Wild type	61(63.5)	G=143(75)
GA	Heterozygous mutant	22(22.9)	
AA	Homozygous mutant	13(13.5)	A = 49(25)

DISCUSSION

The effect of genetic variations in the CYP2C9 and VKORC1 alleles on a person's susceptibility to VTE is still unknown (Kumari *et al.*, 2019). This research aim to, assess the relationship between venous thrombotic events and prevalent mutations in these two genes. Individual's susceptibility to disease and drug response is significantly influenced by genetic factors and due to several risk factors like age, sex, vitamin K consumption, and medications. DVT has the potential to be a life-threatening disease (Arunkumar *et al.*, 2017). The anticoagulant medication, warfarin is used to both prevent and cure venous and arterial thromboembolic diseases (Ye *et al*., 2014; Keeling *et al.*, 2011). Bleeding is a frequent adverse effect of warfarin medication, particularly when the drug is first started (Rad *et al.,* 2019). Dosing for warfarin can be difficult because smaller and larger dosages may increase the chance of thrombosis and bleeding, respectively.

The most significant indicator of warfarin dosage is thought to be the VKORC1 gene (Gaikwad *et al.,* 2018; Pedersen *et al.,* 1991). Gene polymorphism in each group is distinct and has a major impact on the safety & effectiveness of medications (Wadelius and Pirmohamed 2007). The findings of the current research on the South Indian population are shown in Table 3 as belonging to the study group with the wild mutant form of allele VKORC1 -1639 G>A. In contrast to the population of this survey, the community of West Maharashtra is represented in their studies by GG 6%, GA 12%, and AA 82%. The other VKORC1 -AA frequency community is similar to the said study (Sajja *et al.*, 2014). Asians making up 90% of the population, Caucasians 40-50% (Ragia *et al.*, 2013), and other groups like Europeans having 23% and Americans having 0%, which is similar to our research (Ross *et al.*, 2010).

Before making treatment decision for patients clinicians should consider the enormous amount of recent data on pharmacogenetic testing and should use it for their treatment. Homozygous mutant (AA) people are extremely sensitive to warfarin and require a reduced dosage in comparison to heterozygous mutant (GA) individuals who are weakly sensitive to warfarin and require an intermediate dose. Individuals with homozygous wild-type GG are resistant to warfarin, necessitating a larger dosage of the medication. Utilizing CYP2C9 and VKORC1 genetic polymorphisms, as well as environmental and clinical variables, to determine warfarin doses may help to lower the risk of overdosing while on warfarin treatment. Before beginning warfarin treatment, it might be essential to perform CYP2C9 SNP and VKORC1 variant checks. The pharmacogenetic data helps physicians improve patient health by lowering the likelihood of negative drug reactions or decreasing the number of hospital admissions to lower treatment expenses (Gaikwad *et al.,* 2018).

CONCLUSIONS

Future medical practice will increasingly focus on individualized care. The frequency of VKORC1-1639 AA is 13.5%, which is close to its 23% frequency in Europeans, but it varies from that of Asians and Caucasians. The identification of these polymorphisms in patients by physicians may be useful before the start of therapy. They may use this information to determine the best course of action to reduce unpleasant drug side effects like bleeding and to enhance the curative impact.

FUTURE SCOPE

Pharmacogenetic information can assist doctors in treating patients more effectively while also lowering total healthcare costs by minimizing hospital stays and adverse drug reactions.

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Conflict of Interest. None.

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