

DO VKORC1 AND CYP2C9 MUTATIONS LEAD TO WARFARIN RESISTANCE? VKORC1 VE CYP2C9 MUTASYONLARI WARFARİN REZİSTANSINA YOL AÇAR MI?

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ÖZET

Çalışmanın amacı, VKORC1 ve CYP2C9 polimorfizmlerinin varfarine dirençli hastalar üzerindeki etkisini belirlemektir. Varfarin direnci, ilaç tipik dozlarda verildiğinde protrombin süresini uzatamama veya INR'yi 2 terapötik aralığa yükseltmemeye olarak tanımlanır. Bazı VKORC1 ve CYP2C9 varyant alellerinin bu durumlarla ilişkili olduğu bilindiği için polimorfizmler bir rol oynayabilir. Çalışmamıza 15 mg/gün'den fazla varfarin kullanan, INR değeri 2.1'in altında olan ve varfarin kullanırken tromboembolik olay gelişen 28 hasta alındı. 28 hastanın 15'inde VKORC1 geninde heterozigot mutasyon tespit edildi. Yedi hastada CYP2C9 geninin heterozigot mutasyonu vardı ve bu, varfarinin ultra hızlı metabolizmasına karşılık gelebilir. VKORC1 ve CYP2C9 polimorfizmi, hastalar arasındaki farklı doz gereksinimine katkıda bulunur, ancak diğer olası faktörler farklı ırklarda rol oynayabilir. Hekimlerin varfarin tedavisine başlamadan önce bu testleri kullanmalarını ve tedavi sürecini bu sonuçlara göre şekillendirmelerini öneriyoruz.

Anahtar Kelimeler: Genler, Mutasyon, İlaç Direnci, Varfarin

ABSTRACT

The objective of this study was to determine the influence of VKORC1 and CYP2C9 polymorphisms on warfarin resistant patients. Warfarin resistance is described as the inability to prolong the prothrombin time or raise the INR up to the 2 therapeutic range when the drug is given at typically doses. Polymorphisms may play a role as some VKORC1 and CYP2C9 variant alleles are known to be associated with these circumstances. 28 patients who were taking warfarin more than 15 mg/day and had INR values below 2.1 and had thromboembolic events while using warfarin was enrolled in this study. Heterozygote mutation in the VKORC1 gene was identified in 15 of 28 patients. Seven patients had heterozygote mutation of CYP2C9 gene, and that may correspond to ultrarapid metabolism of warfarin. VKORC1 and CYP2C9 polymorphism contribute to the difference dose requirement amongst the patients, but other additional possible factors may play a role in different races. We suggest that medicians may use this tests before starting warfarin therapy and shape the treatment course according to this results.

Keywords: Genes, Mutation, Drug Resistance, Warfarin

1. INTRODUCTION

Warfarin, a coumarin derivative and the most frequently prescribed oral anticoagulant agent globally, is characterized by a narrow therapeutic index and wide inter-individual dose-response variability. Achieving the optimal balance between increased risk of anticoagulation and bleeding against low anticoagulation and clotting risk requires a careful therapeutic management strategy.

The safety and effectiveness of warfarin therapy are followed with prothrombin time expressed as the international normalized ratio (INR). Warfarin resistance has been described as the inability to prolong the prothrombin time or raise the international normalized ratio (INR) into the therapeutic range when the drug is given at typically doses.⁽¹⁾ Patients who require more than 105 mg per week (15 mg/day) for maintaining a therapeutic INR should be considered warfarin resistant. Warfarin resistance can be practically classified as acquired or hereditary.⁽²⁾ Hereditary factors are caused by genetic factors that result either in faster metabolism of the drug (pharmacokinetic resistance) or lower activity of the drug (pharmacodynamic resistance). Polymorphisms may play a role as some vitamin K epoxide reductase complex subunit 1 (VKORC1) and cytochrome P450 2C9 (CYP2C9) variant alleles are known to be associated with these circumstances.⁽³⁾

The objective of this study was to determine the influence of VKORC1 and CYP2C9 polymorphisms on warfarin resistant patients.

2. MATERIALS AND METHODS

All patients gave informed consent. The study was conducted in accordance with the policies and procedures of the Education and Planning Committee of our hospital. Thirty-three patients who were taking warfarin more than two months and more than 15 mg/day and had INR values below 2.1 and had thromboembolic events while using warfarin was enrolled in this study. All clinical data of the 33 patients were collected. A full drug, diet and concomitant disease history of patients (hepatic dysfunction, cancer, advanced heart failure, liver disease, renal disease, hypothyroidism, hyperthyroidism, and diseases with bleeding tendency) were explored. 5 patients who were receiving any of the following medications that could potentially interact with warfarin were also excluded from the study: antiepileptic including phenytoin(1 patient); antifungal including fluconazole (1 patient); antiarrhythmic including amiodarone (2 patients) and oral contraceptive (1 patient). Finally, 28 patients enrolled in the study. The time to therapeutic INR (INR of the prothrombin time within the range of 2.5–3.5 for valve replacement groups, and the range of 2.0–3.0 for the other indications [deep vein thrombosis and atrial fibrillation]) were recorded. Ethics committee permission is available with the number E-92198657.02 and the date 07.10.2021

2.1. Laboratory testing

Before the patients were placed on warfarin treatment, blood was drawn for CYP2C9 and VKORC1 genotyping, INR was measured, and clinical variables were recorded. INR was measured using Thromborel S (Dade- Behring, Marburg, Germany), with an international sensitivity index of 0.91. Deoxyribonucleic acid (DNA) samples from these patients were genotyped for polymorphisms in VKORC1 and CYP2C9 genes. According to our clinic's cardiovascular risk panel screening protocol the following mutations were studied in each patient routinely: FV G1691A (Leiden), FV H1299R (R2), Prothrombin G20210A, factor XIII V34L, β - Fibrinogen –455 G-A, PAI-1 4G-5G – 5G/5G, GPIIIa L33P (HPA-1)- 1a/1a, MTHFR C677T, MTHFR A1298C , ACE I/D – D/D, ApoB R3500Q, ApoE (E2,E3,E4) – E3/E3.

2.2. Statistical analysis

SPSS (version 15.0, SPSS, Chicago, Illinois, USA) is used for the statistical analysis. Quantitative variables were expressed as the mean value \pm SD or median (minimum-maximum), and qualitative variables were expressed as percentages (%).

3. RESULTS

The present study included 28 patients receiving warfarin therapy for valvular heart diseases, AF, and DVT. The demographic characteristics of patients are shown in Table. There were 18 male and ten female patients, and the mean age was 54 ± 13 years. 12 patients used warfarin for mechanical heart valves (7 patients for aortic valve replacement and five patients for mitral valve replacement),

nine patients used for atrial fibrillation and other 7 used warfarin for deep venous thrombosis. Ten patients had embolic cerebrovascular events, and five patients had stuck heart valves and the other 6 six patients had transient ischemic attacks under warfarin treatment. The other seven patients had recurrent pulmonary embolic events. Mean INR of patients was 1.9 ± 0.7 while using mean dose 22 ± 4 mg of warfarin. Heterozygote mutation in the VKORC1-1639 G>A gene was identified in 15 of 28 patients. This type of mutations can be classified as pharmacodynamic warfarin resistance. Seven patients had heterozygote mutation of CYP2C9 gene, and that may correspond to ultrarapid metabolism of warfarin. This type of mutations in CYP2C9 gene may be associated with higher than normal activity and may be classified as pharmacokinetic resistance. Duplication or multiplication of cytochrome P450 enzyme genes has been described as contributing to the phenotype of ultrarapid metabolism.⁽⁴⁾

Additionally, none of the genetic mutations related to cardiovascular risk analysis protocol were described.

Table: Characteristics of the study group

Age (Years)	54±13
Daily Warfarin Dose (mg)	22±13
Male	18
Female	10
Warfarin Indications	
*AF	9
*DVT	7
*AVR	7
*MVR	5
CYP2C9 gene Heterozygote mutation	7
VKORC 1-1639 gene Heterozygote mutation (G>A)	15

4. DISCUSSION

Along with the advances in the field of pharmacogenetics, individualized treatment and dose approaches have been the subject of considerable interest in recent years. A complete control over the occurrence of hemorrhagic or thromboembolic complications during warfarin treatment can potentially be achieved through pharmacogenetic studies. Thus, in the present study, INR levels and thromboembolic complications have been monitored and described in a group of patients requiring warfarin treatment and the association between the polymorphisms of the genes responsible for warfarin metabolism, i.e. VKORC1 and CYP2C9, and warfarin resistance observed in certain patient groups has been examined. All participants had complete negative set of markers for thrombophilia.

Warfarin is a specific inhibitor of vitamin K epoxide reductase (VKOR) encoded by the VKORC1 gene. The anticoagulant action of warfarin occurs through its ability to inhibit the capacity of VKORC1 in producing the reduced form of vitamin K from its epoxide form. Reduced vitamin K is an essential cofactor for the enzyme gamma-glutamyl carboxylase (GGCX), which is responsible for the post-translational gamma-glutamyl carboxylation of the vitamin-K related coagulation factors, i.e. II, VII, IX, and X. In this way, warfarin reduces coagulation by preventing the functional maturation of vitamin K-associated coagulation factors. Functional abnormalities of VKORC1 are known to represent a form of resistance to coumarin-type anticoagulant agents (warfarin resistance). A number of different genetic polymorphisms may occur in VKORC1. One such is represented by the 1639 G >A polymorphism in the promoter region of the vitamin K-epoxide reductase complex subunit 1 gene, leading to an alteration in warfarin sensitivity of the individual and increased dose requirements.^(5,6,7) The study population consisted of a total of 21 patients requiring warfarin due to cardiac causes (mechanical valve replacement) as well as seven

patients requiring warfarin due to vascular conditions (DVT). All patients were on regular warfarin therapy. Our results have shown that 15 patients (53.6%) were GA heterozygous for VKORC1-1639 polymorphism. Also, there was an association between thromboembolic complications and VKORC1 mutation in this patient group.

INR assessment is utilized to achieve and monitor an efficient and safe dose range during warfarin treatment.⁽⁸⁾ Marusic et al. reported an 80-year old patient with deep venous thrombosis who had no increase in INR despite 21 mg of daily warfarin after LMWH. 1173C>T-C/C and -1639G>A- G/G alleles were detected upon examination of VKORC1 gene mutation due to suspicious warfarin-resistance, and only after acenocoumarol treatment was commenced the desired INR could be reached.⁽⁹⁾ In our study, patients without an increase in INR had the same mutation. Treatment was continued with LMWH, and no thrombosis or bleeding episode was observed during the follow-up.

Warfarin is a mixture of the R- and S- enantiomers, which are metabolized by the hepatic cytochrome enzymes. Under stable conditions S- and R- enantiomers are responsible for approximately 60 to 70% and 30 to 40% of the anticoagulant response, respectively. The primary enzyme metabolizing S-warfarin is CYP2C9, while R-warfarin is mostly metabolized by CYP3A4, 1A2, and 1A1. Genetic variations in CYP2C9, 3A4, 1A2 and 1A1 may result in individual variations for potentially useful warfarin doses and isomer CYP2C9 represents the most studied of this enzyme family.⁽¹⁰⁾ The direct association between CYP2C9 genotype and anticoagulant status or bleeding was initially reported by Higashi et al.⁽¹¹⁾ In a subsequent meta-analysis, patients with CYP2C9*2 or CYP2C9*3 allele were found to require lower warfarin maintenance doses.⁽¹²⁾

In this study, a total of 7 patients, who had a thromboembolic event and in whom warfarin treatment was indicated, CYP2C9 mutations were detected. CYP2C9 gene mutations are mostly known to be associated with increased sensitivity to warfarin but in our study we found that its mutations are also associated with warfarin resistance. Parallel to our study there are several studies that suggest possession of a variant allele is associated with an increased risk of warfarin resistance.^(13,4)

In the present study, while individual patients were sustaining a thromboembolic event during warfarin treatment had CYP2C9 mutations, others had VKORC1 G1639A gene mutations. Therefore, thromboembolic events seem to have occurred as a result of both pharmacokinetic and pharmacodynamics resistance mechanisms involved the metabolic pathways of warfarin.

Warfarin is a widely used anticoagulant that has a narrow therapeutic range because of both genetic and environmental factors. Genetic factors make a significant contribution to the wide interindividual variation in warfarin dose requirement. VKORC1 and CYP2C9 polymorphism contribute to the difference dose requirement amongst the patients,⁽¹⁴⁾ but other additional possible factors may play a role in different races. In our study, the diagnostic kit we used for identifying these mutations costs 7 euros per patient. We suggest that medicians may use this tests before starting warfarin therapy and shape the treatment course according to this results. If the patients have hereditary warfarin resistance, there are alternative treatment options for increasing the warfarin doses until the prothrombin time and INR are in the therapeutic ranges or using other types of anticoagulants.

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