The Influence of Genetic Polymorphisms on Warfarin Dosage Requirements in Cardiac Valve Surgery Patients

Abstract

Aim: Warfarin, a widely prescribed anticoagulant, exhibits considerable variability in patient response, making its clinical use challenging due to a narrow therapeutic window. This study aimed to evaluate the prevalence of CYP2C9 and VKORC1 gene polymorphisms in a cohort of 87 Turkish patients who underwent cardiac valve surgery and received warfarin therapy, as well as to assess their impact on warfarin dosage requirements.

Methods: The frequencies of CYP2C9 and VKORC1 polymorphisms were analyzed, and patients were stratified based on the presence or absence of mutations affecting warfarin dosing.

Results: Revealed that patients carrying at least one CYP2C9 or VKORC1 polymorphism required a significantly lower weekly warfarin dose to achieve the optimal international normalized ratio (INR).

Conclusion: This study highlights the critical role of genetic factors in determining warfarin dosage and supports the integration of pharmacogenetic testing into clinical practice to personalize warfarin therapy. Such an approach has the potential to enhance treatment outcomes and minimize the risk of adverse events. Further research involving larger sample sizes and diverse patient populations is warranted to validate these findings and refine the current understanding of the genetic determinants of warfarin dosing.

Keywords: Warfarin; CYP2C9; VKORC1; genetic polymorphisms; cardiac valve surgery; Turkish population.

Özet

Giriş: Yaygın olarak reçete edilen bir antikoagülan olan varfarin, hasta yanıtında önemli farklılıklar göstererek, dar terapötik pencere nedeniyle klinik kullanımını zorlaştırmaktadır. Bu çalışmanın amacı, kalp kapak ameliyatı geçiren ve warfarin tedavisi alan 87 Türk hastadan oluşan bir kohorttaki CYP2C9 ve VKORC1 gen polimorfizmlerinin prevalansını ve bunların warfarin dozaj gereksinimleri üzerindeki etkisini değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: CYP2C9 ve VKORC1 polimorfizmlerinin sıklıkları analiz edildi ve hastalar, warfarin dozunu etkileyen mutasyonların varlığı veya yokluğuna göre sınıflandırıldı.

Bulgular: En az bir CYP2C9 veya VKORC1 polimorfizmi taşıyan hastaların, optimal uluslararası normalleştirilmiş orana (INR) ulaşmak için önemli ölçüde daha düşük haftalık warfarin dozuna ihtiyaç duyduğu ortaya çıktı.

Sonuç: Bu çalışma, warfarin dozajının belirlenmesinde genetik faktörlerin kritik rolünü vurgulamakta ve varfarin tedavisini kişiselleştirmek için farmakogenetik testlerin klinik uygulamaya entegrasyonunu desteklemektedir. Böyle bir yaklaşımın tedavi sonuçlarını iyileştirme ve olumsuz olay riskini en aza indirme potansiyeli vardır. Bu bulguları doğrulamak ve varfarin dozajının genetik belirleyicilerine ilişkin mevcut anlayışı geliştirmek için daha büyük örneklem boyutlarını ve farklı hasta popülasyonlarını içeren daha fazla araştırma yapılması gerekmektedir.

Anahtar kelimeler: Varfarin; CYP2C9; VKORC1; genetik polimorfizmler; kalp kapak ameliyatı; Türk popülasyonu.

1.Introduction

Warfarin, a commonly prescribed anticoagulant worldwide, is administered to prevent and manage thromboembolic incidents. Roughly 0.5-1.5% of individuals are estimated to receive this medication.[1-3]

However, its clinical use is complicated by considerable individual variability in response, a narrow therapeutic window, and the risk of severe bleeding or stroke events, which are influenced by a myriad of environmental and genetic factors.[4-6] Although various models have been proposed for calculating warfarin dosage, incorporating both clinical and genetic markers (VKORC1 and CYP2C9 genotypes), their practical application in a clinical setting remains a subject of ongoing debate.[7,8]

Variations in warfarin response among individuals can be traced back to genetic polymorphisms present in the CYP2C9 and VKORC1 genes.[9] Accounting for gene polymorphisms, age, and body surface area, daily dosage requirements for individuals can deviate by approximately 50%.[8,10] Warfarin is comprised of two enantiomers, S and R, with S-warfarin being the more potent enantiomer, contributing to 60-70% of the anticoagulant effect.[11]

Genetic variations in the CYP2C9 enzyme can affect drug metabolism by altering its ability to convert S-warfarin into inactive metabolites.[12] The gene responsible for encoding this enzyme is located on chromosome 10q24.2 in humans [13], and eight distinct single nucleotide polymorphisms (SNPs) have been identified within it, all of which reduce CYP2C9 activity levels.[14] Patients possessing the *2 or *3 variants of this gene require lower warfarin dosages due to their decreased enzymatic activity, resulting in less efficient S-warfarin metabolism overall.[15] Additionally, CYP2C9 polymorphisms *5, *6, *8, and *11 have been shown to significantly delay S-warfarin metabolism.[16]

The crucial role of the vitamin K cycle is fulfilled by the enzyme VKORC1, which facilitates the restoration of reduced vitamin K (KH2) and plays a vital part in clotting factor synthesis.[17] Warfarin exerts its anticoagulant effect by impeding the enzymatic function of VKORC1, thereby obstructing the apt maturation process of vitamin K-dependent clotting factors.[18]

VKORC1 is located on chromosome 16p11.2 [19], and several genetic variants have been linked to altered warfarin metabolism.[20]

The -1639G > A polymorphism is observed to decrease the expression of VKORC1, resulting in a reduction in both warfarin metabolism and coagulation factors. This phenomenon has been duly noted.[9]

This study aimed to investigate the occurrence rates of genetic variations in CYP2C9 and VKORC1 among patients who received warfarin treatment after undergoing surgery for heart valve disorders. Additionally, it sought to evaluate the potential influence of these gene variations along with pertinent clinical factors on the necessary dosage of warfarin.

2. Materials and Methods

2.1 Study subjects:

A total of 87 patients (41 females, 46 males) under warfarin maintenance therapy for cardiac valve operation in a tertiary state hospital between January 2018 and January 2022 were included in the study. The study was approved by local Clinical Research Committee. Written informed consent was obtained from all patients included in the study. Blood samples were taken from each patient and transferred to Department of Medical Genetics for genetic analysis. Clinicians were not aware of the patients' CYP2C9 and VKORC1 genotypes prior to anticoagulant therapy initiation. Beta adrenergic receptor blockers, statins and proton pump inhibitors were the frequent additional therapies used by the study population. Patients with liver and kidney dysfunction, malignancy, pregnancy and lactation, or those taking medications known to interfere with warfarin metabolism were excluded from the study.

2.2 Genotype analysis:

Genomic DNA was extracted from peripheral blood sample using HiPure Blood DNA Mini Kit according to the manufacturer's protocol. Next Generation Sequencing (NGS) test was performed using Illumina MiSeq NGS platform (Illumina Inc., San Diego, CA, USA). The test platform screened targeted mutations on *CYP2C9* named 430C>T (haplotype CYP2C9*2), 1075A>C (haplotype

CYP2C9 *3), 1076 T>C (haplotype CYP2C9 *4), 1080C>G haplotype (CYP2C9 *5), 817delA (haplotype CYP2C9 *6) and c.1003C>T (haplotype CYP2C9*11) and also mutations on VKORC1 gene named c.173+1000 (haplotype VKORC1*2), c.492+134 (haplotype VKORC1*3) and c.173+525 (haplotype VKORC1*4). The variants that passed through the filters were analyzed with Sequencing Analysis Viewer (SAV) Software, Illumina and The Integrative Genomics Viewer (IGV) according to the pathogenicity scores and *in-silico* prediction tools.

2.3 Statistical analysis:

Descriptive statistics were used to present the baseline characteristics of the study population. The frequencies of CYP2C9 and VKORC1 polymorphisms were calculated, and the Hardy-Weinberg equilibrium was assessed. Patients were grouped according to the presence or absence of mutations requiring a reduction in warfarin dose. Comparisons of the median weekly warfarin doses for reaching the ideal INR between the two groups were performed using an independent samples t-test. The statistical significance was determined by a p-value lower than 0.05, with the aid of a commercially accessible software package used for all analyses.

3. Results

The study comprised 87 patients, consisting of 46 males, who had cardiac valve replacement between January 2018 – January 2022. Were included in the study. The baseline clinicopathological characteristics of the patients are listed in Table1. The mean age of the patients was 55±13 years (range: 19–80 years). The mean BMI of the patients was 28.5±4.5 (range: 19.1–42.3). Among the patients, 30 had hypertension (23%), 30 had diabetes (34.5%), 16 had atrial fibrillation (18.4%). Regarding the type of cardiac valve replacement surgery, 46 (52.9%) had aortic valve replacement (AVR), 32(36.8%) had mitral valve replacement (MVR), and 9 (12.3%) had both AVR and MVR. The mean weakly warfarin dose for reaching ideal INR was 37.47±17.12 mg (range: 8.75-85 mg).

The CYP2C9 and VKORC1 gene polymorphism frequencies for the whole study group are given in Table 2. The observed frequencies of CYP2C9 *1/*1, *1/*2, *1/*3, *2/*2, *3/*3 and *1/*11 genotypes were respectively as 60.9% (n = 53), 17.2% (n = 15), 17.2% (n = 15), 1.2% (n = 1), 2.3%

(n=2) and 1.1% (n=1) (Table 2). VKORC1 frequencies were 25.3% (n=22) for *2/*2, 31% (n=27) for *2/*3, 19.5% (n=17) for *3/*4 (Table 2).

Normal coumadin dose is ordered for *1/*1 CYP2C9 mutation while decreased dose is ordered for other mutations. For VCORC1 mutations dose decrease is ordered for *2 mutations. So, patients were grouped according to dose requirements. 19 of the patients had mutations of both genes who did not need dose decrease while 23 patients had mutations in both genes requiring dose decrease.

Patients with and without mutations in any gene that required a reduction in warfarin dose were analyzed in two groups. 19 of the patients had none of the mutation (Group 1) while 68 of the patients had at least one mutation (Group 2). Median dose for reaching ideal INR was compared between two groups and it was 50.39±15.62 mg/week vs 33.86±15.81 mg/week: p=0.00 (Figure 1).

4. Discussion

This study aimed to determine the frequencies of *CYP2C9* and *VKORC1* polymorphisms in patients who underwent cardiac valve surgery and received warfarin treatment, as well as to assess the potential effects of these genetic variants and clinical factors on the required warfarin dose. According to the findings, individuals with one *CYP2C9* or *VKORC1* polymorphism necessitated a notably lower weekly dose of warfarin to achieve the optimum INR. This underscores the criticality of factoring in genetic components when determining warfarin dosage.

In our cohort, the most frequent *CYP2C9* genotype was *1/*1, followed by *1/*2 and *1/*3. These findings are consistent with previous studies conducted in different populations, where the wild-type *CYP2C9* *1/*1 is the most common genotype, and *1/*2 and *1/*3 are the most frequent variants.[21] The VKORC1 genotype distribution in our study is also in line with previous research, which demonstrated a high prevalence of *VKORC1* *2/*2 and *2/*3 genotypes among patients taking warfarin.[22,23]

Our study found that patients with at least one *CYP2C9* or *VKORC1* polymorphism (Group 2) required a significantly lower weekly warfarin dose to achieve the target INR compared to patients without any mutations (Group 1). This observation is consistent with previous reports indicating that carriers of

CYP2C9 *2 and *3 alleles, as well as *VKORC1* variant alleles, have reduced enzyme activity and consequently require lower warfarin doses to avoid excessive anticoagulation and related complications.[24] Our findings underscore the importance of genotyping patients for *CYP2C9* and *VKORC1* polymorphisms to optimize warfarin dosing and minimize the risk of adverse events.

In addition to genetic factors, our study also considered clinical factors that could influence warfarin dosing, such as age, BMI, comorbidities, and type of cardiac valve replacement surgery. These factors have been reported to impact warfarin dose requirements and treatment outcomes in previous studies.[25] Further research is needed to elucidate the complex interplay between genetic and clinical factors in determining the optimal warfarin dose for individual patients.

There are some limitations to our study. First, the sample size was relatively small, which may have limited the statistical power to detect small differences in warfarin dose requirements among different genotype groups. Second, our study population was restricted to patients who underwent cardiac valve surgery, so the results may not be generalizable to other patient populations receiving warfarin therapy. Finally, other genetic polymorphisms not assessed in this study may also contribute to warfarin dose variability and warrant further investigation.

In conclusion, our study demonstrates the significant impact of *CYP2C9* and *VKORC1* polymorphisms on warfarin dose requirements in patients who have undergone cardiac valve surgery. These findings support the integration of pharmacogenetic testing into clinical practice to personalize warfarin therapy, thereby improving treatment outcomes and reducing the risk of adverse events. Further studies with larger sample sizes and diverse patient populations are warranted to validate our findings and refine the current understanding of the genetic determinants of warfarin dosing.

5. References

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Table 1. The distribution of clinicopathological characteristics of the study group.	
Variables	**n =87**
Sex, Males (%)	46(52.9%)
Age (years)	55± 13 (19–80)
BMI (kg/m^2)	28.5± 4.5 (19.1–42.3)
Cigarette use, n (%)	33(37.9%)
INR	2.6± 0.5 (2.1–3.7)
Dose mg/month, n (%):	37.47± 17.1 (8.75–85)
Concomitant disease, n (%):	
Hypertension	20(23%)
Type 2 DM	30(34.5%)
Atrial fibrillation	16(18.4%)
MVR	32(36.8%)
AVR	46(52.9%)
AVR-MVR	9(12.3%)
Concomitant medications, n (%):	
PPI	32(36.8%)
Statins	46(52.9%)
Metoprolol	55(65.2%)
INR: International normalization ratio of prothrombin time, AVR: aortic valve replacement, MVR: mitral valve replacement	

Table 2. CYP2C9 and VKORC1 genotype frequencies of patients.	
Genotype	**Genotype frequency, n (%)**
CYP2C genotype	
***1/ *1**	**53(60.9%)**
***1/ *11**	**1(1.1%)**
***1/ *2**	**15(17.2%)**
***1/ *3**	**15(17.2%)**
***2/ *2**	**1(1.1%)**
***3/ *3**	**2(2.3%)**
VKORC1 genotype	
***1/ *3**	**2(2.3%)**
***1/ *4**	**2(2.3%)**
***2/ *2**	**22(25.3%)**
***2/ *3**	**27(31%)**
***2/ *4**	**8(9.2%)**
***3/ *3**	**5(5.7%)**
***3/ *4**	**17(19.5%)**
***4/ *4**	**4(4.6%)**

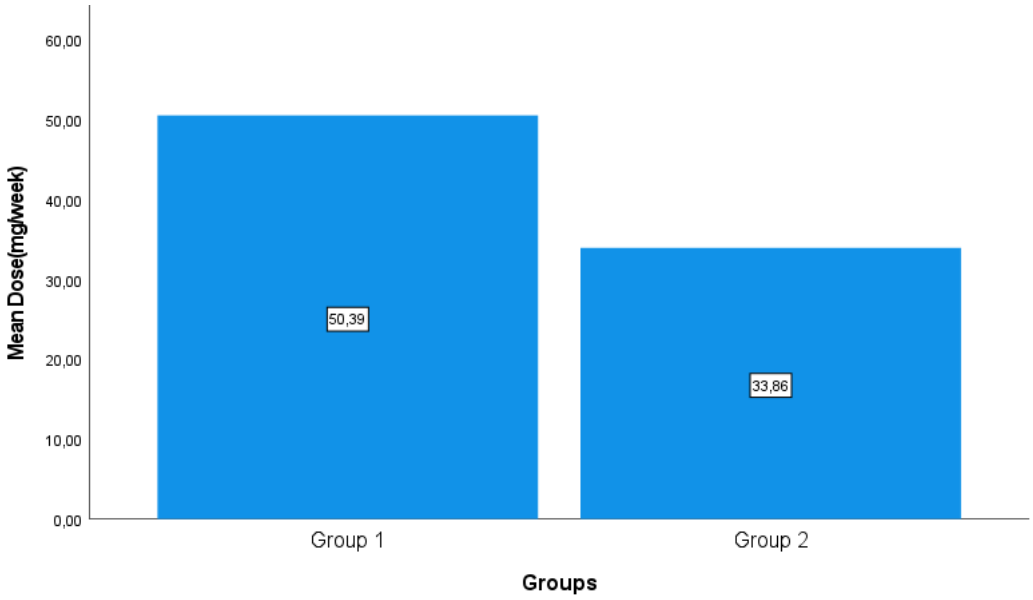

Figure 1: mean weekly doses of groups