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Research

The Relationship Between Warfarin Resistance and CYP2C9*2 and CYP2C9*3 Variatıons

Varfarin Direnci CYP2C9*2 ve CYP2C9*3 Varyasyonlarıyla İlişkili Olabilir

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ABSTRACT

Objective: Warfarin is one of the most widely used anticoagulants worldwide. Some patients need >15 mg/day of warfarin to get their therapeutic international normalized ratio (INR). This condition is known as warfarin resistance (WR). WR is related to enzyme deficiencies, which play a role in warfarin metabolism. One of the most important enzyme-related drug metabolism is the cytochrome P450, family 2, subfamily C, member 9 (*CYP2C9*). Therefore, this study aimed to investigate the relationship between *CYP2C9* variations and WR.

Methods: To find patients with WR, 650 patients who used warfarin for at least 6 months were screened. Then, patients were grouped into two according to the INR values, wherein 30 patients with INR levels not reaching the therapeutic range (<2) despite using 15 mg of warfarin per day were included in the non-responder group and 30 randomly selected patients who received low-dose warfarin, whose INR levels were within the therapeutic range (2-3), were included in the responder group. After the genomic deoxyribonucleic acid isolation from the peripheral blood, *CYP2C9*2* and *CYP2C9*3* variations were investigated using the real-time polymerase chain reaction. Results were statistically evaluated.

Results: Heterozygous genotype of *CYP2C9*3* was statistically high in responders (33.3%), whereas the wild-type genotype was statistically high in nonresponders (90%) ($p<0.05$). In addition, the T allele of *CYP2C9*2* (18.3%) and the C allele of *CYP2C9*3* (16.7%) were statistically high in responders ($p<0.05$).

Conclusion: Patients with gene variations that reduced the *CYP2C9* activity are termed poor metabolizers. These individuals metabolize warfarin more slowly and require smaller doses of the drug to reach the therapeutic INR values. Therefore, adjusting the warfarin dose is possible depending on the genotype of patients.

Keywords: Warfarin resistance, *CYP2C9*, RT-PCR

ÖZ

Amaç: Varfarin, dünyada en yaygın kullanılan antikoagülanlardan biridir. Bazı hastalar, uluslararası normalize oranlarını (INR) terapötik aralığa getirmek için günde 15 mg'dan fazla varfarine ihtiyaç duyar. Bu durum, varfarin direnci (VD) olarak bilinir. VD, varfarin metabolizmasında rol oynayan enzim eksiklikleri ile ilişkili olabilir. İlaç metabolizması ile ilgili en önemli enzimlerden biri sitokrom P450, aile 2, alt aile C, üye 9'dur (*CYP2C9*). Bu nedenle bu çalışmada, VD ile *CYP2C9* varyasyonları arasındaki ilişkinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: VD olan hastaları bulabilmek için en az 6 ay varfarin kullanan 650 hasta tarandı. Daha sonra hastalar içerisinden INR değerlerine göre 2 grup oluşturmak için hasta seçimi yapıldı. Günde 15 mg varfarin kullanmasına rağmen INR düzeyi terapötik aralığa gelmeyen (<2) 30 kişi ilaca yanıt vermeyen gruba, düşük doz varfarin kullanarak INR düzeyleri terapötik aralığa (2-3) gelen ve tüm hastalar arasından rastgele seçilen 30 hasta ise tedaviye yanıt veren gruba dahil edildi. Periferik kandan genomik DNA izole edildikten sonra, *CYP2C9*2* ve *CYP2C9*3* varyasyonları gerçek zamanlı polimeraz zincir reaksiyonu (GZ-PZR) kullanılarak araştırıldı. Sonuçlar istatistiksel olarak değerlendirildi.

Bulgular: Tedaviye yanıt veren grupta *CYP2C9*3* heterozigot genotipinin (%33.3), tedaviye yanıt vermeyen grupta ise yabani tip genotipin istatistiksel olarak yüksek olduğu (%90) belirlendi ($p<0,05$). Ek olarak, tedaviye yanıt veren grupta *CYP2C9*2* T alleli (%18.3) ve *CYP2C9*3* C alleli (%16,7) istatistiksel olarak yüksek bulundu ($p<0,05$).

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Sonuç: Hastalarda *CYP2C9* aktivitesinde azalmaya neden olan gen varyasyonları varsa, bunlar zayıf metabolize ediciler olarak adlandırılır. Bu kişiler, varfarini daha yavaş metabolize ederler ve terapötik INR değerlerine ulaşmak için daha küçük dozlarda ilaca ihtiyaç duyarlar. Bu nedenle hastaların genotipine bağlı olarak varfarin dozunun ayarlanması mümkündür.

Anahtar Kelimeler: Varfarin direnci, *CYP2C9*, GZ-PZR

INTRODUCTION

Atrial fibrillation (AF) is one of the most known abnormal heart rhythm disorders that affects >30 million people worldwide. Patients with a high risk of AF usually take anticoagulants, such as warfarin (1). The risk of embolism, especially the risk of ischemic stroke, increases in AF associated with thrombus formation in the left atrium (2). Pulmonary embolism (PE) is a life-threatening condition and is one of the most common causes of cardiovascular death, which is a known complication of deep venous thromboembolism (DVT) (3,4). Mechanical heart valves are implanted in patients with long life expectancy, which are more durable than bioprostheses. However, the disadvantage is the lifelong requirement of anticoagulants, such as warfarin, due to their higher thrombogenicity (5).

Warfarin is one of the most used anticoagulants worldwide, which reduces vitamin K formation by inhibiting vitamin K epoxide reductase, a multiprotein enzyme complex that activates vitamin K. Prothrombin and clotting factors VII, IX, and X have reduced coagulation ability without adequate active vitamin K. Anticoagulant protein S and C are also inhibited in this process. Thus, blood clotting decreases (6). Age, gender, body mass index, smoking, drug therapy, concurrent hepatic or renal disease, and dietary vitamin K intake also play a role in warfarin metabolism (7-9).

The international normalized ratio (INR), developed by the World Health Organization in the early 1980s, is widely used to monitor oral anticoagulation and evaluate patients with coagulation disorders, which was designed to eliminate problems in oral anticoagulants (10). It is the test of choice for patients taking vitamin K antagonists (VKA). INR is also used to assess patients' risk of coagulation or bleeding. Patients receiving oral anticoagulants need to monitor the INR values to adjust their VKA dose, as this value differs between patients (11). INR value is around 1 in healthy individuals. INR ratio differs according to each disease. INR value should be between 2-3 in patients with cerebro-cardiovascular occlusion or with DVT/PE and 2.5-3.5 in patients with heart rhythm disorders, such as heart valve disease or AF (12). Patients usually receive 5 mg of warfarin daily; however, some patients require >15 mg of warfarin daily to get the INR value into the therapeutic range. This condition is defined as warfarin resistance (WR) (13). WR is associated with enzyme deficiencies involved in warfarin metabolism (14). cytochrome P450, family 2, subfamily C, member (*CYP2C9*) is one of the cytochrome p450 enzymes involved in the metabolism of many drugs, which is also involved in warfarin metabolism (15). *CYP2C9*2* and *CYP2C9*3* variations are frequently encountered in the *CYP2C9* gene encoding the *CYP2C9* enzyme. *CYP2C9*2* (rs1799853) variation causes Arg144Cys amino acid change due to a C430T nucleotide change in exon 3. *CYP2C9*3* (rs1057910) variation causes Ile359Leu amino acid change due to the A1075C nucleotide change in exon 7. Both variations decrease enzymatic activities (16). The variations in the *CYP2C9* gene are associated with WR, thus this study aimed to investigate the relationship between the *CYP2C9* gene variations and WR.

METHODS

Study Population

WR is a rare condition. Therefore, 650 patients who applied to the cardiology clinic of Haydarpaşa Numune Training and Research Hospital and used warfarin for at least 6 months and had AF, mechanical aortic prosthetic valve, and DVT or PE were screened to find those with WR. Then, patients were grouped into two according to the INR values, wherein 30 patients, whose INR level did not reach the therapeutic range (<2) despite using 15 mg of warfarin per day, were included in the non-responder group and 30 randomly selected patients who received low-dose warfarin, with INR levels within the therapeutic range (2-3) were included in the responder group. Patients who have liver disease, heart failure, or hematologic disorders were excluded from the study. Patients who used aspirin, non-steroidal anti-inflammatory drugs, antifungals (fluconazole), antibiotics, statin group drugs, tricyclic antidepressants, and thyroid drugs within the last week were excluded from the study, together with those alcoholic and pregnant. The study, which is compatible with the Helsinki Declaration, was approved by the Clinical Research Ethics Committee of Haydarpaşa Numune Training and Research Hospital (approval date: december 30, 2019; approval number: 2019/166-1096). Each individual was informed about the study and written informed consent was obtained from each participant.

Blood Sampling and Genotyping

Whole blood samples were obtained from all patients. Deoxyribonucleic acid (DNA) was extracted from 200 µL of peripheral blood using commercially available kits (Qiagen, Foster City, USA) according to the manufacturer's instructions. DNA purity and concentrations were determined by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). Real-time polymerase chain reaction (RT-PCR) reactions for *CYP2C9*2* and *CYP2C9*3* were carried out on 7500 fast RT-PCR System (Applied Biosystems). The reaction was performed according to the manufacturer's instructions.

Statistical Analysis

Statistical Package for the Social Science 25.0 was performed for statistical analysis (IBM Corp. released 2017, IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.). Continuous variables are expressed as mean ± standard deviation and discrete variables are expressed as counts or percentages. Normal distribution assumption was checked with the Kolmogorov-Smirnov test. Two independent samples t-test was used to compare continuous variable means between two groups, which were normally distributed. The Kruskal-Wallis tests were performed to investigate the difference between genotypes and risk factors (which are not normally distributed). The Mann-Whitney U test was performed for pairwise comparison in statistically significant differences, and Bonferroni correction was applied to the p-values. The p-values of <0.05 ($p<0.05$) were considered statistically significant.

RESULTS

Study Population

The demographic characteristics of patients are shown in Table 1. Demographic characteristics comparison between groups revealed a statistically significant relationship between age and INR values.

CYP2C9 Genotyping

Table 2 shows the nucleotide distributions among the groups. The genotype distribution comparison between the groups revealed a statistically high heterozygous genotype of *CYP2C9*3* (33.3%) in responders, whereas a statistically high wild-type genotype was found in nonresponders (90%) ($p<0.05$).

Allele Frequencies of CYP2C9 Variations

Table 3 shows the allele frequencies of groups. The group comparison in terms of allele frequencies revealed a statistically high T allele of *CYP2C9*2* (18.3%) and C allele of *CYP2C9*3* (16.7%) in responders ($p<0.05$).

Table 1. Demographic characteristics of patients

Demographic characteristics		Groups: Non-responders (n=30)	Groups: Responders (n=30)	p
Age (year)		53.17±13.48	66.4±12.46	<0.001**
Average INR value		1.96±0.39	2.59±0.24	<0.001**
Warfarin indication	Atrial fibrillation	11	18	0.118
	Deep vein thrombosis or pulmonary embolism	6	6	
	Mechanical aortic prosthetic valve	13	6	
Gender	Female	21	17	0.284
	Male	9	13	

**p<0.001, INR: International normalized ratio

Table 2. Distribution of nucleotide variations between groups

Genes, genotypes, nucleotide variations	Groups: Non-responders (n=30)	Groups: Responders (n=30)	p
CYP2C9*2			
CC	27(90%)	22 (73.3%)	0.135
CT	3 (10%)	5 (16.7%)	
TT	0 (2.8%)	3 (10%)	
CYP2C9*3			
AA	*27 (90%)	20 (66.7%)	0.029*
AC	3 (10%)	*10 (33.3)	
CC	0 (0%)	0 (0)	
*p<0.05			

Table 3. Allele frequencies of groups

Alleles	Groups: Non-responders (n=30)	Groups: Responders (n=30)	p
CYP2C9*2			
C	57 (95%)	49 (81.7%)	**0.012***
T	3 (5%)	*11 (18.3%)	
CYP2C9*3			
A	57 (95%)	50 (83.3%)	**0.037***
C	3 (5%)	*10 (16.7%)	
*p<0.05			

DISCUSSION

Warfarin is frequently used for venous thromboembolism treatment after mechanical heart valve implantation and thromboembolic complication prevention in AF (15). It is metabolized by *CYP2C9*, one of the cytochrome P450 enzymes, to the 7-hydroxylated form. Many variants were identified in the *CYP2C9* gene which encodes the CYP2C9 enzyme. *CYP2C9*2* and *CYP2C9*3* are best-characterized variations of *CYP2C9* (13). Individuals with *CYP2C9*2* and *CYP2C9*3* genotypes have 12% and 5% lower enzyme activity than the wild type, respectively, and are prone to bleeding after warfarin treatment (17). *CYP2C9*2* reduced warfarin metabolism by 30% and *CYP2C9*3* by 80% compared with *CYP2C9*1*, which is a wild-type variant of *CYP2C9* (18). Individuals carrying the *CYP2C9*2* and *CYP2C9*3* variants reach the targeted INR level with lower amounts of warfarin (13). Ozer et al. (19) found that individuals with the *CYP2C9*1*1* genotype had a higher daily warfarin dose than those with the *1*3/*2*3 genotype. The same study revealed a similar result of undetected *1*2 genotype. Another study revealed no significance between the WR and *CYP2C9* variations (13). Dilge Taşkın et al. (18) showed that *CYP2C9* variants were detected at the rates of *1/*1 (54.6%), *1/*2 (16.4%), *1/*3 (24.2%), *2/*3 (2.9%), and *3/*3 (1.9%). The same study concluded that patients with variations needed lower warfarin doses (18). Another study found that *CYP2C9*2* and *CYP2C9*3* variations of *CYP2C9* decrease the rates of warfarin clearance. Another study found a significant association between *CYP2C9*3* with warfarin therapy (20). The allele frequency comparison between the groups in our study revealed a statistically high T allele in *CYP2C9*2* (18.3%) and C allele in *CYP2C9*3* (16.7%) in responders ($p<0.05$). In addition, the genotype distribution comparison between the groups revealed a statistically high CYP2C9*3 (33.3%) variation in responders ($p<0.05$). The wild-type genotype of *CYP2C9*3* was significantly high in nonresponders ($p<0.05$).

The limitation of our study is the small number of patients (n=60). However, considering that WR is an extremely rare condition, it is acceptable to screen approximately 650 patients and include 30 patients with WR in the study.

CONCLUSION

In conclusion, individuals with *CYP2C9* homozygous wild-type genotype required higher daily warfarin dose compared to the *2 and *3 variants. Particularly, patients with gene variations reduced *CYP2C9* activity, which is termed as poor metabolizers. These individuals metabolize warfarin more slowly and require smaller doses of the drug to reach the therapeutic INR values. Therefore, adjusting the warfarin dose is possible depending on the genotype of patients.

ETHICS

Ethics Committee Approval: The study, which is compatible with the Helsinki Declaration, was approved by the Clinical Research Ethics Committee of Haydarpaşa Numune Training and Research Hospital (approval date: december 30, 2019; approval number: 2019/166-1096).

Informed Consent: All patients and/or legal guardians included in the study provided their written informed consent.

Authorship Contributions

Surgical and Medical Practices: A.E.Y., Concept: D.K., A.E.Y., Design: D.K., A.E.Y., E.Ç.A., Data Collection or Processing: D.K., A.E.Y., T.D., E.Ç.A., Analysis or Interpretation: D.K., A.E.Y., T.D., E.Ç.A., Literature Search: D.K., T.D., Writing: D.K., T.D., E.Ç.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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