ORIGINAL ARTICLE

The relationship between CYP2C9 gene polymorphisms and upper gastrointestinal bleeding in patients who used warfarin

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ABSTRACT

Aim Oral anticoagulants are the most common used substance for treatment and prophylaxis of warfarin venous and arterial thromboembolic disorders in the world. Therapeutic index of warfarin is narrow. CYP2C9 is a hepatic microsomal enzyme and has a primary role in metabolism of warfarin and genetic variations of CYP2C9 may cause a serious effect on the response to warfarin in patients. The aim of this study was to determine the efficiency of CYP2C9 gene polymorphisms on drug metabolism in patients who had upper gastrointestinal system bleeding while using warfarin.

Methods There was a total of 67 patients in this study, 37 of whom had upper gastrointestinal system bleeding when INR was above 3 while using warfarin (group 1), 30 of whom had no bleeding and INR was stable under 3 (group 2).

Results There was no difference in terms of warfarin dose used among the groups (p>0.05). Mutant genotype, INR and aspirin usage were found significantly different in the group with bleeding (p<0.05). When analyzed in terms of drug interaction, there was no difference between the two groups (p>0.05). Logistic regression analysis was made in order to determine the risk factors that may cause bleeding. Aspirin usage (p= 0.016) and genetic polymorphism (p= 0.024) were related to the increased risk of bleeding.

Conclusion CYP2C9*2 and CYP2C9*3 polymorphisms were related to the increase of excessive anticoagulation and bleeding risk in the patients who used warfarin.

Key words: warfarin, bleeding, CYP2C9, aspirin
INTRODUCTION

At the present time, it is known that many factors which are not genetic such as age, gender, organ function and drug interaction have an effect on drug metabolism (1-4). It is guessed that genetic difference may compose 20-95% of the variability on the effect of drugs (5,6). In the recent years, many genetic factors which are effective on drug metabolism have been identified with the molecular studies on genetic science. It was shown in many studies that the changes in the genes which code the enzymes metabolizing the drugs, drug deliveries and drug objectives may cause interpersonal differences on response to drugs (7-9).

In this new period, pharmacogenomics evaluates the effects of the response that the patient gave to the drug with effect of many genes (10-12). Even though pharmacogenomics notion entered the literature in 1950s, it has been promising in the last 10 years especially with developments in molecular medicine and in terms of modifying the treatment for the diseases in which chronic drug is used.

Oral anticoagulant is the most commonly used substance for treatment and prophylaxis of warfarin venous and arterial thromboembolic disorders in the world (13). As the therapeutic range of warfarin is narrow and its interpersonal effect shows differences, it is quite hard to continue the treatment (14). It may cause serious bleeding with inconvenient doses, especially in the initial periods of the treatment or insufficient protection for preventing thromboembolism. Although there are many reasons for interpersonal efficiency difference, it was shown in the recent years that the differences in pharmacokinetics and pharmacodynamics of variable drug are the main reason in the basis of genetic differences. After the discovery that warfarin is metabolized from cytochrome P 450 (CYP450) enzyme system, it was shown that polymorphic changes in this enzyme affect drug response and even side-effects. It was shown that s-warfarin, which is a more active form of especially warfarin, decelerates warfarin metabolism of polymorphic nature of 9 polypeptide (CYP2C9) enzyme of IIC subfamily from cytochrome P450 family responsible for metabolism at the rate of 80-85% (15,16).

The aim of this study was to determine the efficiency of CYP2C9 gene polymorphisms and the effective factors for bleeding in the patients who had upper gastrointestinal system (GIS) bleeding while using warfarin and in this way to provide the anticipation of drug dose according to genotype of the patient before starting the warfarin treatment and the evaluation of risk factors.

PATIENTS AND METHODS

Patients

This study, which was planned as prospective, was made between October 2007 and November 2009 in Cumhuriyet University Health Care Application and Research Hospital Gastroenterology Clinic. Permission was received from Cumhuriyet University Medicine School Presidency of Ethics Authority and all of the patients were requested to sign the disclosure and consent form.

A total number of 37 patients (19 males, 18 females) with upper gastrointestinal system bleeding when Internasyonel Normalized Ratio (INR) was above 3 while using warfarin were included in the study (group 1). As a control group, 30 people (14 males 16 females) who used warfarin, lived in the same area, did not have bleeding and INR was stable under 3 were included in the study (group 2). INR values at the time of application, additional drugs used, aspirin and nonsteroidal anti-inflammatory drug (NSAID) usage, accompanying diseases, smoking, weekly warfarin dose taken were registered for all patients who were included in the study. The patients who had liver and renal failure or cognitive function disorders due to cerebrovascular diseases were left out of the study.

Genetic polymorphism assay

Two mL peripheral blood samples were taken from patients for DNA extraction. Extraction was made from peripheral blood with DNA isolation kit (Spin Blood DNA) according to the protocol of the manufacturer (K1820-02, Invitrogen Life Technologies, Carlsbad, CA, USA). The CYP gene was amplified with polymerase chain reaction (PCR). Primers were prepared based on gene sequences from the data source http://www.ncbi.nlm.nih.gov for amplification of CYP gene. Amplification kit (ViennaLab PGX-HIV PZR, Gaudenzdorfer Gürtel 43-45 1120 Vienna, Austria) was used for the amplification of the gene according to the protocol of the manufacturer. Obtained products as a result of PCR with each primer were controlled by performing marker DNA at 1.5 % agarose gel.
Ten PZR products, from which successful amplification was obtained, were used for SouthernBlot analysis. Reverse-hybridization ProBlot T48 (Tecan Group Ltd., Seestrasse 103, 8708 Männedorf Switzerland) hybridization device was used for SouthernBlot analysis. Reverse-hybridization was made by using strip test technique. At the end of the study, strips were placed in the evaluation table (Collector). The genotype of the patient was determined by comparing the control tapes with the tapes that were obtained at the end of the study.

Statistical analysis

While evaluating the data, momentousness test of the difference between two averages was used in 2x2 orders. Chi-square test was used in multispan orders, fischer exact Chi-square and logistic regression analysis were used and ODS values were found. The data was shown on the tables by stating arithmetic mean ± standard deviation, number and percentage of people and mistaking percentage was taken as 0.05.

RESULTS

Thirty seven patients who had upper gastrointestinal system bleeding when INR was above 3 while using warfarin, were taken for our study (group 1). Nineteen (51.4%) of them were males, and the rest 18 (48.6%) were females. As a control group, 30 people, 14 (46.7%) males and 16 (53.3%) females using warfarin, who lived in the same area, with no bleeding and INR stable under 3, were included in the study (group 2). The mean age in the group 1 was 64.43 ± 12.05 and it was 58.53 ± 14.48 in the group 2 (p>0.05). Weekly warfarin dose was 31.92 ± 6.76 mg in the bleeding the group 1 and 33.91 ± 5.48 mg in the group 2 (p>0.05) (Table 1). When the distribution of INR values in the groups was analyzed, the average INR values of the group 1 were 7.58±4.80 and 2.38 ± 0.28 in the group 2 (p<0.05) (Table 1).

When the groups were compared in terms of the existence of additional disease, the difference was found significant (p<0.05) (83.8% in the group 1 vs. 56.7%) when the patients who were included in the study were compared in terms of usage of additional drugs, the difference was found significant. While all patients (n=37, 100%) of the group 1 were using additional drugs, 21 (70%) patients of the group 2 were using additional drugs. A significant difference was determined in aspirin usage, no difference was determined between the groups in terms of usage of other drugs or in terms of smoking (p>0.05). When two groups were compared in terms of the effects of the drugs used by the patients on INR, statistical difference was insignificant (p>0.05). For the group 1, CYP2C9 *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, *3/*3 polymorphism distribution was found as 37.8% (14), 32.4% (12), 21.6% (8), 2.7% (1), 5.4% (2) and 0.0%, respectively. For the group 2, *1/*1, *1/*2, *1/*3 polymorphism distribution was found as 63.3% (19), 20.0% (6), 16.7%, respectively. Other polymorphisms were not followed (Table 2). Genetic results of all the patients included in the study were identified as normal (wild type) for CYP2C9 *1/*1 and they were identified as mutant for other types. Normal allele number was 14 (37.8%) and mutant allele number was 23 (62.2%) in the group 1; they were 19 (63.9%) and 11 (36.1%), respectively in the group 2. The difference was statistically significant (p<0.05) (Figure 1).

![Figure 1. Genotype distribution in groups]({})

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>INR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.58 ± 4.80</td>
<td>2.38 ± 0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Weekly dose (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.92 ± 6.76</td>
<td>33.91 ± 5.48</td>
<td>0.197</td>
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</tbody>
</table>

*Average used to±SD.
INR, International Normalized Ratio; Group 1, Patients with bleeding when using warfarin; Group 2, control group.
Logistic regression analysis was made in order to determine the risk factors that may cause bleeding. Aspirin usage (p = 0.016) and genetic polymorphism (p = 0.024) were related to the increased risk of bleeding. Gender, age and NSAID usage were not followed as risk factors (Table 3).

**DISCUSSION**

It was reported that especially genetic polymorphisms in CYP2C9 and CYP450 family have a significant effect on warfarin metabolism (17). It was reported in a few studies that racial difference is a factor affecting warfarin dose (18, 19). In a study of Higashi et al, it was reported that arising risk of INR from targeted value and life-threatening bleeding risk increased in the patients who had at least one variant when compared to wild type (20). As a result of another study researching the relationship between warfarin dose need of CYP2C9 genetic polymorphism and bleeding risk, a strong relationship was reported among variant alleles, low warfarin dose needed and major bleeding (21).

It was shown in a systematic composition and meta-analysis which researches the effect of CYP2C9 genotype on needed warfarin dose that when compared to wild type in CYP2C9*1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 carriers 19.6%, 33.7%, 36.0%, 56.7% and 78.1% respectively, a need for a smaller dose occurred (22). It was seen in our study that mutant genotype frequency was higher in the group 1 who had upper GIS bleeding than the group 2 who did not have bleeding. However, warfarin doses used by these two groups were similar. Moreover, INR was seen higher in the group whose mutant genotype frequency was higher and had upper gastrointestinal bleeding and were treated with the same warfarin dose as the other group. Our study showed that inappropriate INR increase and bleeding complication may occur when warfarin dose is not decreased in patients who have mutant genes. Having mutant genotype has been identified as a risk factor in terms of bleeding development. Bleeding risk has increased 3.88 times in the patients who have mutant gene and use warfarin.

In a meta-analysis of Francesco et al., which include 10 randomize clinical researches, it was shown that there was an association between aspirin usage together with oral anticoagulant and increased bleeding risk (23). It was observed in our study that aspirin usage together with warfarin was much higher in the patient group who had upper GIS bleeding. Using aspirin with warfarin increases the risk of bleeding. It was determined in our study that aspirin usage increases the risk of upper GIS bleeding 4.33 times in the patients who use warfarin.

As a result, there was no difference in terms of age and drug interaction between the groups in our study. Although the same warfarin dose was used in the patient group who had bleeding, higher INR level existed. It has been thought that genetic polymorphism can cause this situation when genetic differences between the groups were considered. The screening of CYP2C9 genotype survey of the patients to whom oral anticoagulant treatment will be applied and determination of high risk patients in variant group can help the clinicians develop new dosages and following protocols. The early determination of high risk patients who may respond to the treatment immoderately, choosing low warfarin dosages, increasing the safety and efficiency of the treatment by making more frequent clinical and laboratory monitoring in these patients can be useful in decreasing the life threatening bleeding complications. Besides this, alternative treatment suggestions can be made in the patients who were determined as high risk as a result of genotyping and thought to have elective anticoagulant.

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**TRANSPARENCY DECLARATIONS**

Competing interests: none to declare.
REFERENCES