J Cardiovasc Disease Res., 2020;11(1):09-11.
A Multifaceted Peer Reviewed Journal in the field of Cardiology
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Review Article

The relation between CYP2C9 gene polymorphism and warfarin dosing in an Iranian population

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Submitted: 23-11-19
Revision: 20-12-19
Accepted date: 14-01-20

DOI: 10.5530/jcdr.2020.11.03

ABSTRACT

Background: Warfarin is a common anticoagulant agent which is widely used in many countries. However, Warfarin is one of those drugs which requires close monitoring and dosing while inappropriate could lead to hemorrhagic epoxides. Achieving a desirable international normalized ratio (INR) range depends on various demographic factors and genetic factors are now becoming an emerging area of interest in cardiovascular research. CYP2C9 is a gene encoding a member of cytochrome P450 enzyme which metabolizes many drugs. Genetic variation in this gene has been linked to abruption in Warfarin drug response. In present study the effect of genetic polymorphisms in CYP2C9 gene and its response to Warfarin is investigated.

Methods: Total of 95 patients who had cardiovascular diseases and were using Warfarin for at least 3 months were enrolled in this cross sectional study. Their DNA was extracted and the genetic polymorphisms in CYP2C9 gene (RS1799853 as CYP2C*2 and RS1057910 as CYP2C9*3) were evaluated by using polymerase chain reaction and sequencing. Kruskal-Wallis test were used for comparing the Warfarin values between the genotype groups. The SPSS software 22v was used for data analysis.

Results: Among the study population the prevalence of genotypes were as follow: CC/AA: 61.1, CT/AA: 17.9, CT/AC: 1.1, CC/AC: 3.6, TT/AA: 2.3, CC/CC: 0%. The CC/AA and CC/AC variants were related to Warfarin dose ($P<0.05$). These patients require lesser doses of Warfarin in order to achieve the desired INR range.

Conclusion: Determination of genetic polymorphisms of effective genes in Warfarin response such as CYP2C9 gene is important in initiating the Warfarin therapy and reducing possible complication of Warfarin. Considering genetic polymorphisms in every population alongside with other effective factors on warfarin dosing should be considered in initiation of anticoagulant therapy.

Keywords: Anticoagulants; Genotype; Polymorphism, Genetic; Warfarin

INTRODUCTION

Cardiovascular disease is one of the main leading causes of death worldwide. Approximately more than 30% of annual death globally is because of this disease and a remarkable burden is also prominent[1,2]. As same as other parts of the world, our country is also dealing with this global concern. It has been reported that 46% of Iranian population has cardiovascular diseases[3]. Similar to other diseases, there are various medications available for treating different types of cardiovascular problems. Anticoagulants are a well-known treatment option in managing specific cardiovascular diseases such as atrial fibrillation and valvar heart diseases[4]. Warfarin is an old example of anticoagulants which is widely used in many countries. Warfarin can prevent thrombotic events in susceptible patients by inhibition of coagulant factors related to vitamin K and, C and S proteins[5]. Despite of wide therapeutic effect on many cardiovascular diseases, Warfarin could also be potentially a harmful drug. Maintain a constant therapeutic dose of warfarin is hard to achieve will it has a narrow therapeutic window. Slight changes in Warfarin doses may result in hemorrhagic events. So, careful dosing and monitoring of warfarin has been always a big concern for clinicians[6]. The Warfarin dosing is mainly based on monitoring the patient's INR. Despite of many known effective factor on Warfarin dosing such as patient's age, weight, gender, drug history and previous bleeding episodes; genetic factors are also acting as an emerging factor. CYP2C9 is one of the first genes which were related to Warfarin metabolism[7-11]. This gene encodes a member of cytochrome P450 enzyme family and appropriate function of this cytochrome P450 enzyme. Disrupted function of this protein which may be caused because of genetic variations may disrupt the function of this enzyme. There are some single nucleotide polymorphisms (SNP) reported to be effective on enzymatic activity of CYP2C9. The reduced enzymatic activity will result in reduced drug metabolism rate and therefore will reduce the required dose of Warfarin[11]. Allelic variants of CYP2C9 codes different enzymes with variable enzymatic activity in different populations. In CYP2C9*2 and CYP2C9*3 allelic variants shows 12% and 5% of enzymatic activity. Approximately one fourth of White Americans have at least one copy of CYP2C9*2 and CYP2C9*3 allelic variants while this ratio is lesser in African Americans and Asians. It has also been reported that heterozygote patients for CYP2C9*2 and CYP2C9*3 alleles requires 20% to 30% of Warfarin dose compared to those with wild alleles. Moreover, the homozygote patients will require 50% to 70% of Warfarin dose[11]. According to such variation in every population, it seems that adjusting the Warfarin dose can be also based on genetic variants such as CYP2C9[6]. Regarding to this fact, we have conducted our study to determine the CYP2C9 SNPs relation with the Warfarin response in North east of Iran.

MATERIAL AND METHODS

The present cross-sectional study took place in Mashhad, Iran and was approved by Islamic Azad University, Neyshabur Branch ethic committee (). The study population was chosen from patients who were referred to Jawad Al-Aime Cardiology and Charity Hospital in a 2-year period starting from 2015 to 2017. According to Awad et al. study which have had evaluated the warfarin dose for patients with or without CYP2C9 polymorphism, the sample size of presented study was calculated as 61 patients (male: female ratio of 1 with mean age of 54.3 years) (Power and confidence rate of 90 and 95 respectively). The

patients who were receiving warfarin because of any cardiovascular disease for at least 3 months were considered eligible to enroll in present study (INR ranging from 1 to 3.5). Also, none of these patients had taken the drugs which have major interaction with warfarin (Amiodarone, statins, omeprazole or non-steroid anti-inflammatory drugs), history of hematologic disease, peptic ulcer, liver, thyroid or kidney dysfunction, autoimmune or infectious disease, malignancies, non-compensated heart failure and smoking more than 10 cigarettes per day. Those patients who were eligible to enroll in present study according to these criteria had full filled an informed consent form. Patient's medical and demographic data including gender, age, ethnicity, height, weight, initial and maintenance warfarin dose was recorded. In order to determine the patient's genotype for the desired polymorphism, 5ml of venous blood sample were taken in EDTA tubes. The tubes were stored in -20°C and the DNA were extracted by use of taking a QIAamp DNA Mini Kit (Yekta Tajhiz Teb, Iran) and were stored in -20°C. In order to examine the quantity and purity of extracted DNAs, UV absorption in 280nm and 260/280nm ratio were assayed by UV-spectroscopy (Nanodrop™ 2000/2000c)(12).

In order to identify the polymorphisms, two pairs of primers were designed for the desired polymorphisms. The forward primer for CYP2C9*2 (rs1799853) was 5'-GGGGAGGATGGAAAACAGAG-3' and the reverse primer was 5'-CCCCTGAAATGTTTCCAAGA-3'. The forward primer for CYP2C9*3 (rs1057910) polymorphism was 5'-CCCCTGAATTGCTACAACAAA-3' and the reverse primer was 5'-ACCCGGTGATGGTAGAGGTT-3'. PCR and sequencing was used to detect the desired polymorphisms as follow: 1ul of DNA was added to 10ul of PCR master mix (Yekta Tajhiz Teb, Iran) and 0.75ul of each forward and reverse primers (Dena Zist, Iran) were added to the mixture. The final volume was diluted to 25ul. The denaturing temperature was considered as 94°c for 60 seconds, 59 °C and 62 °C for annealing of C9 and VCR primer sets for 30 seconds respectively and 70oC for 25 minutes were considered as extension cycle. Five microliter of the PCR product were loaded on 2% agarose gel and after 60 minutes gel electrophoresis (50v DC), specific bands were visualized by RedSafe DNA stain 20000 X (Chembio Ltd, United Kingdom) in InGenius3 (SYNGENETM, USA) gel documentation system. The PCR product were send for sanger sequencing which performed by method protocol by selective incorporation of chain-terminating dideoxynucleotides in ABI Prism 3100 genetic analyzer (ABI, USA) and then the results were interpreted.

Mean values and standard deviation were used for describing the study variables. K-independent sample t-test (Kruskal-Wallis test) were used for comparing the Warfarin values and the genotypes. The SPSS software version 22 was used for data analysis and P values less than 0.05 was considered as statistically significant.

RESULTS

Total number of 95 patients enrolled in present study. The mean(SD) of patients age was 54.3(19.6) years and most of the patients were female (51.6%). The patient's demographic data is summarized in table 1. The mean dose of warfarin was 29.6 mg and the dose distribution in study population is summarized in Figure 1. Most of the patients were suing warfarin for their heart valve replacement surgery (60 patients, 63.2%). Heart failure (22 patients, 23.2%) and venous thrombosis (13 patients, 13.7%) were the other reasons for using this drug. More than half of the patients have experienced hemorrhagic episodes (55 patients, 57.9%). The distributions of the co-incidence of CYP2C9 polymorphisms are summarized in table 2. Our data demonstrated that there was also a significant relation between patients genotype and warfarin dose (P<0.05) (Figure 2). Patients with CC/AA genotype have higher Warfarin dose with respect to patients with CC/AC genotype which indicated that these patients need to lesser doses of Warfarin (P=0.001).

Table 1. Patients demographic and genotype data.

Variable	Mean (Standard deviation)	
Age	54.3(19.6)	
Weight (kg)	69.3(17.2)	
Height (cm)	164.2(15.9)	
Warfarin dose (mg)	31.4(13.1)	
Genotype status		Number
CYP2C9*2	TT	3
	CT	18
	CC	66
	Unknown	8
CYP2C9*3	CC	0
	AC	9
	AA	82
	Unknown	4

Table 2. The distribution of genotypes of in study participants.

CYP2C9*2/CYP2C9*3	Number	Percent	
CC/AA	58	61.1	Kruskal Wallis Test=22.94 P=0.0001
CT/AA	17	17.9	
CC/AC	6	6.3	
TT/AA	3	3.2	
CT/AC	1	1.1	
CC/CC	0	0	
TT/CC	0	0	
unknown	10	10.5	

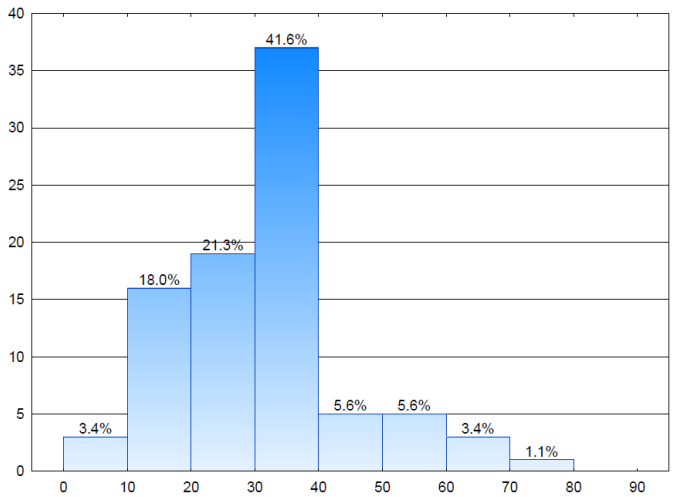

Figure 1. Distribution warfarin dose among the study population

DISCUSSION

Warfarin is one of the Comarines derivate which is widely used in our country because of its low price and availability[12]. Some patients depending on their personalized factors such as genetic polymorphisms require different doses of Warfarin in order to control their INR[13]. Our study indicated that CYP2C9*2 and CYP2C9*3 polymorphisms are related to Warfarin response. In our population those patients who are carrying CC/AA and CC/AC variants requires lesser doses of Warfarin. It has been reported that response to Warfarin is 6% to 10% dependent on CYP2C9 gene. CYP2C9 inhibits the cytochrome p450 and therefore the drug metabolism in liver will be reduced. CYP2C9*3 heterozygote variant (AC) and CYP2C9*3 homozygote variant (CC) will reduce the Warfarin metabolism to 40% and 80% respectively[14]. Also, CYP2C9 (rs1799853) homozygote variant (TT) and heterozygote variant (CT) will reduce the Warfarin metabolism to 40% and 20% respectively. The prevalence of genetic variants in CYP2C9 (CYP2C9*2 and CYP2C9*3)

is similar to European and Asian regions, especially the Italians and Greeks[15-18]. A study from center of Iran has reported that CC/CC is the least common and CC/AA is the most common variant[15]. Another study from west of Iran has reported that the CC/AA and CT/AC are the most and least common genetic variants[16]. Our study has revealed that CC/CC and CC/AA genetic variants are the least and most common findings. A Chinese study has demonstrated that the CC/AA genotype is the most prevalent one as same as our study, however, they have not reported any TT/AA, CT/AC or CT/AA genotype[14].

The main finding of present study was the relation between CYP2C9 genotype and required Warfarin dose. A similar study for North West of Iran has demonstrated that reduced enzyme function due to CYP2C9*2 and CYP2C9*3 polymorphisms in comparison to wild allele will result in reduced Warfarin dose[17]. Also, another study from Hungary has demonstrated same results as our study regarding to the effect of CC/AC variant in CYP2C9 gene. They have stated that their patients with CC/AC genotype will also require lower doses of Warfarin because of reduced enzyme activity[18]. This finding is in line with other studies which suggest lower doses of Warfarin in patients with CYP2C9*3 and CYP2C9*2 in order to reduce the chance of bleeding[19-22].

CONCLUSION

According to present study results, the polymorphism in CYP2C9 gene is effective adjusting the Warfarin doses. Determination of such polymorphisms in every population will be effective in reducing the possible complications related to higher doses of Warfarin The study will done in wider sample sizes.

Strength and limitation

A major limitation of this study could be the limited sample size which was because of limited time and financial resources available for conducting this study. One the other hand, present study has used PCR-sequencing for all patients instead of using other molecular methods.

ACKNOWLEDGMENT

This project is funded by the Dr. Shahinfar Medical Faculty-Islamic Azad University of Mashhad. The authors would like to express their gratitude to the Jawad Al-Aime Cardiology and Charity Hospital and Genetics Laboratory Counseling Center and University of Khorasan Razavi for their cooperation and assistance.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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Cite this article : Sadr-Nabavi A. The relation between CYP2C9 gene polymorphism and warfarin dosing in an Iranian population. J Cardiovascular Disease Res. 2020; 11(1): 09-11.