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Research Article
Impact of Cytochrome P450 2C9 Polymorphism on Warfarin Therapy in Saudi Population

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Abstract

Background and Objective: Individualization of therapy based on patient's genetic profile is particularly important with certain drugs. Warfarin is one of vitamin K antagonists (VKA) that is widely used anticoagulant with inter and intra-individual dosage variability depending on many non-genetic and genetic factors. This study aimed to evaluate the effect of cytochrome P450 2C9 isoform (CYP2C9) polymorphism on the dosage variability and therapeutic efficacy of warfarin in a subset of Saudi patient. **Materials and Methods:** The study included 112 patients on regular warfarin therapy for various causes. Genomic DNA of all patients was isolated and quantified. The DNA samples were genotyped for CYP2C9*2 and CYP2C9*3 alleles by TaqMan allelic discrimination genotyping method. The primary outcome was time in therapeutic range (TTR). Data were compared utilizing one-way ANOVA, independent measures t-tests and the corresponding non-parametric test and Fisher's exact test. **Results:** The dose of warfarin was less for patient expressing either genotype variant alleles of CYP2C9. Time in therapeutic range was not significantly different utilizing one-way ANOVA test when evaluating CYP2C9 genotype. Patients homozygous for *2 allele had less TTR (50.0%, p = 0.10) and lower average weekly dose than the others. **Conclusion:** CYP2C9 polymorphism influences warfarin dosage and efficacy among a subset of Saudi population and tends to be a good clinical practice particularly in patients experiencing excessive bleeding or patients with less TTR.

Key words: Warfarin, CYP2C9*2, CYP2C9*3 polymorphism, anticoagulant, time in therapeutic range (TTR)

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Personalized medicine is growing to be the trend for future medicine[1]. Coagulation disorders and vitamin K antagonists (VKA) use to overcome this defect are accompanied by several unfavorable events[2]. Thus, personalized therapy could offer the required knowledge to optimize patient care.

There is an established knowledge of two known sequence variants in the CYP2C9 gene NG_ 008385. 1: g. 8633C>T or *1/*2 and NG_008385.1: g.47639A>C or *1/*3[3] and their effect on the patient response to commercially available warfarin[4-6]. Vitamin K-antagonist targets vitamin K-epoxide reductase (VKOR) enzyme but S-warfarin (the most potent enantiomer in the commercial preparations) is metabolized by the cytochrome P450 enzyme 2C9[4]. Various types of VKA dose requirements are similarly impacted by VKORC1 sequence variant; however, warfarin is highly impacted by both VKORC1 and CYP2C9 genotypes[7].

When VKA get initiated for any patient who has any enzyme polymorphism, they will have a supra-therapeutic effect[8-11]. Published trials of limited subject focusing on genotyped-based warfarin dosing failed to confirm such theory on period while at an acceptable therapeutic range of INR or excessive bleeding during initiation phase[12,13]. Recently, a study indicated that dosing VKA based on pharmacogenetics revealed less low INRs and more time in the acceptable therapeutic range during the first 12 weeks[14]. It is not clear if genotyping assessment prior to dose initiation is of any clinical value especially during first days on VKA[8,9]. But, CYP2C9 *2 and *3 variant alleles are linked to risk of bleeding during initiation phase[15,16].

This study designed to evaluate the effect of cytochrome P450 2C9 isoform (CYP2C9) SNP polymorphism on the dosage variability and therapeutic efficacy of warfarin in a subset of Saudi patient.

MATERIALS AND METHODS

Design: The subjects in this study were recruited from PSAU hospital and Prince Sultan Military hospital cardiology and internal medicine units. All patients were adult as per selection criteria and must provide a signed consent. They were already on a selected dose for continued warfarin therapy (at least 4 weeks in therapy). Dose initiated and maintained was performed by medical staff and all patients' lab values were tracked and recorded manually. No genetic variability was provided to the treating staff at the initiation of therapy. Initially, 119 patients were part of this study between January, 2016 and December, 2016. Only 112 accepted to participate in this prospective study. Patient refusal to be included was not due to any particular obvious reason other than lack of interest.

Patients included were part of the regular care provided to all other patients who did not participate in the study. The usual standard period for INR evaluation was based on patient clinical status. At the anticoagulation clinic, critical levels are seen sooner than stable cases, 2-6 weeks intervals. For the purpose of this study analysis, at least two consecutive INR values must be available. The INR goals were 2-3 for all patients; beyond this range was not included in this study to avoid the need for additional analysis. INR values, a diagnosis that requires warfarin therapy and prior treatment data were available from the clinic own records. Patient demographics, characteristics and other patient-related information were obtained partially through clinic records and patients themselves during inclusion period[17].

All participants were evaluated for a maximum of 12 months and the median days in the study were 310 (289-365) due to project time frame. Any subject who left or failed to be assessed was excluded from the analysis. The study participants received warfarin due to specific indications and the duration of follow-up was illustrated in Table 1. The study was ended in April, 2017.

Ethical committee approval was obtained before the commencement of this project. All healthcare workers were instructed and educated on Helsinki declaration which was followed during the study.

Genotypes and variables: Genomic DNA from mouthwash samples was isolated by FlexiGene DNA Kit (Qiagen, Valencia,

Table 1: Demographic characteristics of the participants

Characteristics	All participants (n = 112)
Gender	
Male	59 (52.68)
Female	53 (47.32)
BMI (kg m^{-2})	28.80±3.40
Indication for warfarin	
AF	40 (35.71)
DVT	67 (59.82)
Mechanical valve	5 (4.46)
Age (years)	54.66±9.10
Duration for taking warfarin (years)	4.48±3.25
Duration of follow-up	
<6 months	12 (10.71)
>6 months	99 (88.39)
INR range	
2-3	107 (95.53)
2.5-3.5	5 (4.47)

AF: Atrial fibrillation, DVT: Deep venous thrombosis, INR: International normalized ratio. Data are presented as n (%) or Mean±SD

CA, USA) and the concentration and 260/280 quality ratio for all isolated DNA samples were determined using the Nanodrop spectrophotometer (Wilmington, DE, USA). DNA samples were genotyped for CYP2C9*2 allele (430C>T, rs28371674) and CYP2C9*3 allele (1075A>C, rs1057910) on StepOnePlus Real-Time PCR System by TaqMan allelic discrimination genotyping method (Applied Biosystems/Thermo Fisher Scientific, Foster City, CA, USA). The PCR primers and probes for CYP2C9*2 allele (430C>T, rs28371674) and CYP2C9*3 allele (1075A>C, rs1057910) TaqMan assays were purchased from Applied Biosystems/Fisher Scientific (Thermo Fisher Scientific, Foster City, CA, USA) and based on previous publication[18]. The genotyping assays were performed and analyzed according to the manufacturer's recommendations (Applied Biosystems/Fisher Scientific, Foster City, CA, USA).

Outcome variables evaluation: Our primary outcome was the time patient stays in therapeutic range (time in therapeutic range, TTR) as outlined in the study protocol (INR value of 2.0-3.0). This data were obtained directly from laboratory system available at participating sites and the clinic database. Other external software like DAWN could be used to employee Roosendaal's method but that was not available at the time[19]. The dose used during each visit was also obtained from pharmacy records and/or the clinic database. In order to expedite the project, manual entry for all records was done to create clinic database. Patients' visits were retrieved and noted. A total of 620 visits was encoded into the database. This database generates all required records like a number of high doses (> 3 INR) or low doses (<2 INR), the number of visits of each patient, average days between visits, the number of times warfarin dose was changed for each and every patient and days of follow-up. Average days off between each visit was obtained by days off between visits over the number of visits. INR range above limits or below limits were obtained from clinic protocols. The average dose of warfarin between genotype groups was obtained from warfarin dose patient uses during inclusion time.

Events registration: All events categorized under the jurisdiction of the anticoagulation clinic were reported utilizing the hospital-wide forms. Such forms must be critically reviewed by the principal investigator and medical staff running the clinic. Major bleeding criteria (ISTH criteria) were implemented[20]. No life-threatening or serious events were encountered during this study period.

Statistical analysis: All statistical assessment and analysis were carried out with IBM SPSS 24.0 (SPSS Inc., Chicago, IL). The level of significance was set at 0.05. Alignment with Gaussian distribution was also looked at by inspecting Q-Q plots. The variables here were listed as Mean±SD (standard deviation) but other variables are listed as median (quartiles) except for high INR readings that encountered less than low INR readings. High INR or low INR were expressed as numbers and percentage. Results were compared utilizing one-way ANOVA, independent measures t-tests and the corresponding non-parametric test was used as applicable. The risk of overdosing between two CYP2C9 variant alleles and other patients was assessed by Fisher's exact test.

RESULTS

All of the 112 Saudi patients receiving warfarin therapy were included in the study and their demographic data were shown in Table 1. The study involved 59 men and 53 women with a mean age of 54.66±9.10 years. Warfarin was prescribed due to different medical conditions, mostly due to deep venous thrombosis (n = 67, 59.82%). The INR target was 2-3 in most patients (n = 107, 95.53%); only 5 patients with mechanical valve have INR goal of 2.5-3.5 (n = 5, 4.46%).

Genotype data of CYP2C9 to all patients were presented in Table 2. Among the 112 patients, there were 80 patients wild-type homozygous *1/*1, 30 patients heterozygous variant type (*1/*2 or *1/*3) and 2 patients homozygous *2/*2 variant type. As it was anticipated, chronic dose of warfarin was significantly less for patient expressing either genotype variant alleles ($p<0.01$, Table 2). Time in therapeutic range (TTR) was not significantly different utilizing one-way ANOVA test when evaluating CYP2C9 genotype. There was no difference between genotype CYP2C9 after testing the range of aforementioned variables for which no adjustment for any strong bias was carried out in our analysis (Table 2).

CYP2C9 sequence variants were evaluated in comparison with outcomes observed. Chronic dose was higher without a *2 allele (n = 80) than those with a *2 allele (n = 18) which was our anticipation. But no statistical significance was observed. Comparable, subjects with one *3 alleles (n = 12) needed fewer doses than those without a *3 allele. For *3 allele patients, TTR was significantly different (25% vs. 71.25%, $p<0.05$).

Figures which reflect less stability of warfarin dosing in patients confirmed to express CYP2C9 genotypes *2/*2 were illustrated in Table 2; which were in comparison with other patients in this study. Even though there were only two subjects with such polymorphism, results showed less TTR than others (50-55.6%) and that is expected. The average weekly warfarin consumption for this group was between 7.9-16 mg/week. This dose average is below the entire

Table 2: Results of CYP2C9 genotype for patients on warfarin therapy and INR 2.0-3.0

CYP2C9 Multiplex	Maintenance dose (mg/week)	TTR of INR (%)	Days between visits days	Dose changes (n)	INRs <2.0 n (%)	INR >3.5 n (%)
*1/*1	33.5	71.25%	23.8	4.5	4	19
(n = 80)	(28.0-46.5)		(20.4-30.0)	(2-6)	(5%)	(23.75%)
*1/*2	27.4	55.6%	22.5	4	3	5
(n = 18)	(20.0-39.9)		(16.8-26.2)	(1-6)	(16.6%)	(27.8%)
*1/*3	23.4	25%	20.0	3	2	7
(n = 12)	(20.0-30.2)		(17.7-23.6)	(1-6)	(16.7%)	(58.3%)
*2/*2	14.0	50%	15	4	0	1
(n = 2)	(7.9-16.0)		(11.9-19.9)	(2-7)	(0%)	(50%)
P	<0.01	0.10	0.56	0.65	0.67	0.78

Statistical tests: Kruskal-Wallis test or one-way ANOVA as appropriate

group median (29.5 mg/week). Statistically, patients without any CYP2C9 expression or one allele (110 patients) had higher TTR than those with two variant alleles, (61.5% vs 73.4%, p = 0.014) and showed excessive anticoagulation effect p = 0.04 (Fisher's exact test). All factors were comparable between groups in this study.

Few patients (five patients) with target INR range of 2.5-3.5 had an average TTR of 69.9% and all five patients were found to express CYP2C9 genotype *1/*1. In comparison with the majority of patients (n = 107) with INR range of 2-3, TTR was 69.1% with no statistical difference between the two ranges concerning TTR (p = 0.39).

With relation to patient medical condition during follow-up period; there weren't any clinical events that had been reported.

DISCUSSION

This study aimed to explore the effect of CYP2C9 allelic variant on warfarin dose requirement in a subset of Saudi patients and concluded that the presence of *2 and *3 heterozygous alleles predict lower warfarin dose requirement and lower time in therapeutic range (TTR). Additionally, the presence of CYP2C9 *2/*2 homozygous alleles require administration of a very low weekly warfarin dose as depicted in Table 2.

The finding here were not different from the results of other studies on VKA and CYP2C9 gene sequence variants that greatly affected patient's TTR. Verhoef *et al.*[11] reported the occurrence of INR values >3.5 in patients with CYP2C9*3 (62%) or CYP2C9*2 (52%) allele than in wild-type patients (40%) receiving phenprocoumon. No possible correlation of such finding other than CYP2C9 functionality is negatively affected and therefore warfarin will accumulate potentiating its effect and eventually more unpredicted interactions. Another study showed that TTR was predicted by CYP2C9 sequence variants as well as non-genetic factors including alcohol consumption and perceived stress. Patients with high-stress score at the initiation of therapy showed lower TTR. In addition, patient age and duration of treatment at recruitment phase were perceived to be predictors[21].

Thus, the CYP2C9 gene group alleles affecting warfarin therapy with supra-therapeutic INR values and more tendency for bleeding hazards than wild-type. This in agreement with Hummers-Pradier *et al.* study that demonstrated CYP2C9*3 variants were linked to more bleeding risk in 185 German patients on phenprocoumon anticoagulation[15]. In another cohort study, CYP2C9 variant allele was accompanied with a higher risk of major bleeding in patients receiving acenocoumarol but not phenprocoumon[16]. So, it is recommended that patients who need less maintenance dose of warfarin to be evaluated for CYP2C9 alleles. If a genetic variant was confirmed, other means of safer anticoagulation might be explored. Although CYP2C9 sequence variants are of more clinical importance, it is important not to disregard other genes that might influence warfarin effect (e.g., CYP4F2 or EPHX1)[1]. The rs2108622 polymorphism of CYP4F2 is coupled to varied hepatic metabolism of vitamin K1 that requires greater warfarin dose to obtain the same therapeutic effect and may account for 4-12% of warfarin dose variability particularly in European[22]. Moreover, the rs2292566 variant of EPHX1 is linked to 1.7% of the variability of warfarin dose and also requires dose reduction[23]. Although this study results showed that the effect of CYP2C9 gene sequence variants on INR levels was not extreme, CYP2C9 genes variants alleles could disturb the outcomes of chronic warfarin therapy even when patients are tightly followed up.

SIGNIFICANCE STATEMENTS

This study explored the influence of CYP2C9*2 and *3 alleles on the efficacy of warfarin therapy in Saudi patient. This will help the researchers to uncover the importance of CYP2C9

genetic testing in the context of time in therapeutic range assessment in patients receiving warfarin therapy.

CONCLUSION

It is concluded that, it is imperative for all anticoagulation clinics to establish a reliable monitoring system (database) in order to keep track of difficult patients or patients that spend less TTR. Healthcare workers of anticoagulation clinic should consider such database and continuously review it. Patients that experience excessive response to usual warfarin doses or those spend less TTR should be evaluated for the presence of CYP2C9 variant alleles, this is in particular with one or more CYP2C9 *3 alleles. Such practice will be extremely beneficial for patient safety and better overall care.

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