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Research Article
Influence of Vitamin K Epoxide Reductase Complex 1 Polymorphism on Warfarin Therapy in a Cohort Study of Saudi Patients

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Abstract

Background and Objective: Precision medicine is a new field of medicine aimed to individualize patient therapy through identifying patient's genotype specifically with narrow therapeutic index medications. Warfarin is a widely used vitamin K antagonists (VKA) that showed great inter- and intra-individual dose variability depending on many genetic and non-genetic factors. This study was designed to assess the effect of 1173C>T SNPs of vitamin K epoxide reductase complex 1 (VKORC1) on the dose variability and patient response to warfarin in a cohort of Saudi population. **Methodology:** Initially, 190 patients from internal medicine and cardiology units of Prince Sattam bin Abdulaziz University "PSAU" hospital and Prince Sultan Military hospital were recruited over 12 months. Only 175 accepted to take part in this study. Patients who failed to be followed up were excluded (n = 11). Genomic DNA of all patients was isolated and quantified, then genotyped for rs9934438 variants (6484C>T or 1173C>T SNP) of VKORC1 gene by TaqMan allelic discrimination genotyping. The primary outcome was the time patients' INR value in therapeutic range (TTR). **Results:** The study participants (n = 164) were on chronic warfarin therapy for different indications. The minor allele frequency (MAF) of 1173C>T SNP in Saudi patients (0.259) is greatly different from other ethnic groups particularly Asian (0.90). Patients who have either genotype variant alleles showed significantly less warfarin dose. TTR was significantly lower in T/T homozygous genotype (68.4%) than C/C wild-type or heterozygous C/T genotype. **Conclusion:** VKORC1 1173C>T SNP is associated with lower warfarin dose in a cohort of Saudi patient and screening for this SNP tend to identify patients at risk of bleeding or patients that may have higher INR target.

Key words: Warfarin, vitamin K epoxide reductase complex 1, 1173 C>T single nucleotide polymorphism, therapeutic range, international normalized ratio

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

INTRODUCTION

Pharmacogenomics is a growing field and an approach to precision medicine and future therapeutics[1]. Coagulation disorders are managed successfully with vitamin K antagonists (VKA) however, their use was associated with several adverse events[2]. Precision medicine, through studying the genetic polymorphism related to a particular drug effect/side effect, could extend the available knowledge to optimize patient therapeutics.

Warfarin is one of VKA that is widely prescribed worldwide. However, its narrow therapeutic window and greater inter-individual dose variability limited its successful use without the hazard of adverse events. Several studies reported non-genetic and genetic factors that could influence the warfarin dose and so its efficacy among different ethnic groups[3]. The genetic factors may be related to warfarin pharmacokinetics or pharmacodynamics. Warfarin commercial preparation is a mixture of two enantiomers. The S-warfarin (the most potent form) is metabolized by the cytochrome P450 enzyme 2C9 and genetic polymorphism in CYP2C9 (*2 or *3) is associated with reduced warfarin dose[4].

The pharmacodynamics factor is related to the enzymatic target of warfarin known as vitamin K epoxide reductase (VKOR) and its gene product VKOR complex 1 (VKORC1)[5]. After cloning of VKORC1 in 2004, several single nucleotide polymorphisms (SNPs) were identified, studied and proved to affect warfarin dose requirements in different ethnic groups[6,7]. Among those SNPs, there is a well-established knowledge of a known sequence variants rs9934438 (6484C>T or 1173C>T) SNP in intron 1 of VKORC1 gene[8]. This SNP was the earliest one related to lower-dosage warfarin therapy[6]. Moreover, the importance of 1173C>T SNP is that it can be used a marker SNP to detect other SNPs with high linkage disequilibrium (LD) or haplotypes containing this 1173C>T SNP[9].

When warfarin therapy get started for any patient who has enzyme polymorphism, he/she will have a supra-therapeutic effect[10-12]. Unfortunately, previously published clinical trials focusing on genotyped-based warfarin dose-adjustment failed to confirm such assumption while patients are at acceptable therapeutic INR range but excessive bleeding during the initial phase of therapy[13, 14]. Recently, a study indicated that pharmacogenetic-based dosing of warfarin led to less low INRs and more time in the acceptable therapeutic range during the first 12 weeks[15].

Whether warfarin administration remains in therapeutic range or not in association with VKORC1 rs9934438 (6484C>T or 1173C>T) sequence variants in Saudi patient was evaluated in the current study.

MATERIALS AND METHODS

Study design: The study subjects were recruited from internal medicine and cardiology units of Prince Sattam bin Abdulaziz University "PSAU" hospital and Prince Sultan Military hospital. The patients were receiving a particular dose of continued warfarin therapy (minimum 4 weeks on therapy). Warfarin dose was started and maintained by the hospital medical staff. All patients' laboratory values were recorded manually throughout the study. No previous genetic variability related to warfarin therapy were reported to the treating staff. Patients were adults (age 18 years or more) and must provide a signed informed consent.

Preliminary, 190 patients were recruited to the study over 12 months (January-December, 2016). Only 175 accepted to take part in this prospective study; the other patients refused to be involved due to lack of interest and not because of any medical problem.

All involved patients received regular medical care offered to all other patients attending the specified hospital units and did not participate in the study. At the local anticoagulation clinic, the patients were followed by the International Normalized Ratio "INR" over a standard period of evaluation based on patient clinical condition with a minimum of two successive INR values must be available. The INR goal during this study was 2-3 for all patients. Beyond this INR target was not included in this study to avoid additional analysis. Any patient who failed to be followed up was excluded from the study (n = 11). The study was ended in March, 2017.

Ethics approval: The study protocol was approved by the local ethics committee before the beginning of this project. All healthcare personnel were trained and instructed to follow Helsinki declaration during the commencement of the study.

VKORC1 genotyping: Genomic DNA was isolated from mouthwash samples by FlexiGene DNA Kit (Qiagen, Valencia, 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 VKORC1-1173C>T (rs9934438), 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 VKORC1-1173C>T (rs9934438), TaqMan assays were purchased from Applied Biosystems/Fisher Scientific (Thermo Fisher Scientific, Foster City, CA, USA). The genotyping assays were performed and analyzed according to

the manufacturer's recommendations (Applied Biosystems/ Fisher Scientific, Foster City, CA, USA)[16].

Study outcome: The primary outcome was the time patient stays in therapeutic range (TTR) as outlined in the study protocol. These data were recorded directly from laboratory system then to the anticoagulation clinic archive. The warfarin dose prescribed during each follow-up visit was also obtained from pharmacy records then recorded to clinic archives. In order to accomplish the project, a separate project database with a manual entry for all records was created. Patients visits to the clinic were retrieved and documented. A total of 890 visits were recorded into the project database. This database included all required details like a number of high INR (>3.5) or low INR (<2), the number of visits of each patient, average days between visits, the number of times warfarin dose changed for every patient and days of follow-up. Average days off between each visit were obtained by days off between visits over the number of visits. The average warfarin dose between the different genotype groups was obtained from the initial warfarin dose during inclusion time.

Events recording and processing: The hospital-wide forms were used to record all project events categorized under the domain of the anticoagulation clinic. Such forms were critically reviewed by the principal investigator and the clinic medical staff. Major bleeding criteria (ISTH criteria) were implemented[17, 18]; however, no life-threatening events were encountered during this study period.

Statistical analysis: Statistical analyses were carried out with IBM SPSS 24.0 (SPSS Inc., Chicago, IL). The level of significance was set at a p-value of 0.05. Gaussian distribution of the variables was tested by inspecting Q-Q plots. The normally distributed variables were reported as mean±standard deviation (SD), the other variables were reported as median (quartiles) except for high INR readings that encountered less than low INR readings. For that, dichotomous axis for patients experiencing a high INR or low INR was used. The groups were compared utilizing one-way ANOVA, independent measures t-tests and corresponding nonparametric test as applicable.

RESULTS

The demographic and clinical characteristics of the study participants (n=164) are shown in Table 1. All patients were Saudian that included 99 males and 65 females with a mean age of 58.73±6.12 years. Warfarin therapy was indicated due to a variety of medical conditions, with the most frequent was deep venous thrombosis "DVT" (n = 93, 56.7%). Some patients had more than one indication of warfarin therapy as noted in Table 1. The patient involved in this study were on warfarin therapy for a mean of 5.22±1.53 years and most of them were followed for more than 8 months (n =137, 83.54%) over the project duration. More than 90% of the patient had INR range of 2-3 but, eleven patients (6.71%) had higher target INR of 2.5-3.5. With relation to patient medical condition during follow-up period. There weren't any clinical events that had been reported.

Table 1: Demographic and clinical characteristics of the study participants

Characteristics	Study participant (n = 164)
Ethnicity	
Saudian	164 (100)
Sex	
Male	99 (60.37)
Female	65 (39.63)
Other properties	
Wight (kg)	80.93±7.20
Height (m)	1.67±0.38
Age (years)	58.73±6.12
Medical condition requires warfarin therapy[a]	
DVT	93 (56.71)
AF	69 (42.10)
Mechanical valve	10 (6.10)
Duration for taking warfarin (years)	5.22±1.53
Follow up duration	
<8 months	27 (16.46)
>8 months	137 (83.54)
INR value	
2-3	153 (93.29)
2.5-3.5	11 (6.71)

[a]Patients might have more than one medical condition that requires warfarin therapy. Data are reported as n (%) or mean±SD. DVT: Deep venous thrombosis, AF: Atrial fibrillation, INR: International normalized ratio

The genotyping data of VKORC1 1173C>T are in Hardy-Weinberg equilibrium as shown in Table 2. The minor allele frequency (MAF) of the studied Saudi population were contrasted with other ethnic groups (Asian, Caucasian, European, African-American) according to reference data obtained from NCBI dbSNP and PharmGKB. The results suggest that the MAF of VKORC1 1173C>T SNP in Saudi population is not similar or close to any ethnic group. Contrasting with Asian ancestry (Japanese and Chinese) that have a high MAF, Saudi patient showed low MAF of VKORC1 1173C>T SNP reaching 0.259, even lower than the Caucasian and European.

Genotype of VKORC1 (rs9934438) with warfarin dose variations shown in Table 3. As anticipated, the chronic dose of warfarin was significantly less ($p<0.01$) for patient expressing either genotype variant alleles C/T or T/T. The only major difference for warfarin effect was for VKORC1 as

Table 2: Minor allele frequency (MAF) among the study population and other ethnic groups

VKORC1 SNP rs9934438	Genotyping	Frequency n (%)	Hardy-Weinberg equilibrium frequency	Minor allele frequency (MAF) Allele	Saudian (current study)	Different ethnic groups[a] Asian	Caucasian	European	African-American
1173C>T	CC	81 (49.39)	0.55	T	0.259	0.90	0.42	0.39	0.13
	CT	81 (49.39)	0.38						
	TT	2 (1.22)	0.07						

[a]Data from NCBI dbSNP (http://www.ncbi.nlm.nih.gov/) and PharmGKB (http://www.pharmgkb.org/)

Table 3: Results for patients on warfarin, INR 2.0-3.0 processed for VKORC1 genotype

VKORC1 rs9934438	Maintenance dose (mg/week)	TTR of INR (%) (SD %)	Days between visits days	Dose changes	Number of INRs* <2.0	INR>3.5 n (%)
C/C (n = 81)	44.0 (31.5-52.0)	72.5 (12.6)	23.9 (17.9-28.6)	2.5 (1-5)	3 (1-4)	16 (20)
C/T (n = 81)	29.0 (21.5-39.5)	70.1 (12.1)	20.6 (16.1-21.4)	3.5 (2-5)	3 (1-4)	26 (32)
T/T (n = 2)	19.0 (12.7-23.3)	68.4 (11.9)	20.5 (15.0-23.0)	2.0 (1-5)	2 (1-4)	1.0 (50)
P	<0.01	0.02	0.70	0.51	0.38	0.31

*Data are presented as median (min-max). VKORC1: Vitamin K epoxide reductase complex 1, TTR: Time in therapeutic range, INR: International normalized ratio

observed in time in therapeutic range. T/T genotype patients experienced less time in therapeutic range as shown in Table 3. There was a significant difference between VKORC1 genotypes when compared with each other only for the time in therapeutic range (68.4 vs. 72.5%, p<0.05), this was not experienced with other variables. There was no difference between genotype VKORC1 after testing the range of aforementioned variables for which no adjustment for any strong bias was carried out in this analysis.

Few patients (eleven patients) with target INR range of 2.5-3.5 who had an average TTR of 69.9% (14.2). In comparison with the majority of patients with INR range of 2-3, TTR was 69.1% (14.9). There is no statistical difference between the two ranges concerning TTR (p = 0.39). All the eleven patients were found to express VKORC1 genotype C/C.

DISCUSSION

The current cohort study explored the association of VKORC1 SNP rs9934438 (6484C>T or 1173C>T) with warfarin dose requirement and the time the patients remain in therapeutic range (TTR) among 164 patients from KSA. Although the 1173C>T SNP is believed to be an inert SNP, we decided to genotype it because it is used as a marker SNP and it is in close linkage disequilibrium with other VKORC1 SNPs (G3673A, T381C, G6853C, C7566T) in Asian ancestry[19]. The haplotype containing the minor alleles of these 5 SNPs is associated with lower warfarin maintenance dose compared to the haplotype containing the major alleles[20].

Comparing the minor allelic frequency (MAF) of the VKORC1 1173C>T SNP of Saudi patient to other ethnic groups showed greatly different pattern especially with Asian population (0.259 vs. 0.90, respectively) and inconsistent pattern with the Caucasian, European and African-American (Table 2). This result denotes that Asian population is heterogeneous groups that may have mixed ancestries over the centuries and pharmacogenomics research is very important to identify specific population genetic markup. In one study by Pathare *et al.*[21] the authors reported the MAF of VKORC1 (1639G>A) SNP in a 199 Omani patient of 0.36 that is quite close to our result.

The results of the current study showed that patients with their genotype expressing either variants alleles of 1173C>T SNP (C/T or T/T) had significantly lower warfarin dose than the wild-type. In addition, homozygous genotype (T/T) had the lowest TTR (68.4 vs. 72.5%, p<0.05) compared to the C/C homozygous wild-type. In agreement with our findings, some studies reported similar results with the VKORC1 1639AA and 1173TT genotypes constantly linked to lower chronic warfarin dose in Asian patients[19, 22].

Even though this study group gene sequence variants effect on INR levels was not extreme, VKORC1 genes sequence variants could disturb the outcomes of chronic warfarin dosing even when patients are tightly followed up. Patients with none (C/C) or one(C/T) VKORC1 variant alleles had better control on warfarin therapy than those with two variant allele (T/T). The latter had less time in therapeutic range. Therefore, in this study, we noticed no correlation between a number of alleles and time spent in the therapeutic range. In the T/T genotype group, the number of patients was n = 2 and they showed a lower TTR denoting that it is of low incidence.

Regarding the occurrence of supratherapeutic INR (>3.5) in relation to 1173C>T SNP, our results showed that the INR >3.5 was high with the T/T (50%) and C/T (32%) genotypes versus C/C wild-type (20%); although it did not research statistical significance (p = 0.31). The finding here is not different from the results of Verhoef *et al.*[12] study on VKA "phenprocoumon" and VKORC1 gene sequence variants

(1173C>T) that greatly affected patient's INR reporting supra-therapeutic INR in 33% of the wild-type patients (C/C), compared to 48% (p<0.001) and 66% (p<0.001) in patients with C/T or T/T variants, respectively.

As VKORC1 variants may explain some of the inter-patient warfarin dose variability, it is important not to neglect the combined influence of other genes particularly CYP2C9[12], CYP4F2 and EPHX1[1]. The variability of VKORC1 together with CYP2C9 account for up to one-third of inter-individual dosage variability. The study of Verhoef *et al.*[12] demonstrated that patients expressing CYP2C9*3 and CYP2C9*2 alleles had INR target >3.5 in high percentage than wild-type patients (62, 52 vs. 40%, respectively). The rs2108622 SNP of CYP4F2 is associated with altered vitamin K1 hepatic metabolism that necessitates larger dose of warfarin to obtain the desired therapeutic effect and may underlie about 4-12% of warfarin dose inconsistency especially in European[23]. In addition, the rs2292566 SNP of EPHX1 is coupled to 1.7% of warfarin dose variability that also entails lower dose[24].

It is really vital to create a reliable database system in the anticoagulation clinics to allow follow-up difficult to manage patients or those that record less TTR. In well-established point of care clinics that are centered on controlled outcomes, variables like drug interaction and vitamin K intake might not be of significant influence on warfarin effect and TTR, but gene sequence variants are. Patient age and duration of treatment at recruitment phase were perceived to be also predictors[25].

SIGNIFICANCE STATEMENT

The current study investigated the impact of VKORC1 rs9934438 SNP (6484C>T or 1173C>T) on warfarin therapy in a subset of Saudi patient and proved it is clinically relevant. This will facilitate to establish the value of pharmacogenomic testing in the relation to warfarin therapy. To our knowledge, this study is one of the rarest studies that prove the importance of VKORC1 1173C>T SNP in relation to warfarin therapy in Saudi patient, in addition, it is the first study to report the SNP minor allele frequency (MAF).

CONCLUSION

The current study highlights the importance of pharmacogenomics testing of VKORC1 SNPs to patients undergoing chronic warfarin therapy in order to adjust the warfarin dosage according to the patient genotype and avoid sub-therapeutic or supratherapeutic INR value. Patients that encounter excessive bleeding to the traditional warfarin doses or those spend less TTR should be assessed for the presence of VKORC1 variant alleles. Such procedure will be very useful for patient safety and better overall health care.

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