



Original article

Genetic risk assessment towards warfarin application: Saudi Arabia study with a potential to predict and prevent side effects



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ABSTRACT

Warfarin doses are greatly affected by polymorphism altering cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) gene. This study evaluated the prevalence of alleles (either single or double) and carriers of single nucleotide polymorphisms (SNPs) in both genotypes CYP2C9 and VKORC1 in alkharij area, Saudi Arabia and its association with warfarin use risk. Total 112 samples were collected and genotyped using FlexiGene DNA Kit for isolation and StepOnePlus Real-Time PCR System by TaqMan allelic discrimination methods. The results indicated the frequency of 11%, 8% and 45% for CYP2C9 *2 *3 and VKORC1-1639 G > A polymorphism. And as a combination genotype it was 15.18% For both CYP2C9 and VKORC1 polymorphism, 27.67% for CYP2C9 and 42.86% for VKORC1. Non-carriers rate came to be at 30.3%. According to previously published dosing changes in warfarin for polymorphism carriers (single-double-triple). The predicted warfarin doses reduction in order of 1–1.6, 2–2.9, 2.9–3.7 mg/day. It was found that 72.3% of the study population was carrier of a type of polymorphism, 15.18% for two types of polymorphisms. These findings predict changes in warfarin metabolism and eventually dosing alteration among patients on warfarin. Both genotypes (CYP2C9 and VKORC1) require different dosing of warfarin than non-carriers in order to minimize the risk of warfarin overdosing and avoidance of the drug-related problems (DRPs).

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1. Introduction

Warfarin is recognized as a widely used oral anticoagulation drug in several anticoagulation clinics worldwide (Ansell et al., 2004). It is a very effective anticoagulant used to manage certain disorders, e.g. venous thromboembolism risks. (Lim et al., 2018; Kearon et al., 2016). According to the statistics, more than 23 million warfarin prescriptions were written in the United States in 2007 (Top 200 drugs, 2007). The dosing-response curve of warfarin greatly varies among patients and often results in various unwanted responses like excessive bleeding episodes and sometimes stroke (Hirsh et al., 2001). Expected warfarin therapy outcomes are deeply reliant on several factors like age, race, body weight, international normalized ratio (INR) control, daily dose, dietary habits, and concurrent medication (Hylek, 2001). Due to

its narrow therapeutic index, warfarin therapy is always closely monitored utilizing the INR and dose adjustments are made accordingly (Ansell et al., 2004; Al-Eitan et al., 2019).

Undeniably, patients' genetics plays a significant role in exerting warfarin therapy outcomes in warfarin patients. Initially, CYP2C9 was thought to be attributed to the warfarin dose-response curve changes. It produces its effect via modification of S-warfarin molecule metabolism by CYP450 enzyme (Linder et al., 2009). Around 25–35% of the studied individuals carry CYP2C9 variant, which eventually alter the warfarin dose response. Based on literatures, patients' sensitivity to initial estimated dose could be greatly affected (Scordo et al., 2002).

Whereas, maintenance dose will not be achieved easily as seen in non-carriers therefore side effect as bleeding disorders will be manifested at higher percentages (Linder et al., 2002; Scordo et al., 2002). The projected mechanism of such changes in dose-repose is due to reduced enzyme activity of CYP2C9 (*2 to 70% and *3 to 5%) of normal rate due to polymorphism that takes place at gene levels (Rettie et al., 1994; Voora et al., 2005). Reduced rate of clearance will result in warfarin accumulation that leads to severe blood thinning effect and excessive bleeding complications (Higashi et al., 2002). The impact of CYP2C9 polymorphism is

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substantial and is reported to affect several populations and that is approximately 15–20% in warfarin dose variance (Scordo et al., 2002; Zhu et al., 2007; Hillman et al., 2004).

VKORC1 gene products are inhibited by warfarin in order to produce its anticoagulation property (Li et al., 2004). If someone is found to be a carrier of VKORC1 polymorphism genotype (–1639 G > A), then less dose is required at the early stages of therapy as compare to the chronic patients (Yuan et al., 2005). 20–25% of warfarin dose requirement is determined by VKORC1 promoter sequence –1639 G > A and its related haplotype (Li et al., 2004). Both aforementioned genotypes (CYP2C9 and VKORC1) as a combination would interpret up to 45% of warfarin dose response variability (Wadelius et al., 2007).

Implied clinical practices at anticoagulation clinics are not enough to prevent warfarin side effects during initial therapy stages. These practices are mainly attributed to available recommendation on initial warfarin dosing pattern that is usually decided to be 5–10 mg per day, which is re-evaluated in few days later. In several warfarin practice settings, individualized warfarin therapy approach according to genetic variability is not considered. Personalized therapy is a unique opportunity for all anticoagulation clinicians to tailor initial doses and avoid unfavorable adverse events. Tailoring initial and maintenance doses may result in less side effects, diminish hospital admissions and shorter hospital stays and decreased adverse events, which can further reduce warfarin therapy costs.

Fatal adverse events due to CYP2C9 and VKORC1 polymorphism had warranted the Food and Drug Administration (FDA) in 2007 to change warfarin labeling to clearly reflect these risks that occur at a rate of 7.2 and 1.3 per hundred patient-years respectively (Wattanachai et al., 2017; Linkins et al., 2003). Such incidents would easily be avoided if patients' genotype is identified beforehand. Around 85,000 incidents of major bleeding and 17,000 S annually in the United States can be prevented if warfarin patients are properly screened for possible genotypes prior to warfarin therapy initiation. This could save more than \$1 billion healthcare costs in the USA (McWilliam et al., 2006). Likewise, in most of western countries, several non-profit organizations are currently sponsoring warfarin-guided dosing protocols after reviewing the benefits of prior genotype screening for warfarin patients.

The aim of this study was to estimate the frequencies of CYP2C9 and VKORC1 polymorphism in alkharij area, Saudi Arabian cohort. This study also evaluates and compares findings with previously published studies regarding doses adjustments as a result of presence or absence of these genotypes in alkharij area, Saudi population.

2. Materials and methods

2.1. Study cohort

A total of 112 saliva samples of the patients visiting practice sites at alkharij university hospital between the period June 2017–December 2017. For patients who accepted to participate, samples

were processed and their genotypes were identified for the purpose of this study.

2.2. Laboratory analysis

Genomic DNA from mouthwash samples was isolated by FlexiGene DNA Kit (Qiagen, Valencia, CA, USA) and the concentration with 260/280 quality ratio for all isolated DNA samples were determined using the Nanodrop spectrophotometer (Wilmington, DE, USA). The DNA samples were genotyped for VKORC1 –1639G > A (rs9923231), 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 VKORC1 –1639G > A (rs9923231), 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). The genotyping assays were performed and analyzed according to the manufacturer's recommendations (Applied Biosystems/Fisher Scientific, Foster City, CA, USA).

2.3. Statistical analysis

Determining the rate of frequencies of selected alleles in Saudi Arabia was for loci interest. To estimate the magnitude of prevalence of warfarin dosing changes due to polymorphism in Saudi Arabian population. We subcategorized them into groups as per the presence or absence of VKORC1 and CYP2C9 genotypes and related subunits that we elected to evaluate for the purpose of this study. Available warfarin dosing equations for each genotype carrier was used (Zhu, et al., 2007). We estimated our dose according to person age of 55 years, sex, and ideal weight of 27 kg (KG). Two values were generated for each genotype we are evaluating. Dose changes (reduction) between our predicted dose against each genotype and the estimated dose for non-carriers was then calculated.

3. Results

Table 1 illustrates genotype CYP2C9 SNPs. It reflects 11% and 8% for alleles *2 and *3 respectively. Frequencies of VKORC1 SNPs (–1639A) are shown in Table 1 as well with 45% frequency. Non-carriers rate became to be at 30.3% of study population. Single gene polymorphism in our study sample was found to be 27.67% for CYP2C9 and 42.86% for VKORC1. Patients expressing one gene only of each genotype (CYP2C9 and VKORC1) were 52.67%. The lowest among all was the rate of subjects carrying triple genes which was zero%.

All genotypes were in Hardy-Weinberg equilibrium (HWE). Few samples were not processed due to low DNA concentration. Minor Allele frequencies (MAFs) of the SNPs study were compared to other populations as published in various studies (Asian, Caucasian, and African-American). The outcome of the analysis suggests great similarity with Caucasian in variant alleles VKORC1

Table 1
CYP2C9 and VKORC1 alleles.

Gene	Polymorphism	Allele count	Frequency %	Single gene carrier	Non-carrier
CYP2C9 Multiplex	*1/*1	80	71.4	27.67%	30.3%
	*1/*2	12	11		
	*1/*3	9	8		
	*2/*2	2			
VKORC1	–1639A	50	45	42.86%	

Table 2
Comparison of CYP2C9(*2, *3) and VKORC1 (–1639G > A (rs9923231 G/A)) minor allele frequencies in Saudi, Caucasian, and African-American population.

Gene	Polymorphism	Minor allele	Racial groups			
			Saudi	Asian ^a	African-American ^a	Caucasian ^a
VKORC1	3673G > A(rs9923231)	A	0.449	0.12	0.12	0.43
CYP2C9	*2C > T (rs1799853)	T	0.11	0.00	0.02	0.10
	*3 A > C (rs1057910)	C	0.08	0.03	0.00	0.06

^a Information from NCBI dbSNP (<http://www.ncbi.nlm.nih.gov/>).

(rs9923231 G/A) that came to be 0.449. Our compared results were for Caucasians 0.43, Asians 0.12, and African Americans 0.12. Variant alleles CYP2C9 results for our studied population were *2 0.11 and *3 0.08. Additionally, the closest population to our analysis outcome was the Caucasians population results. All data are shown in Table 2.

Both genotypes (VKORC1 and CYP2C9) carriers' warfarin dosing changes projection was according to published equations (Zhu et al., 2007). The effect of genotypes on clinical practices at anticoagulation clinics was estimated by calculating the regular dose which then compared with dosing for carriers of the CYP2C9 and/or VKORC1. Both genders and age were taken into consideration, in addition to physical attributes utilizing available algorithm of previous studies (Zhu et al., 2007).

It was noticed that 40.5% of our studied population are carriers of a single genotype (heterozygous) CYP2C9 (24.10% *1/*2 or *1/*3), VKORC1 (36.61% GA). These patients would require minimal dose reduction that did not exceed 1–2 mg. In contrast, patients that are double carriers (homozygous) (15.18%) in our study population, are required greater dose reductions (2–3 mg). It was obvious that more polymorphism would result in greater dose reduction.

4. Discussion

Even though this study was of limited subjects. The results showed that 72.3% of study population was carrier of one or more of studied polymorphisms that lead to changes in warfarin clearance (CYP2C9) and/or sensitivity (VKORC1). Presence of such polymorphism in a population often put individuals at a greater risk while administering warfarin. This greater risk might manifest as excessive bleeding due to warfarin accumulation in the body of the affected patients. Such risks are clearly observed if polymorphism carriers are provided with standard recommended warfarin doses. This warrants a properly tailored warfarin dosing for each patient after being evaluated for presence or absence of CYP2C9 or VKORC1 polymorphism. The studied population expressed single polymorphism of either studied types (55.35%), and double polymorphism of either studied types (15.18%) whereas none showed to have three/triple polymorphism.

This study also compared the obtained findings with other studies done in the region. It was observed that noted frequencies of studied population polymorphism were lower than earlier regional studies evident in the literature. It is understood that frequencies of genotype variants are different in each ethnicity (Al-Eitan et al., 2019). European and Native Americans polymorphism prevalence is observed at a rate of 60% and 20% respectively (Salari et al., 2005; Choudhry et al., 2006). The observation from this study seems to be not so different from Caucasian population studies (Higashi et al., 2002).

It was noted that there is great similarity with HWE. HWE is usually applicable to populations with limited diverse ethnicity. Most of the Saudi population is of known and local backgrounds that does not go beyond the Arabian Peninsula in many instances, especially in the central region where we collected our samples. Even though there is a favor for subpopulations among different

tribes residing the Arabian Peninsula, all of which have related ancestors one way or another. In this regard, it would be better to sample larger number of subject from different regions in Saudi Arabia, to evaluate for deviations, if any exists.

Our findings in the studied population especially with patients that carry more than one genotype, revealed a valuable information towards individualized therapy avoiding over dosing of warfarin. Patients with higher frequencies will have greater benefit of personalized therapy applications (Al-Eitan et al., 2019). We were able to estimate the average dose for daily warfarin requirement to be between 2 and 3 mg/day instead of 5 mg/day as the standard dose. The great difference between the findings and the standard dose will be enough to prevent major bleeding side effects of warfarin overdosing that is very risky and costly. It is worth mentioning that, in warfarin clinics nationwide, patients should be evaluated for any genetic variabilities once adverse events are reported.

As an acceptable number of samples were collected and 112 were used for genotyping for the most common CYP2C9 and VKORC1 genes that are of significant importance and clinical implication in actual practice settings. Earlier published studies also demonstrated a greater risk of adverse events in patients with African ethnicity and carrier of CYP2C9 variants (Schwarz and Stein, 2006). In other studies, Asians were found to have less warfarin requirements when VKORC1 is present as the genotype (Veenstra et al., 2005). In these studies warfarin dosing was suggested according to genotype which definitely can be very useful to avoid various unfavorable adverse events in warfarin patients (Womack, 2005). On the other hand, Caucasian populations with CYP2C9 polymorphism are having more risk as of warfarin overdosing that increases bleeding events 2 to 3 folds during the early phases of drug initiation that was not the same as the maintenance phase (Visser et al., 2004; Taube et al., 2000). It was also suggested that when both genotype polymorphism exist, the chances for adverse drug events might be severe or life-threatening mainly due to increased bleeding episodes as a result of warfarin overdosing (Schalekamp et al., 2006).

Personalized therapy is an evolving practice in several western countries where healthcare is very advanced but concerned practitioners are still struggling to persuade their officials for such importance. Positive clinical outcomes on the community are tremendous (Kearon et al., 2016). These days, more clinical settings are exploring personalized medicines in order to improve patients' outcomes by targeting individual drug therapies with sensitive genotypes in an effort to tailor appropriate warfarin therapy. Various warfarin dosing protocols are currently far-away from gene-guided dosing which ultimately increase the risks of warfarin overdosing and extensive bleeding that is very obvious in the early stages of drug initiation (Veenstra, et al., 2005).

5. Conclusion

As seen in this research and many other related data, the warfarin dosing and general anticoagulation therapies must be tailored to each patient needs based on their genotyping pattern.

In addition, each patient should be evaluated for possible polymorphism before the initiation of intended anticoagulation therapy. The greater risk resides during therapy initiation phase regardless of the responsible gene. As genotype-guided pharmacotherapy definitely provides better therapy outcomes, hence it should be a standardized “standard of care” (Ruano, 2004).

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