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REVIEW ARTICLES

Validation of Pharmacogenetic Testing Before Initiation of Warfarin Therapy

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Abstract:

Warfarin is an oral anticoagulant used to prevent or treat clotting disorders associated with venous thrombosis, pulmonary embolism, atrial fibrilation, cardiac valve replacement, stroke and acute myocardial infarction. It is a vitamin K antagonist composed of S- and R- isomers. The more potent S-warfarin is metabolized by cytochrome 450 isoenzyme 2C9 (CYP2C9), encoded by CYP2C9 gene. Warfarin exerts its anticoagulants effect by inhibitingits target enzyme vitamin K epoxide reductase (VKOR), encoded by vitamin K epoxide reductase subunit 1 (VKOR1) gene. Genetic variation in the CYP2C9 and VKOR1 gene can affect warfarin efficacy and dose required to achieve stable International Normalization Ratio (INR). Specifically two variants in the CYP2CP gene (CYP2C9*2 and CYP2C9*3) result in an enzyme with reduced activity, leading to increased active warfarin levels. A variant in the VKORC1 gene (VKORC1-1639 G>A) can lead to reduced gene expression resulting in decresed level of VKOR. Together these three variants can account for 40-70% of the variability of warfarin dose. Carriers of variant alleles are at higher risk for bleeding complications, particularly at the induction of warfarin therapy. So, genotype-guided dosing algorithms would be better approximate for maintenance of warfarin dose than fixed-dose algorithms.

Key words: Warfarin; Genetic polymorphism; Dose.

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Introduction:

Warfarin is an oral anticoagulant prescribed for the prevention and treatment of thrombotic disorders such as atrial fibrillation, heart valve replacement, history of thrombosis, and post-orthopedic surgery.[1] It is clinically available as a racemic mixture of R and S isomers. The S-isoform is 2–5 times higher potent than the R-isoform and is metabolized mainly by the CYP/CYP450 2C9 enzyme (*CYP2C9*) in the liver.[1,2] Warfarin act as an anticoagulant by deactivation of the vitamin K epoxide reductase C complex (*VKORC1*) and regenerating the reduced form of vitamin K that is necessary for the activation of coagulation factors.[1,3,4] The efficacy and safety of warfarin depend mainly on the maintenance of prothrombin time, represented by the international normalized ratio (INR), within the therapeutic range. Analysis of many studies has shown that higher INR values are related to an increased risk of hemorrhage, while a lower INR value leads to a high risk of thromboembolism or stroke.[5–8] However, reaching the target INR may take weeks and increasing dose to achieve target INR may increase the risk of adverse drug events during the initial phase of therapy.[9] Knowing the extent to which genetic and environmental factors affect the anticoagulant response might help predict more individualized warfarin doses, resulting in a more accurate and safe anticoagulation therapy.[10]

In the late 2000s, the United States Food and Drug Administration announced that the warfarin label stat (which describes patients' therapeutic dosage) had to focused its pharmacogenetic activity, particularly in patients with *VKORC1* 1, 639G>A, *CYP2C9* *2, and *CYP2C9* *3 polymorphisms.[11–13] Polymorphisms in genes encoding metabolic enzymes, transporters, and drug receptors can modulate warfarin response.[14] Correspondingly, pharmacogenetic analysis of two genes, *CYP2C9* and *VKORC1*, suggests that their genetic variants strongly influence the individual response to warfarin.[3] *CYP2C9* metabolizes almost 25% of all clinical medications, and genetic variation in *CYP2C9* gene can

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have a significant effect on the outcome of treatment, particularly for drugs with a narrow therapeutic index like warfarin.[15] Polymorphisms of *CYP2C9* coding region have been extensively studied and over 30 alleles have been recognized.[16] The most common *CYP2C9* allele, *CYP2C9* *1 (wild type), *2 and *3. Both the *CYP2C9* *2 and *3 alleles play an important role in the metabolic activity of warfarin.[17] In 2007 the Food and Drug Administration (FDA) issued a labeling change advising physicians to consider the use of 'genetic tests to improve their initial estimate' of warfarin dose.[18]

The goal of pharmacogenetics is to predict patient's response to a specific drug, based on the genetic variations, in order to deliver the best possible medical treatment. Prediction of the drug response of an individual can increase the success of therapies and reduce the incidence of adverse side effects.

Objectives

In order to summarize the current evidence supporting the use of warfarin pharmacogenetics, we performed a review of articles, published in esteemed journals that compared a dose-selection strategy that used pharmacogenetic information to one that did not.

Method

Data Sources We searched PubMed, EMBASE and the International Pharmaceutical Abstracts through May 5, 2019. We examined the reference lists of included articles and professional reviews, and contacted experts to identify other potentially relevant studies.

We included numerous observational and prospective studies and randomized controlled trials that compared clinical outcomes among a pharmacogenetic dosing group, using common genetic variants of CYP2C9 and/or VKORC1, to a dosing algorithm that did not incorporate genetic testing. Eligible studies enrolled adult, warfarin-naïve patients with any indication for warfarin therapy, including atrial fibrillation, venous thromboembolic disease, recent orthopedic surgery and valvular disease.

Review Of Literature

Gaikward et al. 2018 was carried out a study in the King Edward Memorial Hospital, Mumbai, BYL Nair Charitable Hospital, Mumbai, as well as clinics located in Mumbai and other parts of India. The main aim of this study was to screen various genetic and nongenetic factors that are known to alter warfarin response and to generate a model to predict stable warfarin maintenance dose for Indian patients. The study was comprised of 300 patients who were prescribed warfarin for various clinical conditions. Followed by extensive literature review, 10 single-nucleotide polymorphisms, that is, VKORC1-1639 G>A, CYP2C9*2, CYP2C9*3, FVII R353Q, GGCX 12970 C>G, CALU c.*4A>G, EPHX1 c.337T>C, GGCX: c.214þ597G>A, GGCX: 8016G>A, and CYP4F2 V433M, and 5 nongenetic factors, that is, age, gender, smoking, alcoholism, and diet, were selected to find their association with warfarin response. The univariate analysis was carried out for 15 variables (10 genetic and 5 nongenetic). Five variables, that is, VKORC1-1639 G>A, CYP2C9*2, CYP2C9*3, age, and diet, were found to be significantly associated with warfarin response in univariate analysis. These 5 variables were entered in stepwise and multiple regression analysis to generate a prediction model for stable warfarin maintenance dose. The generated model scored R2 of 0.67, which indicates that this model can explain 67% of warfarin dose variability. The generated model will help in prescribing more accurate warfarin maintenance dosing in Indian patients and will also help in minimizing warfarin-induced adverse drug reactions and a better quality of life in these patients.[19]

Al-Eitan et al. 2019 conducted a study in the period of January 2014 to November 2015 in Queen Alia Heart Institute under Jordan University of Science and Technology in Irbid and the Royal Medical Services in Amman, Jordan. Patients taking *CYP2C9*-inducing drugs or those receiving concomitant treatment known to interact with warfarin or recorded clinical data lost, pregnant women, and alcohol abuse were excluded. In total, 416 subjects (205 healthy controls free of any cardiovascular disease and 211 cardiovascular patients) who were aged ≥18 years, who had been receiving warfarin for at least 3 months. They found that the genotypic and allelic frequencies differ significantly between patients and healthy individuals; therefore, their results suggest that this polymorphism is associated with cardiovascular disease in the Jordanian population. Moreover, during the initiation phase of therapy, 20% of warfarin-sensitive patients were homozygous for a short allele (p-VNTR-S), and 12.2% were heterozygous for this allele (p-VNTR-M/p-VNTR-S). During the stabilization phase, no significant differences were found between these groups and their genotypic frequencies. Additionally, they did not confirm any relationship between the *CYP2C9* p-VNTR polymorphism and warfarin response during either the initiation or the stabilization phases of therapy. They conclude their study that, significant difference between the *CYP2C9* p-VNTR polymorphism and risk of cardiovascular disease, in addition to significant association between this polymorphism and sensitivity to warfarin at the initiation phase of therapy in a Jordanian population. However, there is no correlation between this polymorphism and warfarin response, international normalized ratio (INR) values, or required warfarin dose to achieve a target INR either at the initiation or stabilization phases of therapy. Finally they recommended for further study with a larger number of samples and different ethnic groups.[20]

A study done by Galvez et al. 2018 in a tertiary level hospital named Rosario University's Teaching Hospital (Méderi), Bogota, Colombia, where 152 patients enroled in this study. They genotyped *CYP2C9**2 (c.430C > T), *CYP2C9**3 (c.1075A > C), *CYP4F2* (c.1297G > A), and *VKORC1* (-1639 G > A) polymorphisms in all patients who received warfarin. They evaluated the impact on the variability of patients' warfarin dose requirements. Multiple linear regression analysis, using genetic and non-genetic variables, was used for creating an algorithm for optimal warfarin maintenance dose. Median weekly prescribed warfarin dosage was significantly lower in patients having the *VKORC1*-1639 AA genotype and poor *CYP2C9*2/*2,*2/*3* metabolizers than their wildtype counterparts. They found a 2.3-fold increase in mean dose for normal sensitivity patients (wild-type *VKORC1/CYP2C9* genotypes) compared to the other groups (moderate and high sensitivity); 31.5% of the patients in this study group had warfarin sensitivity-related genotypes. The estimated regression equation accounted for 44.4% of overall variability in regard to warfarin maintenance dose. The algorithm was validated, giving 45.9% correlation ($R2$=0.459). This study results describe and validate the first algorithm for predicting warfarin maintenance in a Colombian mestizo population and have contributed toward the understanding of pharmacogenetics in a Latin American population subgroup.[21]

A meta analysis of Randomized Controlled Trials (RCT) was carried out by Feifei et al. 2019 in China, where intotal 2137 participants from 14 RCTs were included in the meta-analysis. Primary analysis showed that both bleeding events [odds ratio (OR) = 0.24; 95% confidence interval (CI), 0.11–0.52; P = 0.0003] and adverse events (OR = 0.60; 95% CI, 0.43–0.83; P = 0.002) were significantly lower in the genotype-guided group than in the clinical or standard group. The percentage of patients who received a warfarin-stable therapeutic dose during follow-up was increased in the genotype-guided group compared with the percentage in the clinical or standard group (OR = 2.68; 95% CI, 1.82–3.95; P < 0.00001). In the genotype-guided group, the time to a stable therapeutic dose (mean difference = "7.98; 95% CI, "9.08 to "6.87; P < 0.00001) and the time to the first target value (mean difference = "1.87; 95% CI, "3.41 to "0.32; P = 0.02) were shortened compared with those of the clinical or standard group, but there was no difference for international normalized ratio >4, between the 2 groups (OR = 0.42; 95% CI, 0.14–1.25; P = 0.12). This metaanalysis concluded that, genotype-guided warfarin-dosing algorithms could improve the efficacy and safety of warfarin anticoagulation in the Chinese population.[22]

Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update recommended that pharmacogenetic warfarin dosing be accomplished through the use of the pharmacogenetic dosing algorithm (Figure-1).[23]

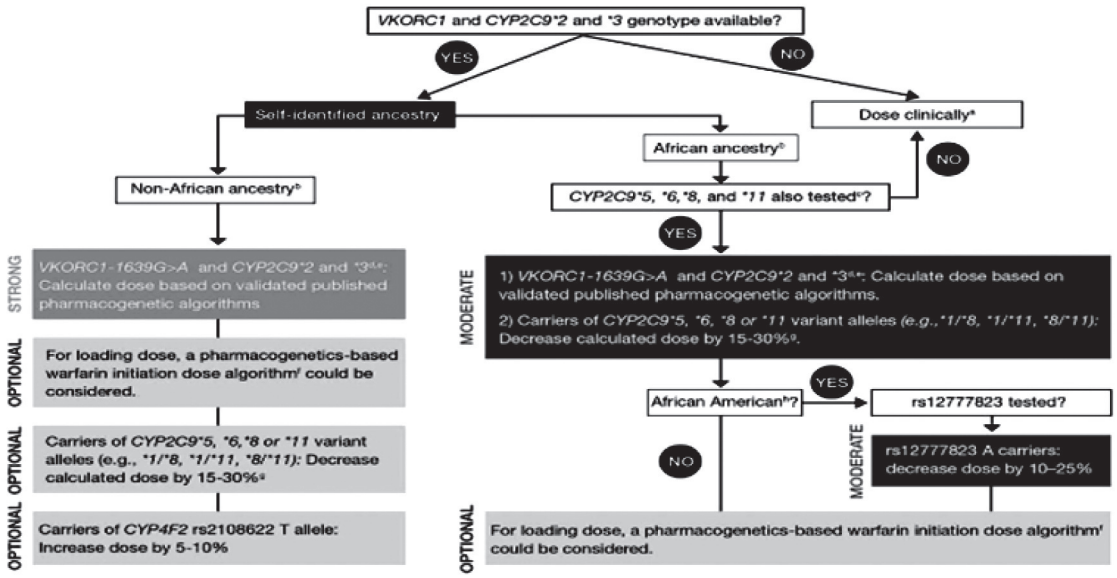

Figure-1: Dosing recommendations for warfarin dosing based on genotype for adult patients. (a) "Dose clinically" means to dose without genetic informa-tion, which may include use of a clinical dosing algorithm or standard dose approach. (b) Data strongest for European and East Asian ancestry populations and consistent in other populations. (c) 45–50% of individuals with self-reported African ancestry carry CYP2C9*5, *6, *8, *11,or rs12777823. IfCYP2C9*5, *6, *8,and*11 were not tested, dose warfarin clinically. Note: these data derive primarily from African Americans, who are largely from West Africa. It is unknown if the same associations are present for those from other parts of Africa. (d) Most algorithms are developed for the target INR 2-3. (e) Consider an alternative agent in individuals with genotypes associated with CYP2C9 poor metabolism (e.g.,CYP2C9*3/*3, *2/*3, *3/*3) or both increased sensitivity (VKORC1A/G or A/A) and CYP2C9 poor metabolism.[23]

FDA Approved Warfarin Dose:

The FDA (2017) Drug Label for Warfarin. Three ranges of expected maintenance warfarin doses based on CYP2C9 and VKORC1 Genotype.

VKORC1	CYP2C9				
	*1/*1 Normal/Extensive metabolizer	*1/*2 Intermediate metabolizer	*1/*3 and *2/*2 Slow metabolizer	*2/*3 Slow metabolizer	*3/*3 Very slow metabolizer
GG Low sensititviy	5.0-7.0 mg	5.0-7.0 mg	3.0-4.0 mg	3.0-4.0 mg	0.5-2.0 mg
AG Medium sensitivity	5.0-7.0 mg	3.0-4.0 mg	3.0-4.0 mg	0.5-2.0 mg	0.5-2.0 mg
AAHigh sensitivity	3.0-4.0 mg	3.0-4.0 mg	0.5-2.0 mg	0.5-2.0 mg	0.5-2.0 mg

Ranges are derived from multiple published clinical studies. The *VKORC1, c.–1639G>A* (rs9923231) variant is used in this table. Other co-inherited *VKORC1* variants may also be important determinants of warfarin dose. Patients with *CYP2C9 *1/*3*, *2/*2*, *2/*3*, and *3/*3*may require more prolonged time (>2–4 weeks) to achieve a maximum international normalized ratio (INR) effect for a given dosage regimen than patients without these *CYP* variants.

Please see Therapeutic Recommendations based on Genotype for more information. This table is adapted from the FDA-approved drug label for warfarin.[24]

Conclusion:

Our review study demonstrated that genotype-guided warfarin-dosing algorithms could improve the efficacy and safety of warfarin anticoagulation. This study also recommended that further clinical study can be carried out among Bangladeshi population to find out the validation of pharmacogenetic testing before initiation of warfarin therapy.

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