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Original Article

Effect of *VKORC1* Gene Polymorphism on Warfarin Response in Razavi Khorasan Province Cardiovascular Patients

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ABSTRACT

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Background and Aims: Warfarin is an anticoagulant agent used for many years in treating various clinical conditions such as thromboembolisms in cardiovascular disease. Some patients require different doses of warfarin to reach the therapeutic international normalized ratio ratio. These patients have specific demographic characteristics. Genetic polymorphisms in specific genes have been reported to be an essential factor in response to warfarin. The present study investigated the effect of these polymorphisms of genes on warfarin dose necessities in pediatric of *VCORC1* gene in patients.

Material and Methods: Ninety-five patients with cardiovascular disease, who were receiving warfarin for at least three months, enrolled in the present cross-sectional study. Their genomic DNA was extracted from their peripheral blood, and the *VKORC1* (*rs9923231*) polymorphism was evaluated by polymerase chain reaction and sequencing.

Results: Among the study population, 48 patients (50.5%) had TC genotype and, 21 (22.1%) and 9 (9.5%) patients have TT and CC genotype, respectively. There was no significant relation between Warfarin dose and *VCORC1* genotype in our population ($p<0.05$).

Conclusions: The *VKORC1* polymorphism (*rs9923231*) did not significantly affect the warfarin required for cardiovascular disease patients. Further studies evaluating other genes such as *CYP2C9* polymorphisms in our population are warranted.

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Introduction

Cardiovascular disease is responsible for more than 30% of annual deaths, leading to 17.7 million deaths annually [1]. According to the latest world health organization report, the prevalence of cardiovascular disease in Iran is 46%. A notable portion of cardiovascular diseases such as chronic heart failure and atrial fibrillation require long term anticoagulant therapy to reduce the mortality rate [2]. Warfarin is a commonly used anticoagulant agent that is a coumarin derivate [3]. This drug will inhibit the synthesis of coagulate factors related to vitamin K, including II, VII, IX, and X factors in the pathway, and C and S proteins in the intrinsic pathway [4]. Alongside the beneficial effects of warfarin, this drug is well known because of its narrow therapeutic window. This property causes the prescription and monitoring of the anticoagulant effects of warfarin more complicated. Prescribing the same dose of warfarin in different patients may provide various effects and require careful international normalized ratio (INR) monitoring. Despite careful dosage according to INR status, still in many cases the therapeutic goal couldn't be achieved [5]. Some individual factors such as age, gender, bleeding history, drug interaction, and genetic factors are considered to be important in adjusting the warfarin dose according to INR status. Among genetic factors, CYP29 is one of the first genes which has been associated with warfarin sensitivity [6-10]. *VKORC1* gene is located on the short arm of 16 chromosomes and code a membrane protein consisted of 163 amino acids. VKOR complex converts inactive Vitamin K 2, 3-epoxide to activated vitamin K. Vitamin K is a cofactor for carboxylation of residual glutamic acid from gamma carboxylase of coagulating enzyme's. Allelic variations in *VKORC1* will result in different responses to warfarin therapy [11, 12]. A single nucleotide polymorphism (SNP) in the *VKORC1* gene (*rs9923231* (1639 G→A). This SNP will result in lesser enzyme products and is mostly seen in the Asian and European populations. The lower amount of enzyme could lead to increased sensitivity to warfarin. Patients carrying this SNP can tolerate a higher risk of warfarin toxicity and bleed while using the same dose as the rest of the population [13]. Recently, the international warfarin pharmacokinetics consortium has proposed the effect of *VKORC1* polymorphism in their guidelines and stated that some studies had suggested their guidelines for their specific populations [14]. Such reports emphasize the importance of genetic polymorphisms in response to different drug therapies. In the present study, the prevalence of a *VKORC1* polymorphism in an Iranian population using warfarin was investigated.

Materials and Methods

Study design

This cross-sectional study was performed in Mashhad, Iran. The Nishaboor Azad medical university ethic committee has approved the present research, and all the participants have filled an informed consent form. The study population was selected from patients referred to Mashhad's Javad Al-Aeme hospital in 2 years,

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starting from 2015 to 2017. The sample size of the presented study was calculated as 78 patients (power and confidence: 90 and 95, respectively). The cardiovascular patients receiving warfarin for at least three months enrolled in the present study. None of the patients had taken the drugs which have primary interaction with warfarin (Amiodarone, statins, omeprazole or non-steroid anti-inflammatory drugs), history of hematologic disease, peptic ulcer, thyroid, liver or kidney dysfunction, autoimmune or infectious disease, malignancies, non-compensated heart failure and smoking more than ten cigarettes per day. The patient's medical and demographic data and maintenance warfarin dose were documented [15].

DNA extraction and polymerase chain reaction (PCR) method

To determine the patient's genotype for the desired polymorphism, 5ml of the whole blood sample was collected in Ethylenediamine-tetraacetic acid tubes. The tubes were stored at -20˚C, and the DNA was extracted using a QIAamp DNA Mini Kit (Yekta Tajhiz Teb, Iran). Two pairs of primers were designed in Table 1. PCR and sequencing were used to detect the desired polymorphisms: 1 µl of DNA was added to 10 µl of PCR master mix, and 0.75 µl of each forward and reverse primers (Dena Zist, Iran) were added to the mixture. The final volume was diluted to 25 µl. The denaturing temperature was considered as 94˚C for 1 minute, 59˚C, and 62˚C for annealing of primer sets for 30 seconds, respectively, and 70˚C for 25 minutes were considered as extension cycle. Five microliters of the PCR product were loaded on agarose, and the presence of the specific band was checked by gel electrophoresis for 60 minutes (50V DC). The PCR product was sent for sequencing, and the results were interpreted [16].

Statistical analysis

Mean values and standard deviation were used for describing the study variables. Chi-square and Fisher's exact test and regression tests were used to define the nominal variables and describe the relationship between genotypes. The SPSS software version 22 was used for data analysis, and P values less than 0.05 were considered statistically significant.

Results

Among the study population, the mean and standard deviation (SD) age was 54.3(19.6), and most of the participants were female (49 patients, 51.6%). The mean (SD) of height and weight of the patients was 162.2 (15.9) and 69.3 (17.2), respectively (Table 2). The mean (SD) of warfarin dose was 31.4 (13.1) mg with maximum and minimum values of 8.8 mg and 75 mg, respectively. Cardiac valve replacement surgery, heart failure, and vein thrombosis were the indications for receiving warfarin according to their prevalence among our population. Most of the patients were not receiving Amiodarone (68 patients, 71.6%). Fifty-five (57.9%) patients experienced hemorrhagic events. Patient characteristics are presented in Table 3.

Frequencies of *VCORC1* alleles and genotypes

The frequencies of the *VCORC1* (*rs9923231*) alleles and genotypes are listed in table 4.

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Among the study population, only warfarin dose and gender were significant. The patient's genotype was not significantly related to warfarin dose (p=0.801).

Table 1. SNP, primer sequences

***SNP* name**	**Primer sequences**
VCORC1	F= 5' CTCCAGGGTTCAAGTGGTTC 3'
(rs9923231)	R= 5' ACAGACGCCAGAGGAAGAGA 3'

Table 2. Characteristics of study populations

Variables	**Number 95 (%)**
Sex	
Male	46 (48.4%)
Female	49 (51.6%)
Age (year)	
Minimum	5
Maximum	80
Average	54.3
Weight (kg)	
Minimum	18
Maximum	105
Average	69.3
Height (cm)	
Maximum	190
Minimum	100
Average	162.2
Disease distribution (month)	
Maximum	420
Minimum	3
Average	70.9

Table 3. Distribution of study variables and their relation with warfarin dose (mg)

Study variables		**Warfarin dose (mg)**				**p**
		Minimum	**Maximum**	**Average**	**Standard deviation**	
Age (Year)	Younger than 30	12.5	61.3	35.3	17.6	0.062
	30-50	17.5	75.0	41.0	16.9	
	51-70	8.8	52.5	29.3	10.1	
	Older than 70	8.8	35.0	25.8	9.4	
Gender	Male	8.8	75.0	35.1	15.5	0.012
	Female	8.8	50.0	27.7	9.0	
Weight (kg)	Lower than 50	8.8	52.5	26.5	15.6	0.538
	50-70	10.9	61.3	31.6	10.6	
	71-90	8.8	52.5	30.9	11.0	
	More than 90	8.8	75.0	38.1	25.1	
Height (cm)	Shorter than 160	8.8	52.5	28.7	12.0	0.179
	160-170	17.5	61.3	29.9	10.0	
	Higher than 170	8.8	75.0	36.1	17.0	

Table 4. Distribution of genotypic characteristics of study participants for *VKORC1* polymorphism

GENE	**SNP**	**Genotype**	**Frequency number (%)**
VKORC1	*rs9923231*	Unknown	17 (17.9)
		TT	21 (22.10)
		TC	48 (50.52)
		CC	9 (9.5)

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Discussion

As an inexpensive anticoagulant for managing many cardiovascular diseases, warfarin has become a common drug of choice in many countries [17]. However, the narrow therapeutic window of this drug is the main challenge for physicians yet. Careful dosing of this drug will reduce the risk of further complications, such as bleeding [18]. In the present study, the possible effect of genetic polymorphism in warfarin response was evaluated. *VKORC1* gene is an essential factor in warfarin metabolism. According to the results, a *VKORC1* polymorphism (*rs9923231*) is prevalent in Iran's northeast, even though this SNP is not related to warfarin dose. In the population, 9.5% of patients had wild type homozygote genotype, and 50.5% were heterozygote. In a similar study with a slightly greater sample size from Shiraz and Brigand cities, most of the patients had a heterozygous genotype (57.1% and 45.9%, respectively). However, our population had a 9.5% wild type homozygote genotype as the least prevalent genotype, while the study from Birjand indicated that the non-wild type homozygote genotype is the least pervasive [19]. A possible reason for this different finding could be related to the study population. In Razavi et al. study, they have chosen their sample size among the patients referred for checking their prothrombin time and partial thromboplastin time while we have chosen our patients among those with cardiovascular diseases [20]. Another study conducted on 29 patients who were receiving warfarin in the north of China has shown that the TT genotype was the most common (89.7%) [21]. While our study fails to establish a statistical correlation between warfarin dose and different *VKORC1* genotypes, the T allele required a lesser warfarin dose to achieve the stable INR. McKinney et al. study has shown that the C allele requires higher doses of warfarin [22]. Yuan et al. have reported that the wild-type homozygote patients will require higher doses of warfarin while the TT and CT genotype did not show any difference in terms of the necessary warfarin dose [23]. A study on the Ashkenazi population has demonstrated that the heterozygote genotype will require more warfarin [24]. Also, the non-wild type homozygote patients face a reduced amount of coagulate factors related to Vitamin K. While putting the possible effects of the same SNP in different populations aside, the next leading probable cause of varying response to warfarin could be explained because of other genes. A Japanese study has shown that the *VKORC1* is not related to warfarin response and demonstrated that *CYP2C9* and CYP4F2 genes are related to warfarin response in their population [25]. There are some other genes that are responsible for warfarin response that can be seen in other studies as well. For example, in an Iranian patient who was resistant to great doses of warfarin (more than 100mg warfarin per day), the plasma dose of warfarin was higher than the therapeutic limit (22.8 mg/lit) [26]. The authors didn't find any mutation in *VKORC1* gene and proposed that

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the genetic effect may be in other genes responsible for response.

Conclusion

The present study could not find a significant relation between warfarin dosing and *VKORC1* polymorphism in an Iranian population. While the response to warfarin has been reported to be related to other genetic polymorphisms, the next step would be evaluating polymorphisms in other genes such as *CYP2C9* in our population.

Conflict of Interests

The authors declare no competing financial and non-financial interests.

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References

[1]. Kasper D, Fauci A, Hauser S, Longo D, Jameson J, Loscalzo J. Harrison's principles of internal medicine, 19th ed. New York; McGraw-Hill; 2015, p. 1442.
[2]. Taghadosi M, Memarian R, Ahmadi F. Experiences of warfarin use among cardiac valve-replaced patients in Iran. Iran J Critic Care Nurs. 2014; 6(4): 213-22.
[3]. Tabatabaeifar SM, Monfaredan A, Bargahi N. CYP2C9 gene polymorphism and warfarin dosage in thrombotic events study form in northwest of Iran. Med J Tabriz Univ Med Sci. 2012; 34(2): 75-80.
[4]. Chen J, Shao L, Gong L, Luo F, Wang Je, Shi Y, et al. A pharmacogenetics-based warfarin maintenance dosing algorithm from Northern Chinese patients. PLoS One 2014; 9(8): 1685-693.
[5]. Wadelius M, Pirmohamed M. Pharmacogenetics of warfarin: current status and future challenges. Pharmacogenom J. 2007; 7(2): 99-111.
[6]. An S, Lee K, Chang B, Gwak H. Association of gene polymorphisms with the risk of warfarin bleeding complications at therapeutic INR in patients with mechanical cardiac valves. Journal Clin Pharmacy Therapeut. 2014; 39(3): 314-18.
[7]. Lu Y, Yang J, Zhang H, Yang J. Prediction of warfarin maintenance dose in Han Chinese patients using a mechanistic model based on genetic and non-genetic factors. Clin Pharmacokinet. 2013; 52(7): 567-81.
[8]. Nguyen N, Anley P, Margaret YY, Zhang G, Thompson AA, Jennings LJ. Genetic and clinical determinants influencing warfarin dosing in children with heart disease. Pediatr Cardiol. 2013; 34(4): 984-90.
[9]. Ye C, Jin H, Zhang R, Sun Y, Wang Z, Sun W, et al. Variability of warfarin dose response associated with CYP2C9 and VKORC1 gene polymorphisms in Chinese patients. J Int Med Res. 2014; 42(1): 67-76.
[10]. Cullell N, Carrera C, Muiño E, Torres N, Krupinski J, Fernandez-Cadenas I. Pharmacogenetic studies with oral anticoagulants. Genome-wide association studies in vitamin K antagonist and direct oral anticoagulants. Oncotarget 2018; 9(49): 29238.
[11]. Banavandi MJS, Satarzadeh N. Association between VKORC1 gene polymorphism and warfarin dose requirement and frequency of VKORC1 gene polymorphism in patients from Kerman province. Pharmacogenom J. 2020; 1(1): 1-5.
[12]. Kurnik D, Qasim H, Sominsky S, Lubetsky A, Markovits N, Li C, et al. Effect of the VKORC1 D36Y variant on warfarin dose requirement and pharmacogenetic dose prediction. Thrombos Haemostas. 2012; 108(10): 781-88.
[13]. Consortium IWP. Estimation of the warfarin dose with clinical and pharmacogenetic data. New England Journal of Medicine 2009; 360(8): 753-64.
[14]. Lane S, Al-Zubiedi S, Hatch E, Matthews I, Jorgensen AL, Deloukas P, et al. The population pharmacokinetics of R-and S-warfarin: effect of genetic and clinical factors. British journal of clinical pharmacology 2012;73(1):66-76.
[15]. Caldwell MD, Awad T, Johnson JA, Gage BF, Falkowski M, Gardina P, et al. CYP4F2 genetic variant alters required warfarin dose. Blood 2008; 111(8): 4106-112.
[16]. Shirkani A, Mansouri A, Abbaszadegan MR, Faridhosseini R, Azad FJ, Gholamin M. GATA3 gene polymorphisms associated with allergic rhinitis in an Iranian population. Reports of biochemistry & molecular biology 2017; 5(2): 97.
[17]. Mahtani KR, Heneghan CJ, Nunan D, Bankhead C, Keeling D, Ward AM, et al. Optimal

[DOI: 10.18502/ijml.v7i4.4795]

loading dose of warfarin for the initiation of oral anticoagulation. Cochrane Database of Systematic Reviews. 2012(12): CD008685.

[18]. Azarpira N, Namazi S, Hendijani F, Banan M, Darai M. Investigation of allele and genotype frequencies of CYP2C9, CYP2C19 and VKORC1 in Iran. Pharmacological Reports 2010; 62(4): 740-46.

[19]. Lacut K, Larramendy-Gozalo C, Le Gal G, Duchemin J, Mercier B, Gourhant L, et al. Vitamin K epoxide reductase genetic polymorphism is associated with venous thromboembolism: results from the EDITH Study. Journal of Thrombosis and Haemostasis 2007; 5(10): 2020-2024.

[20]. Razavi FE, Zarban A, Hajipoor F, Naseri M. The allele frequency of CYP2C9 and VKORC1 in the Southern Khorasan population. Research in Pharmaceutical Sciences 2017; 12(3): 211.

[21]. Liu R, Cao J, Zhang Q, Shi XM, Pan XD, Dong R. Clinical and genetic factors associated with warfarin maintenance dose in northern Chinese patients with mechanical heart valve replacement. Medicine. 2017; 96(2): 5658.

[22]. McKinney A, Dailey L, McMillen J, Rowe AS. Impact of obesity on warfarin reversal with fixed-dose factor VIII inhibitor bypassing activity. Critical Care Medicine 2020; 48(1): 251.

[23]. Yuan HY, Chen JJ, Lee MM, Wung JC, Chen YF, Charng MJ, et al. A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity. Human molecular genetics 2005;14(13):1745-51.

[24]. Scott SA, Edelmann L, Kornreich R, Desnick RJ. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. Am J Human Genet. 2008; 82(2): 495-500.

[25]. Takahashi H, Wilkinson GR, Nutescu EA, Morita T, Ritchie MD, Scordo MG, et al. Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra-and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. Pharmacogenet Genom 2006; 16(2): 101-10.

[26]. Malekzadeh M, Pazoki M. Warfarin resistance and recurrent thrombosis in an Iranian patient. Practice 2018; 3(3): 91-4.

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