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Original Article

The allele frequency of *CYP2C9* and *VKORC1* in the Southern Khorasan population

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Abstract

The genetic factors are determinants in required dosage changes of warfarin among which are polymorphisms of *CYP2C9* and *VKORC1* genes. The present study aimed to determine the allele and genotype frequency of *CYP2C9* and *VKORC1* genes in Birjand population. This study was conducted on 120 individuals who referred to Imam Reza and Vali-Asr hospitals for PT/INR test. After extracting the genomic DNA, the considered sequences were amplified by PCR, and restriction fragment length polymorphism analysis was done by *AvaII* and *KpnI* enzymes to determine allele polymorphisms. Moreover, related sequences of *VKORC1*, after amplification, were sequenced for determining the genotype. Allelic and genotypic frequencies as well as Hardy-Weinberg equilibrium, observed heterozygosity, expected heterozygosity, and polymorphism information content were calculated by PowerMarker V 3.25 software. Amongst 120 individuals in this study with the mean age of 58.12 ± 12.7 years, 80.8%, 9.1%, and 10% exhibited the alleles of 1, 2, and 3 *CYP2C9* gene, respectively. The genotype frequencies of 1/1, 1/2, 2/2, 3/1, 3/2, and 3/3 of this gene were found to be 64.1, 15.8, 0, 17.5, 2.5, and 0 %, respectively. In -1639 G>A region, *VKORC1* had normal homozygote genotype (GG) and in 1173 C>T region, heterozygote (CT) with the frequency of 48.7% and 45.9% had the most prevalence. Compared with other populations, there is a considerable difference between the allele frequency of *CYP2C9* and *VKORC1* genetic variance. Since 35.8% of the selected populations carry an abnormal allele causing sensitivity to warfarin, the specialists at medical centers must be informed about the genotypes of patients before prescribing warfarin.

Keywords: Warfarin; Coagulant; *CYP2C9*; *VKORC1*; Polymorphism

INTRODUCTION

Coumarin-based drugs prevent the production of vitamin K-dependent coagulation factors and thereby reduce the risk of blood clots formation (1). Warfarin is the main member of coumarin family and is the most prescribed oral anticoagulant drug across the world (1-3). Studies show that one patient out of every 100 patients taking warfarin encounters fatal bleeding annually (4) and up to 15% of patients experience mild bleeding (5). Therefore, physicians use international normalized ratio (INR) to adjust the medication in order to put prothrombin time (PT) in the proper range for the patient (2-6). Because of individual differences such as age, diet, smoking, weight, and genetic differences, the required dose of anticoagulant is not the same for different patients (7-9). According to Food and Drug Administration (FDA), pharmacogenetics testing for dosage adjustment is necessary for warfarin treatment (2,10).

Of pharmacogenetic factors affecting the diversity of the required dose of warfarin, polymorphism, a subunit gene of *VKORC1* [MIM608547] producer (Vitamin K epoxide reductase complex subunit 1) is considered as a pharmacodynamics and polymorphisms of cytochrome *CYP2C9* [MIM601130] and regarded as pharmacokinetic factors (11).

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Six single nucleotide polymorphisms (SNPs) which influence the activity of *CYP2C9* gene have been identified of which *CYP2C9*1* represents the normal allele. However, the allele *CYP2C9*2* (rs1799853) is caused by changing Arg-144-Cys in exon 3 and allele *CYP2C9*3* (rs1057910) by changing in Ile-359-Leu in exon 7 of this gene (3,12). Generally, about 40% of the diversity in warfarin dose is due to the changes in genes involved in the metabolism of warfarin. For instance, polymorphism in *CYP2C9* gene is responsible for 5-22% variations in response to warfarin in different individuals (13,14). Besides, in Iran, European and American countries, the variants of *CYP2C9*2/*3* have shown the greatest effect on *CYP2C9* enzyme activity (12,15,16).

Also, studies of *VKORC1* gene with 6-37%, responsible for the diversity in response to warfarin in different individuals, have shown that two SNPs affect warfarin oral dose of -1639 G>A (rs9923231) and 1173 C>T (rs9934438) (17,18). Polymorphism -1639 G>A in promoter and 1173 C>T in intron of a *VKORC1* gene, by reducing expression of this gene, reduce the required dosage and warfarin sensitivity (12,19). In ethnographic studies, ethnicity is also an important factor in maintenance dose and warfarin sensitivity such that Asians have high sensitivity and African-Americans have less sensitivity to coumarin drugs (20).

This study aimed to determine the prevalence of alleles of **1*,**2*, and **3* of *CYP2C9* gene and polymorphisms of -1639 G>A and 1137 C>T of *VKORC1* in Birjand population in order to both determine the prevalence rate of the mentioned genetic variants in the region and represent the practical value of the test.

MATERIALS AND METHODS

Subjects

In order to determine the allele and genotype frequencies of *CYP2C9* and *VKORC1* in this study, citrated blood samples of 120 patients referring to Imam Reza and Vali-Asr hospitals of Birjand city, during the first 6 months of the year 2015 (end of March to end of December) with the request for PT and INR tests were used. The study protocol was approved by the Ethics Committee of the Birjand University of Medical Sciences (code No. Ir.bums.1394.89).

DNA extraction

First, by salting out of 2 mL citrated blood, the genomic DNA of the samples was extracted in the laboratory of Birjand University of Medical Sciences. The concentration of purified DNA samples of each individual were evaluated by Nanodrop Specterophotometer Bio Tek, USA EPOCH, and samples with appropriate concentration and optical density were selected for the next stages.

Determination of genotype

To analyse the alleles of *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910) in exon of 3 and 7 of *CYP2C9* gene and to determine the frequency of polymorphisms of -1639 G>A (rs9923231) promoter and 1173 C>T (rs9934438) in intron of a *VKORC1* gene, the primers were designed by Primer 3 Software. After the blast and confirmation by the National Center for Biotechnology Information (NCBI), they were sent for the synthesis to South Korea, Bioneer. The sequences of primers used in this study are shown in Table 1. After the synthesis, sequencing primers were amplified by thermocycler machine (Eppendorf AG.22331, Hamburg, Germany, No. 5345-015844) in the laboratory of Birjand University of Medical Sciences with the following applications.

PCR cycling condition

Temperature programs for manipulating the reactions of two alleles of *CYP2C9*2* and *CYP2C9*3* are as follows: *CYP2C9*2*: 95 °C for 2 min followed by 32 cycles (95 °C for 60 s), 62 °C to 30 s, and 72 °C for 1 min and the final extension was performed at 72 °C for 2 min. *CYP2C9*3*: 95 °C for 5 min followed by 35 cycles (95 °C for 15 s), 65 °C in 20 s, and 72 °C for 30 s and the final extension time was done at 72 °C for 2 min. Master Mix compositions in this study are shown in Table 2.

Table 1. Master Mix compositions in this study.

Materials	**Volume (µL)**
Buffer PCR 10x	5
dNTP	1.5
MgCl2	3
Primer F	1
Primer R	1
Taq DNA polymerase	0.2
Template	1
Distilled Water	38.8

Table 2. Sequence of primers used in this study.

primer	**Sequence**		**Annealing**
*CYP2C9*2*	F R	5′-GGAGGATGGAAAAGAGAGACTTA-3′ 5′-TGAGCTAACAAGGAGGACTCAT-3′	62 °C
*CYP2C9*3*	F R	5′-GCTGTGGTGCAGCTCGTCCAGAGATG-3′ 5′-ACACACACCGCCAGACACTAGG-3′	65 °C
VKORC1 (-1639 G>A)	F R	5′- GCCAGCAGGAGAGGGAAATA-3′ 5′-AGTTTGGACTACAGGTGCCT-3′	60 °C
VKORC1 (1173 C>T)	F R	5′- TGACATGGAATCCTGACGTG-3′ 5′- GAGCTGACCAAGGGGGAT-3′	57 °C

F, Forward; R, Reverse.

Table 3. Cutting region and digested fragments after RFLP affecting by *AvaII* and *KpnI* enzymes.

***CYP2C9*2* (RFLP) (exon 3)**	***CYP2C9*3* (RFLP) (exon7)**
AvaII: G^GACC	*Kpnl*: GGTAC^C
*CYP2C9**2 GGAC (MUT = T) C	AA (Normal): 291
TT(RESTANT, HOMOZYGOTE): 397 bp	AC (Heterozygote): 291 + 270
TC(semiresistant,heterizygote): 397 + 223 + 173 bp	CC (Homozygote): 270
CC (Wild type): 223 + 173 bp	

In order to confirm the amplification of the regarded fragment and non-amplification of other regions and the formation of primer dimer, the PCR products on a 1.5% agarose gel were electrophoresed besides the size markers of 50 bp. Afterwards, the gel affected by UV light was imaged by Gel Documentation Machine (UVITEC CAMBRIDGE, United Kingdome).

The products obtained by PCR were cut under the enzymes *AvaII* (Jena Biosience, Cat. No EN-119-csTM) and *KpnI* (Sinaclon Bioscience, Cat.No PR 911710). Table 3 shows the cutting and the fragments after the restriction fragment length polymorphism (RFLP) analysis.

Under the condition of mutation emergence, the cutting site for *AvaII* enzyme disappears, but it appears for *KpnI* enzyme. The cutting condition by *AvaII* and *KpnI* enzymes is applied in the following order:

Controlled PCR product (10 µL) was combined with 18 mL of distilled water along with 2 mL buffer R (or buffer *Kpn*) and 1 µL of *AvaII* and *KpnI* enzymes. This compound was then incubated at 37 °C for 5 h and the obtained cutting product was electrophoresed on 3% agarose. After imaging the gel, the obtained pattern was examined.

To determine the frequency of polymorphisms -1639 G>A promoter and 1173 C>T in intron of a *VKORC1* gene, at first, the respective sequences were amplified by both synthesized primers and thermocycler instruments with the following temperature program: 94 °C for 5 min followed by 35 cycles (95 °C for 30 s), annealing temperature 60 °C in 20 s, 72 °C for 30 s and final extension time was performed at 72 °C for 2 min.

After the electrophoresis of PCR products on 1.5% agarose gel and making certain about using correct and specific polymerase chain reaction, the samples were sent to Kowsar Biotech Company (Iran) for sequencing.

Data analysis

The raw results of sequencing the samples were examined with the help of appropriate ChromasLite 2.1.1 Software.

Available possible variants of *VKORC1* gene were identified after alignment and data analysis.

Finally allelic and genotypic frequencies as well as Hardy-Weinberg equilibrium (HWE), observed heterozygosity (H_o), expected heterozygosity (H_e), minor allele frequencies (MAF) and polymorphism information content (PIC) were calculated by PowerMarkerV 3.25 Software.

RESULTS

The results show that from among the 120 patients, 62 individuals were men (51.6%) and 58 women (48.3%) whose age means were 58.12 ± 12.7 years respectively. The mean intake time of warfarin in patients was 6 ± 2 months whereby the patients reached relatively stable INR. Among the population, 76.2% were from Imam Reza hospital and 23.8% from Vali-Asr hospital. The results of the allele variants of *CYP2C9*2* and *CYP2C9*3*, after applying PCR and RFLP, are shown in Figs. 1 and 2.

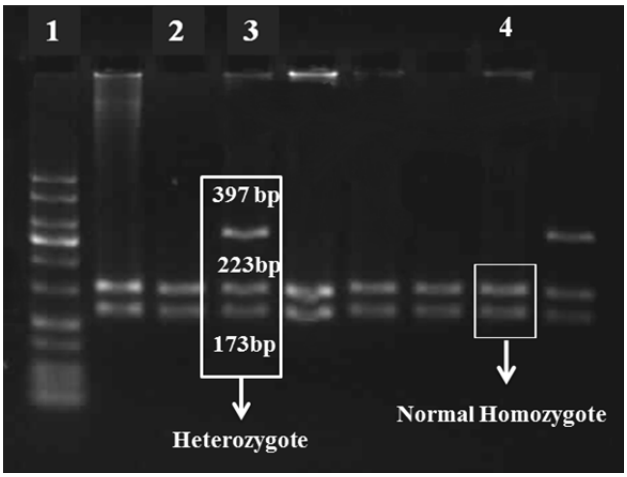

Fig.1. The image of the gel and produced bands with *AvaII* enzyme. Raw 1, marker 50bp; Raw 2 and 4, normal homozygote *CYP2C9**2; Raw 3, heterozygote *CYP2C9**2.

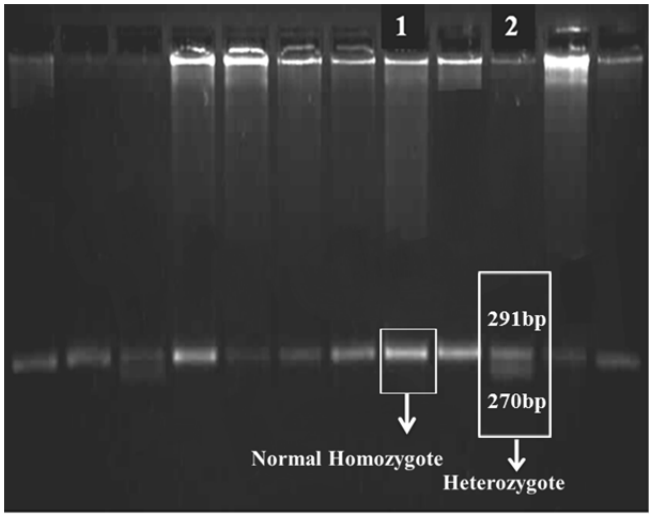

Fig.2. *KpnI* enzyme. Raw1, normal homozygote *CYP2C9**3;Raw 2, heterozygote *CYP2C9**3.

Genotyping

Normal genotype of *CYP2C9*1/*1* shows that the individuals did not exhibit any kind of mutation for *CYP2C9*2* and *CYP2C9*3* alleles which indicates the presence of *CYP2C9*1* allele. Genotype of *CYP2C9*1/*2* allele includes those whose *CYP2C9*3* is wild type but have mutant heterozygote for *CYP2C9*2*.

Individuals with *CYP2C9*3* allele and mutant heterozygote of *CYP2C9*2* have 2/2 genotype, and individuals with wild type *CYP2C9*2* allele and mutant heterozygote *CYP2C9*3* have 1/3 genotype. Genotype of 3/3 and 3.2("/" means division and heterozygote) are reported in individuals with wild type *CYP2C9*2* and mutant homozygote of *CYP2C9*3*, respectively, and for *CYP2C9*2* and *CYP2C9*3* alleles are both reported as mutant heterozygote. The method of determining the genotypes of *CYP2C9* gene by enzymes with limited effect used in this study is presented in Table 4.

The allele frequency of *CYP2C9*2* was 9.1% in the studied population. After RFLP analysis, *CYP2C9*2* allele, normal homozygote genotypes, and heterozygote showed the frequency of 83.3%, 16.6%, and 0%, respectively, and the frequencies in terms of gender were 84.7 %, 15.3%, and 0% in men and 75.9%, 24.1%, and 0% in women .The allele frequency of *CYP2C9*3* in the sample was 10%. As for *CYP2C9*3* allele, normal homozygote and heterozygote genotypes were found to be 82.8% and 17.5%, respectively. The allele frequencies of these genotypes were 85.55% and 11.3% in men and 76.7% and 23.3% in women. For *CYP2C9*3* allele, no mutant homozygote was observed in the individuals. The genotype frequency of *CYP2C9* gene is shown in Table 5. Heterozygosity, Hardy–Weinberg equilibrium and minor allele frequencies and their frequencies for the three polymorphisms tested are given in Table 6. Allele frequencies of all the markers were in HWE ($P > 0.05$).

Table 4. Determining *CYP2C9* genotype by *AvaII* and *KpnI* enzymes.

Genotype											
1/1		**1/2**		**1/3**		**2/2**		**2/3**		**3/3**	
KpnI	***AvaII***	***KpnI***	***AvaII***	***KpnI***	***AvaII***	***KpnI***	***AvaII***	***KpnI***	***AvaII***	***KpnI***	***AvaII***
291	223	291	397	291	223	291	397	291	397	291	223
173		223		270	173			270	223	270	173
		173						173			

*Restricted enzyme protocols and parts length obtained from Digest.

Table 5. Genotype frequency of *CYP2C9* gene in the present study.

Sex		**Genotype**						***P* value**
		CYP2C9*1*1	***CYP2C9*1*2***	***CYP2C9*2*2***	***CYP2C9*1*3***	***CYP2C9*2*3***	***CYP2C9*3*3***	
M	**P**	76.8%	15.3%	0%	11.7%	0.71%	0%	*P >.0.05*
	F	42	9	0	7	1	0	
W	**P**	62.1%	17.2%	0%	23.3%	1.6%	0%	*P > 0.05*
	F	35	10	0	14	2	0	

M, men; W, women; P, Percent; F, frequency.

Table 6. Heterozygosity, Hardy-weinberg equilibrium, polymorphism information content, and allele frequencies.

Marker	**Obs H**	**Pred H**	**HWP**	**MA**	**MAF**	**PIC**
*CYP2C9**1	0.28	0.24	0.12	C	0.80	0.21
*CYP2C9**2	0.14	0.13	1	T	0.09	0.12
*CYP2C9**3	0.16	0.14	0.56	C	0.10	0.13
VKORC1-639G>A	0.40	0.44	0.24	A	0.32	0.34
*VKORC1*1173C>T	0.47	0.46	0.84	T	0.37	0.35

Obs H, observed heterozygosity; Pred H, predicted heterozygosity; HW, Hardy-weinberg equilibrium; MA, minor allele; MAF, minor allele frequency; PIC, polymorphism information content.

Alleles and genotypes

Among the 120 individuals in this study, *CYP2C9*1/*1* genotypes has the highest prevalence with a frequency of 64.1%, and genotypes have the lowest frequency of 2/2 and 2/3 with 0%. Finally, the normal allele of *CYP2C9*1* in both genders showed highest presence with the frequency of 83.3%, while the two alleles of **3*, **2* with respective frequencies of 10% and 9.1% had the lowest prevalence. Overall, 35.8% of the participants had an abnormal allele. Despite differences in allele frequencies in the two genders, the differences were not statistically significant.

After PCR and sequencing -1639 G>A region of *VKORC1* gene (g.8853 G>A), genotype distribution of the current population showed that there was normal homozygote (GG) with a frequency of 48.7% in 55 individuals, heterozygote (AG) with a frequency of 38.9% in 44 individuals, and mutant homozygote (AA) with a frequency of 12.4% in 14 individuals. Moreover, in terms of gender, statistics for normal homozygote genotype, heterozygote and mutant homozygote of men in -1639 G>A region were 40.3%, 30.5%, 17.5%, respectively and in women, 50%, 36.7%, and 5%, respectively. Allele frequency of-1639 A and allele of -1639 G in the population are 31.9% and 68.1%, respectively. Some electropherogram images of -1639 regions are shown in Fig. 3A. Amplification and sequencing of *VKORC1* gene with the related primer of -1639 G>A region of its promoter, showed two novel mutations in this region which include g.3660 G>A heterozygote mutation in 3 individuals (1 man and 2 women) with a frequency of 2.7% and g. 3565 G>T heterozygote mutation in one woman with the frequency of 0.9% in the whole population.

Further, the results of PCR and sequencing of 1173 C>T region of *VKORC1* gene showed that in this population heterozygote genotypes (CT), normal homozygote (CC), and mutant homozygote (TT), as for gender, had the frequencies of 52.6%, 40.4%, 7% in woman and 38.9%, 38.9%, and 22.2% in men, respectively. Allele and genotype frequency of *VKORC1* gene is shown in Table 7. Electropherogram image of 1173 C>T region is shown in Fig. 3B.

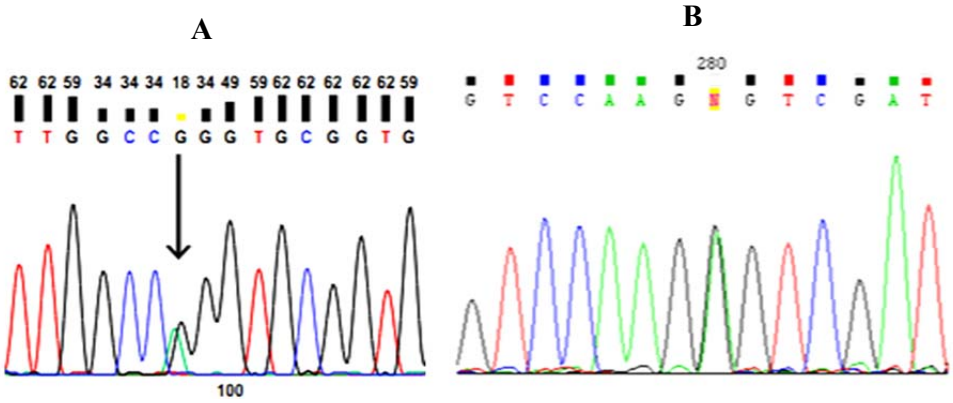

Fig. 3. Electropherogram images of heterozygote genotype of (A) *VKORC1* gene, and (B) 1173T region of *VKORC1* gene.

Table 7. Allele and genotype distribution of individuals' *VKORC1* gene.

Polymorphism	Genotype	Frequency (%)	Alleles	Frequency (%)
-1639G>A	AA	14 (12.4%)	-1639A	72 (31.9%)
	AG	44 (38.9%)		
	GG	55 (48.7%)	-1639G	154 (68.1%)
1173C>T	CC	44 (39.6%)	1173C	139 (62.6%)
	CT	51 (45.9%)		
	TT	16 (14.4%)	1173T	83 (37.4%)

DISCUSSION

This study shows the prevalence of 9.1% and 10% of variant alleles of *CYP2C9*2* and *CYP2C9*3* in Birjand population. Comparison of genotype frequencies of *CYP2C9* gene in this study showed that the majority of individuals have *CYP2C9*1/*1* genotype with the frequency of 64.1%. Also, *CYP2C9*2/*2* and *CYP2C9*3/*3* genotypes in this population had no frequency. Amongst 120 individuals studied for -1639 G>A region of *VKORC1* gene, normal homozygote with a frequency of 48.7% in 55 individuals had the highest prevalence and mutant homozygote genotype had the lowest prevalence with 12.4% frequency. Furthermore, heterozygote genotype of 1173 C>T region of *VKORC1* gene with a frequency of 45.9% in 53 individuals was dominant and showed mutant homozygote with 14.4% frequency.

The presence of allele variants of *CYP2C9*2* and *CYP2C9*3*, -1639A, *VKORC1*, and allele of 1173 T of *VKORC1* gene, as the cause of the patients' sensitivity to warfarin, indicates that the formula to determine the daily dose of warfarin in which the genetic variants of *CYP2C9* and *VKORC1* are significant is imperative and crucial variables can be clinically implemented with greater certainty (9,14,21,22).

Warfarin dose (mg/day) = $[0.628 - 0.0135 \times (\text{Age}) - 0.24 \times (\textit{CYP2C9*2}) - 0.37 \times (\textit{CYP2C9*3}) - 0.241 \times (\textit{VKORC1}) + 0.0162 \times (\text{height})]^2$ (22).

(Wild type, heterozygous and homozygous genotypes of CYP2C9*2 and *3 were coded as 0, 1, and 2, respectively, while that of VKORC1 were coded as 1, 2, and 3, respectively).

Geographically, Iran is located between both European and Asian populations. Thus, the diversity of ethnic and genetic variants in Iran as a result of migration and population flows seems probable. Thus, access to distribution patterns of genetic variants in different parts of Iran with regard to the effect on the sensitivity to warfarin dose is of great importance.

In Shiraz city, Azarpira, *et al*. determined the presence of normal allele of *CYP2C9*1* about 64.88% and allele variants of *CYP2C9*2* and *CYP2C9*3* with the frequencies of 25.3% and 9.8%, respectively (15). Contrary to Shiraz, variant of allele *CYP2C9*3* (with a frequency of 10%) in Birjand population is higher than *CYP2C9*2* allele (9.1%). In addition, genotype frequency of *CYP2C9*2/*2* in Birjand population was zero compared to Shiraz population which was 1.37%. (15,23,24).

Tabatabae, *et al*. reported that 200 patients with cardiovascular diseases from northwest of Iran which includes Azari population showed frequencies of 69% and 1% for *CYP2C9*1/*1* and *CYP2C9*2/*2* genotypes, respectively. *CYP2C9*3/*3* genotype in this population was zero but genotype frequency of *CYP2C9*2/*2* in Azari population was reported to be 1% (21). In northwest of Iran, similar to Shiraz population, *CYP2C9*2* allele appeared to be the most prevalent allele with 31.3% frequency.

In another study by Zand, *et al*. conducted on 200 healthy Iranians, showed that the prevalence of *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* alleles were 87.2%, 12.7%, and 0%, respectively (25). Therefore, the population of Birjand city is different from northwestern and southern populations of Iran in terms of genetic frequency variant of *CYP2C9.* The allele frequency of *CYP2C9*3* is lower in Birjand as compared to Shiraz (9.8%) and northwest of Iran (zero). In contrast to Birjand population, most of the individuals in Shiraz and northwest of Iran showed more variant allele of *CYP2C9*2* (25.3% and 31%, respectively) (15,21). These differences can be attributed to their ethnic background.

In 2011, in a study conducted on 111 Russian patients with thrombosis showed that the prevalence of *CYP2C9*1/*1* genotype was 84.7%. For heterozygote genotypes, frequencies of *CYP2C9*1/*2* and *CYP2C9*1/*3*, were reported to be 4.5% and 10.8%, respectively. However, no genotypes of **2/*2* and **3/*3* was observed which is in agreement with the findings of our study (26).

Allele frequency of *CYP2C9*2* and *CYP2C9*3* in Birjand population, compared with Korea, Japan, China, France, Germany, and Brazil populations, showed a considerable

difference. In Korea, by analyzing 574 individuals, allele frequency of *CYP2C9*2* and *CYP2C9*3* were found to be 0% and 1.1%, respectively (27); allele frequency of *CYP2C9*2* in 147 Japanese individuals was zero, and allele of *3* was about 0.7% (28). There was no allele frequency of *CYP2C9*2* in 130 Chinese patients, but allele frequency of **3* was about 12.2% (29). In France, in a population of 151 individuals, allele frequencies of *CYP2C9*2* and *CYP2C9*3* were about 15% and 8%, respectively (30). In Germany, analysis of 118 individuals showed that the allele frequencies of *CYP2C9*2* and *CYP2C9*3* were 14% and 5% and in 780 Brazilian individuals, allele frequencies were 12.4% and 5.6% (31,32). There was a kind of similarity of allele frequency of *CYP2C9*2* and *CYP2C9*3* between Birjand and 499 Turkish individuals. Both populations had similar allele frequency of **3* (about 10%); however, allele frequency of *CYP2C9*2* in the Turkish population was greater than in Birjand population (33).

Krajčíová, *et al*. analyzed 112 healthy Slovak individuals and concluded that for *CYP2C9*2* allele in that population, the frequencies for normal homozygote, genotypes (CC), and mutant homozygote (TT) were 81%, 17%, and 2%, respectively. Also, for allele of *CYP2C9*3*, the percentage of frequencies of normal homozygote, mutant heterozygote and homozygote were 58%, 15% and 0%, respectively (3).

Kamal El-Din, *et al*. reported 8.5% and 12% allele frequency for *CYP2C9*2* and *CYP2C9*3* in 41 children in Egypt. The results of *CYP2C9* alleles in Egypt correlate with the results of this study suggesting that the highest presence of variant allele in Birjand population is also related to *CYP2C9*3* allele (16).

By enlarging the scope of studies, it turns out that *VKORC1* gene also has an important role in ethnic differences in terms of sensitivity to warfarin (35), and polymorphisms of -1639 G>Aand 1173 C>T region of this gene have significant roles in interpersonal changes in response to warfarin (36).

Salehifar, *et al.*, examined 29 patients in Mazandaran in order to analyze their polymorphisms of -1639 G>A and 1173 C>T of *VKORC1* gene. Finally, for -1639 region, the individuals showed mutant homozygote genotype (AA) with allele frequency of 86.2%. Genotypes of AG and GG had the same frequency of 6.9%. In analyzing 1173 C>T intron of one *VKORC1* gene, 26 individuals had 89.7% mutant homozygote genotype (TT), 6.9% had normal homozygote genotype, and 3.4% had heterozygote (36). In this study, contrary to Mazandaran population, normal homozygote genotype and heterozygote for -1639 region was 48.7% and 38.9%, respectively which were the highest prevalence, and individuals with mutant homozygote encompassed a small percentage of the population which is 12.4%.

By studying 150 individuals of Shiraz population in 2010, frequency genotype of -1639 G>A was 57.14% and -1639 AA genotype was about %73 which shows that, similar to Mazandaran population, mutant homozygote genotype is more prevalent (15). Moreover, allele of 1173T of *VKORC1* gene in this study showed the frequency of 56% while for Birjand population it was 37.4%. Accordingly, sensitivity to warfarin in Shiraz population like Mazandaran population is more than Birjand population. Therefore, the need for a daily dose of warfarin will presumably be lower for Shiraz and Mazandaran populations.

By studying the frequency of polymorphism of -1639 G>A of *VKORC1* gene in 200 individuals of northwestern of Iran in 2012 (East and West Azerbaijan provinces and Ardabil), the prevalence of mutation in this region was observed. Genotype frequency of normal homozygote, heterozygote, and mutant homozygote of -1639 G>A in these regions were 21.6%, 53.7%, 24.5% in women and 6.3%, 60.22%, and 17.02% in men, respectively (37). By analyzing genetic variants of *VKORC1* for -1639 G>A region in 112 healthy Slovak individuals, Krajčíová, *et al*. reported the presence of GG, GA, and AA genotypes with the percentage of 73%, 52%, and 12% (3). It is worth noting that the observed percentage of genotypic frequencies in 1173 C>T of *VKORC1* gene of Slovak population is exactly the same as -1639 G>A region.

In a study which has been conducted on 148 healthy Greek-Cypriots in 2014, allele frequency of *CYP2C9*2*, *CYP2C9*3*, and -1639 A of *VKORC1* were found to be 16.2%, 11.2%, and 53%, respectively. The percentage of allele frequency of *CYP2C9*3* in Cyprus population is somehow in accordance with the present study. Nonetheless, the prevalence of *CYP2C9*2* allele in this population is more than **3* allele, contrary to Birjand population. Similar to Mazandaran and Shiraz population, Cyprus population has a higher frequency of -1639 A of *VKORC1*, all more than that of Birjand population (38).

In another study conducted on 557 English patients in 2012, it was observed that the individuals with genotype AA pertaining to -1639 G>A of *VKORC1* gene who had a frequency of 14.5% required a lower dose of warfarin in order to achieve the targeted INR, where they had more bleeding and therefore, more sensitivity to warfarin. Comparing these findings with those reported from Iran indicates a lower prevalence of this genotype (-1639 AA) in this European population, similar to Birjand population, but higher prevalence in Mazandaran population (86.2%) and northwestern of Iran (21%) (39). Therefore, the individuals of Mazandaran population followed by northwestern of Iran are more sensitive to warfarin than the English population and are at risk of more bleeding. However, individuals of Birjand population with12.4% frequency of AA-1638 genotype, compared to other three populations, have a lower sensitivity to warfarin.

As a result, because of the frequency of polymorphisms in -1639 G>A region of *VKORC1* gene, our population is similar to many far populations and different from closer populations. Genotype -1639 AA in Birjand population has the frequency of 12.4% which is different from Mazandaran with 86.2% (36), Shiraz 55.6% (15), northwestern of Iran 21% (37), Chinese 69% (40), Cyprus 53% (38), and Argentina populations 20% (41). But it bears close similarity to Slovak population with 12% (3) and England with 14.5% frequencies (39). These findings confirm that -1639 A allele has dominance in Asia with a prevalence of 90% (42). However, due to the fact that in Birjand population, the results are completely different and most of the individuals have -1639 G allele; therefore, the difference among ethnic peoples in the same geographic region must be considered.

In a study conducted on 93 individuals in Japan in 2007, the allele frequency of 1173 T of *VKORC1* gene is about 11.3%. This finding resembles the percentage of allele frequency in China (12.8%) found in a study conducted on 133 individuals in 2010 (23,29). Since the prevalence of this allele in our 120 individuals is much higher than the two populations in East Asia, i.e., 37.4%, therefore, compared with Chinese and Japanese populations, people in Birjand are at a higher risk of bleeding and warfarin sensitivity.

Variant allele frequency of this gene in some of the European and Asian countries are more similar to our results. For instance, Bodin, *et al*. analyzed 222 individuals in France among which 42% had allele of 1173 T of *VKORC1* gene (43). This allele frequency in Slovak population was reported as 50.5% (3,43). Furthermore, Kamal El-Din, *et al.* reported the allele frequencies of 1173T of *VKORC1* gene and -1639 A of *VKORC1* gene in 41 Egyptian children as 52% and 54%, respectively. Thus, allele of 1173 T *VKORC1* gene has a higher frequency in these European and Asian populations than Birjand population. Meanwhile, in 2013, genotype of 1173 C>T of *VKORC1* gene of 103 Indian individuals were determined; normal homozygote genotype, heterozygote and mutant homozygote were 76%, 22%, and 2%, respectively (14).

CONCLUSION

The comparison of the frequency of genetic variants of *CYP2C9* and *VKORC1* in this study with results from other parts of the world confirms that by moving from East to West Asia, the number of carriers of alleles of *CYP2C9*2* and *CYP2C9*3*, unlike -1639 A *VKORC1*, is increasing. However, in terms of distribution pattern, in countries such as Iran, Oman, India, and Russia, an extreme and unbalanced diversity of *CYP2C9* and *VKORC1* genotype and allele can be observed (20).

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