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THE FREQUENCY OF VITAMIN K EPOXIDE REDUCTASE COMPLEX–1639G>A GENETIC VARIANT AMONG HEALTHY UNRELATED JORDANIAN VOLUNTEERS

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Abstract

Substitution of guanine to adenine at the promoter region of Vitamin K epoxide Reductase Complex (VKORC) gene has an impact on warfarin response and cardiovascular diseases. There is no report regarding VKORC1 – 1639 G > A genotype among Jordanian population. Therefore, the present study aimed to determine the frequency of VKORC1 – 1639 G > A genetic variant among 90 healthy unrelated Arabic Jordanian volunteers by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. The results showed that the frequency of VKORC1– 1639 G and A alleles among Jordanians were 0.47 and 0.53, respectively, which were similar to Middle Eastern Iranian, Turkish and Egyptian populations but significantly higher (χ2-test, p value <0.05) than European Caucasian and Saudi Arab populations. The VKORC1 – 1639 G > A genotype frequencies among healthy Jordanian volunteers were wild GG (0.17), heterozygote GA (0.60) and homozygote AA (0.23). It is concluded from this study that Jordanian population has high frequency of VKORC1 – 1639 G > A variant. This finding may increase our understanding of the inter-ethnic variation in warfarin response and predisposing to cardiovascular diseases.

Keywords: VKORC gene, Jordanian, genetic polymorphism, PCR-RFLP

Introduction

Vitamin K epoxide Reductase Complex (VKORC) enzyme plays a major role in blood coagulation through activation of vitamin K (1), which is important in the synthesis of coagulation factors II, VII, IX, and X (2). Pharmacologically, VKORC enzyme is a target of the anticoagulant warfarin in treatment of cardiovascular and thrombotic diseases (3).

The VKORC enzyme is encoded by VKORC1 gene (4). This gene is located on human chromosome 16 with gene size of around 5 kilo base pair (Kbp) and containing 3 exons. The VKORC1 gene is polymorphic. More than 30 single nucleotide polymorphisms (SNPs) were identified on VKORC1 gene among different ethnic populations in 1000-Genome project (5). Genetic variants on VKORC1 gene may influence on clotting factor synthesis and predisposition to cardiovascular diseases. It is found that VKORC1 rs2359612 and rs9923231 genetic variants were associated with susceptibility to cardiovascular and cerebrovascular diseases (6). In addition, genetic variants on the coding and promoter region of VKORC1 gene showed to influence on the anticoagulant efficacy of warfarin drug (4). The most common VKORC1 SNP affected on warfarin response is rs9923231 (4, 7). This SNP was identified with substitution of guanine to adenine nucleotide on the promoter region of VKORC1 gene (– 1639 G > A). It is found that VKORC1 – 1639 G > A altered the VKORC's RNA transcription levels where G allele had higher activity by 44% over the A allele (8). Therefore, VKORC1 – 1639 G > A increases the sensitivity to warfarin which needs to decrease the warfarin dose to avoid the bleeding unwanted site effect of warfarin (9).

There is an interethnic differences in the frequency of VKORC1 –1639G>A. It is reported that VKORC1– 1639 G>A is high among Chinese and Japanese Asians (>74%) (10), while it is low among Africans (9%) (11). Among Middle Eastern non-Arabic populations, it is found that the frequency of VKORC1–1639 G>A among Turkish and Iranian populations was 64.8% and 51%, respectively (12, 13). Among Arabic Middle Eastern populations, studies reported that Saudi and Egyptian populations have VKORC1 –1639A allele frequency of 22.9% and 46%, respectively (14, 15).

However the frequency of VKORC1 –1639G>A genetic allele and genotype had been identified in many ethnic populations, no published study about VKORC1 –1639G>A variant in Jordanians. Therefore, the present study aimed to determine the allele frequency and genotype of VKORC1–1639G>A variant among healthy unrelated Arabic Jordanian volunteers.

Methods

Sample collection

A total of 90 healthy Jordanian volunteers (30 males and 60 females, with an average age of 23± 3 years) agreed to participate in the study and signed an informed consent. The study was approved by the College of Pharmacy, Al-Zytoonah University, Jordan. From each volunteer 3 ml of venous blood were obtained in EDETA tubes. Individuals were Arab Jordanian, unrelated and healthy volunteers, as judged by the physical examination with no history of chronic diseases.

DNA extraction

The genomic DNA was extracted using Wizard DNA extraction kit (Promega, Madison, WI, USA) according to the manufacturer's method. Briefly, leukocytes were isolated from whole blood through 3000 rpm centrifugation and then incubated with cell lysis solution. After that, the cell nucleus pellet was lysed through lysis solution. Then, the proteins in the samples were precipitated. After that, the genomic DNA was precipitated by isopropanol and washed with %70 ethanol. Lastly, the DNA was dissolved in nuclease free water and was stored at -20˚C until used.

Amplification of fragment of *VKORC1* gene

Fragment of the promoter region of *VKORC1* gene was amplified using polymerase chain reaction (PCR) with the following reaction system : a sample of 50 ng of genomic DNA was reconstituted in a total 50 µl reaction mixture containing 1 µl of 10 mM dNTPs; 2 µl of 25mM $MgCl_2$; 10µl of 10X Taq polymerase buffer, 1 unit of Taq DNA polymerase and 10 pmole from each of The following forward (5'-CCAGCAGGAGAGGGAAATA-'3) and reverse (5'-AGTTTGGACTACAGGTGCCT-3') PCR primer sequences (16).

After that, the PCR mixture was heated to 94 °C for 5 min, and then it incubated in 35 thermal cycles of: denaturation step at 94°C for 50 seconds, annealing step at 57 °C for 1 minute and elongation step at 72 °C for 30 seconds. Then, the PCR product was completed by incubating it at 72 °C for 5 min as a final step of elongation.

Restriction fragment length polymorphism

A sample of 1 µg DNA from PCR product was subjected to Msp1 restriction enzyme analysis (new England bio labs, England) (16). Breifly, 1 µl of Msp1 and 5 µl (x1) of 10x NEBuffer were added to 10 µl PCR product then incubated it at 37 °C for overnight to detect the *VKORC1* – 1639 G > A variant.

The digested PCR products were separated on 2% agarose gel after gel staining with ethidium bromide.

Statistical analysis

Chi-square (χ^2 test) was used to determine whether the *VKORC1* – 1639 G > A allele distribution was in Hardy-Weinberg equilibrium. A p-value < 0.05 was used to reject the null hypothesis and z- core test was used for comparison of *VKORC1* genotypes frequency among Jordanians with other populations.

Results

The current study amplified a fragment of the promoter region of *VKORC1* gene from the isolated genomic DNA of the volunteers using PCR technique. The size of the PCR product, as shown after gel electrophoresis on 2% agarose gel, was 290bp (Figure 1).

The Msp1 restriction enzyme cut the PCR product of the *VKORC1 – 1639G* wild allele to 2 fragments with size of 150 and 140bps, while couldn't cut the *VKORC1 – 1639A* mutant allele, as represented on Figure 2. The heterozygote samples were represented by three bands (290, 150 and 140bp) on 2% gel electrophoresis.

The frequency of *VKORC1*– 1639 G and A alleles among healthy unrelated Jordanians were 0.47 and 0.53, respectively (Table 1).

The *VKORC1* – 1639 G > A genotype frequencies among healthy Jordanian volunteers were wild GG (0.17), heterozygote GA (0.60), homozygote AA (0.23) as presented in Table 2. The data showed that heterozygote GA genotype is the most common genotype with frequency of 60% of the total *VKORC1* – 1639 G > A genotypes among these healthy volunteers. As it is shown on Table 2, all of *VKORC1* – 1639 G > A genotype frequencies were within Hardy-Weinberg equation.

In comparison with other ethnic populations, the current study found that the frequency of *VKORC1*–1639 G > A among Jordanians was similar to Middle Eastern Iranian, Turkish and Egyptian populations, while it was higher than European Caucasians, African and Saudi populations and significantly (χ2-test, p value <0.05) lower than Japanese, Chinese and Indonesian Asians (Table 3).

Discussion

Although *VKORC1 –1639 G >A* variant is clinically significant in warfarin dosing and predisposing to cardiovascular diseases (4, 6), no published study about the frequency of *VKORC1 –1639 G>A* among Jordanian population. In this study, the frequency and genotype of *VKORC1 –1639G>A* were determined among healthy Jordanians. It is found, in this study, that *VKORC1 –1639A* allele was relatively high among Jordanian population and similar to most Middle Eastern populations. This result may explain, at least in part, the inter-ethnic variation in the response to warfarin therapy.

The FDA updated the warfarin label with the recommendation of genotyping *CYP2C9* and *VKORC1* to determine the initial dose ranges (25). In addition, the algorithms of warfarin dosing among different ethnic populations use *VKORC1 –1639G>A*, *CYP2C9*2* and *CYP2C9*3* SNPs to predict the warfarin dose (26, 27). It is recommended to reduce the warfarin dose from 5mg/day to 3mg/day in patients with double carrier of *CYP2C9* and *VKORC1 –1639G>A* genetic variants (28). The frequencies of major *CYP2C9* SNPs were determined Among Jordanians. Yousif et al (2012), found that *CYP2C9*2* and *CYP2C9*3* frequencies were 0.14 and 0.07, respectively (29). To the best to our knowledge, no study published the frequency of *VKORC1 –1639G>A* in Jordanians. The present study is the first study reported the *VKORC1–1639 G>A* genotyping among Jordanians. As *VKORC1–1639 G >A* was associated with the warfarin adherence (30), high prevalence of *VKORC1–1639 G >A* among Jordanians may explain, at least in part, the reported non-adherence to warfarin treatment in Jordanian population (31). Therefore, it is recommended to investigate the influence of *VKORC1–1639G>A* on warfarin adherence and bleeding among Jordanian population.

However *VKORC1–1639 G >A* affected clinically on warfarin dosing, there are other SNPs on *VKORC1* gene (such as VKORC1 Asp36Tyr substitution) which reported to influence on warfarin response (32). The structure and haplotype of *VKORC1* gene was investigated among different ethnic groups and novel functional SNPs were identified (22, 33). Therefore, it is recommended to sequence *VKORC1* gene among Jordanian population to identify the haplotype structure and novel SNPs which may influence on warfarin response.

The prevalence of cardiovascular diseases is high among Jordanians (34). Genetic variants were showed to affect on the predisposition the cardiovascular diseases (35). As *VKORC1–1639 G>A* was associated with cerebrovascular diseases, we are planning to investigate the association of *VKORC1–1639 G>A* with cardiovascular patients in Jordan.

The present study found that the frequency of *VKORC1–1639 G>A* was generally different than Europeans, Asians, Indians and Africans (Table 3). Among Middle Eastern populations, the frequency of *VKORC1–1639 G >A* among Jordanians was higher than Saudi but similar to Iranians, Turkish and Egyptians. This inter-ethnic variation in the allele frequency of *VKORC1–1639 G >A* may affect on the inter-ethnic variation to the warfarin drug response (36).

Jordanian population is admixture of different ethnic populations, including Arabs, Kurds, Circassians and Armans (37). The present study genotyped Arab

Jordanians, which form the majority, and excluded the minor groups (Armans and Kurds) through direct questioning of the volunteers about their paternal and maternal ancestors. Therefore, the results of the present study were applied on the Arabic Jordanians but not on other minor Jordanian ethnic groups.

In conclusion, the present study determined the frequency and genotype of *VKORC1–1639 G >A* genetic variant among healthy Jordanian volunteers and found that its frequency is close to Middle Eastern Iranians and Egyptians. This finding may increase our understanding of the inter-ethnic variation of drug response and predisposing to cardiovascular diseases.

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Table 1. The allele frequency of *VKORC1* – 1639 G > A genetic variant among healthy Jordanian population

Allele	Frequency observed	95% confidence
G	0.47	0.40-0.54
A	0.53	0.45- 0.60
Total	1	

Table 2. The *VKORC1 – 1639 G > A* genotype frequency among healthy Jordanian population

Genotype	Observed number (frequency)	Predicted number (frequency)	χ^2 test †
Wild (G / G)	15 (0.17)	20 (0.22)	>0.05
Heterozygote (G / A)	54 (0.60)	45 (0.50)	
Homozygote (A / A)	21 (0.23)	25 (0.28)	
Total	90 (1)	90 (1)	

† All of *VKORC1* – 1639 G > A genotype frequencies were within Hardy-Weinberg equation

Table 3. The comparison between *VKORC1 –1639 G > A* allele frequencies among healthy Jordanians with other ethnic populations

Ethnic group	VKORC1 –1639 G frequency	VKORC1 –1639 A frequency	Different than Jordanian (χ^2 test)	Is there statistical difference in comparison with Jordanian population	Reference
African					
African-American	0.9	0.1	>0.05	Yes	17
African-American	0.91	0.09	>0.05	Yes	18
Asian					
Japanese	0.08	0.92	>0.05	Yes	19
Indonesian	0.23	0.77	>0.05	Yes	20
Malay	0.44	0.56	>0.05	NO	21
Chinese	0.26	0.74	>0.05	Yes	21
Tamilian	0.9	0.1	>0.05	Yes	22
Indian	0.96	0.04	>0.05	Yes	21
Caucasian					
European	0.70	0.30	>0.05	Yes	22
Romanian	0.84	0.16	>0.05	Yes	23
Hispanic	0.67	0.33	>0.05	Yes	24
Middle Eastern					
Saudi	0.77	0.23	<0.05	Yes	(14)
Egyptian	0.54	0.46	>0.05	NO	(15)
Turkish	0.36	0.64	>0.05	NO	(12)
Iranian	0.77	0.56	>0.05	NO	(13)
Jordanian	0.47	0.53	-	-	This study

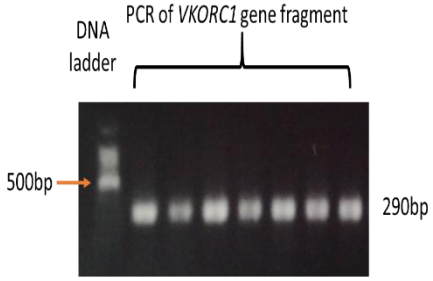

Figure 1. Represented gel-electrophoresis of *VKORC1* gene fragment amplification for healthy Arabic Jordanian volunteers using PCR technique

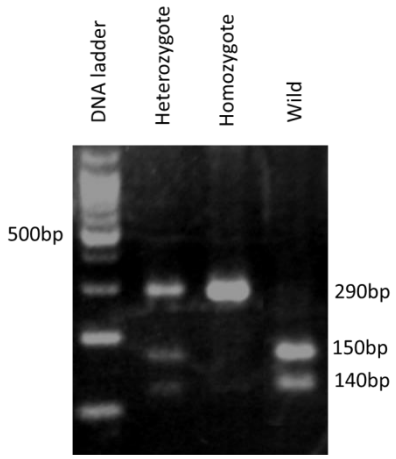

Figure 2. Represented gel-electrophoresis of *VKORC1* – 1639 G > A genotype among sample of healthy Arab Jordanian volunteers