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Impact of Factor VII Polymorphism on Responsiveness to Warfarin Anticoagulant Therapy

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Oral anticoagulants (OAs) are drugs prescribed to reduce the risk of stroke and systemic embolism in non-valvular atrial fibrillation patients, and to treat and decrease the incidence of deep vein thrombosis and pulmonary embolism. We aimed to clarify the impact of factor VII (FVII) polymorphism on the extent of anticoagulation exerted by warfarin therapy.
Methods: The study was performed on 30 patients on warfarin therapy with international normalized ratio (INR) 2-3, 21 of them for cardiac causes, 2 of them for orthopedic causes and the remaining 7 patients for vascular causes. They were subjected to warfarin therapy (Marevan), laboratory investigations (Prothrombin time (PT) and INR, FVII activity by coagulometric method, plasma warfarin level (by high performance liquid chromatography), and detecting FVII polymorphism (-401G/T & -402 G/A) by polymerase chain reaction).

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Results: Regarding the coagulation indicators of the patients with various FVII- 402 genotypes, PT time was significantly lower in homozygous mutant than heterozygous (P=0.032). Also, it was non significantly lower than that of wild type. PT time was non significantly lower in wild type than heterozygous. PT activity was significantly higher in homozygous mutant than heterozygous (P=0.032). Also, it was non significantly higher than that of wild type. PT activity was non significantly higher in wild type than heterozygous. There was significant difference as regard warfarin sensitivity index (WSI) between wild type and homozygous mutant. Marevan dose was significantly lower in wild type than homozygous mutant and heterozygous. Regarding the coagulation indicators of the patients with various FVII -401 genotypes, WSI was lower in wild type than heterozygous and homozygous mutant, however, it was significantly different between wild type compared to homozygous and heterozygous genotypes (P<0.001). Marevan dose was significantly higher in wild type than homozygous mutant (P<0.001), and it was non significantly higher in heterozygous than homozygous mutant.
Conclusions: Marevan dose was significantly lower in wild type than homozygous and heterozygous mutant as regard FVII -402 G/A, while regarding FVII -401 G/T, marevan dose was significantly higher in wild type than homozygous mutant. Polymorphisms in FVII genes may play a significant role in modulating the warfarin's anticoagulant effect.

Keywords: Factor VII polymorphism; warfarin anticoagulant therapy; oral anticoagulants; non-valvular atrial fibrillation; vitamin K antagonists.

KEY FINDINGS

- Clarify the impact of FVII polymorphism on the extent of anticoagulation exerted by warfarin therapy.

What is known and what is new?

- Warfarin's efficacy is impacted by genetic variants.
- We confirmed that polymorphisms in factor VII genes may play a significant role in modulating the anticoagulant effect of warfarin.

What is the implication, and what should change now?

Use genetic profiling in cases of increased risks of bleeding complications or thrombosis.

1. INTRODUCTION

Oral anticoagulants (OAs) are medications prescribed to reduce the risk of systemic embolism and stroke in non-valvular atrial fibrillation patients (NVAF), and to treat and decrease the incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE) [1].

OAs are classified into vitamin K antagonists (VKAs) (warfarin) [2] and direct oral anticoagulants (DOACs) such as dabigatran, apixaban, rivaroxaban and edoxaban [3].

Despite of its numerous applications, the management of oral anticoagulation therapy with VKAs is a delicate issue, as warfarin has narrow therapeutic index and wide inter-individual variability [4]. Over 50 years ago, the initial dose of warfarin, which ranged from 2-10 mg/day depending on the indication and clinical factors, was determined through trial and error [5]. However, the World health organization WHO's Expert Committee on Biologic Standardization in 1982 developed International Normalized Ratio (INR) [6] which is "the ratio of a patient's prothrombin time to a control sample" and it is used to monitor the warfarin effectiveness and thus measures the blood coagulation pathway [7].

Warfarin responsiveness varies depending on the host's age, sex, race, weight, and body mass index as well as environmental factors such nutrition and the use of other medications [8].

Genetic polymorphism has also been reported to modulate warfarin responsiveness and sensitivity [9]. Warfarin's efficacy is impacted by genetic variants via a pharmacokinetic or pharmacodynamic mechanism [10].

Factor VII (FVII) is a vitamin K-dependent inactive protease. Upon coming into contact with tissue factor (TF), it transforms into an active state and activates factor X until the formation of a thrombus [11].

In the promoter region of the FVII gene, guanine to thymine (G/T) base substitution at the -401st

position results in decreased gene transcription and lower plasma FVII levels, whereas guanine to adenine (G/A) base substitution at the -402nd position in the same region increases gene transcription and the FVII level. The variance in the plasma concentration and coagulation activity of FVII that is caused by the two polymorphisms together by 18% and 28%, respectively [12].

Patients with the FVII -401GG genotype receive significantly more warfarin on a daily basis than do individuals with the FVII -401TT genotype [13]. While the FVII -402 AA genotype is associated with higher daily doses of warfarin than the FVII -402 GG genotype.

The potential benefit of using genetic profiling is likely substantial in cases of increased risks of (i) bleeding complications or (ii) thrombosis, and (iii) when therapeutic warfarin dosages must be reached quickly [14].

1.1 Objective

The aim of this work is to clarify the impact of FVII polymorphism on the extent of anticoagulation exerted by warfarin therapy.

2. METHODS

The present trial was performed on 30 cases on warfarin therapy with INR 2-3 were selected from outpatient clinic of Tanta University hospitals, 21 patients were taking warfarin anticoagulant for cardiac causes [atrial fibrillation (AF), mitral valve replacement (MVR), and coronary artery disease (CAD)], 2 patients for orthopedic causes and 7 patients for vascular causes [DVT, pulmonary embolism (PE) and stroke] at clinical pathology department in Tanta University after the approval of the ethical committee (Approval code: 33373/09/19) in the period from January 2020 to June 2021. Informed written consent was obtained from all cases to participate in this trial.

All patients were subjected to the followings:

- Thorough history taking (age, gender and clinical indications).
- Warfarin therapy (Marevan).
- Laboratory investigations including:

1. Prothrombin time (PT) and INR.
2. Factor VII activity by coagulometric method.
3. Complete blood count (CBC).
4. Plasma warfarin level measured by high performance liquid chromatography (HPLC).
5. Detection of FVII polymorphism (-401G/T & -402 G/A) by PCR.

Inclusion criteria: Patients on warfarin therapy with INR range from 2.0 to 3.0.

Exclusion criteria: Patients on oral anticoagulant other than warfarin.

2.1 Sampling Procedures

Five ml peripheral blood samples were obtained by venous puncture from patients, 2.7 ml were added to sodium citrate tube to test PT, INR, and factor VII activity, and 2 ml were added to EDTA tube for CBC analysis, plasma warfarin level and PCR.

2.2 Prothrombin Time Test (PT)

Determination of PT was performed by Thromborel® S kit (Siemens, Germany) on automated coagulation analyzer (Sysmex CA-1500, serial number F9278).

2.2.1 Principle of the method

The coagulation process was triggered by incubation of plasma with the optimal amount of thromboplastin and calcium. The time to formation of a fibrin clot was then measured.

2.2.2 Reagents

Thromborel® S reagent: lyophilized human placental thromboplastin (≤ 60 g/L).

2.2.3 Procedure

1. The content of the Thromborel® S reagent vial was dissolved with 4 mL of distilled or deionized water use and mixed well by inverting the vial 8- 10 times. Then it was warmed at 37 °C for at least 30 minutes.
2. One hundred (100) µL of citrated plasma was added into a test tube and incubated for 1 minute at 37 °C.
3. Then 200 µL of Thromborel® S reagent (prewarmed to 37 °C) were added.
4. On addition of Thromborel® S reagent, timer on the coagulation analyzer was started to determine the coagulation time.

2.3 Statistical Analysis

Statistical analysis was performed using SPSS statistics software v20. Categorical variables

were described using frequencies and percentages, and analyzed for association using Chi-square test and Monte Carlo test. Quantitative data were described using mean and standard deviation as well as median, range (minimum and maximum). Mann-Whitney test was used to compare two independent groups and Kruskal-Wallis test was used to compare more than two independent groups. Any significant Kruskal-Wallis comparison was followed by adjusted post-hoc pair-wise comparisons. Spearman's correlation was used for testing correlations between quantitative variables. Statistical significance was accepted as $p < 0.05$. All applied statistical tests of significance were two-tailed. The allelic frequencies were estimated by gene counting, and genotypes were scored. Deviations from Hardy-Weinberg equilibrium (HWE) were assessed by the goodness-of-fit chi-square test.

3. RESULTS

Out of 30 patients, 18 (60%) of them were males and 12 (40%) were females (male to female ratio is 3:2). The mean age of the patients was 63.63± 8.89 years (range 26- 79 years). Average warfarin dose requirements ranged from 3- 14mg / day (5.85± 2.68). 21 (70 %) of the patients were taking warfarin (marevan) for cardiac causes, 12 patients (40%) with AF, 8 patients (26.7%) had CAD, and 1 patient (3.3%) with MVR, 2 patients (6.7%) for orthopedic causes and 7 patients (23.3%) for vascular causes, 5 patients (16.7%) with DVT, 1 patient (3.3%) with PE and 1 patient (3.3%) with stroke (Table 1, Fig. 1).

Table 1. Characteristics of the studied patients

			N= 30
Gender, n (%)	Male		18 (60)
	Female		12 (40)
Age, years	M ± SD		63.63± 8.89
	Min-Max		26- 79
Marevan dose, mg	M ± SD		5.85± 2.68
	Min-Max		3- 14
Clinical indications, n (%)	Cardiac causes	AF	12 (40)
		CAD	8 (26.7)
		MVR	1 (3.3)
	Orthopedic causes		2 (6.7)
	Vascular causes	DVT	5 (16.7)
		PE	1 (3.3)
		Stroke	1 (3.3)

Data is presented as mean ± SD, Min-max, or frequency (%). AF: Atrial fibrillation, CAD: Coronary artery disease, MVR: Mitral valve replacement, DVT: Deep vein thrombosis, PE: Pulmonary embolism

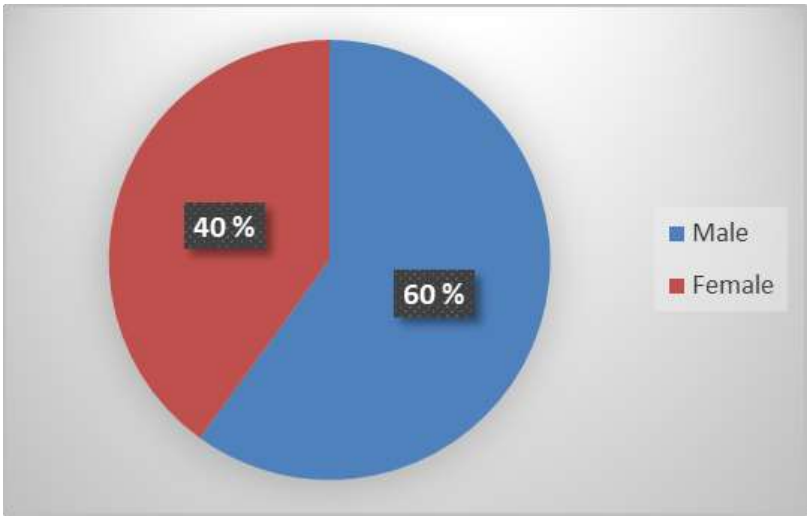

Fig. 1. The percentage of the gender in the studied patients

The median PT time (sec) was statistically significantly lower in homozygous mutant (21.7 sec) than heterozygous (26.1 sec). Also, it was lower than that of wild type but this was not statistically significant. The median PT time (sec) was lower in wild type (25.2 sec) than heterozygous (26.1 sec) but this was not statistically significant (Table 2; Fig. 2).

The median PT activity (%) was statistically significantly higher in homozygous mutant (35.5%) than heterozygous (28.5%). Also, it was higher than that of wild type but this was not statistically significant. The median PT activity (%) was higher in wild type (30.8 %) than heterozygous (28.5%) but this was not statistically significant (Table 2; Fig. 3).

Table 2. Coagulation indicators of the patients with various FVII- 402 genotypes

Coagulation indicators	FVII -402 (G/A) genotypes			H	P
	Heterozygous GA	Homozygous Mutant AA	Wild Type GG		
	n= 11	n=6	n= 13		
	Mdn (Min- Max)	Mdn (Min- Max)	Mdn (Min- Max)		
PT time, sec	26.1[a] (21.7- 32.5)	21.7[b] (21.5- 31.9)	25.2 [a,b] (21.7- 31.9)	6.878	0.032*
PT activity, %	28.5 [a] (21.8- 35.5)	35.5 [b] (22.7- 35.5)	30.8 [a,b] (22.7- 35.5)	6.890	0.032*
INR	2.34 (2- 3)	2.1 (2- 3)	2.32 (2- 3)	4.921	0.085
Warfarin sensitivity index	0.39 [a,b] (0.27-0.60)	0.27 [a] (0.14-0.40)	0.61 [b] (0.41-1)	18.523	<0.001*
Marevan dose, mg	6.0[a] (5- 7.5)	7.5[a] (7.5- 14)	4.0[b] (3- 5)	22.969	<0.001*
Plasma warfarin level, µg/mL	0.3 (0.2- 0.5)	0.45 (0.2- 0.6)	0.4 (0.2- 0.7)	5.331	0.070
FVII activity, %	27 (20.5- 30.9)	34.3 (9.6- 39)	23.8 (5.2- 49.5)	1.089	0.580

*Data is presented as median (min-max), H: Krukal-Wallis test, *Statistically significant, Medians with differing superscripts within rows are significantly different at the adjusted p < 0.05 based on post hoc paired comparisons. FVII: Factor VII, PT: Prothrombin time, INR: International normalized ratio*

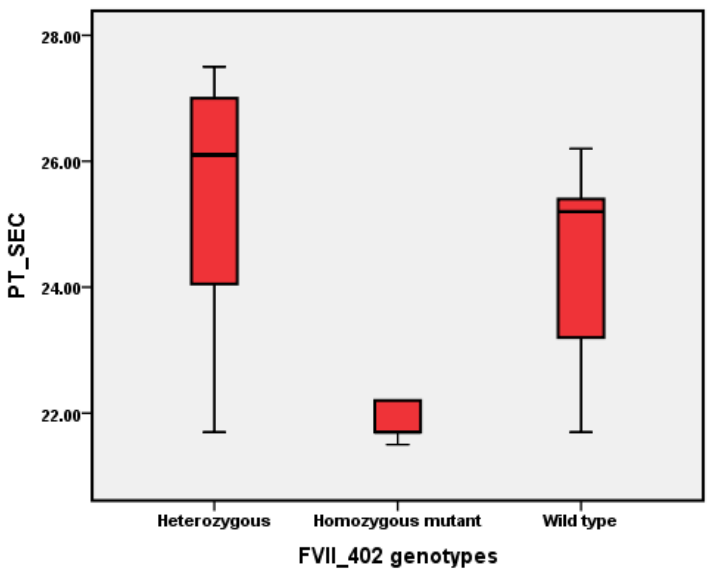

Fig. 2. The median PT time (seconds) between various FVII -402 genotypes

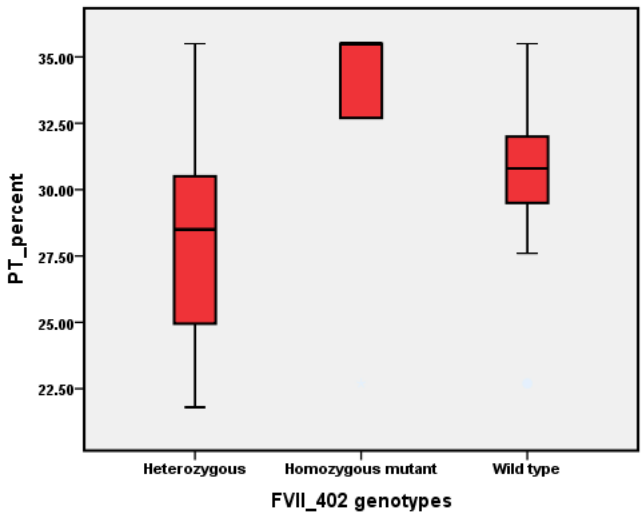

Fig. 3. The median PT activity (%) between various FVII -402 genotypes

The median INR was 2.34 ranging from 2 to 3 in heterozygous, 2.1 ranging from 2 to 3 in homozygous and 2.32 ranging from 2 to 3 in wild type which was not statistically different between various FVII -402 genotypes (Table 2).

The median WSI was higher in wild type (0.61) than heterozygous (0.39) and homozygous mutant (0.27). There was statistically significant difference as regard WSI only between wild type and homozygous mutant (Table 2; Fig. 4).

The median marevan dose (mg) was statistically significantly lower in wild type (4.0 mg) than homozygous mutant (7.5 mg) and heterozygous (6.0 mg) (Table 2; Fig. 5).

The median plasma warfarin level (µg/mL) was not statistically significant between heterozygous, homozygous mutant and wild type. The median FVII:c (%) was higher in homozygous mutant (34.3%) than heterozygous (27%) and wild type (23.8%). However, it was not statistically significant between various factor VII- 402 genotypes (Table 2).

The median PT time (sec), PT activity (%) and INR were not statistically significantly different among the different factor VII- 401 genotypes (Table 3).

The median WSI was lower in wild type (0.39) than heterozygous (0.57) and homozygous mutant (0.67). However, the median WSI was statistically significantly different between wild type as compared with homozygous and heterozygous genotypes (Table 3; Fig. 6).

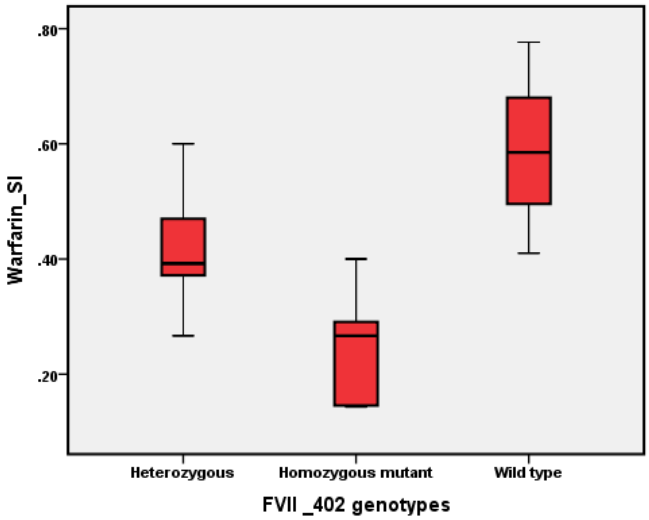

Fig. 4. The median warfarin sensitivity index between various FVII -402 genotypes

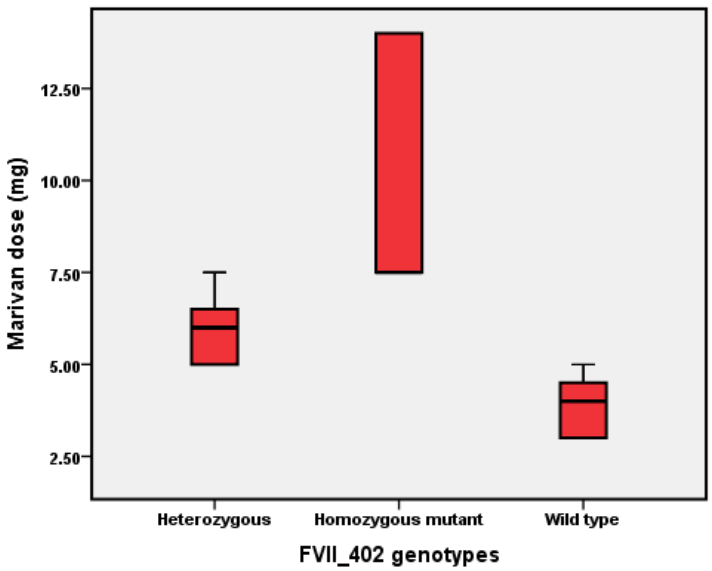

Fig. 5. The median marevan dose (mg) between various FVII- 402 genotypes

Table 3. Coagulation indicators of the patients with various FVII -401 genotypes

Coagulation indicators	FVII -401 (G/T) genotypes				
	Heterozygous GT	Homozygous Mutant TT	Wild Type GG	H	P
	n= 3	n=7	n= 20		
	Mdn (Min- Max)	Mdn (Min- Max)	Mdn (Min- Max)		
PT time, sec	23.4 (23.2- 26.1)	25.2 (21.7- 31.9)	24.6 (21.5- 32.5)	0.136	0.934
PT activity, %	30.8 (23.2-26.1)	30.8 (22.7- 35.5)	30 (21.8-35.5)	0.099	0.952
INR	2.23 (2.2- 2.3)	2.32 (2- 3)	2.24 (2- 3)	0.013	0.994
Warfarin sensitivity index	0.57[a] (0.44-0.78)	0.67[a] (0.52-1)	0.39[b] (0.14-0.60)	15.604	<0.001*
Marevan dose, mg	4.5[a] (3- 5)	3.0 [a, b] (3- 4.5)	6.25 [a, c] (4.5- 14)	18.359	<0.001*
Plasma warfarin level, µg/mL	0.4 (0.4- 0.4)	0.6 (0.2-0.7)	0.35 (0.2- 0.6)	3.456	0.178
Factor VII activity (FVIIc), %	23.5 (21- 28.5)	23.3 (5.2- 32.2)	28.5 (9.6- 49.5)	1.784	0.410

*Data is presented as median (min-max), H: Krukal-Wallis test, *Statistically significant, Medians with differing superscripts within rows are significantly different at the adjusted p < 0.05 based on post hoc paired comparisons, FVII: Factor VII, PT: Prothrombin time, INR: International normalized ratio*

The median marevan dose (mg) was statistically significantly higher in wild type (6.25 mg) than homozygous mutant (3.0 mg). The median marevan dose was higher in heterozygous (4.5 mg) than homozygous mutant. However, there was no statistical significant difference in the median marevan dose between heterozygous and homozygous mutant (Table 3; Fig. 7).

The median plasma warfarin level (µg/mL) was not statistically significant between heterozygous, homozygous mutant and wild type. The median FVII:c (%) was higher in wild type (28.5%) than heterozygous (23.5%) and homozygous mutant (23.3%). However, it was not statistically significant different between various factor VII-401 genotypes (Table 3).

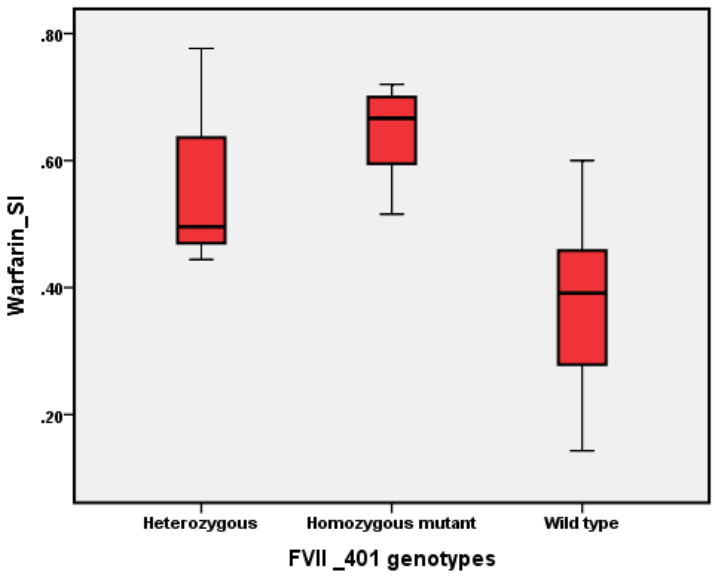

Fig. 6. The median warfarin sensitivity index between various FVII -401 genotypes

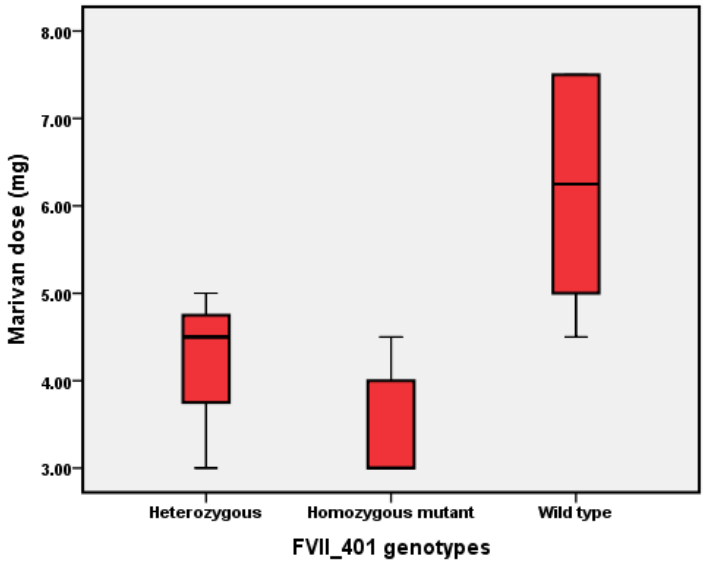

Fig. 7. The median marevan dose in mg between various factor VII- 401 genotypes

4. DISCUSSION

Warfarin is the most widely used oral anticoagulant for the treating and preventing the thromboembolic manifestations that associate multiple conditions. It works as a vitamin K antagonist to inhibit the activation of various vitamin K-dependent coagulation factors, including factors II, VII, IX, and X. Warfarin therapy necessitates frequent dose modifications and careful monitoring due to its narrow therapeutic index and high interpatient variability in order to achieve and maintain a therapeutic anticoagulation effect [15].

Host factors like age and weight as well as environmental factors like diet and the use of interfering medications account for a portion of the variation in warfarin response [16]. FVII gene polymorphisms may alter the responsiveness of warfarin therapy [11]. By combining host, environmental and genetic markers into a single model, some 50–60% of the variability in warfarin maintenance dose could be accounted for [16].

This study aimed to clarify the impact of FVII polymorphism on the extent of anticoagulation exerted by warfarin therapy.

This study was conducted on 30 patients on warfarin therapy (age; 26-79 years old) with INR 2-3. All patients were subjected to thorough history taking (age, gender, clinical indication and dose of warfarin (Coumadin or Marevan or Jantoven), laboratory investigations included prothrombin time (PT), CBC, factor VII activity by coagulometric method, plasma warfarin level measured by high performance liquid chromatography (HPLC) and detection of FVII polymorphism (-401 G/T and -402 G/A) by PCR.

The present study included 30 patients, 18 (60%) of them were males and 12 (40%) were females (male to female ratio is 3:2). The mean age of the patients was 63.63 ± 8.89 years (range 26-79 years). Average warfarin dose requirements ranged from 3- 14 mg / day (5.85± 2.68).

Our results came in line with Yildirim et al. [13] who observed that the mean age of 101 warfarin-treated Turkish patients was 63.74 ±11.43 years (26-80 years). However, their patients consisted of 60 (59.41%) females and 41 (40.59%) men, with a mean daily warfarin dosage of 4.06 ±1.60 mg / day (1.13- 7.86). Different sample size and ethnicity may be appropriate reasons for this difference.

Also, Shikata et al. [17] included 45 patients with 27 males and 18 females and mean age 63.1 years (35 to 82 years). The average warfarin dose was 3.8 mg/d (1 to 8.5 mg/d).

In our study, 21 (70%) patients were taking warfarin for cardiac causes (12 (40%) AF, 8 (26.7%) CAD, and 1 (3.3%) MVR), 2 (6.7%) for orthopedic causes and 7 (23.3%) for vascular causes (5 (16.7%) DVT, 1 (3.3%) PE and 1 (3.3%) stroke.

Our results are in agreement with Yildirim et al. [13] who observed that the indication for warfarin therapy in their patients included: AF (68 patients (67.33%)), MVR (21 patients (20.79%)), aortic valve replacement (AVR) (9 patients (8.91%)), DVT (5 patients (4.95%)), CAD (21 patients (20.79%)). However, some patients may have more than one indication for warfarin therapy.

Also, Caldwell et al. [18] reported that indication for warfarin treatment among 570 patients included: atrial arrhythmias (299 patients (52.5%)), prosthetic heart replacement (114 patients (20%), thromboembolic disease (94 patients (16.5%)), and others (arthropathy, stroke, ect.) (63 patients (11.1%)).

In our study, the mean plasma warfarin level was 0.4± 0.16 µg/mL (0.2- 0.7 µg/mL) and the mean FVII coagulant activity (FVII: c) was 27.78± 10.29% (5.20- 49.50 %).

In the present study, the genotype frequency (distribution) was as follows: 17 patients (11 (36.7%) heterozygotes and 6 (20%) homozygotes showed the A-402 allele and 13 (43%) the G-402 allele (wild type) while 10 patients (3 (10%) heterozygotes and 7 (23.3%) homozygotes showed the T-401 allele and 20 (66.7%) the G-401 allele. Allele frequency of the G-402 allele was 0.62 and of the A-402 allele was 0.38 while that of the G-401 allele was 0.72 and of the T-401 allele was 0.28. The distribution of FVII -402 G/A genotypes were in HWE. However, the frequency of FVII -401 G/T genotypes were deviated significantly from the expected HWE ($P< 0.05$).

Our results came in line with Fuchshuber-Moraes et al. [19] who found that out of 353 patients, 67% were GG (wild type), 29% were GA (heterozygous) and 3% were AA (homozygous) for FVII -402 G/A while 66% were GG (wild type), 29% were GT (heterozygous), and 4% were TT (homozygous) for factor VII 401 G/T.

However, they reported that allele frequency was 0.81 for the G-402 allele and 0.18 for the A-402 allele while that of the G-401 allele was 0.80 and of the T-401 allele was 0.19. Both FVII -401 G/T and -402 G/A were in HWE. The deviation from our results could be attributed to different sample size and populations [19].

Allele distributions can vary significantly in different populations. Kang et al. [20] analyzed 60 CHD Chinese cases, including 33 AMI cases and found allele frequencies 0.03/0.97 for factor VII-401 G/T and 0.48/0.52 for -402 G/A in patients.

In the same context, Dönmez et al. [21] enrolled 83 patients with AMI in southern Turkey area. They observed that FVII -402 G/A genotypes were 5.8%, 30.1%, 24.1% for wild type (GG), heterozygous (GA), homozygous mutant (AA) respectively. While for FVII -401 G/T genotypes were 39.8%, 38.6%, 21.7% for wild type (GG), heterozygous (GT), homozygous mutant (TT) respectively. Allele distribution was 0.61/0.39 for FVII -402 G/A and 0.59/0.41 for FVII -401 G/T and both were HWE.

There was a noticeable difference in the Factor VII- 402 and -401 G/T and allele

frequency between our population and their populations.

Also, Liu and Chen [22] reported that genotype distribution for FVII -402 G/A was 62 (41%), 58 (38%) and 33 (22%) for wild type (GG), heterozygous (GA) and homozygous mutant (AA) among 153 Chinese patients. Allele frequency of the G-402 allele was 0.59 and of the A-402 allele was 0.41.

Additionally, in another study carried out by Lindman et al. [23] they stated that the genotype distribution for both FVII -402 and FVII -401 was in HWE ($p > 0.25$) with allele frequency 0.29 and 0.71 for the A-402 and G-402 allele respectively and 0.12 and 0.88 for the T-401 allele and G-401 allele respectively.

Moreover, Caldwell et al. [18] found that genotype frequency (distribution) in 570 patients was 230 (59.4%) for GG (wild type), 138 (35.7%) for GA (heterozygous), and 19 (4.9%) for AA (homozygous) for FVII -402 G/A while it was 6 (1.6%) for GG (wild type), 82 (21.2%) for GT (heterozygous), and 199 (72.3) for TT (homozygous) for FVII -401 G/T.

In their study, D'Ambrosio et al. [24] reported that out of 147 warfarin receiving Italian patients, 37 patients (35 heterozygotes and 2 homozygotes: 25.2%) showed the A-402 allele and 110 (74.8%) patients showed the G-402 allele (wild type) while 49 patients (44 heterozygotes and 5 homozygotes: 33.3%) the T-401 allele and 98 (66.7%) patients showed the G-401 allele (wild type).

Similarly, Yildirim et al. [13] found that genotype frequency (distribution) in 101 warfarin treated Turkish patients was 47 (46.54%) for GG (wild type), 49 (36.63%) for GT (heterozygous), and 17 (16.83%) for TT (homozygous) for factor VII 401 G/T.

In the present study, regarding CBC, the average white blood cell counts (WBCs) was 2.36- 12.18 x 10^3/µL, red blood cell counts (RBCs) was 3.26- 5.32 x 10^6/µL, and platelet counts (PLT) was 140- 355 x 10^3/µL. the average hemoglobin was 7.3- 15.4 g/dl.

In the present study, as for coagulation profile, the mean prothrombin activity (PT activity) was 30.1± 4.24%, the mean prothrombin time (PT time) was 25.1± 3.27 second, and INR was 2.32 ± 0.31 (2 – 3). Warfarin sensitivity index ranged from 0.14 to 1.

Similarly, Yildirim et al. [13] included Turkish patients with target INR (2.0 – 3.0). Also, Fuchshuber-Moraes et al. [19] involved Brazilian patient with 3 consecutive INR readings ranging from 2.0- 3.5. Caldwell et al. [18] also stated that most of their patients had therapeutic INR (2.0-3.0), except for heart valve patients whose INR ranged from 2.5- 3.5. However, Shikata et al. [17] included 45 patients with average INR 2.32 (1.52 to 3.84).

In the present study, as regard the patients with FVII -402 G/A, the median age was 64 years ranging from 52 to 72 years in heterozygous, 66 years ranging from 65 to 79 years in homozygous and 64 years ranging from 26 to 73 years in wild type. Males were 64%, 83% and 46% in heterozygous, homozygous and wild type respectively. There is no statistical evidence that age and sex are significantly associated with various FVII -402 genotypes.

In the same context, D'Ambrosio et al. [24] stated that for FVII -402 GG (wild type) 59(40.3%) were males and 51(34.5%) were females, while for both FVII-402 GA and AA (heterozygous, homozygous) were 21(14.8%) males and 16(10%) females out of 147 subjects and these results are comparable to ours.

In the present study, as regard patients with FVII -401, the median age was 63 years ranging from 61 to 64 years in heterozygous, 64 years ranging from 26 to 73 years in homozygous and 65 years ranging from 52 to 79 years in wild type. Males were 66.7%, 28.6% and 15% in heterozygous, homozygous and wild type respectively. There is no statistical evidence that age and sex are significantly associated with various FVII- 401 genotypes.

Our results are compatible with Yildirim et al. [13] who stated that the median age for FVII -401 G/T was 65 years ranging from 57.5 to 74.5 years in heterozygous (GT), 64 years ranging from 57 to 72 years in homozygous (TT) and 64 years ranging from 48 to 72 years in wild type (GG). Regarding gender, females were 33.3%, 21.7% and 45% in heterozygous, homozygous and wild type respectively, while males were 41.5%, 9.8% and 48.8% in heterozygous, homozygous and wild type respectively.

In harmony with our findings, D'Ambrosio et al. [24] stated that for FVII -401 GG (wild type) 51(34.6%) were males and 47 (31.9%) were females, while for both FVII-401 GT and TT

(heterozygous, homozygous) were 29 (19.7%) males and 20 (13.6%) females out of 147 subjects.

In the present study, concerning FVII -402 G/A, the median INR was 2.34 ranging from 2 to 3 in heterozygous, 2.1 ranging from 2 to 3 in homozygous and 2.32 ranging from 2 to 3 in wild type. However, the median INR was not statistically different between various factor VII -402 genotypes.

Our results agreed with D'Ambrosio et al. [24] who reported that the mean INR for FVII -402 G/A was 2.55 ± 0.4 and 2.50 ± 0.36 for wild type (GG) and both FVII-402 GA and AA (heterozygous, homozygous) respectively.

In the present study, concerning FVII -402 G/A, the median warfarin sensitivity index was higher in wild type (0.61) than heterozygous (0.39) and homozygous mutant (0.27). However, the median warfarin sensitivity index was statistically significantly different only between wild type and homozygous mutant.

Similarly, Shikata et al. [17] concluded that the mean warfarin sensitivity index for FVII -402 G/A was higher in wild type (3.42± 0.82) than heterozygous (3.09±0.43) and homozygous mutant (2.39±0.18). They calculated the warfarin sensitivity index by the INR response per warfarin plasma concentration.

In the current study, concerning FVII -402 G/A, the median prothrombin time (PT time, sec) was statistically significantly lower in homozygous mutant (21.7 sec) than heterozygous. Also, it was lower than that of wild type, but this was not statistically significant. The median PT time (sec) was lower in wild type (25.2%) than heterozygous (26.1%), but this was not statistically significant.

Our results came in line with Liu and Chen [22], who reported that the mean prothrombin time (PT time) was 12.77± 1.17, 12.60± 1.52 and 12.70± 1.18 seconds for FVII -402 wild type (GG), heterozygous (GA) and homozygous mutant (AA) respectively which showed no statistical significance ($P > 0.05$).

In our study generally, INR was nearly the same among the different FVII -401 genotypes. The median warfarin sensitivity index was lower in wild type (0.39) than heterozygous (0.57) and homozygous mutant (0.67). However, the median warfarin sensitivity index was statistically significantly different only between wild type and homozygous mutant as well as wild type and heterozygous.

In the same line with our findings, D'Ambrosio et al. [24] reported that the mean INR for FVII -401 GG (wild type) was 2.50 ± 0.40 and for both FVII -401 GT and TT (heterozygous, homozygous) was 2.61 ± 0.36. Also, they documented that the groups of patients with the different genotypes did not differ for sex, indication and average INR for both polymorphisms.

Also, Yildirim et al. [13] found that the median INR was 2.40 ranging from 2.01 to 2.79 in heterozygous (GT), 2.37 ranging from 1.86 to 2.66 in homozygous (TT) and 2.41 ranging from 2.13 to 2.77 in wild type (GG).

In our study, we found that regarding FVII -402 G/A, the median marevan dose was statistically significantly lower in wild type (4 mg) than homozygous mutant (7.5 mg) and heterozygous (6 mg). The median plasma warfarin level was not statistically significant between heterozygous, homozygous mutant and wild type.

Similarly, Shikata et al. [17] observed an association of the FVII -402 G/A with warfarin sensitivity in Japanese patients where the mean warfarin daily dose for the wild type (GG) was 3.5 ± 2.0, lower than that for both heterozygous (GA) and homozygous mutant (AA), 3.9 ± 1.9 and 3.8 ± 1.3, respectively.

On the other hand, although, D'Ambrosio et al. [24] found that the two patients carrying the AA-402 genotype were prescribed a higher mean dose (6.9 mg) of warfarin than that prescribed to patients with the GG-402 genotype (5.5 mg) and to the heterozygotes (5.7 mg), these differences were not statistically significant.

Also, Caldwell et al. [18] concluded that the factor VII -402 A/G polymorphism was not associated with differences in the average dose of warfarin.

Similarly, Herman et al. [25] concluded that the factor VII -402 A/G polymorphism was not associated with differences in the average dose of warfarin.

Similarly, Fuchshuber-Moraes et al. [19] showed no association of the FVII -401G/T or -402G/A polymorphisms with the stable warfarin dose requirement in the overall study cohort.

In our study, we found that regarding FVII -402 G/A, the median factor VII activity (FVII:c) was higher in homozygous mutant (AA) (34.3) than heterozygous (GA) (27) and wild type (GG) (23.8). However, it was not statistically significant between various FVII- 402 genotypes**.** This may be due to our low sample size.

Similarly, Carew et al. [26] found that FVII -402 homozygous mutant (AA) is associated with significantly increased FVII:c, (mean 122 ±28.9), which is higher than 112± 27.5 for heterozygous (GA) and 103 ± 27.0 for wild type (GG).

Also, in Lindman et al. [23] study on a Norwegian population of elderly men, they provided evidence that −402 A allele is associated with elevated levels of FVII:c which is higher than homozygous for −402 G allele.

In our study, we found that regarding FVII -401 G/T, the median Marevan dose was statistically significantly higher in wild type (6 mg) than homozygous mutant (3 mg). The median marevan dose was higher in heterozygous (4.5 mg) than homozygous mutant. However, there was no statistically significant difference in the median marevan dose between heterozygous and homozygous mutant. The median plasma warfarin level was not statistically significant between heterozygous, homozygous mutant and wild type.

In agreement with our findings, Yildirim et al. [13] found that the mean daily dose of the patients with the FVII -401 GG genotype [5 (3.93-6.96) mg] was significantly higher than that of the patients with the FVII -401 TT genotype [3.57 (2.5-5) mg] ($p<0.05$). There was no statistically significant difference between the mean daily doses in patients with the FVII -401 GG genotype and patients with the FVII -401 GT genotype ($p>0.05$). They confirmed that the FVII -401 G/T polymorphism only explains approximately 2.2% of the inter-individual variability in the warfarin dose requirement.

Also, D'Ambrosio et al. [24] observed that the average dose of warfarin received was higher among Italian patients with the factor VII -401 (GG) genotype (5.9 mg) than among Italian patients carrying the -401T allele (4.9 mg; p=0.017).

However, this observation was not verified in other studies. Fuchshuber-Moraes et al. [19] and Caldwell et al. [18] as they showed no association of the FVII -401G/T polymorphism with the stable warfarin dose requirement. And, Schelleman et al. [27] who studied 259 warfarin initiators Caucasians and African Americans, showed no association of the FVII -401G/T polymorphism with the stable warfarin dose requirement.

Discrepancies may be primarily attributable to variations in genetic origins, followed by differences in research design, in the criteria used to select the groups, and in the statistical power of the investigations.

In our study, we found that regarding FVII -401 G/T, the median FVII activity (FVII:c) was higher in wild type (28.5%) than heterozygous (23.5%) and homozygous mutant (23.3%). However, it was not statistically significant different between various FVII- 401 genotypes. This may be due to our low sample size.

In agreement with our findings, a study carried out by Lindman et al. [23] on a Norwegian population of elderly men provided evidence that −401 G allele is associated with elevated levels of FVII:c which is higher than −401 T allele.

Also, van't Hooft et al. [12] reported that both polymorphisms (FVII -401 G/T and -402 G/A) accounts for 18% and 28% of the variation in the plasma concentration and coagulation activity of factor VII (FVII:c), respectively.

There are a number of other factors that influence FVIIc including age, body mass index (BMI) and, in women, the use of oral contraceptives and onset of menopause which are all associated with higher levels. In the general population, about 47% of the total variation in FVIIc is related to the difference between individuals [13].

Bivariate analysis of factors (age, sex, INR, clinical condition) that can modulate marevan dose other than factor VII genotypes showed that only age is significantly associated with marevan dose. The possibility that different VII genotypes (FVII- 402 and 401 genotypes) can affect marevan was further investigated in a multiple linear regression model, adjusted for age, sex, INR and clinical condition. A stepwise analysis revealed the independent nature of only factor VII PCR- 401 genotypes (Adjusted R^2: 0.27; p=0.005).

Also, Yildirim et al. [13] reported that increased patient age may lead to a higher sensitivity to

warfarin as there is a negative correlation between age and warfarin clearance. At the same time, as a result of age-related decreases in liver mass, the liver content of vitamin K epoxide reductase is reduced, causing increased sensitivity to warfarin.

5. CONCLUSIONS

Age and sex were insignificantly associated with various FVII -402 G/A and FVII -401 G/T genotypes. Marevan dose was statistically significantly lower in wild type than homozygous mutant and heterozygous as regard FVII -402 G/A, while regarding FVII -401 G/T marevan dose was statistically significantly higher in wild type than homozygous mutant. Age shown a strong association with marevan dosage, but gender, INR, and clinical state exhibited no association. This study confirmed that polymorphisms in factor VII genes may play a significant role in modulating the anticoagulant effect of warfarin.

CONSENT

Informed consent was obtained from all participants in this research. Privacy was maintained by identification of the patient by coded numbers and all the private data about the patients such as name, address, phone number or even photos did not appear in the research instead, a code number was assigned to identify research.

ETHICAL APPROVAL

As per international standard or university standards written ethical approval has been collected and preserved by the authors.(Approval code: 33373/09/19).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cullell N, Carrera C, Muiño E, et al. Pharmacogenetic studies with oral anticoagulants. Genome-wide association studies in vitamin K antagonist and direct oral anticoagulants. Oncotarget. 2018;9: 29238-58.
2. Manolopoulos VG, Ragia G, Tavridou A. Pharmacogenetics of coumarinic oral anticoagulants. Pharmacogenomics. 2010; 11:493-6.
3. Amin H, Nowak RJ, Schindler JL. Cardioembolic stroke: Practical considerations for patient risk management and secondary prevention. Postgrad Med. 2014;126:55-65.
4. Wypasek E, Branicka A, Awsiuk M, et al. Genetic determinants of acenocoumarol and warfarin maintenance dose requirements in Slavic population: A potential role of CYP4F2 and GGCX polymorphisms. Thromb Res. 2014;134: 604-9.
5. Gage BF, Eby C, Johnson JA, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin Pharmacol Ther. 2008;84:326-31.
6. Hirsh J, O'Donnell M, Eikelboom JW. Beyond unfractionated heparin and warfarin: current and future advances. Circulation. 2007;116:552-60.
7. Piatkov I, Rochester C, Jones T, et al. Warfarin toxicity and individual variability-clinical case. Toxins (Basel). 2010;2:2584-92.
8. Klein TE, Altman RB, Eriksson N, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med. 2009;360:753-64.
9. Hughes DA, Pirmohamed M. Warfarin pharmacogenetics: economic considerations. Pharmacoeconomics. 2007; 25:899-902.
10. Johnson JA, Caudle KE, Gong L, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. Clin Pharmacol Ther. 2017;102:397-404.
11. Mlynarsky L, Bejarano-Achache I, Muszkat M, et al. Factor VII R353Q genetic polymorphism is associated with altered warfarin sensitivity among CYP2C9 *1/*1 carriers. Eur J Clin Pharmacol. 2012; 68:617-27.
12. van 't Hooft FM, Silveira A, Tornvall P, et al. Two common functional polymorphisms in the promoter region of the coagulation factor VII gene determining plasma factor VII activity and mass concentration. Blood. 1999;93:3432-41.
13. Yildirim E, Erol K, Birdane A. Warfarin dose requirement in Turkish patients: the influences of patient characteristics and polymorphisms in CYP2C9, VKORC1 and factor VII. Hippokratia. 2014;18:319-27.

14. Helin TA, Joutsi-Korhonen L, Asmundela H, et al. Warfarin dose requirement in patients having severe thrombosis or thrombophilia. Br J Clin Pharmacol. 2019;85:1684-91.
15. Okumura Y, Yokoyama K, Matsumoto N, et al. Three-Year Clinical Outcomes Associated With Warfarin vs. Direct Oral Anticoagulant Use Among Japanese Patients With Atrial Fibrillation - Findings From the SAKURA AF Registry. Circ J. 2018;82:2500-9.
16. Shaul C, Blotnick S, Deutsch L, et al. The impact of R353Q genetic polymorphism in coagulation factor VII on the initial anticoagulant effect exerted by warfarin. Eur J Clin Pharmacol. 2019;75:343-50.
17. Shikata E, Ieiri I, Ishiguro S, et al. Association of pharmacokinetic (CYP2C9) and pharmacodynamic (factors II, VII, IX, and X; proteins S and C; and gamma-glutamyl carboxylase) gene variants with warfarin sensitivity. Blood. 2004;103:2630-5.
18. Caldwell MD, Berg RL, Zhang KQ, et al. Evaluation of genetic factors for warfarin dose prediction. J Clin Med Res. 2007;5:8-16.
19. Fuchshuber-Moraes M, Perini JA, Rosskopf D, et al. Exploring warfarin pharmacogenomics with the extreme-discordant-phenotype methodology: Impact of FVII polymorphisms on stable anticoagulation with warfarin. Eur J Clin Pharmacol. 2009;65:789-93.
20. Kang W, Wang H, Xiong L, et al. [Study on plasma coagulation factor VII (FVII) levels and polymorphisms of FVII gene in patients with coronary heart disease]. Zhonghua Xue Ye Xue Za Zhi. 2002; 23:457-9.
21. Dönmez Y, Hasan K, İçen YK, et al. Factor VII-401 and-402 polymorphisms and acute myocardial infarction in southern Turkey population. Eur J Res. 2019;5:734-9.
22. Liu JW, Chen DQ. Genetic polymorphisms in the FVII gene is associated with lower extremity deep venous thrombosis: A case-control study. J Cell Biochem. 2018; 119:6715-22.
23. Lindman AS, Pedersen JI, Hjerkinn EM, et al. The influence of the -401G/T and -402G/A polymorphisms of the coagulation FVII promoter on plasma levels of FVII. Thromb Res. 2005;116:313-20.
24. D'Ambrosio RL, D'Andrea G, Cappucci F, et al. Polymorphisms in factor II and factor VII genes modulate oral anticoagulation with warfarin. Haematologica. 2004;89: 1510-6.
25. Herman D, Peternel P, Stegnar M, et al. The influence of sequence variations in factor VII, gamma-glutamyl carboxylase and vitamin K epoxide reductase complex genes on warfarin dose requirement. Thromb Haemost. 2006;95:782-7.
26. Carew JA, Basso F, Miller GJ, et al. A functional haplotype in the 5' flanking region of the factor VII gene is associated with an increased risk of coronary heart disease. J Thromb Haemost. 2003;1:2179-85.
27. Schelleman H, Chen J, Chen Z, et al. Dosing algorithms to predict warfarin maintenance dose in Caucasians and African Americans. Clin Pharmacol Ther. 2008;84:332-9.

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