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ESTIMATION OF THE WARFARIN DOSE IN HEART FAILURE PATIENTS WITH IMPLANTED MECHANICAL CIRCULATORY SUPPORT DEVICE

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Abstract

Relevance. Left ventricular assist device (LVAD) is an alternative treatment for heart failure (HF) patients which improves patient's quality of life at their end-stage. Despite to the improvement LVAD is beleaguered with thrombosis/bleeding complications in 70% of HF patients after implantation. An anticoagulant treatment of warfarin is usually prescribed to reduce thrombosis complications. However, due to the incorrect dosage of the treatment thrombosis/ bleeding complications still happen. Warfarin dose could be identified by genetic variants of vitamin K-epoxide reductase complex (*VKORC1*) and the cytochrome P450-2C9 *(CYP2C9*) which account for 50% of dose variability.

Aim. The aim of our investigation is to identify variability of warfarin dose according to the genotype polymorphisms of genes and their difference from the clinical dosage in HF patients.

Materials and methods. The case series study included 98 HF patients (without complications – 74 patients, with complications – 24 patients) with prescribed warfarin treatment after device implantation. Clinical warfarin dosage difference was identified between genotype polymorphisms of rs9923231, rs9934438 in *VKORC1* gene. Furthermore, warfarin dosage was calculated according to the genetic test results and compared with clinical dosage.

Results. Warfarin dosage according to the clinical protocol between three genotypes of polymorphisms in *VKORC1* gene showed significant difference. HF patients were prescribed with higher warfarin dosage with wild type genotype polymorphism of rs9934438 in *VKORC1* gene than with mutant genotype < 2.5mg (3.88 ± 1.25 vs. 2.44 ± 0.81, p = 0.00005).

Conclusion. Genotype-guided warfarin dosing may estimate accurate dose and potentially improve outcomes in LVAD patients.

Keywords: *heart failure, anticoagulant therapy, warfarin, gene polymorphism.*

Резюме

РАСЧЕТ ДОЗЫ ВАРФАРИНА У ПАЦИЕНТОВ С СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТЬЮ С ИМПЛАНТИРОВАННЫМ УСТРОЙСТВОМ МЕХАНИЧЕСКОЙ ПОДДЕРЖКИ КРОВООБРАЩЕНИЯ

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Введение. Вспомогательное механическое устройство левого желудочка (Left ventricular assist device, LVAD) является альтернативным методом лечения пациентов с сердечной недостаточностью (СН), которое улучшает

качество жизни пациентов на терминальной стадии. Несмотря на улучшение, LVAD сопровождается тромбозами/кровотечениями у 70% пациентов с СН после имплантации. Антикоагулянтное лечение варфарином обычно назначают для уменьшения осложнений тромбоза. Однако из-за неправильной дозировки лечения осложнения тромбоза/кровотечения все же случаются. Дозу варфарина можно определить по генетическим вариантам комплекса витамин К-эпоксидредуктазы (*VKORC1*) и цитохрома Р450-2С9 (*CYP2C9*), которые влияет на вариабельности дозы на 50%.

Цель. Целью нашего исследования является выявление вариабельности дозы варфарина в зависимости от генетических полиморфизмов генов и их отличия от клинической дозы у больных СН.

Материалы и методы. В исследование серии случаев включены 98 пациентов с СН (без осложнений – 74 пациента, с осложнениями – 24 пациента), получавших лечение варфарином после имплантации устройства. Выявлена клиническая разница в дозировке варфарина между полиморфизмами генотипов rs9923231, rs9934438 гена *VKORC1.* Кроме того, дозировка варфарина рассчитывалась по результатам генетического теста и сравнивалась с клинической дозировкой.

Результаты. Дозировка варфарина согласно клиническому протоколу между тремя генотипами полиморфизмов гена *VKORC1* показала значительную разницу. Пациентам с СН назначали более высокие дозы варфарина при диком типе полиморфизма rs9934438 гена *VKORC1,* чем при мутантном генотипе <2,5 мг (3,88 ± 1,25 против 2,44 ± 0,81, *p* = 0,00005).

Выводы. Дозирование варфарина с учетом генотипа может дать точную оценку дозы и потенциально улучшить исходы у пациентов с LVAD.

Ключевые слова: *сердечная недостаточность, антикоагулянтная терапия, варфарин, полиморфизм генов.*

Түйіндеме

МЕХАНИКАЛЫҚ ҚАН АЙНАЛЫМЫН ҚОЛДАЙТЫН ҚҰРЫЛҒЫСЫ ИМПЛАНТАЦИЯЛАНҒАН ЖҮРЕК ЖЕТКІЛІКСІЗДІГІ БАР НАУҚАСТАРДА ВАРФАРИН ДОЗАСЫН ЕСЕПТЕУ

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Өзектілігі. Сол жақ қарыншаның көмекші механикалық құрылғысы (Left ventricular assist device, LVAD) жүрек жеткіліксіздігі (ЖЖ) бар науқастарға арналған балама емдеу әдісі болып табылады, ол соңғы сатыдағы пациенттердің өмір сапасын жақсартады. Жақсарғанына қарамастан, 70% ЖЖ пациенттерінде тромбоз/ қан кету асқынулары пайда болады LVAD имплантациясынан кейін. Тромбоздың асқынуын азайту үшін антикоагулянттық емдеу варфаринмен әдетте тағайындалады. Дегенмен, тромбоз/қан кету асқынулары емдеудің дұрыс емес дозасынан туындайды. Дозаның өзгергіштігіне 50%-ға әсер ететін, К витаминінің эпоксид-редуктаза кешенінің (*VKORC1*) және Р450-2С9 цитохромының (*CYP2C9*) генетикалық нұсқалары арқылы варфариннің дозасын анықтауға болады.

Мақсаты. Біздің зерттеуіміздің мақсаты гендік полиморфизмге байланысты варфарин дозасының өзгергіштігін және олардың жүрек жеткіліксіздігі бар науқастардағы клиникалық дозадан айырмашылығын анықтау болып табылады.

Материалдар мен әдістер. Жағдайлар сериясына зерттеуге LVAD құрылғысы имплантациясынан кейін варфаринмен емделген 98 ЖЖ пациенттері (асқынулары жоқ – 74 пациент, асқынулары бар - 24 пациент) қамтылды. Варфарин дозасының клиникалық айырмашылығы *VKORC1* генінде полиморфизмдерінің rs9923231 және rs9934438 генотиптерінің арасында анықталды. Сонымен қатар, варфариннің дозасы генетикалық сынақ нәтижелері бойынша есептелді және клиникалық дозамен салыстырылды.

Нәтижелер. *VKORC1* гендік полиморфизмінің үш генотипі арасындағы варфарин клиникалық дозасы айтарлықтай айырмашылықты көрсетті. ЖЖ бар емделушілер *VKORC1* генінің rs9934438 полиморфизмінде, мутантты генотипке қарағанда (<2,5 мг) жабайы типте варфариннің жоғары дозасын қабылдады, (3,88 ± 1,25 қарсы 2,44 ± 0,81, *p* = 0,00005).

Қорытынды. Варфариннің генотипі бойынша дозаның нақты дозасын анықтауға және LVAD пациенттеріндегі нәтижелерді жақсартуы мүмкін.

Түйін сөздер: *жүрек жеткіліксіздігі, антикоагулянттық терапия, варфарин, гендер полиморфизмі.*

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Introduction

Heart failure (HF) is one of the significant problems in the healthcare which affects 30 million people in the worldwide [7]. The gold standard treatment of the HF patients is heart transplantation at the end-stage which is not available for every patient due to the donor shortage [11]. However, there is an alternative treatment which is known as left ventricular assist device (LVAD). LVAD is implanted at the end-stage of HF patients [18, 15]. Implantation of LVAD device improves patient's quality of life and decreases mortality with survival rate for 80%, whereas heart transplantation performs survival rate for 86%. LVAD could be implanted as a bridge-to-transplantation (BTT) for the short term treatment and as a destination therapy (DT) for the long term support, depending on the patient's medical indication. Device implantation helps to prolong patient's life before availability of the heart donor [11, 4].

Despite to the improvements of the quality life HF patients have increased risk to thromboembolism complications due to the contact of the blood with the non-biologic surface and presence of the high non-physiological shear stress at the blade region of the device [21, 5]. Consequently, to reduce risks of thromboembolic complications and pump thrombosis patients are prescribed with anticoagulant and antiplatelet therapy after LVAD implantation [5, 14].

However, thrombosis and bleeding complications still occur with anticoagulant treatment due to the incorrect dosage of the therapy which causes over- and under-coagulation [18, 12].

Warfarin is an anticoagulant treatment that is usually prescribed for HF patients to prevent thrombosis events by inhibiting synthesis of vitamin K dependent clotting factors [13, 19]. Drug dosing is processed by weakly measurement of the International Normalized Ratio (INR) of individual patient. The initial warfarin dosage might range from 3 – 5 mg per day. However, to achieve stable INR 2-3 level warfarin dose might vary from 1 - 20 mg per day. The identification of correct warfarin dosage can take long period of time from weeks to months which increases risks to the development of the complications with over- and under-coagulation of the drug [10].

On the other hand, dose variability of warfarin could be identified by genetic variants of vitamin K-epoxide reductase complex (VKORC1) and the cytochrome P450-2C9 (CYP2C9) which account for 50% of dose variability. VKORC1 and CYP2C9 genes are involved in the drug metabolism and action [18, 21].

In 2007, Food and Drug administration (FDA) mentioned on the warfarin label that genotypes of VKORC1 and CYP2C9 could be helpful in identification the initial warfarin dose. Furthermore, warfarin dose could be also identified by online algorithm which is available on http://www.warfarindosing.org [10].

Warfarin dosage show 30% of variability in European or Asian populations according to the genome - wide association studies (GWAS) [16].

Investigations found that genotype frequency of VKORC1 and CYP2C9 genes differentiates among European-American and African-American populations. Genotype polymorphisms of VKORC1 and CYP2C9 genes could predict optimal warfarin dosage which will prevent over- and under-coagulation and therefore reduce complications after LVAD implantation [18, 3].

Investigations conclude that warfarin dose may vary according to the genotype frequency of single nucleotide polymorphisms (SNP) in VKORC1 and CYP2C9 genes. Thus, **the aim** of our investigation is to identify variability of warfarin dose according to the genotype polymorphisms of rs9934438, rs9923231 in *VKORC1* gene and difference of the genetic dosage from the clinical dosage of warfarin in HF patients with implanted LVAD device.

Materials and methods

Participant recruitment.

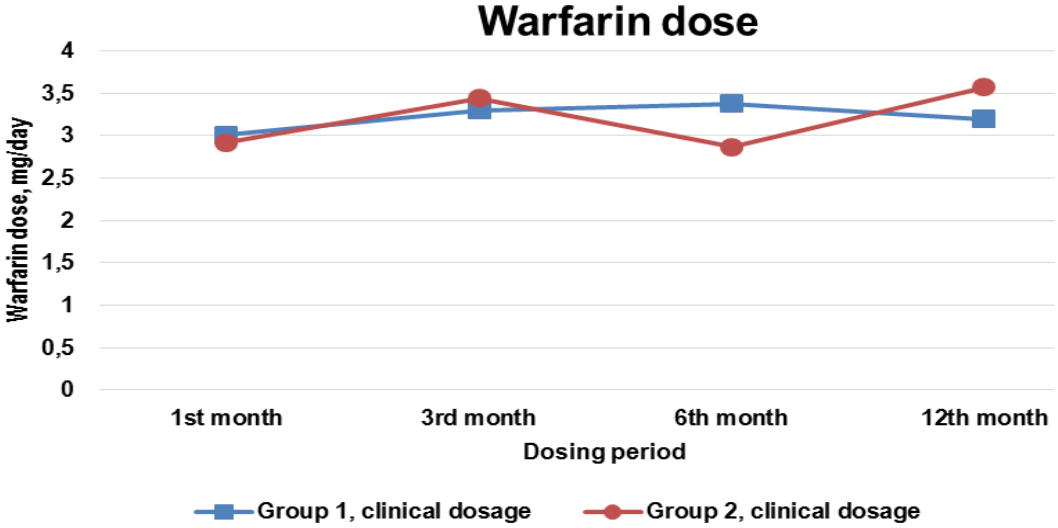
The case series study included 98 patients with end stage heart failure (age ≥ 18) with implanted continuous flow LVAD devices during 2011-2016, treated in National Research Cardiac Surgery Center, Astana. HF patients were implanted with LVAD devices as BTT and DT depending on medical indications. There were implanted three type of devices:

1. HeartWare HVADs (HW);
2. HeartMate II (HM2);
3. HeartMate III (HM3);

Patients were diagnosed with ischemic cardiomyopathy (*n* = 44, ICM), dilated cardiomyopathy (*n* = 40, DCM), hypertrophic cardiomyopathy (*n* = 11, HCM) and valvular heart disease (*n* = 3, VHD). Baseline demographic characteristics of HF patients are summarized in Table 1. Venous blood samples were recruited into sterile vacutainers with K2EDTA at the National Research Cardiac Surgery Center (NRCSC) for genotyping.

Anticoagulant therapy with warfarin were prescribed to HF patients with according to the clinical protocol of the Ministry of Healthcare of the Republic of Kazakhstan. Warfarin dose was corrected to maintain international normalized (INR) ratio range between 2.25-3.25 (2.99 ±

1.15 mg/day). Further, in current manuscript warfarin dosing according to the clinical protocol mentioned as "clinical dosage". "Clinical dosage" of the warfarin was monitored from the initial dose (1st month) then in the 3rd month, 6th month and 12th month (Fig. 1). Patients had LVAD complications such as thrombosis, bleeding and infections. Consequently, HF patients were classified into two groups: Group 1, *n* = 74 (without complications); Group 2, *n* = (with complications); In our previous manuscript, demographic characteristics and biochemical parameters were compared between these two groups (Zhalbinova et al., 2022). Therefore, basic demographic characteristics of all 98 HF patients and comparison between Group 1 and Group 2 are summarized in Table 1. Furthermore, monthly (1st month, 3rd, 6th and 12th) "clinical dosage" of the warfarin was shown between HF patients groups without and with complications (Fig. 1).



Group 1 – without complications, Group 2 – with complications

Figure 1. Clinical dosage of warfarin between HF patients with/without complications.

The study was conducted in accordance with the Declaration of Helsinki. The Ethics Committees of the National Laboratory Astana, Nazarbayev University (No.16 from 11 March 2015) and the National Research Cardiac Surgery Center (NRCC), Astana, Kazakhstan (No.16 from 24 April 2015) approved the research protocol. Written informed consent was obtained from all HF patients.

DNA Extraction and genotyping

DNA samples were extracted from 200 μL blood samples by using the PureLinkTM Genomic DNA Mini Kit (Invitrogen). The concentration and purity of genomic DNA were performed on NanoDrop™ Spectrophotometer (ThermoFisher Scientific). Therefore, DNA samples were genotyped for twenty-one SNPs by using real-time polymerase chain reaction (qPCR) with allele discrimination using TaqMan Real Time PCR Assay on a 7900HT Fast Real-Time PCR System (Applied Biosystems). Method of qPCR was explained in details in previously published manuscript [21].

Selection of SNPs

Overall our study included evaluation of twenty-one polymorphisms in genes that are associated with cardiovascular diseases, blood coagulation system, involved in the metabolism and action of the anticoagulant drug. A list of SNPs and used primers are available elsewhere [21]. Moreover, in this particular study according to the aim, we performed additional analysis for two significant polymorphisms *VKORC1* (rs9934438, rs9923231) out of 4 SNPs (rs9934438; rs9923231 in *VKORC1*, rs5918 in *ITGB3* and rs2070959 in *UGT1A6* genes*)* which were significant in our previous study [21] (Table 2).

Warfarin dosage identification

Warfarin dose was identified by using the algorithm available on http://www.warfarindosing.org. Genotype polymorphisms of rs9923231 in *VKORC1,* rs2108622 in *CYP4F2,* rs11676382 in *GGCX* and rs1799853, rs1057910, rs28371686 in *CYP2C9* genes and patient's demographic characteristics such as age, gender, ethnicity, race type, weight, height, INR level were entered into website to identify initial warfarin dosage. Furthermore, we calculated warfarin dose of the clinical dosage for the 1st month, 3rd, 6th and 12th month as corrected dosage. This corrected warfarin dose was mentioned as "genetic dosage". "Genetic dosage" of warfarin showed the difference from the "clinical dosage". Relationship of "clinical dosage" of the warfarin and "genetic dosage" were shown in line graph between HF patients groups with/without complications (Fig. 2).

Furthermore, we compared clinical dosage of warfarin between genotype polymorphisms of rs9934438, rs9923231 in *VKORC1* gene which influence to warfarin dosage variability, out of 4 significant SNPs (rs9934438; rs9923231 in *VKORC1*, rs5918 in *ITGB3* and rs2070959 in *UGT1A6* genes*)* which were mentioned previously (Table 3).

Statistical analysis

Continuous variables were performed as mean ± standard deviation (SD). Categorical variables were reported as percentages and compared by using chi-square test or Fisher's exact test. Continues variables were assessed for normality of distribution by using a Kolmogorov-Smirnov test ($p > 0.05$). Normally distributed continues variables were compared between two groups by Student's *t*-test and by Mann—Whitney U test for non-normally distributed variables. A one-way ANOVA was performed for comparison normally distributed variables between three groups and by non-parametric Kruskal-Wallis

test for non-normally distributed variables. Hardy-Weinberg equilibrium (HWE) for genetic deviation was performed using chi square test or Fisher's exact test. Statistical significance was considered with *p*-value < 0.05. The statistical analysis was performed in SPSS program version 23.

Results

Data of basic demographic characteristics of HF patients and comparison between HF patients with and without complications are presented in Table 1. Heart transplantation was performed for 10 patients (10.2%) who received LVAD as a BTT during follow up period. On the other hand, 88 patients received LVAD as DT due to the heart donor shortage. Comparative analysis showed that complications were more prevalent in patients with implanted HM2 device in 12 (50%) cases than in 22 patients (29.7%) without complications ($p = 0.01$). Group of patients with complications had development of thrombosis in 13 cases (54.2%), bleeding in 14 cases (58.3%) and infection in 15 (62.5%) cases, respectively ($p < 0.05$). Patients were prescribed with initial warfarin dosage with 2.99 ± 1.15 mg/day. Twenty-seven (27.6) patients died during follow up period and eleven (45.8) of them had complications. On the other hand, seventy-one (72.4) patients achieved outcome and most of them $n = 58$ (78.4) did not have any complications ($p = 0.03$) (Table 1).

Table 1.

Basic demographic characteristics Control group and HF patients.

Characteristic	HF patients, n = 98	Comparison between HF patients		
		Group 1, n = 74	Group 2, n = 24	P value
Age (years)	52.7 ± 11.0	52.5 ± 11.3	53.4 ± 10.1	0.92**
Gender				
Male	92 (93.9)	71 (95.9)	21 (87.5)	0.16
Female	6 (6.1)	3 (4.1)	3 (12.5)	
Ethnicity				
Asian	77 (78.6)	56 (75.7)	21 (87.5)	0.27
Caucasian	21 (21.4)	18 (24.3)	3 (12.5)	
Body weight (kg)	79.8 ± 13.9	80.0 ± 12.2	79.3 ± 18.5	0.86*
Height (cm)	169.8 ± 6.36	170.0 ± 6.08	168.9 ± 7.24	0.46*
BMI (kg/m)	27.7 ± 4.5	27.7 ± 4.10	27.6 ± 5.66	0.97*
Diagnosis				
ICM	44 (44.9)	36 (48.6)	8 (33.3)	0.10
DCM	40 (40.8)	25 (33.8)	15 (62.5)	
HCM	11 (11.2)	10 (13.5)	1 (4.2)	
VHD	3 (3.1)	3 (4.1)	0	
INR				
Basic INR	1.21 ± 0.36	1.19 ± 0.37	1.26 ± 0.33	0.11**
Target INR	2.39 ± 0.26	2.36 ± 0.24	2.46 ± 0.32	0.06**
Device strategy				
BTT	10 (10.2)	6 (8.1)	4 (16.7)	0.25
DT	88 (89.8)	68 (91.9)	20 (83.3)	
Device type				
HW	18 (18.4)	11 (14.9)	7 (29.2)	**0.01**
HM2	34 (34.7)	22 (29.7)	12 (50.0)	
HM3	46 (46.9)	41 (55.4)	5 (20.8)	
Warfarin dose (mg/day)	2.99 ± 1.15	3.01 ± 1.04	2.92 ± 1.46	0.29
Patients' achieved outcome till 2017				
Survived	71 (72.4)	58 (78.4)	13 (54.2)	**0.03**
Not-survived	27 (27.6)	16 (21.6)	11 (45.8)	
Thrombosis				
Yes	13 (13.3)	0	13 (54.2)	**0.0001**
No	85 (86.7)	74 (100)	11 (45.8)	
Bleeding				
Yes	14 (14.3)	0	14 (58.3)	**0.0001**
No	84 (85.7)	74 (100)	10 (41.7)	
Infections				
Yes	39 (39.8)	24 (32.4)	15 (62.5)	**0.015**
No	59 (60.2)	50 (67.6)	9 (37.5)	

Continues variables are presented, mean ± SD and categorical variables as n (%). HF patients, heart failure patients; Group 1, without complications; Group 2, with complications; The significant p value (p < 0.05) is labeled in bold; BMI, body mass index; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; VHD, valvular heart disease; INR, International normalized ratio; BTT, bridge-to-transplantation; DT, destination therapy; HW, HeartWare HVAD; HM2, HeartMate II; HM3, HeartMate III;

Analysis of Genotyping

Cohort of HF patients (n = 98) were genotyped for twenty-one SNPs [21]. Consequently, in this manuscript the distributions of allelic and genotype frequencies of significantly different two SNPs (rs9934438, rs9923231 in *VKORC1* gene) among HF patients with/without complications are summarized in Tables 2. The distributions of allelic frequencies were tested for Hardy-Weinberg equilibrium (HWE). Heterozygote GA genotype polymorphism of rs9934438 in *VKORC1* gene and CT genotype polymorphism for rs9923231 were significantly higher in HF patient group with complications than in patients without complications (70.8% vs. 39.2% and 70.8% vs. 41.9%, $p < 0.05$).

Warfarin dosage. Clinical dosage of the warfarin between group of patients without and with complications was performed in line graph (Fig. 1). Graph represents that warfarin dose level did not show difference from the 1st month till 3rd months in both groups. However, we found that mean warfarin dose level decreased from 3.44 mg/day to 2.87 mg/day (from 3rd to 6th month) in the group of patients with complications whereas warfarin dose in the group of patients without complications was stable without sharp changes in dosage (3.35 mg/day).

Table 2.

The distributions of allelic and genotype frequencies of SNPs between HF patients with/without complications.

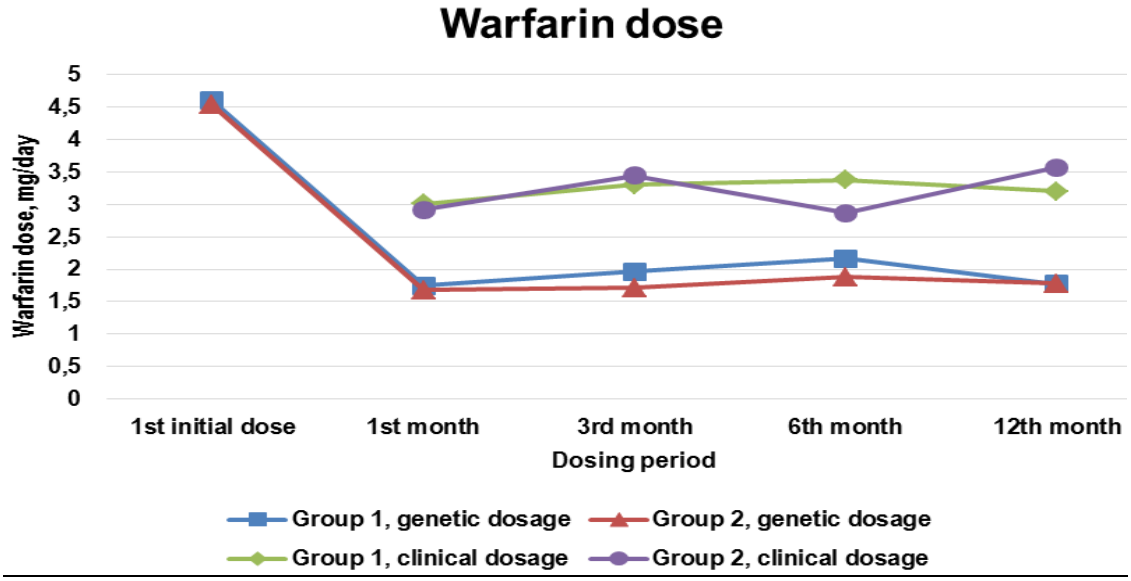
Gene	SNP rs number	Genotype	Group 1, No. (%)	Allele frequency in Group 1	Group 2, No. (%)	Allele frequency in Group 2	P value
*VKORC1*2*	rs9934438	GG	14 (18.9)	G : A = 0.39 : 0.61	0	G : A = 0.35 : 0.65	0.008
		GA	29 (39.2)		17 (70.8)		
		AA	31 (41.9)		7 (29.2)		
		G	57		17		
		A	91		31		
*VKORC1*3*	rs9923231	CC	14 (18.9)	C : T = 0.40 : 0.60	0	C : T = 0.35 : 0.65	0.012
		CT	31 (41.9)		17 (70.8)		
		TT	29 (39.2)		7 (29.2)		
		C	59		17		
		T	89		31		

Group 1, without complications; Group 2, with complications; The significant p value ($p < 0.05$) is labeled in bold.

Furthermore, "genetic dosage" and "clinical dosage" of the warfarin between group of patients without and with complications were summarized in the line graph (Fig. 2). Graph shows that initial "genetic dosage" of the warfarin should be prescribed higher (~ 4.5 mg/day) for both groups of HF patients than it was prescribed with "clinical dosage" (~3.0 mg/day).

Therefore, the sharp decrease of "genetic dosage" (from 4.5 to 1.75 mg/day) illustrates the correction of the "clinical dosage" of the warfarin which could be prescribed for HF patients during treatment starting from the 1st month. Line graph shows that correction of the "clinical dosage" according to the genotyping analysis results recommends decreased level of warfarin dose during follow up time (< 2.16 mg/day) (Fig. 2).

Moreover, "clinical dosage" (1st month) of warfarin showed significant difference between three genotypes of SNPs of rs9934438, rs9923231 in *VKORC1* gene (Table 3). HF patients with wild type genotype polymorphism of rs9934438 in *VKORC1* gene were prescribed with higher warfarin dose than with mutant genotype which was lower < 2.5mg (3.88 ± 1.25 vs. 2.44 ± 0.81, p = 0.00005).



Group 1 – without complications, Group 2 – with complications

Figure 2. Clinical and genetic dosage of warfarin between HF patients with/without complications.

Table 3.

Warfarin dosage between genotype polymorphisms of VKORC1 gene.

SNP of *VKORC1* gene	HF patients, *n*=98	wild type	heterozygote	mutant	*p-Value*
rs9934438	Warfarin dose mg/day	3.88 ± 1.25	3.16 ± 1.15	2.44 ± 0.81	**0.00005**
rs9923231	Warfarin dose mg/day	3.88 ± 1.25	3.17 ± 1.14	2.39 ± 0.76	**0.00002**

Continues variables are presented, mean ± SD;

The p value ($p < 0.05$) numbers with statistical significance are labeled in bold.

Discussion

Our research aimed to investigate difference of warfarin dose in HF patients between "clinical dosage" and "genetic dosage" according to the results of genotype polymorphisms which are involved in metabolism and action of drug. Previously, we did not investigate warfarin dose difference in the group of HF patients with and without complications [21].

According to FDA recommendations and online algorithm of *warfarindosing.org* dosage of warfarin could be estimated by genotype polymorphisms of *VKORC1* and *CYP2C9* in individual patients [10, 16]. Investigation of Finkelman et al., [9] concluded that warfarin dosing according to the pharmacogenetic algorithm was more precise than other methods of dosing. Pharmacogenetic algorithm considered factors such as genotype polymorphisms of *CYP2C9* and *VKORC1* genes, age, body surface area, target INR, amiodarone dose, African American race and warfarin indication. Recommended warfarin dose was better prescribed using pharmacogenetic algorithm [9]. In our investigation we identified influence of polymorphisms genotype on warfarin dose which differentiated from the "clinical dosage" (Fig. 2). We found that the initial "clinical dosage" of warfarin was prescribed with lower amount (3.00 mg/day) compare to the "genetic dosage" which was calculated as initial dosage (4.5 mg/day) without any intake of warfarin. Therefore, we identified that warfarin dose could be decreased according to "genetic dosage" if it would be calculated during intake period to reduce adverse complications. Furthermore, "clinical dosage" of warfarin was decreased (3.44 to 2.87 mg/day) in patients with complications which proves that the reason of the dosage change was development of the complications due to the higher dose.

Genotype polymorphisms of *VKORC1* and *CYP2C9* genes could help to identify patients' sensitivity to warfarin and shorten period of BTT implantation to heart transplantation [18, 3]. However, there are other clinical factors available which cause complications development in HF patients. For instance, apart from warfarin treatments patients take antiplatelet therapy of aspirin which also causes development of thrombosis and bleeding events [5, 21]. On the other hand, LVAD has high non-physiological shear stress which causes platelet receptors' dysfunction and acquired von Willebrand syndrome [3, 6]. Platelet receptors dysfunction also leads to the development of thrombosis/bleeding complications. Patients should be genotyped for other genotype polymorphisms apart from *VKORC1* and *CYP2C9* genes which associated with platelet receptors dysfunction and antiplatelet drugs metabolism to prevent/reduce complications development [8, 21, 9, 20].

Genotype frequencies of polymorphisms in *VKORC1* and *CYP2C9* show variations in different populations such Asian, African-American, European-American and Caucasian [18, 17]. For instance, investigations showed that African-American and Asian populations have significant difference of allele frequency of polymorphism rs9923231 in *VKORC1* ($p < 0.001$). The frequency of mutant allele of polymorphism rs9923231 in *VKORC1* was significantly higher Asian population than wild type allele [17]. On the contrary, the frequency of mutant allele of rs9923231 in *VKORC1* was found to be lower in African-American population [18]. In our investigation, we found that HF patients have higher frequency of mutant allele of polymorphism rs9923231 in *VKORC1* gene. Moreover, heterozygote genotype polymorphisms for rs9934438 and rs9923231 in *VKORC1* gene are significantly higher in the group of patients with complications ($p = 0.01$) [21].

Furthermore, we considered two polymorphisms of rs9923231, rs9934438 in VKORC1 gene and their association with warfarin dosage between three genotypes in HF patients with already prescribed warfarin therapy after implanted LVAD devices. Difference of warfarin metabolism and action depend on genotype polymorphisms of *VKORC1* gene [19]. In our investigation, we identified that there is difference in warfarin dose between three genotypes of rs9934438, rs9923231 in *VKORC1* gene. Topkara et al. [18] compared in their investigation warfarin dose between three genotypes of rs9923231 polymorphism in *VKORC1* gene and identified that patients with mutant genotype was prescribed with lower warfarin dose [18]. Our investigation also found that HF patients were prescribed with lower warfarin dose with mutant genotype of rs9923231 polymorphism in *VKORC1* gene than with wild type genotype (2.39 ± 0.76 vs. 3.88 ± 1.25, $p = 0.00002$). On the other hand, investigations found that mutant genotype of polymorphisms rs9934438 in *VKORC1* gene are significantly associated with lower warfarin dose than wild type genotype [1, 2]. Our investigation also identified that lower warfarin dose was prescribed with mutant genotype of polymorphisms rs9934438 in *VKORC1* than with wild type genotype (2.44 ± 0.81 vs. 3.88 ± 1.25, $p = 0.00005$).

Conclusion

Polymorphisms of rs9923231 and rs9934438 in *VKORC1* gene showed significant association with warfarin dosage in HF patients. Warfarin dose according to genetic test results was different from the clinical dosage which suggest for clinicians to estimate drug dosage according to the genotype polymorphisms results. Estimated warfarin dosage according to genetic test would help to prevent and reduce thrombosis/bleeding complications at pre- and post-LVAD implantation period.

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