ASSOCIATION OF GENE POLYMORPHISMS WITH THROMBOSIS AND BLEEDING IN PATIENTS WITH MECHANICAL CIRCULATORY SYSTEM

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INTRODUCTION

Heart failure continues to be a worldwide epidemic, affecting approximately 23 million people globally and is a major burden for the healthcare system [1,2]. In the United States (US) alone, the cost of caring for 5.8 million heart failure patients in 2010 was $39.2 billion [1]. Despite advances in medical therapy, the disease is progressive and a significant proportion of patients will need advanced heart replacement therapy.

Left ventricular assist devices (LVADs) have been developed as a bridge to heart transplantation or to improve the symptoms of patients ineligible for transplant [1-3]. Over 1500 LVADs are placed in the US annually, and the number is steadily rising [2].

In spite of their success in improving mortality and quality of life, thrombotic and bleeding events remain significant complications [1].

The reason for increased thrombosis is not clear but is likely multifactorial, with gastrointestinal bleeding (GIB), infection, varying anticoagulation bridging strategies, and suboptimal INRs potentially contributing [4]. In addition, knowledge of genetic risk factors – i.e. genetic variations in genes encoding blood coagulation might help to find patients potentially at higher risk of thrombosis and bleedings and to manage patients with mechanical ventricular assist devises.

Aim of the Study

To investigate genetic variants previously identified to associate with thrombosis risk in genes encoding the stages of the blood clotting among patients supported with LVADs.

MATERIALS & METHODS

The study is conducted in accordance with the rules and requirements of the Helsinki Declaration and according to the legislation of the Republic of Kazakhstan (Law of the Republic of Kazakhstan "On protection of public health"). Written informed consent was obtained from all patients included into the study. Research protocol was approved by the ethics committees of National Laboratory Astana, Nazarbayev University and National Research Center for Cardiac Surgery, Astana, Kazakhstan.

Study population

Implantation of LVADs in Kazakhstan is carried out since 2011 in JSC "National Research Center for Cardiac Surgery ", (NRCCS) Astana, Kazakhstan.

All patients were unrelated Kazakhstani individuals treated in NRCCS, Astana, Kazakhstan for heart failure. In total, 100 study participants (patients with implanted LVADs) were recruited in the study group during 2015-2016. The control group consisted of 95 conditionally healthy individuals, corresponding to the patients group by sex, age and nationality.

DNA isolation and genotyping

Genomic DNA was extracted from 200 µL whole blood using PureLinkTM Genomic DNA Mini Kit (Invitrogen, UK) according to manufacturer's standard protocol. The genomic DNA concentration was measured using NanoDrop™ Spectrophotometer (Thermo Fisher Scientific, USA) and adjusted to 10 ng/µl.

Genotyping of polymorphisms: homocysteine metabolism gene: MTHFR (5,10-methylenetetrahydrofolate reductase) C677T; MTHFR A1298C; as well as 6 polymorphisms of hemostasis system genes: F2 (prothrombin CF II) G20210A; F5 (proaccellerin, CF V) G1691A (Leiden's mutation); F7 (prokonvertin, CF VII) G10976A; F13A1 (fibrilnase, CF XIII) G> T; ITGB3 (platelet glycoprotein IIIA) T1565C; PAI-1 (inhibitor of the plasminogen activator 1) -675 5G/4G were performed using RT PCR TaqMan Assay on 7900HT Fast Real-Time PCR System (Applied Biosystems, USA).

Statistical Analysis

Each polymorphism was tested for Hardy Weinberg equilibrium in the study population. *P*-value <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS 19 (SPSS Inc., Chicago, IL).

RESULTS

Demographic and clinical profiles of patients are shown in the Table 1.

Frequency of genotypes and alleles of selected SNPs in groups are presented in the Table. Analysis shows that polymorphism of integrin gene ITGB3 1565 T> C was associated with thrombosis risk in patients with LVAD implantation (Table 2).

Anticoagulation therapy is required for patients with implanted LVADs to avoid thrombotic complications. All patients received standard anticoagulation therapy before and after implantation that included adjusted doses of warfarin to achieve the target INR (in most cases, the target INR value is 2.0-2.5 for HMII and HMIII, 3.0-3.5 for HeartWare HVAD), and antiplatelet therapy with aspirin. This therapy is adjusted individually for each patient to reduce the risk of ischemic stroke and bleeding.

The mean value of the baseline INR before implantation was 1.20, and 2.37 after one month of support on the mechanical device. The prescribed minimum daily dose of warfarin among the subjects was 0.625 mg, and the maximum dose was 6.875 mg.

Table 1. Patient characteristics.

Variables	Subjects (n=100)
Ethnicity: Kazakh	78%
Russian	16%
Other	6%
Average age, years±SD	52,4±11,5
Sex : Male	93%
Female	7%
Weight, kg (range)	79,7±14,0 (48 till 114)
Height, cm (range)	169,9±6,4 (148 till 183)
BMI, kg/m2	27,6±4,6
Smoking, %	58%
Heart Failure etiology: Ischemic genesis	44 %
Non ischemic	56 %
Ischemic cardiomyopathy	44 %
Dilated cardiomyopathy	42 %
Hypertensive cardiomyopathy	9 %
Valvular cardiomyopathy	4 %
Arterial cardiomyopathy	1 %
Type of implanted LVADs:	
• HeartMateIII (Thoratec Corporation, USA)	46%
• HeartMate II (HMII) (Thoratec Corporation, USA)	35%
• HeartWare HVAD (HeartWare Inc., USA)	19%
NYHA class: IIIA+ IIIB	70%
IV	28%
EF, %	21,7 (9 - 41)
LV EDD, mm	70,2 (38 - 88)
Pulse, beats per minute	85,4±17,2
Systolic blood pressure, mm Hg	105,1±15,6
Diastolic blood pressure, mm Hg	87,2±10,9
INR	2.11 ± 0.39 (1.48-3.00)

Table 2. Summary of SNP variations in groups

Group	Genotype			HWE, (χ^2 df=1), p	OR
MTHFR, C677T					
	CC	CT	TT		
Patient group, n=100, abs(%)	44(44.2%)	42(42.3%)	14(13.5%)	0.312	For T allele 1.83, p-value 0.743
Control group, n=95 abs (%)	55(57.9%)	29(30.5%)	11(11.6%)	0.953	
MTHFR, A1298C					
	AA	AC	CC		
Patient group, n=100, abs(%)	54(53.8%)	38(38.5%)	8 (7.7%)	0.235	For C allele 1.17, p-value 0.648
Control group, n=95 abs (%)	29(30.5%)	60(63.1%)	6(6.3%)	0.589	
F2, G20210A					
	GG	GA	AA		
Patient group, n=100, abs(%)	93 (93%)	2(2.3%)	5(4.6%)	0.763	For A allele 0.83, p-value 0.821
Control group, n=95 abs (%)	88(93%)	0	7(7%)	0.686	
F5, G1691A					
	GG	GA	AA		
Patient group, n=100, abs(%)	84 (83.7%)	14(13.9%)	2(2.3%)	0.256	For A allele 1.68, p-value 0.175
Control group, n=95 abs (%)	91(95.3%)	2(2.3%)	2(2.3%)	0.100	
F7, G10976A					
	GG	GA	AA		
Patient group, n=100, abs(%)	40(39.5%)	51(51.2%)	9(9.3%)	0.407	For A allele 1.81, p-value 0.409
Control group, n=95 abs (%)	71(74.4%)	22(23.2%)	2(2.3%)	0.836	
F13A1 rs5985					
	GG	GT	TT		
Patient group, n=100, abs(%)	47(46.5%)	51(51.2%)	2(2.3%)	0.075	For T allele 1.806, p-value 0.409
Control group, n=95 abs (%)	51(53.5%)	44(46.5%)	0	0.046	
ITGB3 T1565C					
	TT	TC	CC		
Patient group, n=100, abs(%)	40(39.5%)	51(51.2%)	9(9.3%)	0.760	For C allele 2.99, **p-value 0.033**
Control group, n=95 abs (%)	75(79%)	18(18.6)	2(2.3%)	0.603	
PAI-1, -675 5G/4G					
	5G/5G	5G/4G	4G/4G		
Patient group, n=100, abs(%)	21(20.9%)	49(48.8%)	30(30.3%)	0.923	For 4G allele 1.83, p-value 0.06
Control group, n=95 abs (%)	21(22.3%)	42(44.2%)	32(34.5%)	0.865	

According to the study protocol, patients are observed within 18 months after the implantation of LVAD.

Cases of active bleeding after implantation of the LVAD were observed in only 14% (18 cases) of the total number of subjects. In the first month after implantation, there were only 5 cases of bleeding: 2 cases in the pleural cavity, 2 cases of gastrointestinal bleeding and 1 nosebleed. During the next 6 months after implantation of the device, 6 cases of active bleeding also occurred in 4 patients, including 4 cases of bleeding from the gastrointestinal tract, one case of gingival hemorrhage, and one – nasal bleeding. After 12 months after the operation, two cases of bleeding from the digestive tract were reported in two patients. After 18 months of mechanical support of the left ventricle, 4 new cases of bleeding were observed, 1 case of repeated bleeding, all of them, 3 cases - from the gastrointestinal tract, 1 – nasal bleeding, 1 - rectal bleeding. During the follow-up period, 12% of patients had a thrombosis of the pump (Table 3).

Table 3. Bleeding and thrombosis complications in patients with LVADs implantation during 18 month follow-up

Complication	Number of events	Number of patients	%
Bleeding	18	14	14
Gastro-intestinal	11	9	9
Nasal	3	3	3
Pleural	2	2	2
Other localization	2	2	2
Pomp thrombosis	12	12	12

CONCLUSIONS

Our study suggested a genetic variant in the ITGB3 gene, T1565C , as a genetic factor that contribute to thrombosis risk in patients with mechanical ventricular support (OR 2.99; 95%CI, 1.05-8.49; p=0.033). Together with non-genetic indicators such as patient age, body weight and body height, gastrointestinal bleeding (GIB), infection, varying anticoagulation bridging strategies, and suboptimal INRs this genetic variant might contribute to inter-individual variability in complication development in patients with mechanical ventricular support..

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