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Investigation of Genetic Polymorphisms in Infective Endocarditis and Artificial Valve Thrombosis

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: Major causes of mortality in patients with valvular disease are endocarditis and valve thrombosis. Oral anticoagulant drugs are used to prevent valve thrombosis. Uses of inadequate doses of medication or absence of medication use are the main reasons for the development of valve thrombosis. In addition to medical treatment or surgical treatment of patients with infective endocarditis has a high mortality rate. In this study, *MTHFR* C677T, Prothrombin (*Factor-II*) G20210A, *Factor-V* Leiden G1631, *PAI-1* 4G/5G and *TNF-α*-308 G>A were determined in patients with infective endocarditis and valve thrombosis groups.

Materials and Methods: 18 patients with infective endocarditis, 12 patients with valve thrombosis and 37 healthy volunteers were included in this study. Polymerase chain reactions (PCR) and restriction fragment length polymorphisms (RFLP) were used to detect the related polymorphism. Chi-square was used to compare groups.

Results: There were no significant differences between the groups regarding *MTHFR* C677T, Prothrombin (*Factor-II*) G20210A, *Factor-V* Leiden G1631, PAI-1 4G/5G polymorphism. In contrast, a significant difference was found in *TNF-α* -308 G>A polymorphism between the groups.

Conclusion: In our study, *TNF-α*-308 G>A results were higher in patients with endocarditis and valve thrombosis than control groups in chi-squares test and there were significant differences between two groups. There is not enough data in literature about the involvement of genetic factors in valve thrombosis. For this reason, larger and more comprehensive studies are needed.

Keywords: Thrombosis, polymorphism, infective endocarditis

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INTRODUCTION

The most frequent causes of death in patients with valvular disease are endocarditis and valve thrombosis. Oral anticoagulants are used to prevent valvular thrombosis. While the main causes of valvular thrombosis include taking an insufficient dose of medication or not taking anticoagulants at all, thrombosis may even occur in patients taking medications at effective doses. In addition, medical or surgical treatment of patients with infective endocarditis is associated with a high risk of mortality (1, 2).

There are few investigations and reports on infective endocarditis and valvular thrombosis in the literature. The aim of the present study was to evaluate the presence of 5 genetic polymorphisms [*MTHFR C677T, prothrombin (Factor-II) G20210A, Factor-V Leiden G1631, plasminogen activator inhibitor (PAI)-1 4G/5G, and tumor necrosis factor-alpha (TNF-α)-308 G>A*], which may be associated with valvular endocarditis and thrombosis, in our patient groups.

MATERIALS and METHODS

Patient Selection

Approval of the ethics committee of Erciyes University Faculty of Medicine was obtained for this study. Eighteen patients with infective endocarditis (10 males and 8 females, age range: 16-72 years; age: 47.59±17.07 years) and 12 patents with thrombosis of artificial valves (4 males and 8 females, age range: 28-81 years, age: 53.33±17.20 years) were included in this study. The control group consisted of 37 healthy individuals (19 males and 18 females, age range: 16-80 years, age: 29.0±8.8 years). Patients and controls were selected from individuals admitted at the Department of Cardiovascular Surgery of Erciyes University Faculty of Medicine.

Genotyping Examinations

After obtaining informed consent from each patient, peripheral blood samples (2 mL) with Ethylenediaminetetraacetic acid (EDTA) were collected. The Roche Magna Pure LC automatic device at the Medical Genetics Department was

used for DNA isolation from the blood samples. The company protocol was used for isolation (Roche, Germany). The amounts of each DNA sample were measured using a nanodrop spectrophotometer (Thermo Scientific, USA), and their quality was checked. All polymorphisms were determined using standard polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) methods. Appropriate PCR reaction solutions (50 µL) were prepared for each gene. For the *Factor-V* gene, 5'-ATAGCACTGGGAGCATTGAAGC-3' forward and 5'-ACCCACAGAAAATGATGCCCA-3' reverse primers were used. For the *MTHFR* gene, 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' forward and 5'-AGGACGGTGCGGTGAGAGTG-3' reverse primers were used. For the *Factor-II* gene, 5'-TCTAGAAACAGTTGCCTGGC-3' forward and 5'-ATAGCACTGGGAGCATTGAAGC-3' reverse primers were used. For the *PAI-1* gene, 5'-CACAGAGAGAGTCTGGCCACGT-3' forward and 5'-CCAACAGAGGACTCTTGGTCT-3' reverse primers were used. For the *TNF-α* gene, 5'-AGGCAATAGGTTTTGAGGGCCAT-3' forward and 5'-TCCTCCCTGCTCCGATTCCG-3' reverse primers were used. Each polymorphism was treated with the appropriate restriction enzyme, and genotypes were determined by agarose gel electrophoresis (3-6). The results are presented in Table 1.

Statistical Analysis

Statistical analysis was conducted using the Statistical Packages for the Social Sciences (SPSS) version 15.0 (SPSS Inc.; Chicago, IL, USA) software; results are presented as "mean±standard error of mean" (mean±SEM). $p<0.05$ was considered as statistically significant. Categorical variables were shown as percent, and polymorphism analysis was conducted using the chi-square test. ANOVA was used for the comparison of age variables.

RESULTS

We searched for polymorphisms in a total of 67 patients (37 patients in the control group, 18 with endocarditis, and 12 with valvular thrombosis). No thrombosis was reported in the medical history of these patients. INR of all the patients was in the effective range.

Artificial valves that had endocarditis were mostly aortic (aortic in 7 patients; mitral in 6; both mitral and aortic in 4; and mitral, aortic, and tricuspid involvement in 1). Artificial valves that had thrombosis were mostly mitral (mitral in 7 patients, aortic in 2, both aortic and mitral in 2, and only tricuspid involvement in 1).

Staphylococcus aureus and *Enterococcus faecalis* were the main microorganisms detected in the patients with infective endocarditis. No tendency of the presence of a particular pathogen was detected in patients with TNF polymorphism. Results for each polymorphism are presented in Table 2 separately.

Factor-V Leiden G1691A Polymorphism Findings

In order to detect Factor-V wild-type (G1691G), heterozygous mutant (G1691A), and homozygous mutant (A1691A) genotypes, the samples were amplified by PCR and imaged in 2% agarose gel at 223 bp. The results are presented in Table 2. The amplified products were treated with *Mnl I* restriction endonuclease enzyme and evaluated in 3% agarose gel. Factor-V G1691A polymorphism results in patients with endocarditis or valvular thrombosis were compared using the chi-square test, and a significant difference was not found between these groups ($p>0.05$).

MTHFR C677T Polymorphism Findings

In order to detect *MTHFR* wild-type (C677C), heterozygous mutant (C677T), and homozygous mutant (T677T) genotypes, the samples were amplified by PCR and was imaged in 2% agarose gel at 198 bp. The results are shown in Table 2. The amplified products were treated with *Hinf I* restriction endonuclease enzyme and evaluated in 3% agarose gel. When *MTHFR* C677T polymorphism results of patients with endocarditis or valvular thrombosis and controls were compared using the chi-square test, no significant difference was found ($p>0.05$).

Table 1. Polymorphism and enzyme cleavage products

Polymorphism Searched	Restriction Enzyme Used	Polymerase Chain Reactions Product	Normal Genotype Enzyme Cleavage Products	Heterozygote Genotype Enzyme Cleavage Products	Homozygote Genotype Enzyme Cleavage Products
Factor-V G1691A	Mnl I	223 bp	104 bp 82 bp	141 bp 104 bp 82 bp	141 bp 82 bp
MTHFR C677T	Hinf I	198 bp	198 bp	198 bp 175 bp 23 bp	175 bp 23 bp
Prothrombin G20210A	Hind III	345 bp	345 bp	345 bp 322 bp	322 bp
PAI-1 4G/5G	Bsl I	98 bp	98 bp	98 bp 77 bp 22 bp	77 bp 22 bp
TNF-α	Nco I	107	87 bp 20 bp	107 bp 87 bp 20 bp	107 bp

bp: base pair

Table 2. Distribution of genotypes among patients and controls

POLYMORPHISM	ENDOCARDITIS (n=18) (%)	VALVULAR THROMBOSIS (n= 12) (%)	CONTROL (n= 37) (%)	p
***FACTOR-V* G1691A**				
GG	14 (77.7)	9 (7.5)	33 (89.2)	p=0.164
GA	3 (16.7)	1 (8.3)	4 (10.8)	
AA	1 (5.6)	2 (16.7)	-	
***FACTOR-II* G20210A**				
GG	17 (94.4)	11 (91.7)	37 (100)	p=0.255
GA	1 (5.6)	1 (8.3)	-	
AA	-	-	-	
***MTHFR* C677T**				
CC	10 (55.6)	7 (58.3)	22 (59.5)	p=0.685
CT	6 (33.3)	5 (41.7)	10 (27.0)	
TT	2 (11.1)	-	5 (13.5)	
***PAI-1* 4G/5G**				
5G/5G	6 (33.3)	6 (50)	10 (27)	p=0.284
4G/5G	10 (55.6)	5 (41.7)	16 (43.3)	
4G/4G	2 (11.1)	1 (8.3)	11 (29.7)	
***TNF-α*-308 G>A**				
GG	6 (33.3)	8 (66.7)	26 (70.3)	p=0.001
GA	5 (27.8)	1 (8.3)	11 (29.7)	
AA	7 (38.9)	3 (25)	-	

Prothrombin G20210A Polymorphism Findings

In order to detect prothrombin wild-type (G20210G), heterozygous mutant (G20210A), and homozygous mutant (A20210A) genotypes, the samples were amplified by PCR and imaged at 345 bp in 2% agarose gel. The results are presented in Table 2. The amplified products were treated with *Hind III* restriction endonuclease enzyme and evaluated in 3% agarose gel. When results of prothrombin G20210A polymorphism in patients with endocarditis or valvular thrombosis and controls were compared using the chi-square test, significant differences could not be found ($p>0.05$).

PAI-1 4G/5G Polymorphism Findings

In order to detect *PAI-1* wild-type, heterozygous mutant (4G/5G), and homozygous mutant genotypes, the samples were amplified by PCR and imaged in 2% agarose gel at 98 bp. The results are shown in Table 2. The amplified products were treated with *Bsl I* restriction endonuclease enzyme and evaluated in 4% agarose gel. When *PAI-1* 4G/5G polymorphism results in patients with endocarditis or valvular thrombosis and controls were compared using the chi-square test, significant differences could not be found ($p>0.05$).

TNF-α-308 G>A Polymorphism Findings

In order to detect *TNF-α* -308 G>A wild-type, heterozygous mutant, and homozygous mutant genotypes, the samples were amplified by PCR and imaged at 108 bp in 2% agarose gel. The results are presented in Table 2. Amplified products were treated with *NcoI* restriction endonuclease enzyme and evaluated in 3% agarose gel. When results of *TNF-α*-308 G>A polymorphism in patients with endocarditis or valvular thrombosis and controls were compared using the chi-square test, significant differences were found ($p<0.05$).

DISCUSSION

Infective endocarditis may be encountered in the form many clinical pictures, such as immunological renal pathologies, arteritis, vasculitis, and skin lesions. In a study by Watkin et al. (7), increases in levels of IL6, IL1 beta, and CRP in patients with IE were detected.

Compared with controls, Rawzczynska-Englert et al. (8) have found increased IL-6 levels without an increase in IL-1 and TNF-α levels in patients with acquired rheumatic valvular disease in whom infective endocarditis had developed.

In a study by Alter et al. (9) on 47 patients with infective endocarditis, IL-2R and IL-6 levels were found to be increased, and the levels later decreased after antimicrobial treatment. In addition, TNF-α, IL-1, and IL-6 transcription and secretion were found to be high

in the monocytes of patients with IE who also had Q fever in the same study (9). In 2 different studies by Rawzczynska-Elart et al. (8) and Kern et al. (10), the expected increase in TNF-α levels could not be shown in patients with infective endocarditis (8). This was thought to be due to the downregulation of immune cells or weak induction of cytokine release. In our study, *TNF-α*-308 G>A polymorphism results of patients with endocarditis or valvular thrombosis and controls were compared using the chi-square test, and a significant difference was observed.

In the study by Musher et al. (11) on patients with endocarditis who had intravenous catheters with bacterial colonization, Factor-V Leiden R506G and Factor-II G20210A polymorphisms were similar in the study and control groups, while *MTHFR* C677T polymorphism was lower in the group with infected catheters than in the control group. In addition, in the same study, in terms of platelet surface antigen polymorphism, the presence of PLA2 and GP1b alpha polymorphism was found to be associated with hypercoagulability (11). In our study, differences were not found in Factor-V Leiden and *MTHFR* C677T results.

Tumor necrosis factoralpha gene polymorphism and increased TNF-α levels were found to be associated with increased postoperative cardiopulmonary morbidity (12, 13). Although we did not observe operative mortality in our study, our sample size is small.

Parlar et al. (14) have investigated the relationship between Factor-V Leiden, prothrombin, IL-6, and TNF-α polymorphisms with regard to mechanical heart valve dysfunctions and have found a statistically significant difference in only IL-6 polymorphism (14). A significant difference was found between the group with valvular thrombosis and the control group in our study in terms of *TNF-α* -308 G>A polymorphism using the chi-square test. However, there is insufficient literature on the relationship between valvular thrombosis and genetic factors.

CONCLUSION

Tumor necrosis factoralpha-α-308 G>A polymorphism findings showed significant differences in both patients with endocarditis and those with valvular thrombosis. However, larger and more comprehensive studies are required on this issue.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erciyes University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Conceived and designed the experiments or case: AT. Performed the experiments or case: ÖNE. Analyzed the data: AT., EFŞ. Wrote the paper: AT., EFŞ. All authors have read and approved the final manuscript.

Conflict of Interest: No conflict of interest was declared by the authors.

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