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The effect of genetic polymorphisms on the mechanical heart valve dysfunction

Mekanik kalp kapağı disfonksiyonunda genetik polimorfizmlerin etkisi

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Background: This study aims to investigate genetic polymorphisms in patients with mechanical heart valve dysfunction.

Methods: Between January 1994 and December 2004, a total of 83 patients including 18 patients who were reoperated due to mechanical heart valve dysfunction (group 1), 15 patients with normal functions with lower international normalized ratio (INR) levels who underwent mechanical valve replacement (group 2), and 50 healthy individuals (group 3) were included. Factor V Leiden, prothrombin, interleukin (IL-6), and tumor necrosis factor alpha (TNF-α) polymorphisms were investigated for possible relationships between these factors and mechanical heart valve dysfunction.

Results: A significant difference in IL-6 polymorphism was found between group 1 and group 3 ($p<0.05$). Other polymorphisms were not significantly associated with mechanical heart valve dysfunction among the groups. The IL-6 G-174C polymorphism was found to be significantly associated with mechanical heart valve dysfunction.

Conclusion: Interleukin-6, the key inflammatory response cytokine, may play an effective role in mechanical heart valve dysfunction possibly through ongoing chronic inflammatory processes.

Key words: Genetic polymorphism; interleukin; mechanical heart valve dysfunction.

Amaç: Bu çalışmada, mekanik kalp kapağı disfonksiyonu olan hastalardaki genetik polimorfizmler araştırıldı.

Çalışma planı: Ocak 1994 - Aralık 2004 tarihleri arasında mekanik kalp kapağı disfonksiyonu nedeni ile yeniden ameliyat edilen hastaların oluşturduğu 18 hasta (grup 1), normal fonksiyonel olan ve mekanik kalp kapağı replasmanı yapılmış, uluslararası normalleştirilmiş oran (INR) düzeyleri düşük olan 15 hasta (grup 2) ve 50 sağlıklı kişi (grup 3) olmak üzere, toplam 83 hasta çalışmaya dahil edildi. Faktör V Leiden, protrombin, interlökin (IL-6) ve tümör nekroz faktör alfa (TNF-α) polimorfizmleri tespit edilerek, mekanik kalp kapağı disfonksiyonu ile olan muhtemel ilişkileri araştırıldı.

Bulgular: İnterlökin-6 polimorfizmi sonuçları, grup 1 ve grup 3 karşılaştırıldığında anlamlı olarak farklı bulundu ($p<0.05$). Diğer polimorfizmler ve mekanik kalp kapağı disfonksiyonu açısından gruplar arasında anlamlı bir farklılık görülmedi. İnterlökin-6 G-174C polimorfizmi mekanik kalp kapağı disfonksiyonu ile anlamlı olarak ilişkili bulundu.

Sonuç: Ana enflamatuvar yanıt sitokini IL-6, muhtemelen devam eden kronik enflamatuvar süreçler ile mekanik kalp kapak disfonksiyonunda etkin bir rol oynayabilir.

Anahtar sözcükler: Genetik polimorfizm; interlökin; mekanik kalp kapağı disfonksiyonu.

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Thrombosis, thromboembolic events, and complications related to anticoagulation remain important causes of morbidity and mortality following heart valve replacement surgery. Mechanical heart valve dysfunction (caused by thrombosis or pannus formation) is a rare but life-threatening complication with an incidence rate of 0.1-6% per patient-year following valve replacement.[1-3]

It is known that there are inherited risk factors that may lead to increases in thrombotic or thromboembolic events, and factor V Leiden G1691A and prothrombin G20210A polymorphisms are associated with thrombosis.[4] In addition, genetic polymorphisms associated with inflammatory response can lead to ongoing chronic inflammation in patients with rheumatic heart valve disease.[5,6]

To the best of our knowledge, no current data is available regarding the role that the factor V Leiden G1691A, prothrombin G20210A, interleukin (IL)-6 G-174C, and tumor necrosis factor-alpha (TNF-α) G-308A polymorphisms play in mechanical heart valve dysfunction. Therefore, this study was designed to investigate the association between these genetic polymorphisms and mechanical heart valve dysfunction.

PATIENTS AND METHODS

Our local ethics committee approved the present study and a total of 83 patients gave their written consent to participate (B.10.4.ISM.4.06.00.15/12376-183). The patients were divided into three groups with group 1 consisting of 18 patients who underwent a reoperation for mechanical heart valve dysfunction. The characteristics of the patients in group 1 are presented in Table 1. Twelve of the 18 patients (66.7%) had isolated bileaflet mitral mechanical heart valve dysfunction, and the remaining six had isolated bileaflet aortic mechanical heart valve dysfunction (33.3%). Group 2 was composed of 15 patients who had low international normalized ratio (INR) levels with functional mechanical heart valves. Their anticoagulation levels were regulated at the outpatient clinic. Group 3 was made up of 50 healthy individuals who served as the controls. Factor V Leiden G1691A, prothrombin G20210A, IL-6 G-174C, and TNF-α G-308A polymorphisms were studied to investigate the possible relationships between these polymorphisms

Table 1. Characteristics of the patients in groups 1 and 2

Variables	Group 1		Group 2		
	n	Mean±SD	n	Mean±SD	*p*
Number of patients	18		15		
Age (year)		47.4±9.2		47.8±13.6	>0.05
Gender					
Male	9		8		>0.05
Female	9		7		
Native valve disease					
Rheumatic	18		15		>0.05
Non-rheumatic	–		–		–
Type of valve					
Mitral valve replacement	10		9		>0.05
Aortic valve replacement	4		4		>0.05
Double-valve replacement	4		2		>0.05
Rhythm					
Sinus	7		7		>0.05
Atrial fibrillation	11		8		>0.05
Interval between					
Valve replacement / MHVD (months)		63.3±39.5		0	–
Previous emboli history					
Yes	2		1		>0.05
No	16		14		>0.05
Average INR levels		1.36±0.5		1.25±0.1	>0.05
Smoking	2		3		>0.05
Aspirine usage	18		15		>0.05

INR: International normalized ratio; SD: Standard deviation; MHVD: Mechanical heart valve dysfunction.

and mechanical heart valve dysfunction. The patients in all of the groups were of the same ethnic origin.

Blood samples of the patients in group 1 were collected before the heart valve reoperation while the blood samples of the patients in groups 2 and 3 were collected at the outpatient clinic. The INR levels of the patients in groups 1 and 2 were low because of the inadequate usage or unwarranted cessation of warfarin. The anticoagulant therapy were then adjusted, and the INR levels were brought to appropriate levels after the reoperation in group 1 while the INR levels in group 2 were regulated at the outpatient clinic. When mechanical heart valve dysfunction was suspected during the clinical examination, a diagnosis was established by echocardiography or cineradiography. Accordingly, the patients who were diagnosed with mechanical heart valve dysfunction were operated on immediately.

Prothrombin, TNF-α, factor V Leiden, and IL-6 Polymorphisms

For analysis of the prothrombin G20210A, TNF-α G-308A, factor V Leiden G1691A, and IL-6 G-174C polymorphisms, the genomic DNA was isolated from the whole blood according to standard procedures. The 3' untranslated region of the prothrombin gene was then amplified using previously reported primers and polymerase chain reaction (PCR) conditions.[7] Next, the PCR products were digested with the enzyme HindIII and analyzed by electrophoresis on 3% agarose gels. The TNF-α gene was also amplified using previously reported primers and PCR conditions.[8] The PCR products were then analyzed by electrophoresis on 2% agarose gels, and exon 10 of the factor V gene was also amplified using previously reported primers and PCR conditions.[9] After this, the PCR products were digested with the enzyme HindIII and analyzed by electrophoresis on 3% agarose gels. In addition, real-time polymerase chain reaction (PCR) analysis for the IL-6 promoter-174G-C genotyping was performed as previously described[10,11] using a LightCycler® real-time PCR instrument (Roche Diagnostics Corporation, Indianapolis, IN, USA).

Statistical analysis

All values are presented as mean ± standard deviation (SD), and the differences between the groups were determined using a chi-square test, the Kruskal-Wallis test, and one-way analysis of variance (ANOVA). The genotypes and allele frequencies were evaluated using a chi-square test, and the allele frequencies were estimated via gene counting methods. A p value of <0.05 was considered to be statistically significant.

RESULTS

The general data regarding the characteristics of the patients in groups 1 and 2 is summarized in Table 1.

The data related to the genotypes and allele frequencies is presented in Table 2, and a statistically significant difference was found between group 1 and group 3 in terms of the IL-6 polymorphism ($p<0.05$) (Figure 1). However, the differences between the groups with regard to the factor V Leiden G1691A, prothrombin G20210A, and TNF-α G-308A polymorphisms did not reach statistical significance (Figures 2, 3, and 4).

Table 2. Genotype and allele frequency data for groups 1, 2 and 3

Mutations	Group 1	Group 2	Group 3	*p*
	n	n	n	
Factor V leiden G1691A				
GA	11.1	13.3	2	NS
GG	88.9	86.7	98	
Prothrombin G20210A				
GA	11.1	6.7	2	NS
GG	88.9	93.3	98	
Interleukin-6 G-174C				
CC	11.1	6.7	–	
CG	38.9	46.6	30	p<0.05
GG	50	46.7	70	
TNF alpha G-308A				
GA	11.1	20	10	NS
GG	88.9	80	90	

NS: Not significant.

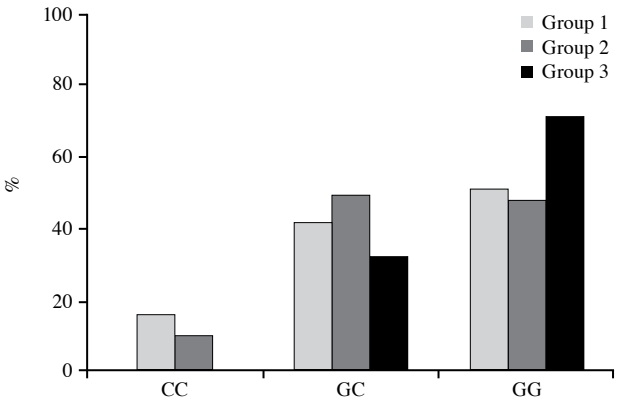

Figure 1. There was a statistically significant between groups 1 and group 3 according to IL-6 G-174C polymorphism ($p<0.05$).

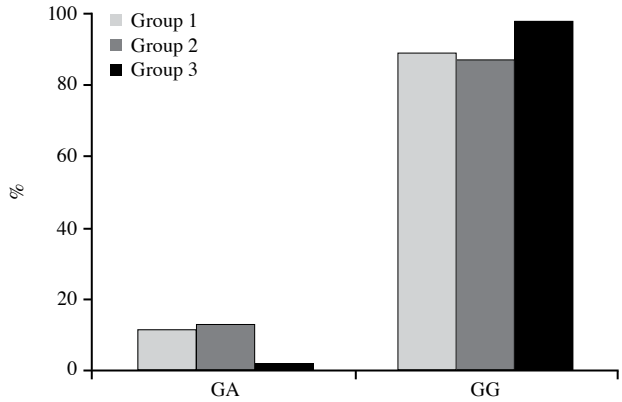

Figure 2. The distribution of factor V Leiden G1691A polymorphism in groups ($p>0.05$).

DISCUSSION

Mechanical heart valve thrombosis remains a rare but significant clinical concern. Its clinical presentation varies from dyspnea and/or cardiogenic shock to cardiac arrest, and the mortality rates associated with this condition are very high.[2,3] Furthermore, the incidence rates for this type of thrombosis were reported to range between 0.1% and 6% per patient-year.[2,3] The most common etiology of obstructive mechanical heart valve dysfunction is insufficient anticoagulation or fluctuations of the INR values within the therapeutic range.[12] Besides insufficient anticoagulation, additional risk factors include recurrent embolism, valve size, atrial rhythm, cardiac failure, sporadic use of drugs, malignancy, incomplete endothelialization of the sewing ring, inflammation, and hypercoagulable states.[13] The current therapeutic approach aimed at preventing mechanical heart valve thrombosis involves anticoagulation therapy or a combination of antiplatelet and anticoagulation therapy. The present recommendation for the use of warfarin is to maintain the INR level between 2.5 and 3.5.[14] Although we aimed to stay within these levels, that was not always possible due to infrequent follow-ups and the fact that the patients were living in rural areas far from the hospital.

Tütün et al.[15] reported that patients with mechanical valve thrombosis along with a deficiency of endogenous anticoagulants, such as protein C, protein S, and antithrombin, had a tendency towards thrombosis, but the levels of endogenous anticoagulants may decrease after two years of warfarin therapy.[15] In the past decade, our knowledge about the genetic polymorphisms that predispose patients to thrombosis has advanced,[4,7,16] and the most common genetic correlations associated with this condition are factor V Leiden and the prothrombin polymorphisms.

Factor V Leiden and prothrombin mutations are found in 2-15% and 1-2% of the normal population, respectively.[4] In addition, factor V Leiden mutation

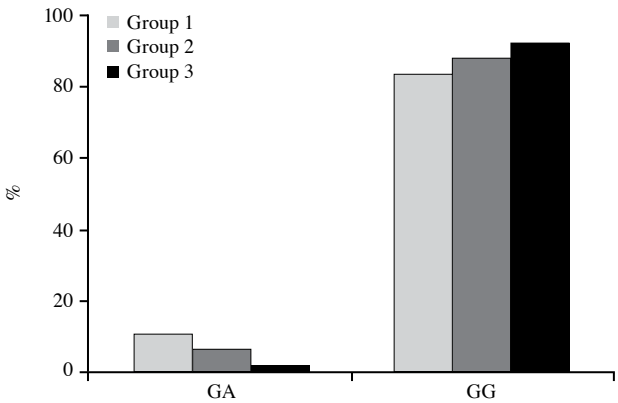

Figure 3. The distribution of prothrombin G20210A polymorphism in groups ($p>0.05$).

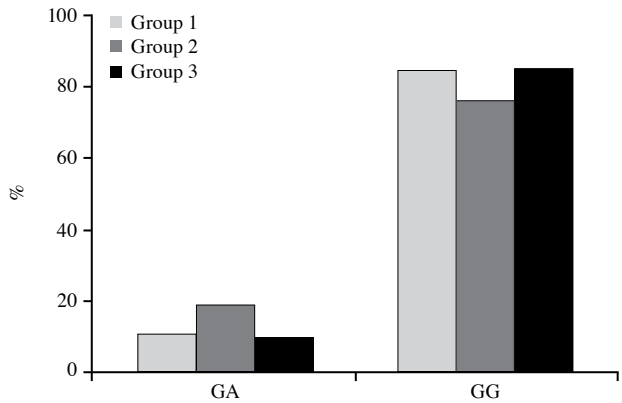

Figure 4. The distribution of tumor necrosis factor alpha G-308A polymorphism in groups ($p>0.05$).

increases the risk of venous thrombosis by three-eight fold for heterozygous carriers and 80-fold for homozygous carriers.[4,16,17] The most common hereditary cause of venous thrombosis stems from a single point mutation in factor V Leiden, a condition known as activated protein C resistance.[17] Prothrombin polymorphisms are found in 6.2% of patients with deep vein thrombosis (DVT) and in 18% of those with a family history of thrombophilia.[4,16] In the Turkish population, factor V G1691A and prothrombin G20210A mutations were higher in patients with venous thromboembolism.[18] To our knowledge, no studies have been conducted on whether or not these factors are associated with thrombophilia and the tendency for thrombi to develop in patients with a mechanical heart valve. However, De Paulis et al.[19] reported a case of early postoperative obstructive prosthetic mitral valve thrombosis in a patient who was double heterozygous for factor V Leiden and prothrombin G20210A.

Histologically, pannus is mainly composed of collagen and elastic fibrous tissue accompanied by endothelial cells, chronic inflammatory cell infiltration, and myofibroblasts,[20] and pannus formation after mechanical valve replacement may be associated with an excessive process of periannular tissue healing via the expression of inflammatory mediators. In addition, several inflammatory gene polymorphisms have been shown to be associated with an increased risk of cardiovascular diseases. For instance, IL-6 appears to have an important role in the pathogenesis of atherosclerosis via the stimulation of endothelial activation, vascular smooth muscle cell proliferation, leukocyte aggregation, and complement activation.[21]

Several studies have focused on the measurement and detection of inflammatory mediators in serum or tissue. In one of these studies, the plasma levels of IL-6 and TNF-α were found to be significantly higher in patients with rheumatic valve disease.[22] The levels of these mediators are affected by many pathological and physiological conditions, such as infections, autoimmune diseases, cancer, trauma, ischemia, and drug treatment.[23] As a result, a single measurement might not be sufficient to predict disease. Moreover, it has recently been discovered that the degree and severity of inflammation may be significantly influenced by genotypes. For instance, polymorphisms in the IL-6 gene strongly predict perioperative plasma levels of IL-6,[24] and the IL-6 G-174C polymorphism has also been associated with atrial fibrillation, pulmonary dysfunction, renal complications, and increased intensive care unit (ICU) stays after cardiac surgery.[5,6,24,25] Similarly, TNF gene polymorphisms have been associated with increased TNF-α levels and cardiopulmonary morbidity after cardiac surgery.[26,27]

Conclusions

The main limitation of this study was the relatively small number of patients who participated, especially in the first group. However, mechanical valve dysfunction is known to be a rare event. In order to avoid confusion, the second group was composed of patients with functional mechanical heart valves who had low INR levels. In light of our experience regarding the replacement of occluded mechanical heart valves,[28] we examined the relationships between factor V Leiden, prothrombin, IL-6, TNF-α polymorphisms and mechanical heart valve dysfunction, which may be the first report of its kind in the literature. When we compared the patients with mechanical heart valve dysfunction with the healthy controls, we found that only the IL-6 polymorphism had a statistically significant difference, and that polymorphism may be associated with the pathophysiology of thrombotic or inflammatory processes related to mechanical heart valve dysfunction.

Declaration of conflicting interests

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