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Relationship of serum HLA-B alleles and TNF-α with rheumatic heart disease

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Background/aim: Acute rheumatic fever and rheumatic heart disease are major causes of morbidity and mortality in developing countries. Genetic studies have determined that the immune response in rheumatic heart disease is genetically controlled and that there is a close relationship between the gene of concern and the class II human leukocyte antigen (HLA) gene. The aim of this study was to evaluate the relationship of serum HLA-B alleles and tumor necrosis factor alpha (TNF-α) with rheumatic heart disease.

Materials and methods: A total of 50 consecutive patients with rheumatic heart disease and 50 controls were enrolled in the study. HLA alleles were analyzed using sequence-specific primer-polymerase chain reaction and nucleotide sequencing.

Results: The HLA-B35 allele was significantly more common in patients with rheumatic heart disease than the control group (P = 0.043). The HLA-B44 allele was significantly more common in control patients than in patients with rheumatic heart disease (P = 0.014). There was a significant inverse correlation between high-sensitivity C-reactive protein and mitral valve area (P = 0.001). There was no correlation between TNF-α levels and mitral valve area (P = 0.066).

Conclusion: Our findings confirmed the association between HLA-B alleles and rheumatic heart disease.

Key words: Rheumatic heart disease, human leukocyte antigen B subgroups, tumor necrosis factor alpha

1. Introduction

Acute rheumatic fever (ARF) is a nonsuppurative multisystem autoimmune disease occurring after upper respiratory tract infection due to Group A streptococci with a common involvement of joints and heart, but rare involvement of the central nervous system, skin, and subcutaneous tissue (1). Although studies conducted in industrialized countries have demonstrated a decrease in the incidence of ARF and rheumatic heart disease (RHD), it is still a medical and public health problem in developing countries. Approximately 15 million people are estimated suffer from rheumatic valvular disease worldwide.

Rheumatic valvular disease is a multifactorial process that appears to be caused by the interaction of risk factors such as genetic, inflammatory, autoimmune, infectious, and oxidative stress; however, the pathophysiology are not completely understood (2). Genetic studies have determined that the immune response in this disease is genetically controlled and that there is a close relationship between the gene of concern and the class II human leukocyte antigen (HLA) gene. However, various HLA-DR studies and studies conducted in ethnic groups have reported that the relationship between the susceptibility to ARF and the class II HLA gene can be quite variable and that a susceptibility gene, rather than a specific allele, located in or near this location may be responsible for this susceptibility (3–5).

However, there have been controversial results regarding the susceptibility or protective alleles (6). Tumor necrosis factor-alpha (TNF-α) is one of the most important cytokines in the pathogenesis of rheumatic diseases. The lack of a definitive diagnosis of the disease and the ongoing existence of morbidity and mortality caused by cardiac involvement are indicative of the necessity to improve new diagnostic and treatment modalities. In this study, we aimed to evaluate whether there is a relationship between rheumatic heart disease and HLA-B and TNF-α.

2. Materials and methods

2.1. Study design

In this study, we enrolled a total of 50 patients with diagnosed RHD and 50 healthy control subjects. The physical examinations and echocardiography assessments of the control subjects were normal. In the medical history of patients, we inquired about the major findings of the

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first episode of ARF, time and age of the first episode, and the existence and number of valvular involvements. The control subjects and the patients with RHD were selected from among patients with no other diseases that may affect the anatomy and function of the heart valves, who signed informed consent forms and agreed to participate as volunteers, in accordance with the European Society of Cardiology Valvular Heart Disease Guidelines (7). Patients with abnormal leukocyte and erythrocyte counts, electrolyte imbalance, or renal and hepatic dysfunction were excluded from the study. The study was approved on 19 June 2012 in accordance with the assessment of our local ethics committee.

2.2. Echocardiographic assessment

Echocardiographic evaluation was performed by using a VIVID 7 Dimension Cardiovascular Ultrasound System (Vingmed-General Electric) with a 3.5-MHz transducer. Echocardiographic examinations were performed in the left lateral decubitus position. Parasternal long- and short-axis views and apical views were used as standard imaging windows. Ejection fraction was calculated by using the modified Simpson method. Mitral valve area (MVA) was calculated by planimetric method in the parasternal short axis. Mitral jet velocity was calculated by Doppler echocardiography. Aortic and tricuspid valve gradients of patients were in the normal range, so these were not specified numerically. All measurements were done according to the recommendations of the American Society of Echocardiography (8).

2.3. Laboratory assessment

HLA genotyping and high-sensitivity C-reactive protein (hs-CRP) and TNF-α measurements were carried out in the laboratory of the Department of Pediatric Nephrology. Genomic DNA from whole blood samples was extracted by a standard method. In the laboratory environment, 200 mL of blood was collected into an EDTA tube for HLA typing. The DNA was isolated with an automated DNA isolation device. The DNA, distilled water, and Taq polymerase enzyme were mixed in suitable proportions and the mixture was collected into 0.5-mL PCR tubes and then placed in a thermal cycler device. DNA amplifications were performed by selecting the appropriate program for HLA-B SSO typing study. The results were processed on a Luminex device after about 4 h of incubation. DNA isolation was performed automatically with Geno Geno M-6 device (GenoVision, QIAGEN). Serum concentrations of TNF-α were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's procedure (Cat No: EK0525, Boster Biological Technology Co., Ltd.). Blood samples were taken into standardized tubes containing dipotassium ethylenediaminetetraacetic acid (EDTA) for complete blood count (CBC). The Coulter Counter LH Series (Beckman Coulter Inc.) was used for CBC and hs-CRP analysis.

2.4. Statistical analysis

Statistical analysis was performed with SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The normal distribution of variables and groups was tested with the Kolmogorov–Smirnov test. The t-test and Mann–Whitney U test were used for the values given as mean ± standard deviation and for the comparison of nonparametric quantitative data, respectively. In addition, the chi-square test was used for nonnumerical categorical variables. Pearson's test was used for the correlation analysis and the r value (correlation coefficient) was calculated. $P < 0.05$ was considered statistically significant.

3. Results

Baseline clinical characteristics and laboratory parameters of the groups are shown in Table 1. The mean age of the patients was 37.8 ± 7.7 years and the mean age of the control group was 35.3 ± 6.3 years. All of the patients had mitral valve involvement, while 44 patients (88%) had both mitral and aortic valve involvement (Table 2). The mean MVA was 2.3 ± 0.8 cm^2, mean diastolic transmitral gradient was 4.6 ± 2.3 mmHg, and the average of maximum diastolic transmitral gradient was 10.8 ± 4.6 mmHg. There was no difference between the two groups in terms of hemoglobin, creatinine, or TNF-α levels, whereas the leukocyte count was significantly higher in patients with RHD than the control group and the platelet count was significantly higher in the control group than the patient group ($P < 0.001$ and $P < 0.001$, respectively). The hs-CRP level was significantly higher in patients with RHD than the control group [1.58 (0.01–9.88) vs. 0.13 (0–2.05); $P < 0.001$] (Table 1).

There was no significant difference in the presence of the HLA-B13, HLA-B27, HLA-B38, HLA-B49, HLA-B51, and HLA-B52 alleles between the two groups. However, the HLA-B35 allele was significantly more common in patients with RHD than the control group, while the HLA-B44 allele was significantly more common in the control group than in patients with RHD (Table 3). There was significant inverse correlation between hs-CRP and MVA in patients with RHD ($r = –0.340$, $P = 0.001$). However, there was no correlation between TNF-α levels and MVA ($r = –0.187$, $P = 0.066$).

4. Discussion

Although the pathogenesis of RHD has not yet been fully clarified, streptococcal virulence factors, individual susceptibility, exaggerated immune response, and tissue damage are thought to play a role in the pathogenesis (9).

There are several studies that indicate the existence of a genetic predisposition in the development of ARF

Table 1. Baseline characteristics and laboratory parameters of the study groups.

Variables	Patient group (n = 50)	Control group (n =50)	P-value
Age, years	37.8 ± 7.7	35.3 ± 6.3	0.079
Mitral valve area, cm^2	2.3 ± 0.8	-	-
Male, n (%)	21 (42)	29 (58)	0.423
Maximum gradient of mitral valve, mmHg	10.8 ± 4.6	-	-
Mean gradient of mitral valve, mmHg	4.6 ± 2.3	-	-
Hemoglobin, g/dL	12.9 ± 1.2	13.1 ± 1.1	0.331
Creatinine, mg/dL	0.6 ± 0.1	0.7 ± 0.1	0.230
White blood cells, $10^3/mm^3$	7.3 ± 1.6	5.8 ± 0.5	<0.001
Platelets, $10^3/mm^3$	237 ± 57	307 ± 50	<0.001
TNF-α, pg/mL*	3.12 (0–58.55)	1.18 (0–11.13)	0.469
hs-CRP, mg/L*	1.58 (0.01–9.88)	0.13 (0–2.05)	<0.001

Data are given as mean ± SD or n (%).TNF-α, Tumor necrosis factor alpha; hs-CRP, high sensitivity C-reactive protein. * Mean (min–max).

Table 2. Valvular involvement in study patients.

Valvular involvement, n	n
Mitral valve	50
Mitral + aortic valve	44
Mitral + aortic + tricuspid valve	3

and RHD. Host genetic factors have been related to susceptibility to ARF and subsequent progression to RHD as earlier studies have shown a low attack rate (up to 3%) of ARF after untreated streptococcal pharyngitis (10), a relatively high concordance rate for rheumatic fever in monozygotic twins (19%) in comparison to dizygotic twins (2.5%) (11), and high familial incidence of ARF (12,13).

There are several studies that investigated the relationship between ARF and HLA antigens. Ayoub et al. reported a statistically significant increase of HLA-DR4 level in Caucasians and of HLA-DR2 level in African Americans in patients with RHD (14). Anastasiou-Nana et al. showed similar results for the relationship between HLA-DR4 and RHD (15). Ahmed et al. observed that the HLA-DRB1*16 allele was statistically increased in patients with ARF (16). Conversely, Maharaj et al. reported no differences in HLA-A, HLA-B, and HLA-DQ frequencies between patients with severe chronic RHD and controls (17,18).

HLA-B27 has been strongly associated with various autoimmune diseases. Toor et al. showed that HLA-B27 was positive in nearly 43% of ARF patients (13). Although patients with the HLA-B27 allele were twofold higher than among control subjects, this was not statistically significant in our study.

There are also conflicting results in Turkey, similar to other worldwide data. Ozkan et al. reported a positive correlation between the expression of HLA-DR3, -DR7, and -DRB16 and RHD development and a negative correlation between the expression of HLA-DR5 and RHD development in adults with chronic RHD (19). Conversely, Karakurt et al. showed that DRB5 and DRB1*15 acted as susceptibility factors for RHD, while DRB4 was a protective factor against RHD (20). Olmez et al. reported that there was a significant correlation between rheumatic fever and HLA-A10 and HLA-B35 and a significant increase in HLA-A10 and HLA-drw11 in patients with cardiac involvement when compared to patients without cardiac involvement (21). Hallioglu et al. reported that the HLA DQA1*03 allele is a protective factor against rheumatic fever in Turkish children (22).

There were no consistent results in the studies that investigated the association between HLA and RHD. This can be attributed to the earlier studies using the serological typing method, which is unable to distinguish allelic subgroups and can produce misleading results, rather than molecular typing methods. These conflicting results can be explained by the ethnic variability of HLA alleles in different populations and the contribution of genes, showing a different linkage disequilibrium pattern with their HLA-DR and HLA-DQ alleles in different populations. The results of the studies investigating genetic susceptibility

Table 3. HLA-B allele distributions of the patient group and the controls.

	Patient group (n = 50)	Control group (n = 50)	P-value
HLA-B13, n (%)	4 (8)	2 (4)	0.400
HLA-B27, n (%)	4 (8)	2 (4)	0.400
HLA-B35, n (%)	18 (36)	9 (18)	0.043
HLA-B38, n (%)	4 (8)	9 (18)	0.137
HLA-B44, n (%)	2 (4)	10 (20)	0.014
HLA-B49, n (%)	7 (14)	3 (6)	0.182
HLA-B51, n (%)	15 (30)	13 (26)	0.656
HLA-B52, n (%)	2 (4)	3 (6)	0.646

against rheumatic fever and rheumatic carditis were also adversely affected by the nonheterogeneity of the clinical modeling of cases of ARF and RHD (6). Regional variations of specific strains of streptococci playing a role in the development of ARF can be considered one of the factors that may affect the results (23).

In the present study, there was not statistically significant difference in the presence of the HLA-B27 allele in patients with RHD when compared to the controls. The HLA-B35 allele was significantly more common in patients with RHD compared with the control group. HLA-B35 is responsible for cytotoxic T-lymphocyte-mediated immune reactions by mobilizing the autoimmune mechanisms (24). Therefore, we believe that there may be a relationship between the HLA-B35 allele and rheumatic mitral valves and the above-mentioned immune mechanism may be responsible for this association. Investigators reported that the incidence of ARF was significantly higher in patients with HLA-B35 (21). This suggests that individuals with the HLA-B35 allele may be more susceptible to development of ARF, and therefore there may be a significantly higher risk of RHD. The level of HLA-B44 allele was observed to be significantly higher in the control group when compared to the group of patients with RHD, which suggests a possible protective role for RHD.

CRP is one of the well-known inflammatory markers. Eastham et al. found that CRP was always present in active RHD and proved to be a useful guide in the management of disease (25). Chiu-Braga et al. also reported high levels of hs-CRP in chronic rheumatic valve disease (26). Karpuz et al. observed that the CRP level was higher in patients with chronic RHD compared with the control group, and they suggested that this increase was an indicator of continuing inflammation (27). We also found that hs-CRP levels were significantly higher in patients with RHD than the control group. The MVA was narrower in patients with higher hs-CRP levels.

TNF-α is a potent immunomodulator and proinflammatory cytokine that mediates various pathological processes (28). Yegin et al. showed an increment in TNF-α levels in ARF patients (29). TNF-α levels were higher in RHD patients with cardiac failure (30). Berdeli et al. investigated the relationship between single nucleotide polymorphisms in the promoter region of TNF-α and ARF in hospitalized patients. No significant difference was found in the frequencies of the TNF-α -308 G and A alleles between the patients and controls (30). There was also no difference in TNF-α levels between the two groups in our study. This result might be attributed to the low number of patients.

Our study has some limitations. First, our study has a small sample size. Second, the study is a single-center study. Further studies including a greater number of patients and control group members should be performed for the confirmation of the sensitivity of HLA-B alleles and TNF-α in patients with RHD.

This study demonstrated that patients with RHD have a higher frequency of the HLA-B35 allele and a lower frequency of the HLA-B44 allele. This suggests that individuals with the HLA-B35 allele may be more susceptible to the development of ARF, and therefore there may be a significantly higher risk of RHD.

There are several studies showing the relationship between HLA-B alleles and RHD. However, there are contradictory results. For this reason, we aimed to investigate the same subject in order to contribute to the literature. Unlike other studies, we researched the relationship between serum HLA-B alleles and TNF-α in patients with RHD at the same time. This is also the first study to investigate the relationship between TNF and RHD in the Turkish population for a wider population than other studies. Yegin et al. researched the TNF-α levels in 15 RHD patients (29). They found that TNF-α levels were higher in RHD patients with cardiac failure. In our

study, we researched TNF-α levels for 50 patients. Berdeli et al. researched TNF-α levels in patients with acute rheumatic fever but not in patients with RHD (30).

Our study provides further information on the genetic predisposition for RHD and the protective immune responses in RHD. Further insight into the molecular mechanisms of the disease will be a useful tool for predicting clinical outcomes in patients with ARF and therefore may potentially offer new means and approaches to treatment and prophylaxis, including a potential vaccine. Therefore, long-term follow-up and large-scale prospective studies are needed to investigate the relationship between RHD and HLA-B alleles.

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