Presence of a D8/17 B lymphocyte marker and HLA-DR subgroups in patients with rheumatic heart disease

Romatizmal kalp hastalarında bir lenfosit belirteci olan D8/17B'nin ve HLA-DR alt gruplarının varlığı

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ABSTRACT

Objective: The aim of our study was to investigate the association of HLA antigens and a non-HLA protein D8/17 with rheumatic heart disease and its pattern of cardiac involvement.

Methods: This cross-sectional observational study included 35 children and 12 adult patients who have rheumatic heart disease and 35 healthy children and 12 healthy adult controls. After physical examination, all patients and control group members were evaluated with 2D and color-coded echocardiography. B-lymphocyte D8/17 expression was tested by a flow cytometry assay. HLA genotyping was performed using polymerase chain reaction sequence-specific primers. In statistical analysis, Chi-square, unpaired t and Mann-Whitney U tests were used for comparison groups.

Results: The percentage of the D8/17-expressing B lymphocytes of the patient group was significantly higher than of the control group (77.3±15.6% vs. 67.7±20.0%, p=0.013). When compared with the control group, the HLA DRB5 (38.6% vs. 13.6%, p=0.007) and HLA DRB1*15 (31.8% vs. 9.0%, p=0.008) expression levels of the patient group were significantly higher and the DRB4 expression of the patient group was significantly lower (29.5% vs. 50.0%, p=0.049).

Conclusion: Our findings support the association between HLA Class 2 subgroups and rheumatic heart disease, and an association between D8/17 expression and rheumatic heart disease. Further studies including higher number of patients and control group members should be performed for the confirmation of our results. *(Anadolu Kardiyol Derg 2011; 11: 314-8)*

Key words: Rheumatic heart disease, human leukocyte antigen DR subgroups, beta-lymphocyte D8/17 antigen, flow cytometry

ÖZET

Amaç: Bu çalışmanın amacı, romatizmal kalp hastalığı olan hastalarda HLA-DR subgrupları ve HLA dışı B lenfosit belirteci olan D8/17 belirteç oranlarını araştırmaktı.

Yöntemler: Bu enine-kesitli gözlemsel çalışmaya romatizmal kalp hastalığı olan 35 çocuk ve erişkin hasta ile kontrol grubu olarak sağlıklı 35 çocuk ile sağlıklı 12 erişkin alınmıştır. Hasta ve kontrol grubunda yer alan bireyler renkli Doppler ve 2 boyutlu ekokardiyografi ile değerlendirildikten sonra D8/17 B lenfosit markeri ve HLA-DR subgrupları için kan örnekleri alındı. B lenfosit D8/17 ekspresyonu akım sitometrisi kullanılarak değerlendirildi. HLA-DR subgupları ise ilgili primerler kullanılarak PCR yöntemi ile değerlendirildi. İstatistiksel analiz için gruplar arası karşılaştırmada Ki-kare, eşleştirilmemiş t ve Mann-Whitney U testleri ile değerlendirildi.

Bulgular: D8/17 eksprese eden B lenfosit yüzdesi hasta grubunda kontrol grubuna göre istatistiksel olarak anlamlı olarak yüksek bulundu (%77.3±15.6' ya karşın %67.7±20.0, p=0.013). HLA DRB5 (%37.6'ya karşın %13.6, p=0.007) ve HLA DRB1*15 (%31.8'e karşın %9.0, p=0.008) ekspresyonu hasta grubunda anlamlı olarak yüksek bulunurken, DRB4 ekspresyonu (%29.5'e karşın %50.0, p=0.049) kontrol grubunda hasta grubuna göre anlamlı olarak yüksek bulundu.

Sonuç: Çalışmamızda HLA DR subgrupları ve D8/17 ekspresyonu romatizmal kalp hastalığı ile arasında ilişki bulunduğunu desteklemektedir. Sonuçlarımızın desteklenmesi daha fazla çalışmaya ihtiyaç vardır. *(Anadolu Kardiyol Derg 2011; 11: 314-8)*

Anahtar kelimeler: Romatizmal kalp hastalığı, insan lökosit antigen DR altgrupları, D8/17 beta lenfosit antigeni, akım sitometrisi

Anadolu Kardiyol Derg
2011; 11: 314-8

Karakurt et al.
D8/17 B lymphocyte marker and rheumatic heart disease   *315*

Introduction

Acute rheumatic fever (ARF) is believed to be a non-suppurative autoimmune complication of a group A beta- hemolytic streptococcal pharyngitis. In the chronic sequela of ARF, rheumatic heart disease (RHD) is the most common cause worldwide of acquired heart disease in children and young adults (1). As living conditions have become more hygienic and less crowded and access to medical care and antibiotics have improved, the incidence of ARF and RHD have become rare in developed countries. ARF and RHD are now largely restricted to developing countries and particular poor populations of wealthy countries (2). According to WHO, at least 15.6 million people have RHD, approximately 300.000 of about 500.000 individuals who acquire ARF every year go on to develop RHD, and 233.000 deaths annually are directly attributable to ARF or RHD (2).

It has long been observed that ARF appears to occur in specific susceptible individuals (3). This is based on evidence of a familial association (4), controlled studies (5, 6) and the fact that only 2-3% of individuals exposed to rheumatogenic group A streptococcal pharyngitis go on to develop ARF (1). Several studies have suggested that genetic predisposition to ARF is linked to human leukocyte antigen (HLA) class 2 alleles (7, 8). However, there has been controversy concerning the nature of the susceptibility or protective alleles (9). A high level of D8/17 binding to B- lymphocytes has been proposed as a susceptibility marker of ARF. Ethnic background apparently does not play a role in the expression of D8/17, hence it may be possible to utilize it as a compelling universal marker of ARF (10).

The aim of our study was to investigate the association of HLA antigens and a non-HLA protein D8/17 with rheumatic heart disease and its pattern of cardiac involvement.

Methods

Study design and patients

This cross-sectional observational study included 47 Turkish patients (35 children, 12 adults) diagnosed with RHD, and followed up at the İnönü University Hospital, Departments of Cardiology and Pediatric Cardiology. The control group consisted of 47 healthy Turkish controls (35 children, 12 adults) that had not been taken any medicine, did not have any diseases and their physical examination and echocardiography analysis were normal. The records of patients were evaluated and the diagnosis of each patient was based on updated Jones criteria. Cardiac lesions were confirmed by echocardiographic evaluation. The records of the first ARF attack including major criteria, involved valves, the number of involved valves, and the severity of the carditis were obtained. Patients with cardiac failure were classified as "severe carditis"; cardiomegaly without cardiac failure were classified as "medium carditis", solely valve involvement were classified as "mild carditis". The history of recurrence, familial history about ARF, and presence of collagen tissue disor-

der were investigated. Group members having abnormal leukocyte and erythrocyte counts, electrolyte imbalance or renal and hepatic dysfunction were excluded from the study.

Ethical evaluation and approval for the study were obtained from our University hospital Ethical Committee assembly on 28.11.2006 (protocol no: 2006/73). The study group members were informed of all issues, and written consent was obtained from each member and parent involved with the study.

Echocardiographic evaluation

Echocardiographic evaluation of the pediatric patients and pediatric controls were established in Department of Pediatrics, Echocardiography laboratory with suitable color-coded and 2D echocardiography (Vivid Pro 7, GE Healthcare, USA); adult patients and adult controls were evaluated in Department of Cardiology, Echocardiography laboratory with color-coded and 2D echocardiography (ATL HDI 5000, Philips, Netherland). In echocardiographic evaluation cardiac functions, chamber dimensions, valvular morphology, valvular regurgitation, valvular stenosis were evaluated. All measurements were done according to the recommendation of the American Society of Echocardiography (11).

HLA genotyping

HLA genotyping was performed in the İnönü University Medical Biology and Genetics Department Laboratory. Genomic DNA from whole blood samples was extracted by a standard method, then isolated DNA samples were stored at -20°C until polymerase chain reaction (PCR) analysis. DNA samples were amplified via Olerup SSP™ DR low resolution typing kit (Olerup SSP AB, Sweden) using an ABI 9600 Thermal Cycler. PCR products were analyzed by agarose gel electrophoresis, screened under UV, where positive bands were separated and evaluated with the SCORE software program.

Flow cytometric analysis

Analysis of D8/17 and CD19 were performed by a flow cytometry method. Obtained whole blood samples were immediately studied in our University Hematology Laboratory Flow Cytometry unit. An aliquot of 20 µL of mouse anti-D8/17 monoclonal antibody (provided by Professor J Zabriskie, Rockefeller University, New York, USA) was added to 100 µL of whole blood. Blood and antibody were co-incubated at 4°C for 40 min and the samples were then washed with phosphate buffered saline (PBS). For the fluorochrome labeling, 10 µL fluorescein-5- isothiocyanate (FITC) labeled goat anti-mouse IgM (µ) antibody (Sigma, St. Louis, MO, USA) and 10 µL phycoerythrin (PE) labeled mouse IgG anti-CD19 (BD Pharmingen, San Diego, CA, USA) were added to the antibody-blood mixture. After 20 min of incubation at room temperature in darkness, red cells were lysed with ammonium chloride lysis solution. Cells were washed with PBS and resuspended in PBS for analysis by flow cytometry. Flow cytometry analysis was performed with a Beckman Coulter Epics Altra (Beckman

316 Karakurt et al.
D8/17 B lymphocyte marker and rheumatic heart disease

Anadolu Kardiyol Derg
2011; 11: 314-8

Coulter, Inc., Brea, CA, USA). For the isotype controls, FITC labeled mouse IgM antibody (Caltag, California, USA) and PE labeled mouse IgG antibody (BD Pharmingen, San Diego, California, USA) were used.

Statistical analysis

The data were analyzed with SPSS for Windows (Statistical Package for the Social Sciences) version 13.0 (SPSS Inc, Chicago, IL, USA). Continuous variables are noted as mean±standard deviation (SD), and categorical parameters were given as numbers and percents. Continuous parameters were tested with the Shapiro-Wilk test for normality. Normally distributed data comparisons were performed with the unpaired t test, where comparison of data not distributed normally were performed with Mann-Whitney U test. Categorical data analysis were made with Pearson Chi-square and Fisher's Chi-square tests. $P < 0.05$ were used for the statistical significance value.

Results

There were 47 patients diagnosed with RHD (35 children and 12 adults), and 47 healthy controls (35 children and 12 adults) selected for this study. The mean age of the patient group was 19.4±17.4 years. The mean age of the pediatric patients was 10.3±2.6 years. The mean age of the adult patients was 49.4±14.2 years. Of the 35 pediatric patients, 19 of them were female (54.2%) and 16 of them were male (44.8%). Of the 12 adult patients, 11 were female (23.4%) and one was male (2.1%). The mean age of the control group was 16.4±12.3 years. The mean age of the pediatric controls was 9.8±3.2 years. The mean age of the adult controls was 35.7±6.6 years. Of the 35 pediatric controls, 10 of them were female (21.2%) and 25 of them were male (53.1%). From the 12 adult patients; 7 of them were female (58.3%) and 5 of them were male (51.7%). Clinical and demographic data of patient and control group are shown in Table 1.

Using the test for the presence of D8/17 positive B cells, our data indicate that the proportion of B cells expressing the D8/17 marker was 77.3±15.6% in the patient group, and 67.7±20.0% in the control group. A statistically significant higher rate of B-cell expression of D8/17 was observed in the patient group when compared to controls (p=0.013). There was no statistical significance between the D8/17 expression rates and type of valvular involvement such as mitral valve involvement, aortic valve involvement and tricuspid valve involvement (p>0.5). When the patient group was subgrouped with designations "one valve involvement" and "more than one valve involvement", and D8/17 expression rates were compared, no statistical difference was observed (p>0.05). There was no observation of significance between the heart failure on the initial attack and D8/17 expression rates (p=0.32).

Although three individuals of the patient samples and control samples were excluded from the study due to technical complications. The frequencies of HLA alleles in the patient groups and

the controls are shown in Table 2. HLA DRB5 (p=0.007), and DRB1*15 (p=0.008) expression in the patient group were significantly higher than that observed for controls, and DRB4 (p=0.049) expression in the patient group was significantly lower than that observed for the control group.

Patients were subgrouped according to specific clinical features, and the occurrence of various alleles was assessed. When the patient group was subgrouped as those possessing "mitral valve involvement" and "without mitral valve involvement" then assessed for expression of DRB5 (p=0.14), HLA DRB15 (p=0.96), HLA DRB4 (p=1.0) alleles, no statistical difference was observed. When the patient group was subgrouped as those possessing "aortic valve involvement" and "without aortic valve involvement" and then assessed for expression of HLA DRB5 (p=0.83), HLA

Table 1. Demographic and clinical data of patient and control groups

Variables	Patient group (n=47)	Control group* (n=47)
Age, years	19.4±17.4 (5-58)	16.4±12.3 (5-47)
Male/female, n	17/30	30/17
Valvular involvement, n(%)		
Mitral valve	21 (44.6)	-
Aortic valve	3 (6.3)	-
Mitral + aortic valve	14 (29.7)	-
Mitral + tricuspid valve	6 (12.7)	-
Mitral + aortic + tricuspid valve	3 (6.3)	-

Data are presented as number(percentage) and mean±SD (range)
*unpaired t test, Mann-Whitney U test, Pearson Chi-square and Fisher's Chi-square tests
Differences are nonsignificant

Table 2. HLA DR allele distributions of the patient group and the controls

Subgroups	Patients (n=44) n	Patients (n=44) %	Controls (n=44) n	Controls (n=44) %	*p
DRB1*01	2	4.5	2	4.5	1.0
DRB1*03	8	18.1	8	18.1	1.0
DRB1*04	10	22.7	14	31.8	0.33
DRB1*07	2	4.5	7	15.9	0.07
DRB1*08	2	4.5	0	0	0.49
DRB1*09	1	2.2	0	0	1.0
DRB1*10	1	2.2	2	4.5	1.0
DRB1*11	20	45.4	23	52.2	0.52
DRB1*13	9	20.4	7	15.9	0.58
DRB1*14	2	4.5	6	13.6	0.26
DRB1*15	14	31.8	4	9	0.008
DRB1*16	5	11.3	3	6.8	0.71
DRB3	31	70.4	37	84	0.126
DRB4	13	29.5	22	50	0.049
DRB5	17	38.6	6	13.6	0.007

Data are presented as number(percentage)
*Pearson Chi-square and Fisher's Chi-square tests

Anadolu Kardiyol Derg
2011; 11: 314-8

Karakurt et al.
D8/17 B lymphocyte marker and rheumatic heart disease   *317*

DRB15 (p=0.97), HLA DRB4 (p=0.68) alleles, no statistical difference was observed. When the patient group was subgrouped as those possessing "tricuspid valve involvement" and "without tricuspid valve involvement" and then assessed for expression of HLA DRB5 (p=0.71), HLA DRB15 (p=0.11), HLA DRB4 (p=1.0) alleles, no statistical difference was observed. Lastly, when the patient group was subgrouped as those possessing "one valve involvement" and "more than one valve involvement" and then assessed for expression of HLA DRB5 (p=0.24), HLA DRB15 (p=0.83), HLA DRB4 (p=0.89) alleles, no statistical difference was observed.

Discussion

While rheumatic fever is observed in only 2-3% of individuals following an untreated episode of acute streptococcal pharyngitis or scarlet fever (1), the incidence of ARF following pharyngitis in patients who had a previous attack of ARF was approximately 50% (1). ARF shows varying prevalence according to geographical regions and ethnic groups (9, 12).

As studies have indicated that host factors are significant in the development of RHD, HLA alleles have become a major focus of attention. The HLA association in ARF/RHD has been investigated in several studies (7, 13-21). Although the data seem to support the hypothesis that such a link exists, a consistent association with specific allele(s)/haplotypes has been lacking (9). For example, Ayoub et al. (22) found that the previously described DR4 association in Caucasian American RHD patients did not hold when HLA was reassessed by DNA typing methods compared to the older results obtained by serological reagents. Inconsistency of the HLA results may also be attributable to the heterogeneous ARF and RHD study groups. Guedez et al. (9) reported that different outcomes of ARF may be associated with distinct genetic elements, and it is important and useful to analyze clinically homogeneous RF categories separately.

When studies investigating the association of RHD and HLA alleles conducted in Turkey were reviewed (22-25) DR3, DR7, DRB16, DRB1*07 were found to be susceptibility factors, and DR5, DRB1*13, DRB5, DRB3, DRB1*11 were found to be protective factors against RHD. However, these results were not confirmed in our study. In contrast with the results of Özkan et al. (23) which showed DR5 as a protective factor against RHD, we observed that DRB5 and DRB1*15 acted as susceptibility factors for RHD, and DRB4 as a protective factor against RHD.

A high level of B lymphocyte D8/17 expression has been proposed as a susceptibility marker for ARF and has been identified in geographically disparate and ethnically diverse populations (10, 26). In 1979, Patarroyo (27) reported that a B-cell alloantigen (called 883) was present with increased frequency in patients with RF, and that this rate did not change with geographic location. The antigen was not related to any of the HLA loci Subsequently, Khanna et al. (10) developed a murine IgM monoclonal antibody, D8/17, which identified a B-cell antigen in 100% of their patients with RF compared to only 14% of the dis-

ease-free controls. It was suggested that the D8/17 antigen may act as a streptococcal binding site on the B cells, and consequently become up-regulated after an infection, with B cell acting as antigen-presenting cells and influencing T-cell cytotoxicity to heart (28).The high rate of expression of the D8/17 marker in patients with poststreptococcic reactive arthritis (PSRA) suggests that RF and PSRA share features of the same genetic susceptibility (29). D8/17 was also found as a highly sensitive marker for a previous study of rheumatic fever (3). The level of positive association to D8/17 was observed at a lower level in a study which was conducted in Northern India (30) These difference may be secondary to methodological, genetic, or environmental variations. In our study, patients with RHD showed significantly higher D8/17 expression on B lymphocytes than observed for the controls, confirming the findings of previous reports.

Study limitations
In our study includes small population of adult patients with rheumatic valvular heart diseases. Further studies including a greater number of adult patients and control group members should be performed for the confirmation of sensitivity of D8/17.

Conclusion

Our findings support the association between HLA Class 2 subgroups and RHD. Although HLA DR subgroups may show genetic predisposition to RHD, most of the studies, which were performed previously showed great diversity due to ethnic variation. In contrast to the HLA DR subgroups, D/17 expression shows no ethnic variation. Our findings showed an association between expression of D8/17 and RHD. Further studies including a greater number of patients and control group members should be performed for the confirmation of these results presented herein.

Conflict of interest: None declared.

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318   Karakurt et al.
D8/17 B lymphocyte marker and rheumatic heart disease

Anadolu Kardiyol Derg
2011; 11: 314-8

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