ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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HLA Type Determination in Patients Diagnosed with Mycosis Fungoides and Sézary Syndrome

Mikozis Fungoides ve Sézary Sendromu Tanısı Alan Hastalarda HLA Tip Tayini

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ABSTRACT Objective: Mycosis fungoides (MF) and Sézary syndrome (SS) represent the most frequently encountered subtypes of primary cutaneous T-cell lymphoma. The human leukocyte antigen (HLA) system was shown to be involved in susceptibility to MF/SS in various studies involving populations of different genetic backgrounds. We aimed to determine the possible association of HLA system with MF/SS in Turkish cases. **Material and Methods:** A total of 30 MF/SS cases underwent genotyping for HLA-A, HLA-B and HLA-DR loci. Samples from 30 healthy subjects were obtained as controls. **Results:** Statistically significant associations were found between MF and HLA-A31, HLA-B51 and HLA-DR3 alleles. A significantly higher frequency of HLA-B35 and HLA-DR4 was noted in healthy controls as compared with the patients. **Conclusion:** This study suggests that HLA genotypes are involved in susceptibility to MF. HLA-A31, HLA-B51 and HLA-DR3 alleles were considered as markers to disease susceptibility whereas HLA-B35 and HLA-DR4 alleles may be protective against neoplastic transformation.

Keywords: Disease susceptibility; HLA antigens; mycosis fungoides

ÖZET Amaç: Mikozis fungoides (MF) ve Sézary sendromu (SS) primer T-hucreli deri lenfomalarının en sık rastlanan formlarıdır. Farklı genetik zeminlerden toplumlarda yapılan çeşitli çalışmalarda hastalığın gelişiminde sınıf I ve II insan lökosit antijenlerinin (HLA) rolü olabileceği gösterilmiştir. Çalışmanın amacı, Türk hastalarda mikozis fungoides ve Sézary sendromu ile HLA sisteminin muhtemel ilişkisinin araştırılmasıdır. **Gereç ve Yöntemler:** Mikozis fungoides ve Sézary sendromu tanısı olan 30 hastaya HLA-A, HLA-B ve HLA-DR lokuslarını değerlendirmek üzere genotipleme yapıldı. Sonuçlar 30 sağlıklı bireyin örnekleri ile karşılaştırıldı. **Bulgular:** HLA-A31, HLA-B51 ve HLA-DR3 alelleri ile MF arasında istatistiksel olarak anlamlı ilişki saptandı. Hastalarla kıyaslandığında, kontrol grubunda HLA-B35 ve HLA-DR4 sıklığında anlamlı yükseklik mevcuttu. **Sonuç:** Çalışmamız HLA genotiplerinin MF'e yatkınlıkta rolü olabileceğini göstermektedir. HLA-A31, HLA-B51 ve HLA-DR3 alelleri hastalığa yatkınlığı göstermekte iken HLA-B35 ve HLA-DR4 alelleri neoplastik dönüşümden koruyucu gibi gözükmektedir.

Anahtar Kelimeler: Hastalığa yatkınlık; HLA antijeni; mikozis fungoides

Cutaneous T-cell lymphomas are a heterogenous group of diseases, with mycosis fungoides (MF) and Sézary syndrome (SS) representing the most frequently encountered subtypes, accounting for 70-75% of all cases.[1-3] Histologically, proliferated atypical CD4+ T cells along with both CD4+ and CD8+ reactive lymphocytes are seen in MF. Clinical manifestations usually start as a non-specific eczematous eruption that evolves into indurated plaques and subsequently into tumours in a subset of patients. Involvement of lymph nodes and viscera may be noted. When peripheral

blood is involved, patients are considered to have SS, an aggressive variant associated with erythroderma and lymphadenopathy. The etiopathogenesis of MF/SS is poorly understood. Genetic and environmental factors have been suggested as culprits. It has been hypothesized that the malignant transformation may arise from a chronic antigenic stimulation to viruses, bacterial superantigens, chemical agents, or yet an unknown antigen.[4-6]

Human leukocyte antigens (HLA) are important mediators of responsiveness in immunity. HLA molecules are cell surface glycoproteins that help the body in differentiating non-self from self. Class I antigens, namely, HLA A, B and C antigens, are found on almost every cell. However, class II antigens (HLA DR, DQ, DP) are confined to specialized cells involved in antigen presentation. Human leukocyte antigens, encoded by major histocompatibility complex (MHC), are among the most polymorphic genes localized at 6p21. Polymorphisms in HLA genes result in variations of the peptide-binding region influencing the bound antigens, which are presented to T cells.[7,8] HLA system associations are studied in many diseases in order to clarify underlying immunopathogenetic mechanisms. To date, susceptibility to many diseases have been linked to different HLA antigens.[9] We aimed to determine the possible association of HLA system with MF/SS in Turkish cases.

MATERIAL AND METHODS

CASES AND CONTROLS

We carried out this study at the Dermatology Department of Cerrahpasa Medical Faculty, Istanbul University. Patient group consisted of 30 Turkish patients (11 men, 19 women) with MF/SS. For each patient, diagnosis of MF/SS was based on clinical features, histopathological findings and immunohistochemical findings. The disease was staged according to revised International Society for Cutaneous Lymphoma staging system.[10] 30 kidney donors without any familial and genetic diseases were recruited as control group. The Declaration of Helsinki protocols were followed. The institutional review board approved the study and informed consent was obtained from all participants.

DNA ISOLATION

From each subject, 2 ml whole peripheral blood was obtained. DNA was extracted using an automated nucleic acid purification instrument (EZ1 Advanced, Qiagen, Hilden, Germany).

HLA TYPING

In all subjects, HLA typing for HLA-A, HLA-B and HLA-DR antigens was performed using polymerase chain reaction-sequence of specific oligonucleotides (PCR-SSO) method (One-Lambda, Canoga Park, CA, USA) and Luminex LABScan 100 flow analyzer (One Lambda, San Diego, CA, USA). The results were assessed in the "Luminex XY Platform" and "Luminex Data Collector Software/HLA Visual Software 2.2.0".

STATISTICAL ANALYSIS

Results were noted as mean± SD or frequencies (number of cases) when appropriate. We compared frequencies of various HLA antigens between MF/SS cases and controls. The calculations were made using the chi-square and Fisher methods. A p value of less than 0.05 was accepted as significant.

RESULTS

Our study comprised 30 MF/SS patients, with an age range from 20 to 86 years, and a mean of 45.9 ± 2 years. Of the 30 patients studied, the male to female ratio was 1:1.7 (male= 11, female= 19). Table 1 shows clinical features of MF/SS cases. Mean age

TABLE 1: Clinical characteristics of the patients.

Variable	Number (%)
Sex	
Male	11 (37%)
Female	19 (63%)
Clinical stage	
Stage IA	10 (33%)
Stage IB	3 (10%)
Stage IIA	12 (40%)
Stage IIB	2 (7%)
Stage III	3 (10%)

of the 30 subjects in the control group was 44±2.1, with an age range from 23 to 60 years.

Numbers of the HLA-A, HLA-B and HLA-DR antigens detected in patients diagnosed with MF/SS are shown in Figure 1, Figure 2, Figure 3, respectively. Table 2 summarizes the results of statistically significant HLA associations.

Among HLA-A antigens, HLA-A2 was the most prevalent allele detected in 15 patients (50%). Statistically significant difference between the patients and controls was a higher number of HLA-A31 in the patients than in controls (4 patients vs 0 controls) (p=0.046). Of the 4 patients with HLA-A31 allele, two were in stage IB, one was in stage IIA and one was in stage III.

As for HLA-B antigens, HLA-B51 was the most prevalent allele detected in 13 patients (43%). HLA-B51 had a statistically significant higher frequency in patients (p= 0.014). Of the 13 patients with HLA-B51 allele, four were in stage IA, three

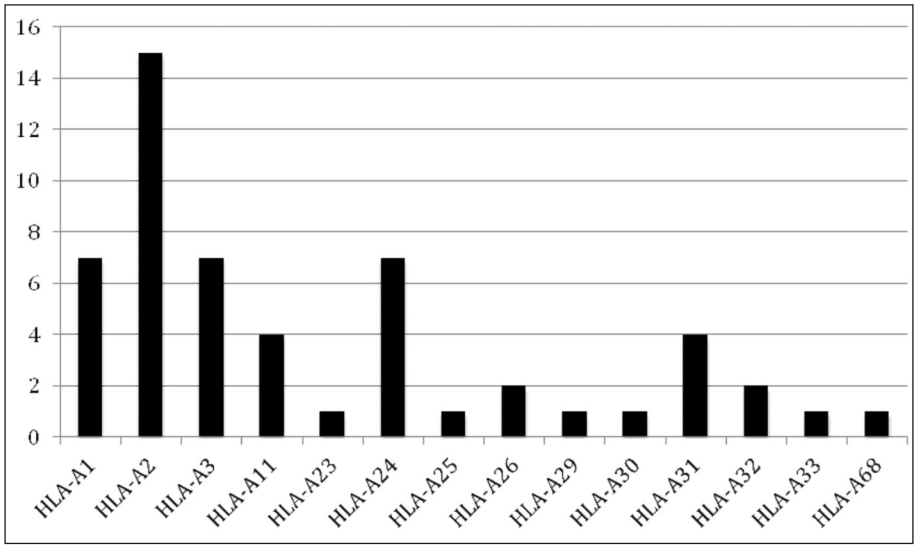

FIGURE 1: HLA-A antigen frequency in patients diagnosed with MF.

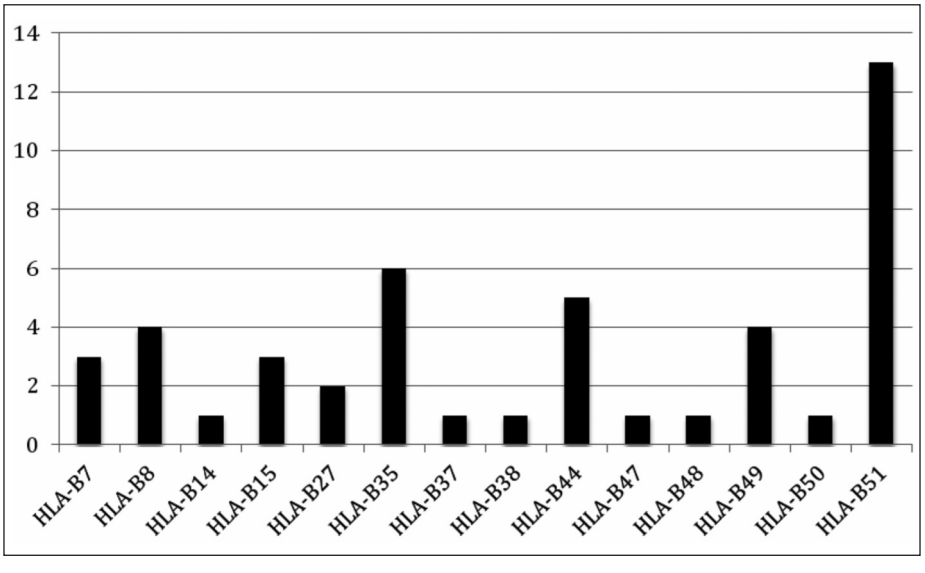

FIGURE 2: HLA-B antigen frequency in patients diagnosed with MF.

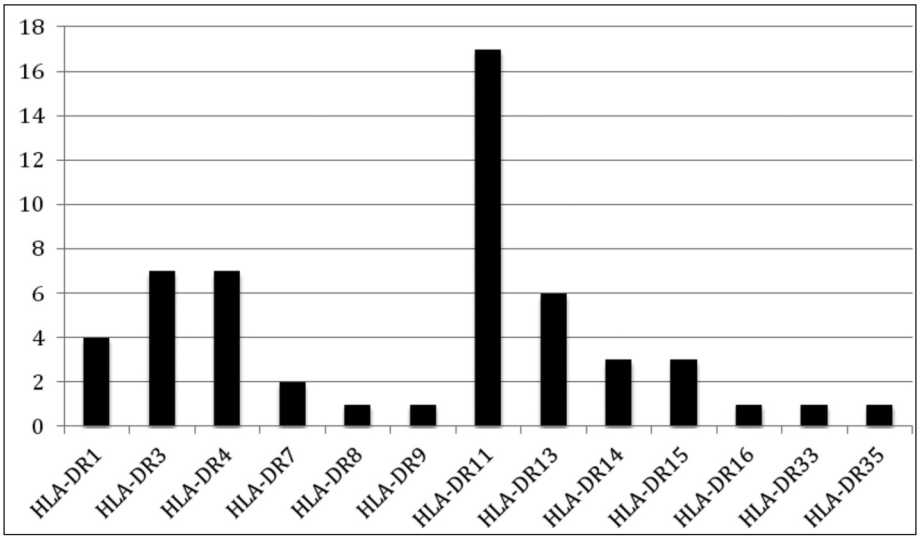

FIGURE 3: HLA-DR antigen frequency in patients diagnosed with MF.

TABLE 2: Statistically significant HLA antigens.

	Patient group	Control group	p value
HLA-A31	4	0	0.046
HLA-B51	13	6	0.014
HLA-DR3	7	1	0.017

in stage IB, four in stage IIA, one in stage IIB and one in stage III. Of note, patient in stage III had also HLA-A31 allele. HLA-B35 allele was more common in controls (6 patients vs 20 controls).

As for HLA-DR series, HLA-DR11 was the most prevalent allele detected in 17 patients (56%). Statistically significant difference between the patient and control groups was a higher frequency of HLA-DR3 in the patient group as compared with controls (7 patients vs 1 controls) (p=0.017). Of the 7 patients with HLA-DR3 allele, three were in stage IA, two in stage IIA, two in stage III. HLA-DR4 antigen was found at a lesser frequency among patients with MF (7 patients vs 19 controls).

DISCUSSION

Familial aggregation and ethnical differences in incidence point to genetic factors in the susceptibility to MF/SS.[11,12] To the best of our knowledge, scientific literature lacks data about HLA polymorphisms in Turkish MF patients. In this study, we found that HLA-A31, HLA-B51 and HLA-DR3 were significantly associated with MF. Significantly expressed alleles in control group, HLA-B35 and DR4, may be considered as protective genotype against malignant transformation.

To date, various studies have evaluated HLA polymorphisms in MF. However, the data of these reports have proved inconsistent. Regarding various HLA class I antigens, the first reports published found that HLA-B8, A19 and C1 alleles were more common in MF patients.[13,14] In a study where seventy-six cutaneous T cell lymphoma cases were investigated for class I antigens; B8 and Bw35 alleles were increased in SS but not in MF patients.[15] A recent study from Italy revealed that HLA-24, A-68, A-69, B-35 alleles were involved in susceptibility to MF.[16] By contrast, a study from Israel and one from North America failed to find any HLA class I susceptibility allele.[17,18]

As for HLA class II associations, since the 1980s, the susceptibility alleles have been analysed in various studies.[17-19] Among 34 cases from North America.Safai et al. reported increased incidence of HLA-DR5 (DRB1*11).[17] In 1996, Jackow et al. detected significantly more DRB1*11 allele in a study involving 47 MF and 23 SS patients. Furthermore, DQB1*03 alleles, were noted as significantly more common in the SS group.[19] HLA-DRB1*11 and DQB1*03 frequency was also shown to be increased in Jewish MF patients.[18] However, DQB1*05 allele

was reported to be more prevalent in an Italian population.[16] In a very recent case-control study evaluating the association between paediatric MF and HLA system, HLA class I and class II allele frequencies did not differ.[20]

Our study was limited by the small sample size. Larger sample trials with subgroup analyses according to family history, ethnicity and clinicopathologic variant are needed.

HLA allele associations in our population demonstrated differences from previously published researches. It could be inferred that in populations with different genetic backgrounds, HLA susceptibility genes may possibly differ. In addition, further research on more patients will better define the associations of HLA genes in MF/SS and confirm our preliminary findings in Turkish patients. Given our small cohort, prognostic conclusions cannot be drawn from our study.

CONCLUSION

In conclusion, we could confirm the role of HLA genotypes in MF/SS pathogenesis. Our data showed that HLA-A31, B51 and DR3 were significantly increased in patients with MF/SS, implicating susceptibility to this neoplastic disease. Significantly expressed alleles in control group, HLA-B35 and DR4, were considered as protective genotype against malignant transformation. Our findings may be used for further analysis of HLA genetic predisposition studies of MF. Further studies are needed to fully assess the utility of HLA polymorphisms in predicting disease progression and treatment outcomes in patients diagnosed with MF.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Ali Rıza Başaran, Burhan Engin, Erkan Yılmaz, Muazzez Çiğdem Oba;* ***Design:*** *Ali Rıza Başaran, Burhan Engin, Zekayi Kutlubay, Server Serdaroğlu;* ***Control/Supervision:*** *Ali Rıza Başaran, Burhan Engin, Erkan Yılmaz, Zekayi Kutlubay, Server Serdaroğlu;* ***Data Collection and/or Processing:*** *Ali Rıza Başaran, Muazzez Çiğdem Oba, Burhan Engin, Zekayi Kutlubay, Server Serdaroğlu;* ***Analysis and/or Interpretation:*** *Ali Rıza Başaran, Burhan Engin, Muazzez Çiğdem Oba, Server Serdaroğlu;* ***Literature Review:*** *Ali Rıza Başaran, Muazzez Çiğdem Oba, Burhan Engin, Erkan Yılmaz;* ***Writing the Article:*** *Ali Rıza Başaran, Muazzez Çiğdem Oba, Burhan Engin, Server Serdaroğlu;* ***Critical Review:*** *Ali Rıza Başaran, Burhan Engin, Muazzez Çiğdem Oba;* ***References and Fundings:*** *Ali Rıza Başaran, Burhan Engin, Zekayi Kutlubay, Server Serdaroğlu;* ***Materials:*** *Ali Rıza Başaran, Burhan Engin, Muazzez Çiğdem Oba, Erkan Yılmaz.*

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