The HLA System And Hodgkin Disease Association Survey

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HLA SİSTEMİ İLE HODGKIN HASTALIĞI ARASINDAKİ İLİŞKİ

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SUMMARY

The reports regarding the association of HLA antigens in increased frequency with a num. ber of disease processes, suggested the possibility of such a link in lymphoreticular malignancies.

This study have been realised with the cooperation of Gulhane Military Medical Academy and University of Ankara, College of Medicine, Hematology-Oncology and Immunology ***Departments.*** *The results of the two study groups have been combined and statistical associations for each HLA subunit defined by chi-square test.*

39 patients with Hodgk in s disease in two ***contributing*** *center were included* ***in the study.*** *HLA typing were done by the* ***method*** *of* ***micor****lymphotoxicity and, results* ***were compared with*** *the HLA findings* ***of 100 healthy individuals as*** *the normal* ***controls, wich may represent the*** *antigenic* ***profile of the Turkish population.***

Comparing ***39 patients*** *to* ***100 normal controls,*** *we have* ***found an increased relative frequency,*** *associated* ***only*** *to* ***BH antigenic subunit in the disease group*** *(X^ = 6.524,* ***p < 0.05).*** *No additional* ***significant*** *correlation* ***have been*** *determined in other antigenic subunits.*

Key words: H L A system Hodgkin disease

ÖZET

HLA antijenlerinin bazdan ile, değişik hastalık gruplarının birlikte görülme sıklığının gittikçe artan oranlarda bildirilmeye başlanması, lenforetiküler malignitelerde de böyle bir olasılığın bulunabileceğini düşündürmüştür.

Bu çalışma Gulhane Askeri Tıp Akademisi ve Ankara Üniversitesi Tıp Fakültesi Hematoloji-Onkoloji ve İmmünoloji Bilim ***Dallarının*** *işbirliği ile* ***gerçekleştirilmiştir. İki*** *grubun aldığı sonuçlar birleştirilerek,* ***istatistik!*** *anlamlılık her bir HLA sublokusu* ***için ki-kare*** *testi ile belirlenmiştir.*

Çalışmaya her iki hastanede tedavi *gören* ***39 Hodgkin*** *hastası alınmıştır. Bu hastaların her* ***birinin HLA*** *yapılan* ***mikrolenfositotoksisite*** *yöntemi* ***ile belirlenerek. Türk toplumumun antijenik profilini*** *yansıtan* ***100*** *kişilik* ***kontrol olgusundan elde edilen*** *değerlerle kıyastandı.*

Hodgkin *hastalısında* ***tüm antjienik*** *stıbgruplar* ***dikkate*** *alındığında,* ***39 olgunun 100*** *olağan* ***kontrol olgusu ile*** *karşılaştırılmasında,* ***sadece*** *I>S* ***antijenik*** *alt* ***tipte*** *hastalığın görülme olasılığını daha fazla bulduk < Y i, = 6.524, serbesttik derecesi:*** *1,* ***X^ = p < 0.05).*** *Diğer* ***antijenik subgruplarda ise*** *anlamlı* ***bir beraberlik tesbil edemedik (Tümünde X^ = p > 0.05).***

Bg subgrubunda Hodgkin hastalığının rölütif görülme sıklığı İse bu *sonuçlardan* ***sonra % 2.96 olarak hesaplandı.***

Anahtar kelimeler: H L A sistemi, Hodgkin hastalığı

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The most polymorphic genetic system known is human so far is the H L A system which consist of a single chromosomal gene complex that codes for the major histocompatibility antigens in all vertebrate species. This gene complex which is present in varying densities in most body tissues with the exception of mature red blood cells, defines the major genetic differences between individuals and is primarily responsible for the recipient's immune response to graft antigens.

H L A antigens are glycoproteins bound to the cell membrane surfaces, and are coded by the genes located in a specific chromosomal segment. This segment is called "major histocompatibility complex" and is a portion of the short arm of the C_6 human chromosome. There are at least four loci within the H L A complex. These are coded by the capital letters of A, B, C and D (1,2,4,5,8,9).

The H L A gene complex consists of several series

of paired alleles which are inherited in a dominant fashion. These are coded as HLA-Aj, HLA-B27 etc. There are numerous subspecificities of A and B and over five C locus specificities known. Antigens which are tentatively accepted by WHO workshop are coded by the letter W after the locus designation (example HLA-D$_{w2}$). Recent studies in mouse species, strongly suggest that the competence of immune response to specific antigens is also controlled by the genes which are most likely located in the same chromosomal segment as HLA antigens. These are called Ir (Immune Response) genes and are probably responsible from individual's resistance or susceptibility to infectious, neoplastic and autoimmune diseases (2, 5, 8, 9, 11). 5 subzones of Ir were identified in murine species (A, B, C, E and J Loci). Helper t-cells are expressed by A sublocus, whereas supressive t-cells are controlled by Jsublocus(2,8,9,12).

When human HLA-A, B and C antigens are injected to another individual or species, cytotoxic complement binding antibodies are produced in response to this antigenic stimulation. Naturally, they can be identified serologically by using lymphocytotoxic test, platelet complement binding or leucoagglutination tests. These antigens associated with loci A, B and C are therefore referred to as SD (serologically determined) antigens (2,8, 9,11).

The antigens governed by locus D (HLA-D), are best identified by a mixed lymphocyte culture reaction (MLR). Principal of the test is the activation of the lymphocytes, thus, the antigen is ofter termed lymphocyte activating determinant (LAD) (2, 8, 9, 11)-

The antigen termed HLA-DR is closely related to but not located on the same D-locus. This antigen is present on B lymphocytes and can be identified by surface double antibody staining techniques (2, 4, 5, 8,9,11).

HLA antigens are polypeptid structures em bedded on the cell membrane surface. Serologically determined antigens (HLA-A, B and C) are composed of two polypeptid chains. The longer heavy chains with 40.000 daltons molecular weight represent the antigenic property. The short chain with 11.800 dalton molecular weight is linked to the heavy chain with a non-covellent bond. This light chain is termed beta-2 microglobulin (8,9).

D-locus antigens (HLA-D) are present in the cytoplasmic membrane having two glycoprotein ch:ins of 29.000 and 34.000 daltons and lacking beta-2 microglobulin subunit (8, 9).

MATERIALS and METHOD

This multicentric study have been conducted with the cooperation of Giilhane Military Academy and University of Ankara Medical School, Departments of Hematology-Oncology and Immunology. 39 cases of Hodgkin's Disease, 22 male and 17 female patients were included in the study, with the average age of 37 ranging from 15 to 56 for male and 43 (22-64) for female cases. 100 healthy individuals were selected randomly as the normal control group. The well recognised method of microlymphocytotoxicity were used for HLA antigenic determination.

1 ul of lymphocyte suspensions prepared from heparinized blood samples, were placed into the wells on Terasaki plates, which contained antilymphocytic antibody and settled for 30 minutes in the room temperature. 5 ul of rabbit complement were added thereafter and the mixture remained in the room temperature for 60 more minutes. 3 ul of %5 eosine solution 8 ul 35-40% formaldehyte at pH 7 were added consecutively into each well, 2 minutes apart. Plates were examined under the inverted phase-contrast microscope. Cytolytic dead cells stained dark and, bright live cells were readly distinguished in the wells. The minimum of 60 percent dead lymphocytes in a well were considered positive for that specific antigenic subunit.

RESULTS

Table -1 depicts the distribution of HLA antigens in 39 patients in comparison with 100 randomly selected healthy subjects as the control group, which we assume to reflect the antigenic profile of the Turkish population.

DISCUSSION

After the striking discovery of Immune Response genes in the contexture of major histocompatibility complex, and, from circumstantial evidence that a number of disease processes, including autoimmune and inflammatory disorders, are positively associated with certain HLA antigens, the search for such associations has also been focused on human malignancies, among which, Hodgkin Disease attracted much attention with its specific immunological determinants (3, 6, 7,10,12).

The most striking and consistent association is the increased frequency of HLA-B$_{27}$ in ankylosing spondylitis (80 to 90 percent of the cases) (2, 5,8,9, 11, 12). Lower degree but clear-cut associations with HLA phenotypes are also reported for myasthenia gravis, gluten enteropathy, psoriasis, dermatitis herpetiformis, chronic active hepatitis, juvenile diabetes mellitus and Addison's disease (2,5,8, 9,11, 12).

Most frequent disease associations with the HLA complex have been described for B and DR locus antigens. Diseases which were shown to have high degree correlation with HLA-D and DR locus antigens

Table -1

HLA Antigenic Distribution in Hodgkin Disease and Normal Controls

HLA Antigens	PATIENTS Positive Cases	PATIENTS % Frequence	CONTROLS Positive Cases	CONTROLS % Frequence	Statistical Significance
A l	11	28.20	41	41	p> 0.05
A_2	17	43.58	52	52	p> 0.05
A_3	5	12.82	12	12	p> 0.05
A_9	13	33.33	38	38	p> 0.05
^10	–	–	1	1	–
A l l	–	–	3	3	
^25	6	15.38	17	17	p> 0.05
^26	11	28.20	35	35	p> 0.05
^28	15	38.46	42	42	p> 0.05
^29	1	2.56	1	1	p> 0.05
A_w23	2	5.16	5	5	p> 0.05
Bö	15	38.46	40	40	p> 0.05
B7	5	12.82	20	20	p> 0.05
B8 (*)	8	2051	8	8	p <0.05 (*
B_{12}	5	12.82	12	12	p> 0.05
B1S	8	20.51	15	15	p> 0.05
»15	2	5.12	6	6	p> 0.05
B17	2	5.12	3	3	p> 0.05
B_{27}	5	12.82	25	25	p> 0.05
B37	2	5.12	7	7	p> 0.05
B40	3	7.69	13	13	p> 0.05
B_w4	14	35.89	47	47	p> 0.05
Bw22,1	1	2.56	1	1	p> 0.05
B_{14}	4	10.25	21	21	p> 0.05
Bw21	2	5.12	10	10	p> 0.05
B_6	2	5.12	–	–	–
Bw44	1	2.56	7	7	p> 0.05
Bw35	11	28.20	27	27	p> 0.05
B_w56	7	17.94	9	9	p> 0.05
C_{w2}	5	12.82	6	6	p> 0.05
cw3	4	10.25	8	8	p> 0.05
C_w4	17	43.58	39	39	p> 0.05

were also known to have immunological abnormalities in their pathogenesis, and more frequently seen in females (2,8,9,11,12).

Supported by aforementioned evidence, it is not surprising to find out that different phenotypes of HLA gene complex may mark susceptibility to leukemias and Hodgkin lymphomas. The HLA antigenic system in patients with a known disease which shows a wide spectrum of clinical course and prognosis in different individuals, drives curiosity of many researchers.

In 1973, Bodmer and his co-workers reported the relative frequency of B_5, B_{15}, Bjg, B_{w35} antigens, which are also termed as 4c combined antigen, in Hodgkin's Disease (3). The data is insufficient however, as to which subunit showed the highest degree of correlation. Later, the analysis of Copenhagen HLA and Disease Association Recording Center, revealed an increased frequency of association with B_5 and B_{18} antigens in Hodgkin's Disease (7).

In 1975, Svejgaard and his colleagues reported Aj and B_8 subunit association in Hodgkin lymphoma (10). Falk and Osoba claimed that A_1 and Bs antigenic carriers run a better prognostic course in Hodg-

kin Disease (6). However, retrospective analysis of the recording center in Denmark disclosed contrary results, revealing even poor prognosis with B_8 association (7).

Greene and his co-workers reported the familial tendency of Hodgkin Disease and emphasized the relative frequency of the disease in B_{w35} and B_{w37} antigenic carriers. Thusfar no circumstantial evidence has been discovered as to the presence of a single HLA related factor coding for the susceptibility or resistance to the disease.

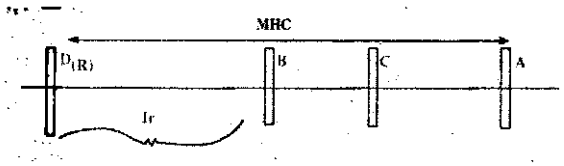

Figure -1. *HLA region and sites of gene loci on chromosome six.*

In our HLA typing and Hodgkin Disease association survey, comparing 39 patients to 100 normal controls, we have found a 2.96% relative frequency linked to B_8 subunit in the disease group ($X^2 = 6.524$, $X^2 = 3{,}841$, $p < 0.05$).

Conclusively, a limited number of surveys investigating the association of HLA antigens and susceptibility to Hodgkin's Disease have been inconclusive thusfar, with conflicting reports from different study groups. Although immune response genes linked to H-2 complex in experimental animals is well recognised, the data with regard to the mechanism of genetic resistance to cancer remains insufficient. It is likely that the development of more precise typing systems searching the D and related regions may realise the immune surveillance against neoplastic disease.

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