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CLINICAL STUDY

Distribution of HLA antigens in breast cancer

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Abstract: Investigation of various tumor-specific markers has a critical role in early diagnosis and treatment of breast cancer. The aim of the this study is to investigate the Human Leukocyte Antigen (HLA) alleles, the molecules that play an important role in immunity and tumor response of the body, and its relationship with breast cancer. In this prospective clinical study, after obtaining approval from the ethics committee of Istanbul University Experimental Medical Research Institute, 22 female patients who have been hospitalized in Istanbul University Cerrahpasa Faculty of Medicine the Department of General Surgery with a diagnosis of breast cancer were selected. In the control group, there were 22 healthy women who had no relationship and were donor candidates for renal transplantation. After collecting blood in 5 ml tubes with EDTA, HLA A, B and DR groups were measured with SSP method using the GenoVision Olerup SSP (Olerup SSP, Stockholm, Sweden) kit in Istanbul University Cerrahpasa Faculty of Medicine Blood Center Tissue Type Determination Laboratory. In patient and control group, totally 53 alleles; 17 alleles of HLA-A gene, 22 alleles of B gene, 14 alleles of DR gene were detected. A statistically significant relationship was determined between HLA-B55:01 and HLA-DRb1*18:01 alleles and the development of breast cancer ($p<0.05$). HLA-B13:01 antigen is determined only in the control group. It was concluded that HLA-B13:01 antigen, determined only in the control group, may be protective for breast cancer and HLA-B55:01 and HLA-DRb1*18:01 antigens, determined only in the patient group, may be a risk factor for breast cancer *(Tab. 5, Ref. 22)*. Full Text in PDF *www.elis.sk.*
Key words: HLA antigens, breast cancer.

Cancer is a serious public health problem in developed countries. According to the World Health Organization's (WHO) data, 12 % of the 56 million deaths are in consequence of identified malignant tumors worldwide in 2000. 5.3 million men and 4.7 million women had malignant tumors and 6.2 million people died as a result of disease (1). Breast cancer is the most common type of cancer in women according to 2002 data. The second most common cause of cancer death in women is breast cancer after cervical cancer in developing countries, and the most common is breast cancer in developed countries (2). Pathogenesis of breast cancer is multifactorial. Breast cancer is thought to develop with genetic predisposition, environmental factors, hormones and even interaction with infectious agents. Today it is known that all species contain a gene part called MHC (Major Histocompatibility Complex), regulating antigen recognition and encoding cell surface glycoprotein by T lymphocytes (3). According to the research HLA (Human Leukocyte Antigen) system, a component of MHC, is not only an important noncompliance factor in transplantation but is also effective in vital biological events such as the immunological recognition, immune response and its relationship with disease. HLA alleles especially play major role in cellular immunity and may be important for the development of breast cancer genetically (3, 4). The relationship between HLA and various pathological disorders have been previously reported. The aim of this study is to demonstrate the association of breast cancer with HLA.

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Materials and methods

After obtaining approval from Istanbul University Institute of Experimental Medical Research Ethics Committee, 22 female patients with breast cancer diagnosis and average age of 48.2, hospitalized at Istanbul University Cerrahpasa Medical Faculty, Department of General Surgery for operations, were selected as the patient group. As the control group 22 healthy unrelated women, with assigned tissue groups because of being donor candidates for renal transplantation and a mean age of 45 were selected. Blood samples were collected in 5 ml tubes with EDTA, and were analyzed at Istanbul University Cerrahpasa Medical Faculty Blood Center Tissue Typing Laboratory. HLA typing was performed using GenoVision Olerup SSP kit (Olerup SSP, Stockholm, Sweden). HLA A, B and DR groups were measured.

Tab. 1. Detected HLA- A alleles.

	Study		Control		p	OR	CI (%95)
	n	%	N	%			
A01:01	6	27,3	3	13,6	0,262	2,37	0,51–11,04
A02:01	9	40	9	40	0,1	0,3	0,3–3,32
A03:01	3	13,6	6	27,3	0,262	0,42	0,09–1,95
A11:01	5	22,7	1	4,5	0,79	6,17	0,65–58,03
A19:01	3	13,6	0	–	0,073	–	0,98–1,36
A23:01	2	9,1	0	–	0,148	–	0,96–1,255
A24:01	6	27,3	6	27,3	1	1	0,26–3,76
A26:01	2	9,1	3	13,6	0,635	0,63	0,09–4,21
A28:01	2	9,1	0	–	0,148	–	0,96–1,25
A29:01	1	4,5	1	4,5	1	1	0,06–17,06
A30:01	1	4,5	2	9,1	0,55	0,47	0,40–5,67
A32:01	3	13,6	3	13,6	1	1	0,17–5,59
A33:01	0	–	2	9,1	0,148	–	0,79–1,03
A68:01	1	4,5	2	9,1	0,55	0,476	0,4–5,67

Tab. 2. Detected HLA- B alleles.

	Study		Control		p	OR	CI(%95)
	n	%	N	%			
B04:01	1	4,5	1	4,5	1	1	0,06–17,06
B05:01	0	–	1	4,5	0,312	–	0,87–1,04
B07:01	2	9,1	2	9,1	1	1	0,12–7,81
B08:01	3	13,6	3	13,6	1	1	0,17–5,59
B13:01	0	–	4	18,2	0,036	–	0,67–0,99
B14:01	0	–	2	9,1	0,148	0,909	0,79–1,03
B15:01	0	–	2	9,1	0,148	–	0,79–1,03
B17:01	1	4,5	0	–	0,312	–	0,95–1,14
B18:01	1	4,5	4	18,2	0,154	0,214	0,02–2,09
B21:01	1	4,5	0	–	0,312	–	0,95–1,14
B55:01	4	18,2	0	–	0,036	–	1–1,48
B27:01	4	18,2	1	4,5	0,154	4,667	0,47–45,6
B38:01	2	9,1	2	9,1	1	1	0,12–7,81
B44:01	6	27,3	5	18,2	0,472	1,688	0,4–7,07
B50:01	1	4,5	0	–	0,312	–	0,95–1,14
B51:01	4	18,2	3	13,6	0,68	1,407	0,27–7,18
B52:01	0	–	2	9,1	0,148	–	0,79–1,03
B53:01	0	–	3	13,6	0,073	–	0,73–1,02

Tissue typing with PCR-SSP methods

3 ml of blood samples were taken from patients and donors sent from other clinics for HLA-A, B and DR typing, and were put into tubes with EDTA. DNA isolation from the stored blood samples was made with standard protocols (5). DNA purity was calculated spectrophotometrically at 260/280 nm wave length, and its concentration was regulated. Twenty-three different primary for HLA-A, 48 for HLA-B, 23 for HLA-DR were used. PCR materials may be received and prepared separately or may be provided as partially ready on commercial basis.

After PCR reaction was installed, it was placed in the thermal cycle device. PCR reaction consists of 3 different phases:

1) DNA denaturation,
2) Matching the primaries,
3) Replication with the Taq polymerase.

This third phase consisted of different temperatures and is counted as 1 cycle. Reaction was terminated after 25–30 cycles. The obtained PCR products were forwarded in a 2 % agarose gel electrophoresis (1XTBE or 0.5 XTBE**).** Then tissue groups were determined by photographing in UV transilluminator. Results were written by grouping according to the allelic or serological numbering system. Internal controls were always checked for proper working (6). Olerup et al have determined that genomic HLA-A, B, C, DR typing is much more accurate and sensitive (99.2 %) than serological methods (6–8).

Statistical analysis

Statistical analysis was performed by using SPSS on Windows computer program. HLA's of patient and control group were compared using Chi square test and Odd's Ratio (OR) that were calculated for HLA's, having difference according to the $p<0.05$ significance level.

Results

In the present study, patient group consisted of 22 women with breast cancer; control group consisted of 22 healthy women. The age of women in patient group ranged between 28 and 79 years and the mean age was 48.2. The age of women in study group

Tab. 3. Detected HLA- DR alleles.

	Study		Control		p	OR	CI(%95)
	n	%	N	%			
DRB1*0101	1	4,5	3	13,6	0,294	0,302	0,03-3,15
DRB1*02:01	1	4,5	0	-	0,312	-	0,95-1,14
DRB1*03:01	1	4,5	4	18,2	0,154	0,214	0,02-2,09
DRB1*04:01	6	27,3	7	31,8	0,741	0,804	0,21-2,94
DRB1*0701	2	9,1	3	13,6	0,635	0,633	0,09-4,21
DRB1*08:01	2	9,1	1	4,5	0,550	2,1	0,17-25
DRB1*11:01	10	45,5	6	27,3	0,210	2,222	0,63-7,82
DRB1*13:01	4	18,2	6	27,3	0,472	0,593	0,14-2,48
DRB1*15:01	6	27,3	3	4,5	0,262	2,375	0,51-11
DRB1*16:01	1	4,5	5	22,7	0,079	0,162	0,01-1,52
DRB1*18:01	5	22,7	0	-	0,018	-	1,03 - 1,62

Tab. 4. HLA antigens, statistically significant.

	Breast Cancer	Control	p<0,05
HLA- B13:01	0	4	0,036
HLA- B55:01	4	0	0,036
HLA- DRB1*18:01	5	0	0,018

ranged between 23 and 60 years and the mean age was 45 years. Pathologies of all patients in the study group were invasive ductal breast cancer. Women in the control group were healthy donor candidates for renal transplantation. All the sub-groups of HLA A, B, DR genes were examined in Blood Center Tissue Type Determination Laboratory in Istanbul University Cerrahpasa Medical School (Tabs 1, 2 and 3). In the study group; 14 alleles of HLA-A gene, 15 alleles of HLA-B gene, 13 alleles of HLA-DR gene were determined. In the control group; 12 alleles of HLA-A gene, 16 alleles, of HLA-B gene, 12 alleles of HLA-DR gene were determined. In both groups; 17 alleles of A gene, 22 alleles of B gene and 14 alleles of DR gene, total of 53 alleles, were detected. HLA-B13:01, was not detected in the patient group, but was detected in 4 women in the control group. The difference was statistically significant ($p < 0.05$). Reliance interval was determined as 0.67–0.99. This antigen was detected only in the control group and was thought to have a protective activity. In patient group, HLA-B55:01 was detected in 4 women and HLA-DRB1*18:01 was also in 4 women. Both antigens were detected in no subject in the control group. Reliance interval of antigens, determined statistically significant ($p<0.05$), was 1–1.48 for HLA-B55:01 and 1.03–1.62 for HLA-DRB1*18:01 (Tab. 4).

Discussion

Breast cancer has a tendency of familial accumulation, suggesting that genetic factors might have a role in the etiology (9). There are many studies emphasizing the relationship between HLA system and some diseases. The aim of the present study was to establish the relationship between HLA antigens and breast cancer (10–13). Several studies (Tab. 5), investigating the relationship between breast cancer and HLA were examined since 1970s (10–14). Patel et al and Iaffaioli et al in their studies determined that there was a significant relationship between HLA B7 and breast cancer and the common characteristics of those who carry these antigens were premenopausal status, the lack of hormone receptors and primary tumors with high histological grade (14–15). In studies of Boulinelle et al, a statistically significant relationship was defined between breast cancer and HLA-A28 and identified that this antigen was common in postmenopausal, especially nulliparous, women (16). Emekcioglu et al determined that HLA-B7, B17, Bw50 and DR4 were associated with breast cancer (4). Casole et al detected a significant relationship between breast cancer and HLA-B7 and DR4 (17), Lavado detected this relationship only with HLA-B7 (18). HLA-DRB1*1101, DQB1*03032 alleles were determined to be protective against breast cancer by Chaudhuri et al (10). Ghaderi et al defined that the frequency of 12.allele on HLA DRB1 was significantly higher (11). Harrath et al established a reverse link among HLA- DRB1*07 and HLA- DRB1*02 alleles and breast cancer (12).

MHC expression of the tumor has been thought to be effective in response to treatment during, before or after immunotherapy. In

Tab. 5. HLA antigens, associated with breast cancer.

HLA antigen	Authors	Association with HLA
HLA B7	Patel (14) and İaffaioli (15)	Determined significant association
HLA A28	Boulinelle et al. (16)	Determined significant association. Especially with postmenapause and nullipars
HLA B7, B17, Bw50 and DR4	Emekcioglu et al (4)	Determined association with breast cancer
HLA B7, HLA DR4	Casoli C. Et al. (17)	Determined significant correlation
HLA B7	Lavado et al. (18)	Determined significant correlation
HLA DRB1, DQB1	Chaudhuri S. et al. (10)	DRB1*1101, DQB1*03032 allelles were determined to be protective for breast
HLA DRB1	Ghaderi A. et al. (11)	12.allele frequency was detected significantly higher in HLA DR B1
HLA DRB1	Harrath AB. et al. (12)	HLA DRB1*07 and HLA DRB1*02 allelles were determined to have a reverse link with breast cancer
HLA class I	Madjd Z. et al. (13)	Total loss of MHC class I was defined to be an independent marker for good prognosis in breast cancer

some studies HLA Class I expression was examined in premalignant lesions or in tissues with high-risk for tumor formation. Thus, in the familial and sporadic colonic carcinogenesis, HLA antigens were found to decrease in the normal mucosa close to adenoma as well as in adenomas (19). Madjd et al determined in their study in women with breast cancer that total loss of MHC class I was an independent marker for good prognosis in breast cancer (13). Effect of hormones might have a role on the expression of HLA antigens and effects the regulation of the behavior of tumor indirectly. In fact, HLA Class I expression is induced in MCF-7 cell lines after the addition of estrogen into the place (20, 21). However Redondo et al. could not define a significant relationship between estrogen receptors and HLA Class I antigens (22). In the present study HLA typing is planned in patients with breast cancer and in healthy donor candidates for renal transplantation. As a result, HLA-B55:01 and HLA-DRB1*18:01 were determined significantly higher in the patient group. It was thought that both HLA antigens cause predisposition for breast cancer, and HLA-B13:01 antigen, detected significantly higher in control group, provides protective activity. The results of the present study do not comprise a common antigen with other studies in the literature, but the reason seems to be because of the low number of patients and lack of studies. When the results were evaluated, HLA DR18 and HLA-B55:01 positive people, having other risk factors, are at risk for breast cancer and they should be considered for examination and follow-up should be planned.

Conclusion

Early diagnosis and treatment of breast cancer is evolving rapidly with advancing technology and progress in the science of genetics. However, breast cancer is still the most common cause of cancer death of women in developed countries. So HLA allele frequencies of different populations, at risk for breast cancer, and various sub-groups of HLA should be investigated for assessing the important alleles, specified as risk factors of development of breast cancer.

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