Article is available online at http://www.webio.hu/por/2002/8/1/0058

ARTICLE

HLA-DQBl Alleles and Susceptibility to Cervical Squamous Cell Carcinoma in Southern Iranian Patients

Alamtaj Samsami DEHAGHANI,[1] Ali AMIRZARGAR,[2] Shirin FARJADIAN,[2] Abbas GHADERI[2]

Departments of [1]Obstetrics and Gynecology and [2]Immunology, Shiraz University of Medical Sciences, Medical School, Shiraz, Iran

The association of HLA class II with various autoimmune diseases has been extensively investigated. Despite the importance and functions of HLA genes in the evolution of cancer, the allele specific association of HLA molecules in cancer patients has not been well investigated. In this study the HLA-class II alleles frequency was investigated in Iranian patients with cervical squamous cell carcinoma. HLA typing was carried out by PCR amplification using sequence specific primers (PCR-SSP). DRB1, DQA1 and DQB1 typing was performed for 23 patients. The allele frequencies were calculated and compared with 36 healthy Iranian female controls. A positive association was observed between the existence of HLA-DQB1*0601 and squamous cell carcinoma of the cervix (p<0.04, RR=1.94). Moreover, analysis of HLA-DRB1, DQA1 and DQB1 haplotypes indicated that none of the putative haplotypes were significantly associated with either patient or control group. Positive association of cervical carcinoma with a single allele of HLA-DQ provides evidence on the importance of HLA class II molecules and the immune response in squamous cell carcinoma of cervix. (Pathology Oncology Research Vol 8, No 1, 58–61, 2002)

Keywords: cervix carcinoma, HLA-DQ, PCR-SSP

Introduction

HLA class I molecules are reported to be expressed by normal, premalignant and a percentage of malignant lesions of cervical epithelium.[l] HLA class II molecules are ectopically expressed in 16-40% of the epithelial ovarian cancers, 25% of endometrial carcinomas and 20% of cervical cancers.[2] An increase in relative risk for squamous cell carcinoma of cervix associated with HLA-DQw3 allele and a significant decrease associated with HLA-DR6 have been reported in Caucasians.[3] However, other studies showed no statistically significant association of any HLA-A, B, C, DR or DQ serologically defined antigens with this carcinoma.[4] Recent findings indicate an increase in relative risk for cervical cancer in African-American women with HLA-DQB1*0303 and DQB1*0604 alleles.[5] The importance of HLA-DQB1*03 and DQB1*06 alleles in cervical carcinoma and/or cervical intraepithelial neoplasia has been disclosed by other investigators in Japanese,[6] Belgian,[7] Tanzanian[8] and British Caucasian women.[9] The present investigation was undertaken to assess the distribution of HLA-DRBl, -DQA1 and -DQB1 alleles and the significance of these alleles in cervical epithelial cell carcinoma among Iranian women.

Received: April 27, 2001; *accepted:* Jan 15, 2002

Correspondence: Dr. Abbas GHADERI, Department of Immunology, Shiraz University of Medical Sciences, Shiraz-Iran, P.O. BOX: 71345-1798. Tel/fax: 0098-711-2334589,
Email: ghaderia@sums.ac.ir

Materials and Methods

Patients and subjects

Twenty-three patients with pathologically proven cervical epithelial cell carcinoma were referred to our laboratory from Tumor Clinic of Shiraz Nemazi Medical Center, Shiraz-Iran. Thirty-six control subjects were randomly selected among female volunteers who attended the Shiraz regional blood transfusion center for routine blood donation. Ten ml of venous blood with EDTA, as anticoagulant, was collected from each patient.

Genomic DNA extraction

Genomic DNA was extracted according to a modified methods of Graham and Miller.[10,11] Blood was transferred to a 50 ml tube and cold red cell lysis buffer I, containing 0.144 M NH_4Cl and 1mM $NaHC0_3$ was added to the final volume of 50 ml. The mixture was centrifuged for 5 min at 2400 x g. Then the supernatant was discarded and the pellet was homogenized by vortexing. This step was repeated until almost all red cells were lysed. The pellet was homogenized and 10 ml of red cell lysis buffer II, containing 0.3 M sucrose, 10 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$ and 1% Triton X-100 were added. The pellet was treated with 4.5 ml of WBC lysis buffer containing 75 µM NaCl and 24 µM Na-EDTA. Subsequently, 125 µl of 10% SDS and 1 ml of 5 M $NaC1O_4$ were added. The mixture was vortexed for 10 seconds and 2 ml of 6 M NaCl were added and vortexed again. The mixture was centrifuged for 5 min at 1500 x g. The supernatant was carefully transferred into a clean 50 ml tube and 7 ml absolute isopropanol were added and mixed gently.

The precipitated DNA was removed. The DNA was washed twice in 1.5 ml of 70% Ethanol. The pellet was dried at room temperature and resuspended in 100-500 µl of double distilled water (ddH20) depending on the yield of the extracted DNA.

Table 1. **HLA-DRB1* allele frequencies in 23 patients with squamous cell carcinoma of the cervix in comparison with 36 normal female subjects**

DRB1	*frequency*	*%*	*frequency*	*%*	*p*
0101	3	6.5	2	2.8	NS
0102	0	0	0	0	NS
1500	8	17.4	5	6.9	NS
1600	5	10.9	5	6.9	NS
0300	0	0	5	6.9	NS
1800	0	0	0	0	NS
0400	6	13	7	9.7	NS
0700	5	10.9	8	11.1	NS
0800	0	0	3	4.2	NS
0900	1	2.2	3	4.2	NS
1001	2	4.3	2	2.8	NS
1101/04	10	21.7	21	29.2	NS
1200	0	0	1	1.4	NS
1301	3	6.5	4	5.6	NS
1302	1	2.2	0	0	NS
1401	2	4.3	5	6.9	NS
1402	0	0	1	1.4	NS

Table 2. **HLA-DQA1* allele frequencies in 23 patients with squamous cell carcinoma of the cervix in comparison with 36 normal female subjects**

DQA1	*frequency*	*%*	*frequency*	*%*	*p*
0101	3	6.5	3	4.2	NS
0102	6	13	9	12.5	NS
0103	6	13	5	6.9	NS
0104	4	8.7	7	9.7	NS
0201	4	8.7	11	15.3	NS
0301/02	8	17.4	6	8.4	NS
0401	0	0	3	4.2	NS
0501/02	13	28.3	28	38.9	NS
0503	2	4.3	0	0	NS

Table 3. **HLA-DQB* allele frequencies in 23 patients with squamous cell carcinoma of the cervix in comparison with 36 normal female subjects**

DQB1	*frequency*	*%*	*frequency*	*%*	*RR*	*P*
0201	6	13	17	23.6		NS
0301/04	17	37	28	38.9		NS
0401/02	0	0	4	5.6		NS
0501/04	12	26	14	19.4		NS
0601	7	15.2	3	4.2	1.94	0.04
0602/03	2	4.4	5	6.9		NS
0604/06	2	4.4	1	1.4		NS

HLA-Class II typing

HLA-DRB1, DQA1 and DQB1 typing were performed by PCR-SSP as previously described.[12] DNA was amplified using 35 PCR reactions for each individual (eighteen for assigning DRB1, eight for DQA1, eight for DQB1 alleles and a negative control) Each reaction was performed in a total volume of 20 µl containing: 17 µl PCR mixture (50 mM KCI, 1 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 0.001% (w/v) gelatin, 200 µM of each dNTPs, 1 µM of specific primers and 0.2 µM of the control primers), 1 µl template DNA and 2 µl Taq DNA polymerase (0.5 U/µl). The mixture was covered with 30 µl of mineral oil and subjected to 30 cycles amplification. Each cycle consisted of denaturation at 94°C for 30 seconds, annealing at 55°C for one minute and extension at 72°C for one minute. The extension was continued for a further 5 minute at 72°C.

PCR products were electrophoresed on 1.5 % agarose gel containing 0.5 µg/ml ethidium bromide and the presence of the specific DNA bands were analyzed under UV light. For HLA subtyping the PCR-SSP method was used as described by Zetterquist and Olerup.[l3]

Statistical analysis

Chi-square test was performed using the EPI INFO version 6.04 statistical software to analyze the observed data.

Results

The frequency of HLA-DRB1, DQB1 and DQA1 in Iranian patients with cervical epithelial cell carcinoma was investigated using PCR-SSP. The results were compared with thirty-six healthy Iranian control subjects (*Tables 1-3*). As indicated, the HLA-DRB1*11, HLA-DQBl*0301/04, and HLA-DQA1* 0501/02 were the most frequent alleles in the patient group. The HLA-DRB1*11, HLA-DQB1 *0301/04 and HLA-DQA1*0501/02 alleles were also represented as the most frequent alleles in the control group. The results revealed a significant association of HLA-DQB1*0601 and epithelial cell carcinoma of cervix (p=0.04, RR=1.94). The haplotype analysis showed that HLA-DRB1*1101/04, DQB1*0301/04, DQA1*O5Ol/02 was the most frequent haplotype in both patient and control groups (Table 4). However, no significant difference was detected between patient and control groups regarding the haplotype frequencies.

Discussion

The frequencies of HLA-DRB1, -DQB1 and -DQA1 alleles were investigated in Iranian patients with cervical epithelial cell carcinoma and results were compared with control subjects.

Statistical analysis of results indicated that there is a positive association between HLA-DQB1*0601 allele and cervical carcinoma in the study group. In 1995, Beck et al[14] reported a strong negative association with antibody titer against SPF66 (a malaria synthetic vaccine) in individuals possessing HLA-DQB1*0601, and recently a positive association of HLA-DQB1*0601 with the susceptibility to cardiac sarcoidosis has been observed.[l5] Results of other investigations indicate an increase in relative risk for cervical cancer and HLA-DQw3 in Caucasians and a decrease in the frequency of the HLA-DR6 in these patients has also been documented.[3] In one study, a highly significant increase in HLA-B7 antigen (p=0.0083) and a decrease in HLA-All, A28, and B12 (p=0.04, p=0.03) in patients affected with ovarian carcinoma has been noticed. [16] In contrast, there are reports indicating lack of association of HLA-A, B, C, DR or DQ with these diseases.[4] The association of HLA-DQB1 *0604 and risk of cervical carcinoma in African-American women has already been reported by others.[5] Since the frequency of HLA alleles (especially allelic subtypes) varies in different normal populations, it is not unexpected to find different significant alleles in different populations. The association of HLA-DQB1*03 with cervical carcinoma in different Caucasian populations has been extensively reported.[3,17,18,19] It is noteworthy that the frequency of HLA-DQB1*03 alleles is already high among normal Caucasian populations (http://histo.chu-stlouis.fr/inserm/stats/bm30114.htm; http://histo.chustlouis.fr/inserm/stats/bm30117.htm, http://histo.chu-stlouis.fr/inserm/statslbm 30122.htm). In a previous study we observed a much higher frequency of HLA-DQB1 *0301 (31.0%) compared to HLA-DQB1 *0601 (7.0%) among 100 Iranian normal subjects.[20] In this regard, the increased frequency of HLA-DQB1*0601 allele among Iranian cervical cancer patients and the similar detection of an increased frequency of HLA-DQB1*06 subtypes in Tanzanian[8] and African-American[5] populations points to the importance of this allele in the development of cervical cancer. Although the etiology of cervical carcinoma is not fully understood, there are a few reports providing evidence that cervical carcinoma is found more fregnently in those subjects with a familial history of cervical cancer[21] suggesting that a genetic background may be required for the development of this tumor. In addition, it has been indicated that cervical cancer is resulted after persistent infection by human papilloma viruses (HPV).[22] In this respect, presence or absence of certain HLA alleles may influence the effectiveness of the immune response against virus. Currently, the involvement of HPV in patients with cervical cancer from southern Iran

Table 4. **The observed HIA-DRB1*, DQA1* and DQB1* haplotypes frequencies in patients with squamous cell carcinoma of cervix in comparison with 36 normal female subjects**

Haplotypes	*P*	*N=23*	*C*	*n=36*	*P*
	F	*%#*	*F*	*%&*	
DRBl*1101/04,DQB1*0301/04,DQA1*0501/02	8	17.4	21	29.2	NS
DRBl*04,DQB1*0201,DQA1*0301/02	1	2.2	2	2.8	NS
DRBl*17,DQB1*0201,DQA1*0501/02	0	0	4	5.6	NS
DRBl*15,DQB1*0601/02,DQA1*0102	1	2.2	0	0	NS
DRBl*16;DQB1*0501/04,DQA1*0102	4	8.7	4	5.6	NS
DRB1*08,DQB1*0402,DQA1*0401	0	0	2	2.8	NS
DRBl*1301,DQB1*0604/06,DQA1*0102	0	0	0	0	NS
DRBl*1401,DQB1*0501/04,DQA1*0104	2	4.3	5	6.9	NS

P= Patients=23; F= Frequency; C= Controls=36; # Percentage of haplotype in all 46 potentially present haplotypes; & Percentage of haplotype in all 72 potentially present haplotypes

possessing HLA-DQB1*0601 is under investigation. Although the low incidence rate of cervical cancer in southern Iran (0.71 % per 100000),[23] has hampered study of a large group of patients, results of this study may provide information for further clarification of the etiology of cervical carcinoma in this region.

Acknowledgement

The authors would like to thank Dr. Mehrnoosh Doroudchi for critical reviewing of the manuscript.

References

1. *Torres LM, Cabrera T, Concha A, et al:* HLA class I expression and HPV-16 sequences in premalignant and malignant lesions of the cervix. Tissue Antigens 41:65-71, 1993.
2. *Reid R:* Immunology and Immunotherapy. In: practical Gynecology Oncology. 1st Edition. Williams and Wilkins Co, 1989.
3. *Wank R, Thomssen C:* High risk of squamous cell carcinoma of the cervix for women with HLA-DQw3. Nature 352:723-725, 1991.
4. *Glew SS, Stern PL, Davidson JA, Dyers PA:* HLA antigens and cervical carcinoma. Nature 356:22, 1992.
5. *Gregoire L, Lawrence WD, Kukuruga D, et al:* Association between HLA-DQB 1 alleles and risk for cervical cancer in African-American women. Int J Cancer 57:504-507, 1994.
6. *Nawa A, Nishiyama Y, Kobayashi T, et al:* Association of human leukocyte antigen-DQB1*03 with cervical cancer in Japanese women aged 35 years and younger. Cancer 75:518-521, 1995.
7. *Vandenvelde C, De Foor M, Van Beers D:* Precision about the association between cervical carcinoma and HLA-DQBl*03 alleles. Lancet 342:553, 1993.
8. *Wank R, Meulen JT, Luande J, et al:* Cervical intraepithelial neoplasia, cervical carcinoma and risk for patients with HLA-DQB1*0602, *0301, *0303 alleles. Lancet 341:1215, 1993.
9. *Odunsi K, Terry G, Ho L, et al:* Susceptibility to human papillomavirus-associated cervical intra-epithelial neoplasia is determined by specific HLA-DR-DQ alleles. Int J Cancer 67:595-602, 1996.
10. *Graham DE:* The isolation of high molecular weight DNA from whole organisms or tissue masses. Anal Biochem 85:609-613, 1978.
11. *Miller SA, Dykes DD, Polesky HE:* A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215-1221, 1989.
12. *Olerup O, Zetterquist H:* HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological DR-typing in clinical practice including donor-recipient matching in cadaveric Transplantation. Tissue Antigens 39:225-235, 1992.
13. *Zetterquest H, Olerup O:* Identification of the HLA-DRB1*04, DRB1*07 and DRB1*09 alleles by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours. Hum Immunol 34:64-74, 1992.
14. *Beck HP, Felger I, Barker M, et al:* Evidence of HLA-class II association with antibody response against the malaria vaccine SPF66 in a naturally exposed population. Am J Trop Med Hyg 53:284-288, 1995.
15. *Naruse TK, Matsuzawa Y, Ota M, et al:* HLA-DQB1*0601 is primarily associated with the susceptibility to cardiac sarcoidosis. Tissue Antigens 56:52-57, 2000.
16. *Illeni MT, Pasquali M, La Monica G, et al:* HLA antigens in ovarian adenocarcinoma patients. Eur J Gynecol Oncol 6:121-125, 1985.
17. *Montoya L, Saiz I, Rey G, et al:* Cervical carcinoma: human papillomavirus infection and HLA-associated risk factors in the Spanish population. Eur J Immunogenet 25:329-37, 1998.
18. *Hamsikova E, Sramkova I, Loudova M, et al:* The frequency of HLA-DRB 1 and -DQB 1 alleles in Cervical Cancer cases in the Czech Republic. Folia Biol 45:31-34, 1999.
19. *Neuman RJ, Huettner PC, Li L, et al:* Association between DQB 1 and cervical cancer in patients with human papillomavirus and family controls. Obstet Gynecol 95:134-140, 2000.
20. *Amirzargar A, Mytilineos J, Farjadian Sh, et al:* HLA Class-II allele frequencies and haplotype association in Iranian normal population. Hum Immunol 62:1234-1238, 2001.
21. *Furgyik S, Grubb R, Kullander S, et al:* Familial occurrence of cervical cancer, stage 0-IV. Acta Obstet Gynecol Scand 65:223-227, 1986.
22. *zur Hausen H, Schneider A:* The role of papillomaviruses in human anogenital cancer. In: The papovaviridae: the papillomaviruses, eds. Salzman NP, and Howley PM, Vol: 2, pp. 245-263, Plenum Press, New York, 1987.
23. *Farahmandbeigi M:* The incidence rate of registered cancer in Fars province. Disease Control Unit, Shiraz University Press, Iran, 2000.