Original Article

STUDY ON HLA-DQB1*03 ALLELE ASSOCIATED WITH WOMEN HUMAN PAPILLOMA VIRUS (HPV) LESIONS

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ABSTRACT

Objectives: The specific HLA alleles associated with HPV differ among various study groups. It has been suggested that women carrying HLA-DQw3 antigen encoded by DQB 1*03, are predisposed to develop cervical cancer. The aim of this study was to determine HLA-DQB1*03 alleles in HPV lesions.

Methodology: In a cross-sectional and analytical study, Hundred women enrolled into the study. Twenty of them had abnormal Pap smear, Fifty patients had condyloma lesions and 30 patients with malignant lesions. HLA–DQB*03 and HPV infection was evaluated by PCR.

Results: PCR of HPV was positive in 50 women with condyloma lesions and 8 women with malignant lesion (p< 0.001). HLA–DQB*03 was positive in 9 women with abnormal Pap smear , 18 women with condyloma lesions and 7 women with malignant lesion(P= 0.261). Positive PCR of HPV was significantly higher in women with condyloma lesions than other women (p< 0.001). Mean age of patients with positive PCR of HPV was significantly lower than patients with negative PCR of HPV (P= 0.001). Mean age of patients with positive HLA–DQB*03 was 42.08 + 11.48 year and mean age of patients with negative HLA–DQB*03 Was 43.83 +11.21 year (P= 0.462). HLA–DQB*03 was positive in 34% of patients.

Conclusion: In this study, we do not see any significant correlation between HPV infection and HLA–DQB*03 in the studied women.

KEY WORDS: HLA-DQB1*03, Human Papilloma Virus, Polymerase Chain Reaction, kondilomaei lesions, Cervical Cancer, Pap smear.

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INTRODUCTTON

Although it is likely that genetic susceptibility plays a role in the development of most human cancers, evidence support-ing this view hitherto has been obtained in only a small fraction of patients who typically carry germ-line mutations in tumor suppressor genes. In addition to the widely recognized role of acquired alterations in oncogenes and tumor suppressor genes, considerable evidence exists to suggest that the immune system might play a protective role in tumorigenesis. Although immune

surveillance is believed to be involved in the elimination of tumors,[1] immunotherapeutic approaches to human cancer by and large have proved unsuccessful.

T cell responses are dependent on the inheritance of specific alleles of the highly polymorphic HLA class I and class II genes. Although weak associations of specific HLA alleles with tumors of viral origin have been described,[2-3] the rele-vance of MHC polymorphisms to the broader category of spontaneous non viral human tumors remains to be estab-lished. Somatic alterations in many tumors can contribute to the down-regulation of HLA class I gene expression in tumor cells.[4] These alterations potentially could contribute to immune evasion and might represent a discrete event in the multistep paradigm of tumorigenesis. Although HLA class I genes are expressed in all cells, immune responses also require the presentation of antigenic peptides to T cells by HLA class II molecules. These heterodimers primarily are ex-pressed by professional antigen-presenting cells such as macrophages, dendritic cells, and B lymphocytes. Somatic alterations in tumor cells cannot influence the expression of HLA class II genes in dendritic cells and other professional antigen-presenting cells. If, indeed, immune surveillance is important during tumorigenesis, certain individuals who in-herit specific alleles of the highly polymorphic HLA class II DPB, DQB, or DRB genes might be resistant to specific types of cancers.[5]

There are contradictory reports regarding the relationship between polymorphic HLA genes and cervical cancer. Although various reports suggest a close link between certain HLA haplotypes and cervical cancer, the specific HLA alleles associated with it differ among various study groups. It has been suggested that women carrying HLA DQw3 antigen encoded by DQB 1*03, are predisposed to develop cervical cancer.[6] While several reports have been put forward in support of this finding[7-8], there are con-tradictory reports that show no such association.[9-10]

The aim of this study was determine HLA-DQB1*03 alleles in HPV lesions.

METHODOLOGY

In a cross-sectional and analytical study, 100 women enrolled in to the study. Twenty of them had abnormal Pap smear, 50 patients with condyloma maei lesions and 30 patients with malignant lesions. All women underwent Pap Smear and cervical colposcopy. HLA–DQB*03 and HPV infection was evaluated by PCR.

Swabs are put in PBS solution wherein and the cells are dissolved. The suspension of the cells are centrifuged, the cellular plate resolved in a proper buffer in 250 ìl volume and the samples are stored in -20^{oc} temperature, until enough number of samples are collected. Condyloma samples are taken by biopsy are sent to the lab in a suitable buffer. Once sufficient samples are collected, they are brought out of freezer. They melt by lysis buffers and what is present in the sample are digested adding the K protease and incubation at 55^{o}c temperature for one hour. Then K protease is deactivated in 95^{oc} via incubation and then it is used in PCR transaction by a proper amount. Primer planning on L1 part of late Region and E part of early region is applied to operate PCR. We used RFLP method to determine genotype of the virus, so that product of PCR was cut by proper enzymes and the size of obtained pieces was determined via measurement in Electroformed jelly compared to DNA marker. At the end, genotype of the virus was determined in comparison to the model of the pieces detained from enzymic digestion by specified enzymes. The samples with positive HPV, genotype of which has been proved, were investigated and compared with HLA-DQB1*O3. HLA alleles in disasters were determined by its specific primes using PCR. PCR-SSP method can be used to detect HLA-DQB10302 Allele. In this method, specific primers of sequence are used. In the case of HLA-DQB10302 Allele the below primes were used:

Sense primer: Primer sequence: 5'-GTGCGT CTTGTGACCAGATA-3'
Annealing position: 70-89
Antisense primer:
Primer sequence: 5'-CTGTTCCAGTACTC GGCGG-3'

Annealing position: 170-188
Product of PCR was a bond with amplion size 118 bp.

Studied variables were PCR of HPV, PCR of HLA–DQB*03, Age, Chief Complaint, Vulva finding, Vaginal examination finding and Colposcopyic finding.

Statistical analysis: All information was entered into computer and SPSS version 11.5 was used and Independent- Samples T test and Chi-Square test were used for analysis of data. $p<0.05$.

RESULTS

Twenty Women with abnormal Pap smear, 50 women with condyloma lesions and 30 women with malignant lesion were enrolled into the study. PCR of HPV was positive in 50 women with condyloma lesions and eight women with malignant lesion. Positive PCR of HPV was significantly higher in women with condyloma lesions than other women ($p< 0.001$).

HLA–DQB*03 was positive in nine women with abnormal Pap smear, 18 women with condyloma lesions and seven women with malignant lesion. Results of HLA–DQB*03 wasn't significantly different between three groups of women (R= 0.261). Mean age of patients with positive PCR of HPV was significantly lower than patients with negative PCR of HPV (P= 0.001). Mean age of patients with positive HLA–DQB*03 was 42.08 + 11.48 year and mean age of patients with negative HLA–DQB*03 Was 43.83 +11.21 year (P= 0.462).

Chief complaint of women in three groups is shown in Table-I. Vulva finding of women in three groups is shown in Table-II. Vaginal examination finding of women in three groups are shown in Table-III while colposcopyic finding of women in three groups are shown in Table-IV.

Table I: Chief Complaint of women in three groups

Chief Complaint	*Groups*		
	Malignant lesions	*Condyloma lesions*	*Abnormal Pap smear*
Vulva itching	0	8	4
Vaginal Discharge	4	8	0
Vaginal Infection	1	1	1
Vulva Lesions	4	8	0
AUB	14	7	10
Hypogastrium Pain	5	7	2
Post Coital Bleeding	1	7	1
Spoting	1	2	1
Dysmenorrhea	0	3	0
Uterine Prolapse	0	0	2

DISCUSSION

Cervical cancer is a preventable disease. While waiting for clinically applicable vaccination programs, strategies to prevent cervical cancer include 1) improved screening covering the widest possible population and using HPV testing, 2) close management and follow-up of women with precancerous lesions.[11]

Host genetic background seems to play a key role in cervical carcinogenesis as only a small subset of women infected with high-risk human papilloma viruses (HPVs) develop cervical cancer.[12] Infection with human papilloma virus (HPV) is the main cause of cervical cancer and its precursor lesion, cervical intraepithelial neoplasia (CIN).[13]

Cervical cancer is strongly associated with infection by oncogenic forms of human papilloma virus (HPV)[14] which is known to play a central role in the development of cervical cancer. Both host and viral genetic factors have been postulated to be important determinants of risk

Table II: Vulva finding of women in three groups

Vulva finding	*Groups*		
	Malignant lesions	*Condyloma lesions*	*Abnormal Pap smear*
Normal	20	32	14
Atrophic	5	4	5
Wart	3	14	0
Dystrophy	1	0	0
Tumor	1	0	0

of HPV progression to neoplasia among infected individuals.[15]

Human papilloma virus infection is an important aetiological agent associated with the development of cervical neoplasia.[16] Human papilloma virus (HPV)-induced malignancies are frequently infiltrated by lymphocytes.[17]

Human leukocyte antigen (HLA) class II alleles have been associated with an increased or decreased risk of developing cervical cancer through infection with oncogenic forms of human papilloma virus (HPV).[18] HLA II DQB1 polymorphisms have been shown to associate with cervical cancer risk, but results vary among different populations.[19] The role of human l eukocyte antigen (HLA) DQB1 alleles and human papilloma virus (HPV) as contributing factors to invasive cervical cancer was investigated.[20]

Certain HLA class II haplotypes (such as DRB1*1501-DQB1*0602) were associated significantly, while DR13 haplotypes were negatively associated with cervical carcinoma. These associations are HPV16-type specific. These results suggest that specific HLA class II haplotypes may influence the immune response to specific HPV-encoded epitopes and affect the risk of cervical neoplasia.[3] These results of Neuman RJ and colleague suggest that the DQB1*0303 allele increases the risk for invasive cervical cancer in women who are HPV-positive.[20]

In the study of Lie AK and colleague the DQBI*0301 allele was significantly more prevalent in CIN III than in CIN II cases. The lesions in two women recurred in the follow-up period, one of whom was carrying the DQB1*0301 allele. Women carrying the HLA-DQB1*0301 allele have an increased risk of developing CIN when infected by HPV 16, although there was not an increased frequency of recurrent disease among women carrying this allele.[21] In contrast, DRB1*1501 alone and in combination with DQB1*0602 was not significantly elevated in cancers overall, but did show some excess in HPV16-positive cancers (2P = 0.05), associated with HPV16-positive cervical cancers.[22]

HLA–DQB*03 was positive in nine women with abnormal Pap smear, 18 women with condyloma lesions and seven women with malignant lesion and HLA–DQB*03 was negative in 11 women with abnormal Pap smear, 32 women with condyloma lesions and 23 women with malignant lesion.

Results of HLA–DQB*03 wasn't significantly different between three groups of women (R= 0.261).

HLA DRB1*13 was associated with cumulative risk of HPV infections (odds ratio [OR], 1.7 [95% confidence interval {CI}, 1.0-2.8]), for oncogenic HPV (OR, 1.6 [95% CI, 0.9-2.8]), and for HPV-16 (OR, 2.0 [95% CI, 0.9-4.4]). DQB1*03 was consistently associated with a lower cumulative risk of HPV infections, but this association was not statistically significant. None of the alleles affected the risk of HPV persistence.[23]

PCR of HPV was positive in 50 women with Kondiloma lesions and eight women with

Table III: Vaginal examination finding of women in three groups

Vaginal finding	*Groups*		
	Malignant lesions	*Condyloma lesions*	*Abnormal Pap smear*
Normal	21	34	14
Inflammation	4	9	5
Wart	2	6	1
Erosive Wart	1	0	0
Tumor	3	0	0

Table IV: Colposcopyic finding of women in three groups

Vaginal finding	*Groups*		
	Malignant lesions	*Condyloma lesions*	*Abnormal Pap smear*
Not performed	4	0	1
Normal	9	18	12
Acetowhite	10	28	5
Coarse Punctuation	6	0	1
Fine Punctuation	1	2	0
Unsatisfactory	0	1	1
Fine Mosaic	0	1	0

malignant lesion and was negative in 20 women with abnormal Pap smear and 27 women with malignant lesion. Positive PCR of HPV was significantly higher in women with Kondiloma lesions than other women (p< 0.001).

A significant association was observed between DQB1 * 03 and CIN (odds ratio 3.03; 95% CI 2.11-3.45). Of women with CIN, 131/178 (73.5%) had HPV (types 16, 18, 31, or 33) infection. There was a significant association between DQB1 * 03 and presence of HPV (odds ratio 3.43; 95% CI 2.25-5.10). Homozygosity for DQB1 * 03 was more strongly associated with CIN than heterozygosity (odds ratios 4.0 and 2.63, respectively); and for the presence of HPV (odds ratio 4.47; 95% CI 2.58-7.77). HLA DQB1 * 0301 was the most strongly associated allele with CIN and HPV (odds ratios 2.53 and 2.63, respectively).[24] Significant association was not found between HLA DQB1 * 0301 and CIN in the studied women (P=0.099).

The DQBI*0301 allele was significantly more prevalent in CIN III than in CIN II cases. The lesions in two women recurred in the follow-up period, one of whom was carrying the DQB1*0301 allele. Women carrying the HLA-DQB1*0301 allele have an increased risk of developing CIN when infected by HPV 16, although there was not an increased frequency of recurrent disease among women carrying this allele.[21] In our study, 19 of women had CIN-III and only seven of them were positive DQB1*0301 but it was not significant. In contrast, DRB1*1501 alone and in combination with DQB1*0602 was not significantly elevated in cancers overall, but did show some excess in HPV16-positive cancers (2P = 0.05), associated with HPV16-positive cervical cancers.[22] Results of our study were the same.

CONCLUSION

Positive PCR of HPV was significantly higher in women with condyloma lesions than other women (p< 0.001). Results of HLA–DQB*03 wasn't significantly different between three groups of women (R= 0.261). Mean age of patients with positive PCR of HPV was significantly lower than patients with negative PCR of HPV (P= 0.001). Mean age of patients with positive HLA–DQB*03 was 42.08 + 11.48 year and mean age of patients with negative HLA–DQB*03 was 43.83 +11.21 year (P= 0.462). HLA–DQB*03 was positive in 34% of patients. In our study, we did not see any significant correlation between HPV infection and HLA–DQB*03 in the studied women.

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