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Evaluation of human leukocyte antigen class-II DRB1 & DQB1 and its association with cervical carcinoma

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Abstract: *Background:* Cervical Carcinoma is the second most leading carcinoma following breast cancer among Indian women. It has been showed that HLA class II DQ is associated in persistent HPV infection and HLA class II DR is responsible for increased clearance of HPV virus from cervix. *Methodology:* In this study we had included total 96 women (53 women in the control, 31 women in CIN group, and 12 women in cervical carcinoma (CC) group. *Result:* Our study showed that HLA DRB1 was associated with the healthy and cervical intraepithelial neoplasia population; whereas HLA DQB1 was associated with the invasive cervical carcinoma. *Conclusion:* HLA DRB1 and HLA DQB1 is useful tool for community based screening and focused HPV vaccination among the risk group to prevent the invasive cervical cancer.

Keywords: Cervical, CIN, Carcinoma, Pap Smear, HLA Class II.

Introduction

Cervical Carcinoma is the second most leading carcinoma following breast cancer among women in India and fourth most common in world [1]. Approximately 122,844 women suffered from cervical cancer and 67,477 died in the year 2017 and 85% among them belong to the low socioeconomic group in India [2]. One of the major causative factors of cervical carcinoma is human papilloma virus (HPV). More than 200 strains of HPV can infect human and 45 serotypes are identified as high risk types and among these types HPV 16, HPV 18 are mostly associated with cervical carcinoma in more than 70% cases [3]. About 90% HPV infections are cleared within two year from the entry of virus in the cervical epithelium. Persistent HPV infections by expressing E6/E7 oncogenes are responsible for development of cervical cytological abnormalities to cervical carcinoma [4].

Several factors may be responsible for the developing HPV infection like early age of coitus, early first child birth, poor hygiene, multiple sexual partners, smoking, but immune tolerance is an upcoming issue [5]. Human leukocyte antigen (HLA) which is present on the chromosome 6p21, encodes for major histocompatibility complex (MHC) proteins for immune modulation. HLA can be classified as class I and class II. Association of HLA Class I molecule and autoimmune diseases has been well cited in many studies [6]. Recently HLA Class II molecule and its loci (DP, DQ, and DR) are emerging as prognostic markers in different carcinoma like colorectal carcinoma, prostate carcinoma, melanoma and in many more [7-9]. It has been shown that HLA class II DQ is associated in persistent HPV infection and HLA class II DR is responsible for increased clearance of HPV virus from cervical epithelium [10-11]. The aim of the study was early identification of such women who may sustain HPV infection to reduce drastically the incidence of cervical carcinoma by focused HPV vaccination among the risk group women.

Material and Methods

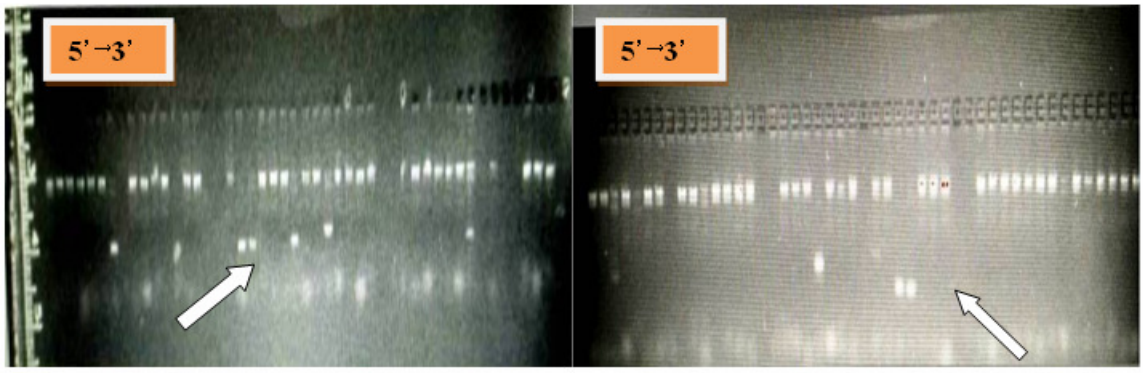
The study has been conducted at the gynecology department in collaboration with

the pathology at IPGMER, Kolkata and College of Medicine & JNM Hospital, WBUHS, Kalyani. The ethical clearance was obtained from the institutional ethics committee (No Inst/IEC/2015/ 217 dated 17.12.2015). The patients of reproductive age group within 15-45 years, married, having at least one child, without any obvious medical complications, and no history of any malignancy were included in the study after obtaining signed informed consent. Women below 15 years or above 45 years, unmarried, married but having no child, suffered from any malignancy, family history of genital malignancy in first degree relative were excluded from the study.

All women were examined for exfoliative cervical cytology by papanicolaou staining, and if no cytological abnormality found they were included in control group. Abnormal cervical cytology was included in cervical intraepithelial neoplasia (CIN) and classified into CIN I, CIN II, CIN III, CIS (carcinoma in situ). We have found 53 women in the control, 31 women in CIN group, and four women in cervical carcinoma (CC) group. Another 8 women already diagnosed with cervical carcinoma and receiving radiotherapy were also included. About 5 ml from antecubital vein was collected in EDTA tube from every woman in our study. After collecting the blood was centrifuged in room temperature for 2 min and plasma discarded. After that it was preserved at -40^{0}C refrigerator for future analysis.

Procedure of HLA typing: The HLA typing was carried out by SSP (sequence specific primer) method. Different markers were used like RING3, D6S26, 6SL00, MICA for analysis of HLA II molecule particularly the DRB1 and DQB1 loci. The forward primer (5'→3') were CCCGATACATAGACATAGG, AATGGATGCTGCATGAGG, CCTCACCCGATACATAGACA, CCTTTTTTTCAGGGAAAGTG and reverse primer (5'→3') were TACCGAAATAAGGCCTCC, TAACCCGAAAGTCCAGCTCTC, AGAAATACCGAAATAAGG, CTTACCATCTCCAGAAACTG for the markers RING3, D6S26, 6SL00, MICA respectively. The four markers were used for all the control, CIN, and CC groups (Figure 1).

Fig-1: PCR amplification using HLA specific locus D6S26 (for primers used please see Table 2 & 3). Lanes showing from lowest to highest bands were of sizes. The band intensities visibly vary indicating that different samples had different alleles.



Panel A – Showing the PCR primer control in the upper lanes and different HLA alleles DRB1 among the healthy populations.
Panel B - Showing the PCR primer control in the upper lanes and different HLA alleles DQB1 among the cervical carcinoma group.
PCR cycle - Initial denaturation: 95°C for 5 minutes
Denaturation 94°C for 30 sec
Annealing: 62°C for 35 Sec
Extension 72°C for 40Sec
Number cycles: 14

Followed by 25 cycles
Denaturation 94°C for 30 Sec
Annealing 55°C for 30 Sec
Extension: 72 °C for 40 Sec

Amplified product of a PCR-sequence-specific primer (SSP) method is shown in above figure

Results

In our study we have analyzed total 96 women who participated in the study. Duration of marriage (years) was significantly higher in CC group (p=< 0.05) and age at marriage (years), age at first child birth were significantly lower in CC group (p=< 0.05) in comparison to control and CIN groups. Laboratory analysis of blood parameters showed that haemoglobin was significantly lower in CC group (p=< 0.05) than the control and CIN groups. Total leukocyte count (per cu.mm), platelet count (per cu.mm), serum urea (mg/dl) and serum creatinine (mg/dl) were comparable between the groups. Whereas erythrocyte sedimentation rate ESR (mm/hr. in first hour) was significantly higher in CC group (p=< 0.05) than control and CIN groups (Table 1).

Table-1: Baseline demographic and laboratory parameters among three groups

Parameters	Control (n=53) (Mean ± SD)	CIN (n=31) (Mean ± SD)	CC (n=12) (Mean ± SD)
Age (years)	35.42 ± 6.48	34.92 ± 4.65	34.56 ± 7.65
BMI (kg/m^2)	26.41 ± 5.23	25.78 ± 6.12	25.94 ± 4.86
Duration of Marriage (years)	5.9 ± 2.93*	6.1 ± 2.72#	9.4 ± 4.34*#
Age at Marriage (years)	26.92 ± 6.54*	25.32 ± 5.32#	20.82 ± 7.38*#
Age at First Child (years)	28.34 ± 5.63*	28.85 ± 4.84#	21.16 ± 5.67*#
Parity (Number)	2.3 ± 1.2	2.5 ± 1.6	2.8 ± 1.3
Haemoglobin (gm./dl)	11.1 ± 0.63*	10.6 ± 0.81#	8.2 ± 0.62*#
Total Leucocyte Count (per cu.mm)	6479.3 ± 2122.7	6670.6 ± 1526.8	6852.4 ± 1723.5
Platelet count (per cu.mm)	208476 ± 11453.5	208548 ± 10751.7	210548 ± 10352.6
ESR (mm/hr. in1st hr)	35.72 ± 12.21*	36.16 ± 11.89#	48.86 ± 12.76*#
Serum Urea (mg/dl)	26.81 ± 2.75	27.36 ± 3.57	28.12 ± 3.47
Serum Creatinine (mg/dl)	0.92± 0.32	0.88 ± 0.47	1.1 ± 0.52

Control- Healthy population, CIN- Cervical Intraepithelial Neoplasia, CC- Cervical Carcinoma

p < 0.05 is considered as significant in comparison to control group * and CIN group # respectively. Analysis of variance (ANOVA) was used to calculate p values.

Only DRB1 Allele 0105 of RING3 gene was common between normal population and cervical intraepithelial neoplasia (CIN) group (Table 2). Among others allele 0109 and 0457 were associated with the RING3 marker of HLA DRB1 in CIN-I group; and allele 0309 of same marker CIN-II group. Allele 0509 and 0607 of HLA DRB1 associated with D6S26 were found in CIN-I group and allele 1101 and 1107 were found in CIN-II and CIN-III groups respectively (Table 2). Allele 0481 and 0708 of 6SL001 marker were seen in CIN-I and allele 1509 in CIN-II groups respectively. Allele 0839, 0702 and 0937 of MICA markers were associated with CIN-I, CIN-II and CIN-III groups respectively (Table 2).

HLA DQB1 loci were consistent with the every patient and different alleles of different markers were found (Table 3). Alleles 1103, 0309 and 1304 of RING3 marker in 3 patients; allele 1607, 1704, 1405 and 0406 of D6S26 in 5 patients; allele 1107, 1501 of 6SL001 marker and 0708, 1809 allele of MICA were found in each single patient (total 12 patients) among the CC group (Table 3).

Table-2: Analysis of HLA allele in normal population (n=53) and Cervical Intraepithelial Neoplasia (CIN) (n=31) group

Markers*	Forward primer (5'→3')	Reverse primer (5'→3')	Allele** *DRB1*	Control group + (n=53)	CIN-I group+ (n=19)	CIN-II group+ (n=10)	CIN-III group+ (n=2)
RING3	CCCGATACATAGACATAGG	TACCGAAATAAGGCCTCC	0103	4 (7.5%)	-	-	-
			0105	5 (9.4%)	3 (9.7%)	-	-
			0107	3 (5.7%)	-	-	-
			0109	-	4 (12.9%)	-	-
			0209	2 (3.8%)	-	-	-
			0304	2 (3.8%)	-	-	-
			0307	3 (5.7%)	-	-	-
			0309	-	-	2 (6.5%)	-
			0457	-	1 (3.15%)	-	-
D6S26	AATGGATGCTGCATGAGG	TAACCCGAAAGTCCAGCTCTC	0509	-	2 (6.5%)	-	-
			0605	2 (3.8%)		-	-
			0607		3 (9.7%)	-	-
			0704	3 (5.7%)	-	-	-
			0407	2 (3.8%)	-	-	-
			0904	2 (3.8%)	-	-	-
			0321	3 (5.7%)	-	-	-
			1101	-	-	3 (9.7%)	-
			1107	-	-	-	1 (3.15%)
			0404	2 (3.8%)	-	-	-
6SL001	CCTCACCCGATACATAGACA	AGAAATACCGAAATAAGG	0109	3 (5.7%)	-	-	-
			0507	4 (7.5%)	-	-	-
			0481	-	3 (9.7%)	-	-
			1509	-	-	3 (9.7%)	-
			0708	-	2 (6.5%)	-	-
			1608	4 (7.5%)	-	-	-
			0706	2 (3.8%)	-	-	-
MICA	CCTTTTTTTCAGGGAAAGTG	CTTACCATCTCCAGAAACTG	1708	2 (3.8%)	-	-	-
			0806	1 (1.75%)	-	-	-
			0702	-	-	2 (6.5%)	-
			0839	-	1 (3.15%)	-	-
			0937	-	-	-	1 (3.15%)
			0901	1 (1.75%)	-	-	-
			0922	3 (5.7%)	-	-	-

+Prevalence among the group was noted as percentage

Table-3: Analysis of HLA allele in Cervical carcinoma (CC) population (n=12)				
Markers**	**Forward primer (5'→3')**	**Reverse primer (5'→3')**	**Allele*** *DQB1***	**Control group + (n=12)**
RING3	CCCGATACATAGACATAGG	TACCGAAATAAGGCCTCC	1103	1 (8.33%)
			0309	1 (8.33%)
			1304	1(8.33%)
D6S26	AATGGATGCTGCATGAGG	TAACCCGAAAGTCCAGCTCTC	1607	1 (8.33%)
			1704	2 (16.7%)
			1405	1 (8.33%)
			0406	1 (8.33%)
6SL001	CCTCACCCGATACATAGACA	AGAAATACCGAAATAAGG	1107	1(8.33%)
			1501	1 (8.33%)
MICA	CCTTTTTTTCAGGGAAAGTG	CTTACCATCTCCAGAAACTG	0708	1 (8.33%)
			1809	1 (8.33%)
+Prevalence among the group was noted as percentage				

Discussion

Human leukocyte antigen (HLA) is a major molecule which takes an important part in immune system. Autoimmune diseases and its relation with HLA for diagnosing and predicting are already established in various studies [12]. Ankylosing spondylosis is diagnosed with the HLA B 27 positivity in the patients who are suffering from bilateral sacroillitis and also predict the prognosis [13]. Likewise the use of HLA molecule in diagnosing and predicting the malignancy is also coming in as an emerging issue.

HLA typing can be done by either serological based (from WBC) or molecular based (DNA) method. Molecular based method can be done by PCR-sequence-specific primer (SSP) method and PCR-sequence-specific oligonucleotide (SSO) probing method. A total 48 cervical cancer patient with the age matched 47 healthy control showed strong susceptible association with the HLA DQB1*0601 in a study performed in South India by the PCR-SSP (sequence-specific primer) method. Analysis of amino acid variation of susceptible allele was diagnosed tyrosine in the beta-9 and beta-37 positions. This tyrosine and non-tyrosine combination increases the risk of cervical carcinoma. A three locus haplotype A*11-B*7-DRB1*04 firstly reported in a study in relation with the susceptible to develop cervical carcinoma [14].

One study involving 80 cervical cancer patients compared with another 80 healthy population among Uyghur women showed the association of HLA DQB1 with the preponderance to develop cervical carcinoma. Total 296 alleles were identified among the 160 women. HLA-DQB1*0325 and HLA-DQB1*0332 were discovered as the cervical cancer susceptible genes [15]. In another study 137 patient with diagnosed cervical carcinoma from stage IIB to IVB were compared with the 175 healthy populations. HLA-DQB1*02 and HLA-DQB1*03 were frequently associated with the HPV 16 positive advanced cervical carcinoma [16].

Chronic HPV infection who fails to clear the virus from the cervical epithelium may have some inherent inability. In a pilot study involving 172 women in Mexico, HLA-DQB1*0501 allele was associated with the susceptibility of HPV re-infection and HLA-

DRB1*14 was reduced in cervical carcinoma group in comparison with the HPV persistent group [17]. Another study involving the North Indian women showed that the HLA B*07:05, B*35:03 and B*40:06 were found in association with the decreased risk for persistent HPV infection and developing cervical carcinoma. HLA-B*08, B*37 and B*58 were associated with the increased risk for chronic HPV infection and cervical carcinoma [18]. Moreover a four locus haplotype A*11-B*07-C*01-DRB1*04 is being discovered in Germany which may be a very specific marker in future for community based screening to identify the high risk women for developing persistent HPV infection and subsequently cervical carcinoma [19].

Conclusion

In our study HLA DRB1 was associated with the healthy and cervical intraepithelial neoplasia population; whereas HLA DQB1 was associated with the invasive cervical carcinoma. It may be useful for community based screening and focused HPV vaccination among the risk group to prevent the invasive cervical cancer. Cost is an issue in our system but if more we can work we may overcome it in future.

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Conflicts of interest: There are no conflicts of interest.

References

1. Tripathi N, Kadam YR, Dhobale RV, Gore AD. Barriers for early detection of cancer amongst Indian rural women. *South Asian J Cancer* 2014; 3:122-127.
2. Bruni L, Barrionuevo-Rosas L, Albero G, Serrano B, Mena M, and Sanjose S et al. ICO Information Centre on HPV and Cancer (HPV Information Centre). *Human Papillomavirus and Related Diseases in India*. Summary Report 30 June 2017. [Date Accessed]
3. Kulkarni SS, Kulkarni SS, Vastrad PP, et al. Prevalence and distribution of high risk human papillomavirus (HPV) Types 16 and 18 in Carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. *Asian Pac J Cancer Prev.* 2011; 12(3):645-648.
4. Fontecha N, Basaras M, Hernáez S, Andía D and Cisterna R. Assessment of human papillomavirus E6/E7 oncogene expression as cervical disease biomarker. *BMC Cancer* 2016; 16:852.
5. Song D, Li H, Li H, Dai J. Effect of human papillomavirus infection on the immune system and its role in the course of cervical cancer. *Oncol Lett.* 2015; 10(2):600-606.
6. Miyadera H and Tokunaga K. Associations of human leukocyte antigens with autoimmune diseases: challenges in identifying the mechanism. *Journal of Human Genetics* 2015; 60: 697-702.
7. Sconocchia G, Eppenberger-Castori S, Zlobec I, et al. HLA Class II Antigen Expression in Colorectal Carcinoma Tumors as a Favorable Prognostic Marker. *Neoplasia (New York, NY).* 2014; 16(1):31-42.
8. Doonan BP, Haque A. Prostate Cancer Immunotherapy: Exploiting the HLA Class II Pathway in Vaccine Design. *J of Clinical & Cellular Immunology* 2015; 6(4):351.
9. Donia M, Andersen R, Kjeldsen JW et al. Aberrant Expression of MHC Class II in Melanoma Attracts Inflammatory Tumor-Specific CD4þ TCells, Which Dampen CD8þ T-cell Antitumor Reactivity. *Cancer Res* 2015; 75(18):1-13.
10. Bernal-Silva S, Granados J, Gorodezky C et al. HLA-DRB1 Class II antigen level alleles are associated with persistent HPV infection in Mexican women; a pilot study. *Infectious Agents and Cancer.* 2013; 8:31.
11. Hu B, Tao N, Zeng F, Zhao M, Qiu L, and Wu X et al. A risk evaluation model of cervical cancer based on etiology and human leukocyte antigen allele susceptibility. *Int J of Infectious Diseases* 2014; 28:8-12.
12. Ravindranath MH. HLA Class Ia and Ib Polyreactive Anti-HLA-E IgG2a Monoclonal Antibodies (TFL-006 and TFL-007) Suppress Anti-HLA IgG Production by CD19+ B Cells and Proliferation of CD4+ T Cells While Upregulating Tregs. *Journal of Immunology Research.* 2017; 3475926.
13. Tedeschi V, Vitulano C, Cauli A et al. The Ankylosing Spondylitis-Associated HLA-B*2705 Presents a B*0702-Restricted EBV Epitope and Sustains the Clonal Amplification of Cytotoxic T Cells in Patients. *Molecular Medicine.* 2016; 22:215-223.
14. Rathika C, Murali V, Dhivakar M, Kamaraj R, Malini RP and Balakrishnan K. Susceptible and Protective Associations of HLA Alleles and Haplotypes with Cervical Cancer in South India. *Asian Pac J Cancer Prev.* 2016; 17(5):2491-2497.
15. Han L, Husaiyin S, Wang L, Wusainahong KD, Fu X, Niyazi M. Analysis of HLA-DQB1 allele polymorphisms in Uyghur women with cervical cancer. *Genet Mol Res.* 2015; 14(4):17252-17261.
16. Liu Y, Zhang J, Jia Z-M, Li J-C, Dong C-H, Li Y-M. The correlation between HLA-DRB1 and HLA-DQB1 gene polymorphisms and cytokines in

HPV16 infected women with advanced cervical cancer. *Int J Clin Exp Med.* 2015; 8(7): 11490-11495.
17. Bernal-Silva S, Granados J, Gorodezky C, Aláez C, Flores-Aguilar H and Barrera-Saldaña HA et al. HLA-DRB1 Class II antigen level alleles are associated with persistent HPV infection in Mexican women; a pilot study. *Infectious Agents and Cancer* 2013; 8:31.
18. Gokhale P, Kerkar S, Tongaonkar H, et al. Variations in immunogenetics, human papillomavirus (HPV) infection & predisposition to cervical cancer in Indian women. *Indian J Med Res* 2014; 140 (Supplement): 36-43.
19. Pingel J, Solloch UV, Hofmann JA et al. High-resolution HLA haplotype frequencies of stem cell donors in Germany with foreign parentage: How can they be used to improve unrelated donor searches?. *Human Immunol* 2013; 74:330-340.

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