RESEARCH ARTICLE

Susceptible and Protective Associations of HLA Alleles and Haplotypes with Cervical Cancer in South India

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Abstract

Background: Human leukocyte antigen (HLA) genes have been implicated in cervical cancer in several populations. Objectives: To study the predispositions of HLA alleles/haplotypes with cervical cancer. Materials and Methods: Clinically diagnosed and PAP smear confirmed cervical cancer patients (n 48) and age matched controls (n 47) were genotyped for HLA-A,-B,-DRB1* and DQB1* alleles by PCR-SSP methods. Results: The frequencies of alleles DRB1*04 (OR=2.57), DRB1*15 (OR=2.04), DQB1*0301 (OR=4.91), DQB1*0601 (OR=2.21), B*15 (OR=13.03) and B*07 (OR=6.23) were higher in cervical cancer patients than in the controls. The frequencies of alleles DRB1*10 (OR=0.22) and B*35 (OR=0.19) were decreased. Strong disease associations were observed for haplotypes DRB1*15-DQB1*0601 (OR=6.56; $p< 3.5\times10^{-4}$), DRB1*14-DQB1*0501 (OR=6.51; p<0.039) and A*11-B*07 (OR=3.95; p<0.005). The reduced frequencies of haplotypes DRB1*10-DQB1*0501 (OR=0.45), A*03-B*35 (OR=0.25) and A*11-B*35 (OR= 0.06) among patients suggested a protective association. HLA-C* typing of 8 patients who possessed a unique three locus haplotype 'A*11-B*07-DRB1*04' (8/48; 16.66%; OR=6.51; p<0.039) revealed the presence of a four locus haplotype 'A*11-B*07-C*01-DRB1*04' in patients (4/8; 50%). Amino acid variation analysis of susceptible allele DQB1*0601 suggested 'tyrosine' at positions β9 and β37 and tyrosine-non-tyrosine genotype combination increased the risk of cervical cancer. Conclusions: Strong susceptible associations were documented for HLA alleles B*15, B*07, DRB1*04, DRB1*15, DQB1*0301, DQB1*0601 and haplotypes DRB1*15-DQB1*0601 and DRB1*14-DQB1*0501. Further, protective associations were evidenced for alleles B*35 and DRB1*10 and haplotypes A*11-B*35 and DRB1*10-DQB1*0501 with cervical cancer in South India.

Keywords: Human leukocyte antigen - alleles/haplotypes - cervical cancer - susceptibility - protection - South India

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Introduction

Globally, cervical cancer accounted for an estimated 528,000 new cases and for 266,000 deaths (http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx). Human papillomavirus (HPV) is central to the development of cervical neoplasia and can be detected in 99.7 percent of cervical cancers (Walboomers, 1999). High-risk papilloma virus type HPV 16 and HPV 18 contribute to over 70% of all cervical cancer cases. In India, cervical cancer is the leading cause of mortality among reproductively active women, which comprised 25% of the global burden (Das et al., 2008).

Earlier studies have reported the association of HLA-DQw3 with cervical cancer in a German (Wank and Thomssen, 1991), Spanish (Montoya et al., 1998), British (Odunsi et al., 1995) and African-American (Gregoire et al., 1994) cohorts. Other HLA alleles that were proposed to have increased risk of cervical cancer include DRB1*1501 (Krul et al., 1999; Montoya et al., 1998), DRB1*04 and DRB1*11 (Odunsi et al., 1996) in different world populations. An increased risk for HPV16 associated cervical cancer was reported for the haplotypes DRB1*1501-DQB1*0602 (Apple et al., 1994), DQA1*0102-DQB1*0602 (Helland et al., 1998) and allele DRB1*0701 (Brady et al., 1999). However, a protective association was documented for the allele DRB1*1501 and haplotype DRB1*1501-DQB1*0602 with HPV16 induced invasive cervical cancer (ICC) in Han Chinese (Hu et al., 2014). A number of alleles affording protection towards cervical cancer include DRB1*1301 (Apple et al., 1994; Krul et al., 1999), DRB1*1302 (Matsumoto et al., 2015), DQB1*0501 (Odunsi et al., 1996) and DQB1*0603 (Montoya et al., 1998). A meta analysis have revealed the protective association of DQB1*02, DQB1*03 and DQB1*0603 alleles and susceptible association of

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DQB1*05, DQB1*0301 and DQB1*0402 alleles with cervical cancer patients from Europe and Asia (Zhang et al., 2015). Significant association was observed for the allele DPB1*05:01 with viral type HPV 16 infection induced squamous cell carcinoma of cervix (Yang et al., 2015). A recent GWAS in the Swedish population has identified a SNP rs3117027 located at HLA-DPB2 region as susceptible and DPB1*0402 allele as protective factors (Chen and Gyllensten, 2014). Since there is no report available on HLA profile of cervical cancer patients in south India, we carried out the present work.

Materials and Methods

Subjects

The cervical cancer patients (n, 48; mean age, 50.7 yrs) were recruited from selected private hospitals from Tiruchirappalli (Tamil Nadu, India) based on the PAP smear positivity. Age matched healthy women with no self or family history of any neoplastic disease was included (n, 47; mean age, 50.95 yrs) as controls. Three ml of peripheral blood was collected in EDTA coated vacutainer tubes. Written informed consent was obtained from all the participants and the study was approved by the Institutional Ethical Committee.

DNA isolation and PCR-SSP typing of HLA class I/ class II alleles

Genomic DNA was extracted from blood samples from patients and controls by the salting out method (Welsh and Bunce et al., 1995). HLA-A/-B/C-DRB1*and -DQB1* alleles were genotyped by PCR with sequence specific primers (SSP) (Olerup and Zietterquist, 1992; Bunce et al., 1995; Tonks et al., 1999; Scola et al., 2008). The HLA-C typing was performed only for the patients (n, 8) who were positive for a 3 locus haplotype (A*11-B*07-DRB1*04). With the protein sequence information of the susceptible HLA-DQB1* (DQB1*0601) alleles, multiple sequence alignment was performed by ClustalW. The amino acid sequence variation of β chain of HLA-DQB1* allele-encoded protein sequences were retrieved from the IMGT/HLA database (http://www.ebi.ac.uk/imgt/hla) for comparison.

Statistical analysis

Allele frequencies were calculated by direct counting and the risk association for each allele was assessed by comparing the allele frequencies between patients and controls. The odds ratios (OR), confidence intervals and P-values were calculated by the Chi-squared test or Fisher's exact test. The haplotype frequencies were determined by the maximum likelihood method using Arlequin Population Genetics Data Analysis Software (v3.11).

Results

HLA-DRB1*/DQB1* genotyping by PCR-SSP method revealed an elevated frequency of DRB1*04 (OR = 2.57; p<0.055) allele in cervical cancer patients than the controls suggesting a susceptible association. The frequency differences of remaining alleles were not statistically significant between patients and controls (Table 1). A positive association with cervical cancer was observed for DQB1*0301 (OR= 4.91; p<0.007) allele. However a weak association was observed for the allele DQB1*0601 (OR=2.21; p<0.082). A decreased allele frequencies were observed for alleles DRB1*10 (OR=0.22; p<0.003) and DQB1*0502 (OR=0.19; p<0.028) than the controls suggesting a protective

Table 3. HLA-A/B Allele Distribution among Cervical Cancer Patients and Controls

HLA- A/ B Allele	Patients (n=48)	Controls (n=47)	Disease association indices			
			OR	95%CI	X^2	P
A*01	6.25 (06)	6.38 (06)	0.98	0.25-3.79	0.000	1.000
A*02	8.33 (08)	7.44 (07)	1.13	0.33-3.93	0.000	1.000
A*03	21.87 (21)	21.27 (20)	1.05	0.43-2.56	0.0000	1.000
A*11	37.5 (36)	43.61 (41)	0.46	0.13-1.43	1.586	0.208
A*23		4.25 (04)				
A*24	3.12 (03)	1.06 (01)	2.33	0.26-77.88	0.219	0.640
A*25		2.12 (02)				
A*26	18.75 (18)	--				
A*29	--	4.25 (04)				
A*33	--	1.06 (01)				
A*68	4.16 (04)	8.51 (08)	0.47	0.10-1.80	0.932	0.334
B*07	38.54 (37)	17.02 (16)	6.23	2.42-17.96	16.134	5.9×10^{-5}
B*08						
B*15	14.58 (14)	1.06 (01)	13.03	2.39-404.71	17.103	0.0001
B*18						
B*35	10.41 (10)	29.78 (28)	0.19	0.06-0.48	13.280	2.68×10^{-4}
B*38		5.31 (05)				
B*40	14.58 (14)	20.2 (19)	0.61	0.242-1.55	0.878	0.349
B*51		7.44(07)				
B*54	4.16 (04)	10.63 (10)	0.36	0.08-1.30	3.220	0.136
B*55	9.37 (09)					
B*57	8.33 (08)	8.51 (08)	0.98	0.29-3.23	0.000	1.000

Table 1. HLA-DRB1/ DQB1*Allele Distribution among Cervical Cancer Patients and Controls

HLA-DRB1/ DQB1 alleles	Patients	Controls	Disease association indices			
	(n =48)	(n =47)	OR	95 % CI	X^2	P
DRB1*01	-	4.25 (4)				
DRB1*03	5.20 (05)	11.70 (11)	0.4	0.10-1.34	2.008	0.156
DRB1*04	20.83 (20)	10.63 (10)	2.57	0.98-7.23	3.674	0.055
DRB1*07	13.54 (13)	13.82 (13)	0.97	0.36-2.63	0	1
DRB1*08	3.12 (03)	2.12 (02)	1.4	0.19-13.60	0	1
DRB1*09	-	--				
DRB1*10	7.29 (07)	22.34 (21)	0.22	0.07-0.62	8.952	0.003
DRB1*11	6.25 (06)	2.12 (02)	2.78	0.54-24.53	1.161	0.281
DRB1*12	1.04 (01)	1.06 (01)	0.98	0.03-37.12	0	1
DRB1*13	6.25(06)	4.25 (04)	1.48	0.38-7.08	0.089	0.765
DRB1*14	8.33 (08)	5.31 (05)	1.62	0.45-6.54	0.309	0.578
DRB1*15	28.12 (27)	19.14 (18)	2.04	0.84-5.12	2.392	0.122
DRB1*16	-	3.19 (03)				
DQB1*0201	9.37 (09)	12.76 (12)	0.65	0.22-1.88	0.427	0.513
DQB1*0202	3.12 (03)	8.51 (08)	0.36	0.06-1.49	1.742	0.187
DQB1*0301	16.66 (16)	4.25 (04)	4.91	1.48-21.22	7.374	0.007
DQB1*0304	--	3.19 (03)				
DQB1*0305	-	1.06 (01)				
DQB1* 0401	-	2.12 (02)				
DQB1*03032	1.04 (01)	--				
DQB1*0501	36.45 (35)	31.91 (30)	1.51	0.59-4.00	0.536	0.464
DQB1*0502	2.08 (02)	10.63 (10)	0.19	0.02-0.86	4.844	0.028
DQB1*0601	31.25 (30)	21.27 (20)	2.21	0.91-5.58	3.032	0.082
DQB1*0602	-	4.25 (4)				

Table 2. Most Frequent HLA A-B-DR haplotypes in Cervical Cancer patients and Controls

Haplotype	Patient (n=48)	Controls (n=47)	Disease association indices			
			OR	95%CI	X^2	P
Two locus (DRB1*-DQB1*)						
DRB1*07-DQB1*0201	5.20 (05)	4.25 (04)	1.22	0.27-6.04	0.000	1.000
DRB1*04-DQB1*0501	9.37 (09)	4.25 (04)	2.32	0.63-10.52	1.330	0.249
DRB1*07-DQB1*0501	5.20 (05)	3.19 (03)	1.61	0.33-9.71	0.114	0.735
DRB1*07-DQB1*0601	2.08 (02)	4.25 (04)	0.52	0.06-3.20	0.201	0.654
DRB1*10-DQB1*0501	5.20(05)	10.63 (10)	0.45	0.12-1.54	1.369	0.242
DRB1*14-DQB1*0501	8.33 (08)	1.06 (01)	6.51	1.08-204.68	4.281	0.039
DRB1*15-DQB1*0601	22.91 (22)	5.31 (05)	6.56	2.18-22.64	12.781	3.5×10^{-4}
DRB1*15-DQB1*0501	2.08 (02)	4.25 (04)	0.52	0.06-3.20	0.201	0.654
Two locus (A-B)						
A*02-B*07	6.25 (06)	3.19 (03)	1.94	0.43-11.43	0.446	0.504
A*03-B*35	2.08 (02)	8.51 (08)	0.25	0.03-1.18	2.913	0.088
A*03-B*40	11.45 (11)	5.31 (05)	2.37	0.71-9.20	1.755	0.185
A*11-B*07	22.91 (22)	8.51 (08)	3.95	1.46-11.96	7.839	0.005
A*11-B*35	1.04 (01)	17.02 (16)	0.06	0.00-0.32	14.405	1.47×10^{-4}
A*11-B*40	1.04 (01)	6.38 (06)	0.20	0.01-1.31	2.560	0.110
Three locus (A-B-DR)						
A*11-B*07-DRB1*07	3.12 (03)	4.25 (04)	0.74	0.12-4.10	0.001	0.977
A*03-B*35- DRB1*10	1.04 (01)	3.12 (03)	0.40	0.01-3.58	0.283	0.543
A*03-B*40-DRB1*10	2.08 (02)	3.12 (03)	0.68	0.07-5.02	0.001	0.981
A*11-B*07-DRB1*04	8.33 (08)	1.06 (01)	6.51	1.08-204.68	4.281	0.039

association of these alleles in cervical cancer.

Analysis revealed a susceptible association for two locus haplotypes DRB1*15-DQB1*0601 (OR=6.56; $p<3.5\times10^{-4}$) and DRB1*14-DQB1*0501 (OR=6.16; p<0.039) in patients. DRB1*10-DQB1*0501 was observed to be the most frequent haplotype in controls suggesting a protection, however, with no statistical significance (OR=0.45; p<0.242) (Table 2).

No significant association was observed for HLA-A alleles with cervical cancer in south India (Table 3). However, analysis of HLA-B locus revealed a very strong disease association of alleles B*15 (OR=13. 03; p<0.0001) and B*07 (OR=6. 23; $p<5.9\times10^{-5}$). An increased frequency of a two-locus haplotype A*11-B*07 (OR= 3.95; p<0.005) in patients have suggested a susceptible association. Further, a three locus haplotype A*11-B*07-DRB1*04 was found in eight cervical cancer patients (n, 8/48; OR=6.51; p<0.039). More interestingly, the HLA-C*typing of these 8 patients have revealed the presence of a 4 locus extended haplotype 'A*11-B*07-

Table 4. Amino acid variations in the peptide binding pockets of the β Chain at position 9 (Pocket 6) and 37 (Pocket 9) of DQB1* allele

Pocket-6		Pocket – 9	
β9 Tyrosine	β9 Non-Tyrosine (Leucine/Phenylalanine)	β37 Tyrosine	β37 Non-Tyrosine (Isoleucine/ Aspartate)
201			0201 (Iso)
202			0202 (Iso)
301		301	
304		304	
	0401(Phy)	401	
501		501	
502		502	
	0601 (Leu)		0601 (Asp)
	0602 (Phy)	602	
305		305	
3032		3032	

Table 5. Comparison of Presence or Absence of Tyrosine at β9 and β37 positions of HLA-DQβ Chain of Patients and Controls

Allele/ Genotype	Patients (2n = 96)	Controls (2n = 94)	Disease association indices			
			OR	95 % CI	X^2	P
β9 allele						
Tyrosine	68.75 (66)	72.34(68)	0.84	0.43-1.65	0.147	0.701
Non-Tyrosine	31.25 (30)	27.65 (26)	1.19	0.61-2.33	0.147	0.701
β37 allele						
Tyrosine	56.25 (54)	74.46(70)	0.45	0.23-0.85	6.173	0.013
Non-Tyrosine	43.75 (42)	25.53 (24)	2.24	1.17-4.40	6.173	0.013
β9 genotype						
Tyrosine-Tyrosine	43.74 (21)	57.44 (27)	0.70	0.34-1.41	0.845	0.358
Tyrosine-Non-Tyrosine	50.0 (24)	19.14 (09)	3.04	1.29-7.85	6.836	0.009
Non Tyrosine-Non Tyrosine	6.24 (03)	23.40 (11)	0.27	0.05-0.99	3.940	0.047
β37 genotype						
Tyrosine-Tyrosine	33.33(16)	46.8 (22)	0.66	0.30-1.42	0.959	0.327
Tyrosine-Non-Tyrosine	45.83 (22)	21.27 (10)	2.43	1.04-6.09	4.273	0.039
Non Tyrosine-Non Tyrosine	20.83 (10)	31.91 (15)	0.62	0.24-1.55	0.837	0.306

C*01-DRB1*04' in 4 patients) 4/8; 50%. The reduced frequency of allele B*35 (OR=0.19; p<2.68×10-4) and the haplotypes A*11-B*35 (OR=0.06; p<1.47×10-4) and A*03-B*35 (OR=0.25; p<0.088) among cervical cancer patients have suggested a weak protective role for these alleles and haplotypes in south India.

*Amino acid variations at peptide-binding pockets of HLA-DQB1**

In an effort to identify the amino acid differences in critical peptide binding sites of the highly susceptible HLA alleles, we carried out ClustalW multiple sequence alignment. Amino acid profile for specific HLA DQB1* alleles were downloaded from the sequence data bank and compared. A classification was made for the amino acid substitutions at β9 (P6 pockets) and β37 (P9 pocket) positions of various HLA-DQB1* alleles. Sequence analysis of amino acids at positions β9 (P6) and β37 (P9) of peptide-binding groove showed a higher level of replacement of 'tyrosine' residue in cervical cancer patients. At β9 position, the amino acid 'tyrosine' was replaced either by 'leucine' or 'phenylalanine', whereas at β37 position it was replaced either by 'isoleucine' or 'asparatate'(Table 4).

The carriers of DQB1* allele with 'leucine' at β9 position and 'asparate' at β37 were found to be susceptible for cervical cancer. Interestingly, HLA- DQB1*0601 is the only allele possess the 'leucine' at β9 and 'asparate' at β37 positions. Our analysis have revealed a moderate frequency differences between patients and controls with references to the presence of 'tyrosine' at β9 position (68.75% vs. 72.34%; OR=0.84; p<0.701). Whereas the frequency differences of 'tyrosine' at β37 position was significant (56.25% vs.74.46%) and affords protection (OR=0.45; p<0.013) (Table 5). Further, the data was stratified into different genotypic combinations such as 'tyrosine/tyrosine' homozygous, 'tyrosine/non-tyrosine' heterozygous and 'non-tyrosine/non-tyrosine' homozygous for both β9 and β37 positions. At β9 position, the patients with heterozygous ('tyrosine/non-tyrosine') genotype was increased in patients and showed significantly elevated risk for the disease (OR=3.04; p<0.004). Similarly, at β37 position too, the frequency of heterozygous combination ('tyrosine/non-tyrosine') was increased in patients and hence increased the risk of developing cancer (OR=2.43; p<0.039). However, a decreased frequency of homozygous combination ('non-tyrosine/non-tyrosine') in the cancer patients have suggested a possible protective association (OR=0.27; p<0.047). Thus, the 'tyrosine' replacement at positions β37 and β9 with a 'non-tyrosine' amino acid (β37 'non-tyrosine' allele: OR=2.24; p<0.013 / and the presence

of heterozygous genotype β37 'tyrosine-non-tyrosine': OR=2.43; p<0.039 β9 'non-tyrosine' allele: OR=1.9; p<0.071 / β9 'tyrosine-non-tyrosine': OR=3.04; p<0.009 are associated with cervical cancer.

Discussion

In the present study, a significant association was documented for alleles HLA-DRB1*04 (OR=2.57) and DRB1*15 (OR=2.04) in cervical cancer patients from south India. This is in agreement with the results published earlier on Swedish (Sanjeevi et al., 1996), Dutch and north Indian (Krul et al., 1999), south-western American Indian with CIN (Schiff et al., 2005) and Caucasian (Yang et al., 2006) women. One previous study showed an increased risk for alleles DRB1*11 (Wang et al., 2001; Duggan et al., 1996) in Costa Rica and North West England population respectively. Further, a higher frequency was observed for allele DRB1*10 among controls and suggested a protective association. HLA allele DQB1*0301 showed a 4 times increased risk of developing cervical cancer (OR=4.91). Many previous reports have documented an increased risk for women carrying this allele to develop squamous cell carcinoma (SCC) of the cervix in United States (Wank and Thomssen 1991), Caucasian (Wang et al., 1992), African-American (Gregoire et al., 1994), Norwegian (Helland et al., 1998) and southern Chinese (Chan et al., 2007). The women homozygous for HLA-DQB1*03 allele from western India were at an increased risk of developing cervical cancer (Bhattacharya and Sengupta, 2005). Various studies in different ethnic populations have documented an association of various HLA alleles such as DR5 (DRB1*11, DRB1*12) (Syrjanen et al., 1996; Odunsi et al., 1995), DQB1*0201 (Gregoire et al., 1994; Helland et al., 1994) and the haplotype DRB1*1101-DQB1*0301 (Odunsi et al., 1996; Allen et al., 1996) with cervical neoplasia. However in our study, the disease association was observed only for alleles DQB1*0301 and DQB1*0601.

The allele DQB1*0601 showed an 2.2 fold increased risk in cervical cancer patients, however without a statistical significance (OR=2.21; p<0.082). Previously it was shown that the allele DQB1*06011 was significantly associated with cervical cancer in Chinese women (Wu et al., 2006). An increased frequency of this allele was documented in Tanzanian (Wang et al., 2001), African-American (Gregoire et al., 1994) and southern Iranian (Dehaghani et al., 2002) women. These published data and results of the present study on south Indian cervical cancer patients reiterated the significance of allele DQB1*06 in the development of cervical cancer in 'Afro-Asiatic' populations. However, studies on north Indian patints have not reported any such association with cervical cancer (Koharr et al., 2009). In this context, it is highly relevant to note the existence of north-south HLA genetic gradients in Indian populations (Pitchappan et al., 1984; Rajasekar et al., 1987; Balakrishnan et al., 2012). Our finding of protective association of allele DQB1*0502 was attested by similar observation among Chinese women (Wu et al., 2006).

In the present study, haplotypes DRB1*15-DQB1*0601 (OR=6.56) and DRB1*14-DQB1*0501 (OR=6.51) were found predominantly in cervical cancer patients. Previously, the haplotype DRB1*1501-DQB1*0602 was reported with a 3 fold risk for invasive SCC cases and with a 5-fold risk for HPV-16 positive cases in Hispanic women (Apple et al., 1995). Further, it has been documented that, DRB1*15 either alone or in haplotypic combination with DQB1*06 is more likely to be found in cervical cancer/ precancer patients infected with HPV16 in different ethnic groups (Gregoire et al., 1994; Apple et al., 1995; Sanjeevi et al., 1996; Maciag et al., 2000). Previous studies on cervical cancer patients have documented high risk haplotypes such as DRB1*0401-DQB1*0301, DRB1*1101-DQB1*0301 and DRB1*1501-DQB1*0602, DRB1*14:03-DQB1*05:03 (Terry et al., 1997; Cuzick et al., 2000; Lin et al., 2001; Gokhale et al., 2014a). Interestingly, it was postulated that the affinity of binding between HPV16 epitope and HLA molecule was lower for susceptible alleles (DRB1*0401) than protective alleles (DRB1*0101) (Odunsi and Ganesan, 2001). Thus, the lower affinity in binding the pathogen derived peptides by certain susceptible alleles and/or haplotypes could lead to the cancer development.

The HLA-A/-B typing revealed the presence of increased risk for alleles B*07 (OR=6.23) and B*15 (OR=13.03) in developing cervical cancer. Previously, a strong association was documented for the allele B*07 (OR=6.23) in a Chinese cervical cancer cohort (Wang and Qiao, 2008). Further, the decreased frequency of HLA-B*35 allele in our study cohort have suggested a protective association with cervical cancer. A recent high resolution typing of north Indian cervical cancer patients have revealed a decreased risk for alleles B*07:05, B*35:03 and B*40:06 and an increased risk for alleles HLA-B*08, B*37 and B*58 (Gokhale et al., 2014b). It is interesting to note the high frequency of haplotype A11-B7, in cervical cancer patients, the most common haplotype in many of the south Indian populations studied earlier (Rajasekar et al., 1987; Pitchappan et al., 1984; Balakrishnan et al., 1996). Further, our report for the first time have suggested a positive association of a 3 locus haplotype (A*11-B*7-DRB1*04; (n, 8/48; OR=6.51; p<0.039) with cervical cancer in south India. Interestingly, HLA-C* typing of all these eight patients revealed the presence of 4 locus haplotype A*11-B*07-C*01-DRB1*04 in 4 patients. It is interesting to note that these 4 locus extended haplotype was reported in German cohort of stem cell donors (Pingel et al., 2013). Such extended haplotypes are highly informative molecular markers for community based screening.

The amino acid sequences analyses of many of the DQB1* alleles have revealed the presence of 'tyrosine' at β9 (P6). However for DQB1*0601, DQB1*0401 and DQB1*0602 alleles, it is replaced by 'leucine' or 'phenylalanine'. Similarly, 'tyrosine' at β37 (P9) was replaced by 'isoleucine' in DQB1*0201 and DQB1*0202 or 'asparatate' in DQB1*0601 allele. Peptide binding affinity within the HLA groove may be influenced by different peptides and/or alternation of aminoacid residues of HLA molecules. Different amino acid residue of the peptide would alter the shape and functionality of

the binding site of HLA-DQ molecule thus impeding the effective binding of the pathogen derived peptides (Evavold et al., 1991). Previous sequence analysis have revealed that DQB1*060101 allele encode 'leucine' at β9 and 'aspartate' at position β37 among cervical cancer patients whereas the other DQB1* alleles encode 'pheylalanine' or 'tyrosine' and 'isoleucine' or 'tyrosine' at these positions respectively. Further, the susceptibility-associated allele DQB1*060101 with 'leucine'/'aspartate' amino acids at positions β9 and β37 of DQB1* respectively are part of predicted antigen recognition sites of class II molecule (Brown et al., 1988). This DQB1*060101 allele also revealed a relatively deeper groove than the protective allele DQB1*050201. Further, the hydrophobic residue (leucine) of the HLA-DQB1*060101 allele can bind pathogenderived peptides with larger hydrophobic side chains. These amino acid changes can alter peptide binding and TCR-HLA interaction efficiencies (Evavold et al., 1995). Thus, the presence or absence of polymorphic amino acids at putative antigen binding residues such as β9 and β37 of HLA-DQB1* allele may play an important role in the development of cervical cancer.

The only shortcoming of the present study was the lack of data on the HPV type (inclusion of patients based only on PAP smear result). A case controlled study with HPV genotyping and high resolution HLA typing will throw more light on the role of host immune response genes in the aetiopathogenesis of cervical cancer. Hence, a population based case-control study is highly warranted in south India for the clear understanding of the disease process, development of strategies for community based molecular diagnosis and vaccine designing.

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