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ORIGINAL ARTICLE

Persistent HPV16/18 infection in Indian women with the A-allele (rs6457617) of HLA-DQB1 and T-allele (rs16944) of IL-1β −511 is associated with development of cervical carcinoma

Sankhadeep Dutta[1] · Chandraditya Chakraborty[1] · Ranajit Kumar Mandal[2] · Partha Basu[2,5] · Jaydip Biswas[3] · Susanta Roychoudhury[4] · Chinmay Kumar Panda[1]

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Abstract The aim of this study was to understand the association of human papillomavirus (HPV) type 16/18 infection and polymorphisms in the HLA-DQB1 (rs6457617) and IL-1β –511 (rs16944) loci with the development of uterine cervical cancer (CaCx). The distribution of HLA-DQB1 G > A and IL-1β −511 C/T polymorphisms was determined in HPV-negative cervical swabs from normal women ($N = 111$) and compared with cervical swabs of HPV-cleared normal women (once HPV infected followed by natural clearance of the infection, $N = 86$), HPV16/18-positive cervical intraepithelial neoplasia (CIN, $N = 41$) and CaCx biopsies ($N = 107$). The A-allele containing genotypes (i.e. G/A and A/A) of HLA-DQB1 was significantly associated with CaCx compared with HPV-negative [OR = 2.56(1.42–4.62), $p = 0.001$] or HPV-cleared [OR = 2.07(1.12–3.87), $p = 0.01$] normal women, whereas the T-allele containing genotypes (i.e. C/T and T/T) of IL-1β showed increased risk of CIN [OR = 3.68(0.97–16.35), $p = 0.03$; OR = 3.59(0.92–16.38), $p = 0.03$] and CaCx development [OR = 2.03(1.03–5.2), $p = 0.02$; OR = 2.25(0.96–5.31), $p = 0.04$] compared with HPV-negative or HPV-cleared normal women. Considering these two loci together, it was evident that the T- and A-alleles rendered significantly increased susceptibility for development of CIN and CaCx compared with HPV-negative and HPV-cleared normal women. Moreover, the T-allele of IL-1β showed increased susceptibility for CIN [OR = 3.62(0.85–17.95), $p = 0.04$] and CaCx [OR = 2.39(0.91–6.37), $p = 0.05$] development compared with the HPV-cleared women, even in the presence of the HLA-DQB1 G-allele. Thus, our data suggest that persistent HPV16/18 infection in the cervix due to the presence of the HLA-DQB1 A-allele and chronic inflammation due to the presence of the IL-1β –511 T-allele might predispose women to CaCx development.

Keywords Uterine cervical cancer (CaCx) · HPV clearance · HPV persistence · Inflammation · HLA-DQB1 G > A polymorphism · IL-1β –511 C/T polymorphism

Abbreviations

AGUS	Atypical glandular cells of undetermined significance
ASCUS	Atypical squamous cells of undetermined significance
CaCx	Uterine cervical cancer
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
HLA	Human leucocyte antigen
HPV	Human papillomavirus
IL	Interleukin
LCR	Long control region

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✉ Chinmay Kumar Panda
ckpanda.cnci@gmail.com; ckpanda@vsnl.net

[1] Department of Oncogene Regulation, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Kolkata, India

[2] Department of Gynecologic Oncology, Chittaranjan National Cancer Institute, Kolkata, India

[3] Department of Surgical Oncology, Chittaranjan National Cancer Institute, Kolkata, India

[4] Cancer Biology and Inflammatory Disorder Division, Indian Institute of Chemical Biology, Kolkata, India

[5] Present Address: Screening Group (SCR), Early Detection and Prevention Section (EDP), International Agency for Research on Cancer (IARC), World Health Organization (WHO), 150 cours Albert Thomas, 69372 Lyon, Cedex 08, France

LD	Linkage disequilibrium
MHC	Major histocompatibility complex
OR	Odds ratio
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SD	Standard deviation
TCR	T cell receptors

Introduction

Cervical cancer (CaCx) is considered to be one of the leading malignancies in women worldwide, and infection with high-risk human papillomavirus (HR-HPV) plays an initiating role during cervical carcinogenesis [1, 2]. The incidence rate of CaCx is lower in developed countries. However, in developing countries such as India, it is the second most frequent cancer among women between 15 and 44 years of age, killing 67,477 women every year [3]. HPV16 and HPV18 constitute more than 84 % of the HR-HPV types present in the transformed cervical epithelium [3]. Being an epitheliotropic virus, HPV16/18 first infects the basal cells of the normal cervical epithelium for propagation and develops different asymptomatic clinical manifestations including inflammation, koilocyte formation and formation of atypical squamous cells of undetermined significance (ASCUS) or atypical glandular cells of undetermined significance (AGUS). Nearly 80 % of such asymptomatic infections are naturally cleared by host immunity [4]. However, persistence of approximately 20 % of asymptomatic infections may lead to development of pre-malignant cervical intraepithelial lesions (CIN) followed by malignant transformation [4]. Therefore, host immunogenetic factors seem to play determining roles in the clearance/retainment of the viral infection from the epithelium.

The human major histocompatibility complex (MHC) is a multi-gene including the highly polymorphic human leucocyte antigen (HLA) class I (HLA-A, HLA-B, HLA-C) and II genes (HLA-DRA, HLA-DRB1, HLA-DRB3-5, HLA-DQA, HLA-DQB, HLA-DPA, HLA-DPB) that code different types of glycoproteins. These glycoproteins are specialised in the presentation of short peptides derived from infectious agents or self-proteins to T lymphocytes, hence playing a key role in both the cellular and humoral immune responses [5, 6]. The peptide-binding groove of HLA class II proteins, which is only expressed on antigen-presenting cells, carries a self- or non-self-peptide derived from proteins sourced from outside the cell by endocytosis [5]. This occurs through signalling to T cell receptors (TCR) of CD4+ (helper) T cells and subsequent initiation of the acquired immune response [7]. The polymorphism in HLA class II genes impacts the peptide-binding groove, varying the amino acids that can be housed within the peptide-binding pockets and is thereby associated with various infectious diseases and malignancies [8–10]. The antigen encoded by the polymorphic A-allele of the HLA-DQB1 gene (G > A, rs6457617, previously known as HLA-DQB1*03) has been heavily studied for its predisposition to CaCx development [11–13]. However, the importance of the A-allele in clearance of HR-HPV is not well studied.

Chronic inflammation is the initial histopathological stage of transformation of normal cervical epithelium by HPV16/18. Cytokines produced by the host monocytes and tissue macrophages modulate both the chronic and acute inflammations and are thereby important players against viral infections [14]. Among the pro-inflammatory cytokines, the increased secretion of IL-1β (interleukin-1 beta) has been reported to be associated with CaCx and other cancers [15, 16]. The promoter region of IL-1β is highly polymorphic, and strong linkage disequilibrium (LD) between the polymorphic loci has been found [17]. Most importantly, the promoter with the −511 T-allele (rs16944) and the −31 C-allele (rs1143627) showed the increased transcription of IL-1β [17]. Interestingly, the presence of the −511 C/T (rs16944) polymorphism has been associated with increased intracellular production of IL-1β and increased risk of CaCx development [18]. Thus, it seems that the HLA-DQB1 G > A and IL-1β −511 C/T-alleles might have importance in the clearance of HR-HPV and remission of HPV-associated inflammation in the cervix. In addition, the synergistic effect of these two polymorphic alleles, if any, in the development of CaCx has not been well studied.

Thus, in this case control study, we aimed to evaluate the effect of the HLA-DQB1 (G > A) and IL-1β −511 (C/T) polymorphisms, either singly or in combination, on the risk of persistent HPV16/18 infection and development of CaCx. Our study showed that HPV16/18-infected women who had minor alleles at the two polymorphic loci were at higher risk of CaCx development.

Materials and methods

Sample collection

Biopsies from 110 women with newly diagnosed, histopathologically confirmed and untreated CaCx were collected from the hospital section of Chittaranjan National Cancer Institute (CNCI), Kolkata, from December 2010 to June 2012. All biological samples were collected and handled according to standard procedures. Pre-neoplastic samples (cervical intraepithelial neoplasia, CIN) were obtained from 105 women who participated in the population-based cervical HPV screening campaigns, which were conducted by the CNCI in 10 different districts of southern West Bengal, India. The mean age of the women who had CaCx or CIN was 45 ± 3.2 (range 28–77) and 34 ± 4.1 years (range 24–62), respectively. The presence of HPV16 and/

or HPV18 and the absence of any medical history of previous cancers and/or previous radiotherapy or chemotherapy were set as inclusion criteria for the cases in this study.

We have included another case group comprised of archival DNA samples from cervical swabs of 86 women who were HPV negative during the follow-up visit but were detected with cervical HPV infection [HPV positive: 86; HPV16: 15/86 (17.4 %); HPV18: 11/86 (12.8 %); HPV16/18 co-infection: 1/86 (1.2 %)] during their first cervical HPV screening (i.e. 3 months before the follow-up screening) [19]. Women in this HPV-cleared group did not use any antimicrobial or antiviral substances during the 3-month follow-up period. They were apparently healthy, cytologically normal, unrelated and ethnicity and age-matched (mean 36 ± 9 years; range 25–59) to the other two case groups.

The control group consisted of 111 archival DNA samples extracted from cervical swabs of apparently healthy, cytologically normal women who had no HPV infection in their cervix [20]. These HPV-negative normal controls were ethnicity and age-matched (mean 40 ± 7.6 years; range 30–65) but unrelated to the cases.

Proper approval from the institutional ethics committee and written informed consent from participants/patients were obtained before commencement of the study.

Microdissection and DNA isolation

Normal cells present as contaminants in the primary CaCx biopsies were removed from the 5-µm cryo-sections by a microdissection procedure under a dissecting microscope (Leica MZ16, Germany) [21]. DNA from the microdissected CaCx samples and cervical swabs was isolated by proteinase-K digestion followed by phenol–chloroform extraction according to the standard procedure [22].

HPV detection and HPV16/18 typing

The prevalence of HPV was tested by PCR using the L1 consensus MY 09/11 primers, and the HPV-positive samples were typed for HPV16 and HPV18 in separate PCRs using type-specific primers from the E6 region of HPV16 and long control region (LCR) of HPV18 [20]. HPV16 and HPV18 typing was further confirmed by Southern blot hybridisation of the PCR product using P^{32}-labelled HPV16/18-specific probes. The same methodology was used for the HPV screening in the cervical swabs of the women from the HPV-cleared group during their first visit. HPV16 and HPV18 plasmids were used as positive controls.

HLA-DQB1 (rs6457617) genotyping

The common sequence of exon 2, 38th codon of the HLA-DQB1 gene, is GCG, and the region contains a G > A polymorphism (rs6457617) located at the last base of 38th codon [23]. This SNP is in strong linkage disequilibrium with other two SNPs (viz. rs9275224, rs9275245) located in this region [24]. Thus, the GCA sequence in exon 2, 38th codon, could commonly be found in DQB1*03, DQB1*04, DQB1*05 and DQB1*06 alleles. All the samples included in this study were screened for genotype distribution of the HLA-DQB1 alleles by PCR amplification followed by restriction fragment length polymorphism (PCR–RFLP) as described by Bhattacharya et al. [25]. PCR amplification with the mutagenesis reverse primer generated a MluI cut site only in the HLA-DQB1 genotypes with a polymorphic A-allele (rs6457617), not in the major G-allele containing genotypes. Thus, overnight digestion of 10 µl of PCR products with 2U of MluI (F. Hoffmann-La Roche Ltd, Basel, Switzerland) at 37 °C followed by 2 % agarose gel electrophoresis generated bands of 120 and 27 bp for the A/A genotype, a single band of 147 bp for the G/G genotype and bands of 147, 120 and 27 bp for the G/A genotype.

IL-1β −511 (rs16944) genotyping

The C/T polymorphism (rs16944) at position −511 was analysed in all of the samples by the PCR–RFLP method according to Achyut et al. [26]. Briefly, 10 µl of the PCR products (155 bp) generated by the specific primer sets were digested with 1.5U of *Alu*I (SibEnzyme, Novosibirsk, Russia) overnight at 37 °C. Upon electrophoresis of the digested products in 2 % agarose gels, the C/C genotype produced two bands of 85 and 70 bp, whereas the polymorphic T/T genotype remained undigested due to the absence of the *Alu*I site. For the C/T genotype, three bands of 155, 85 and 70 bp were obtained.

To further confirm the genotypes, the promoter regions of IL-1β were sequenced (location of forward primer: −686 to −666 nt, and reverse primer: 14–34 nt of the first intron) in 15 % of the samples according to the supplier's protocol on a 3130xl Genetic Analyzer (Applied Biosystems, USA).

Statistical analysis

The distributions of the polymorphic alleles in HPV-negative cytologically normal controls were compared with HPV-cleared normal, HPV16/18-positive CIN and CaCx to identify the risk allele, if any. We also compared the allele's status in the HPV-cleared group to the HPV16/18-positive CIN and CaCx cases to nullify the confounding effect of HPV infection with the host genetic factors.

Age-adjusted risk of HPV16/18-associated CaCx development was calculated as an odds ratio (OR) with 95 % confidence intervals (CI) for all genotypes of HLA-DQB1 and IL-1β in cases and control samples by multiple logistic

regression analysis. For the comparison of proportions, Chi-square tests were performed and Yates' corrected values were considered. A probability value (p value) <0.05 was considered statistically significant. SPSS 10.0 (SPSS, Chicago, IL, USA) was used to perform the statistical analysis.

Results

Prevalence of HPV16/18 and demographic analysis

Among the 110 CaCx samples, 108 (108/110, 98 %) were HPV positive among which 107 were positive for HPV16/18 (HPV16: 98/108, 91 % and HPV18: 9/108, 8 %), whereas among the 105 CIN samples, 75 (75/105, 71.2 %) were HPV positive among which 41 were positive for HPV16/18 (HPV16: 28/75, 37.2 % and HPV18: 13/75, 17 %). Only the HPV16/18-positive samples were included as cases in the present study. The detailed demographic characteristics of the case and control groups are summarised in Table 1. Comparison of the HPV-cleared, CIN or CaCx cases to the HPV-negative normal controls showed that there were no significant differences in the mean age ($p > 0.05$) and use of oral contraceptives ($p > 0.05$). However, the CIN and CaCx cases were married at a significantly earlier age (CIN: $p = 0.04$ and 0.02; CaCx: $p = 0.01$ and 0.004) compared with the HPV-negative normal control and HPV-cleared groups. The CaCx cases also had significantly higher parity ($p = 0.05$ and 0.01) than the HPV-negative normal control and HPV-cleared groups, whereas the parity in CIN cases was significantly higher ($p = 0.04$) compared with the HPV-cleared cases only.

HLA-DQB1 G > A (rs6457617) polymorphism and risk of HPV16/18-mediated CaCx development

The PCR–RFLP analysis of the HLA-DQB1 G > A polymorphism is shown in Fig. 1, and the distribution of HLA-DQB1 alleles among women of different cases and control groups is shown in Table 2.

The frequency of the minor A-allele was significantly higher in the CaCx cases [OR = 2.45 (1.29–4.68), $p = 0.003$] (Table 2a) compared with the HPV-negative normal control group. The age-adjusted logistic regression analysis showed a 2.56 [OR = 2.56 (1.42–4.62), $p = 0.001$] times higher risk of the development of CaCx in women with the A-allele (59/107, 55 %) compared with the HPV-negative normal (36/111, 32 %) women (Table 2b). However, in HPV-cleared [OR = 1.23 (0.66–2.33), $p = 0.58$] and CIN [16/41, 39 %; OR = 1.33 (0.59–2.98), $p = 0.57$] cases, the prevalence of the polymorphic A-allele was comparable to the HPV-negative normal controls.

Similarly, in comparison with the HPV-cleared normal group (32/86, 37 %), the presence of the A-allele in women

Table 1 Demographic and clinico-pathological characteristics of the women in case and control populations

Demographic and clinical variables	Control	Case		
	HPV-negative normal ($N = 111$)	HPV-cleared normal ($N = 86$)	HPV16/18 + ve CIN ($N = 41$)	HPV16/18 + ve CaCx ($N = 107$)
Age (years)				
Range	30–65	25–59	30–60	28–71
Mean ± SD	40 ± 7.6	36 ± 9.0	44 ± 9.5	37 ± 6.8
Age at marriage, mean ± SD (years)	22 ± 4.1	24 ± 3.0	18 ± 3.2	17 ± 4.7
Parity, mean ± SD	2.3 ± 1.2	2 ± 1.45	3.45 ± 1.6	3.7 ± 1.0
Use of oral contraceptives (n)				
Current	15	18	10	37
Not in last 5 years	57	41	22	47
Never	39	27	9	23
Cytology status (n)				
Normal	111	86	–	–
CIN I (LSIL)	–	–	7	–
CIN II–III (HSIL)	–	–	34	–
Tumour stage (n)				
Stage I–II	–	–	–	38
Stage III–IV	–	–	–	69

SD standard deviation

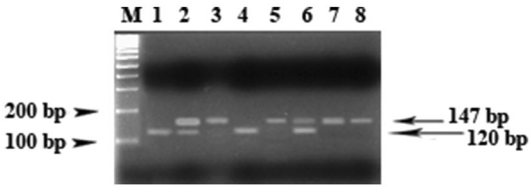

Fig. 1 Determination of HLA DQB1 (rs6457617) genotypes by the PCR-restriction fragment length polymorphism (PCR–RFLP) method. Agarose gel electrophoresis photograph of HLA DQB1 PCR products after digestion with *Mlu*I. M: 100-bp marker; lane 1–2: HPV16/18-positive cervical cancer (CaCx) samples; lane 3–4: HPV16/18-positive cervical swab from women with cervical intraepithelial neoplasia (CIN); lane 5–6: cervical swab from HPV-cleared women; lane 7–8: cervical swabs from HPV-negative women. In lanes 1 and 4, samples were homozygous for the minor A-allele; in lanes 2 and 6, samples were heterozygous, and the samples in lanes 3, 5, 7 and 8 were homozygous for the major G-allele

showed 2.07 times higher risk of the development of CaCx [OR = 2.07 (1.12–3.87), $p = 0.01$] (Table 2c).

Thus, it appears that the presence of the A-allele, in either homozygous or heterozygous combination, predisposes the individual to develop CaCx by compromising the immune clearance of HPV16/18 infection.

IL-1β –511 C/T (rs16944) polymorphism and risk of HPV16/18-mediated CaCx development

The PCR–RFLP analysis of the IL-1β −511 C/T polymorphism is shown in Fig. 2, and concordance of the allele status was evident by sequencing analysis (Supplementary Fig. 1). The distribution of alleles at the −511 locus of IL-1β among women of different cases and control groups is shown in Table 3.

The frequency of the minor T-allele was significantly higher in the CIN [OR = 2.60 (1.38–4.90), $p = 0.001$] and CaCx cases [OR = 1.87 (1.01–3.44), $p = 0.03$] (Table 3a) compared with the HPV-negative normal control group. There was no significant difference [OR = 1.03 (0.49–2.13), $p = 0.94$] in the prevalence of the polymorphic T-allele between the HPV-negative normal (86/111, 77 %) and HPV-cleared (67/86, 78 %) control groups (Table 3b), whereas, the prevalence of the T-allele was significantly higher in CIN (38/41, 93 %) and CaCx (95/107, 89 %) cases with 3.68 [OR = 3.68 (0.97–16.35), $p = 0.03$] and 2.08 [OR = 2.03 (1.03–5.2), $p = 0.02$] times higher risk association compared with the HPV-negative normal groups.

Upon comparison with the HPV-cleared group, the risk of CIN development was 3.59 times higher [OR = 3.59 (0.92–16.38), $p = 0.03$] and the risk of CaCx development was 2.25 times higher [OR = 2.25 (0.96–5.31), $p = 0.04$] (Table 3c). It is noteworthy to mention that in either analysis, the risk association with the presence of the T-allele was nearly double in CIN cases compared with CaCx. Thus, the T-allele of IL-1β confers higher risk of the development of pre-malignant and malignant lesions of the cervix in HPV16/18-infected women.

Table 2 Distribution of HLA-DQB1 G > A polymorphism in case and control populations

Subject groups (*N*)[a]	G-allele frequency	A-allele frequency	Age-adjusted OR (95 % CI) & *p* value
HPV-negative normal ($N = 111$)	0.75	0.25	1.00 (Ref.)
HPV-cleared normal ($N = 86$)	0.72	0.28	1.17 (0.59–2.29), $p = 0.63$
HPV16/18-positive CIN ($N = 41$)	0.78	0.22	0.85 (0.42–1.71), $p = 0.61$
HPV16/18-positive CaCx ($N = 107$)	0.55	0.45	*2.45 (1.29–4.68), p = 0.003*
Subject groups (*N*)[b]	G/G, *n* (%)	G/A + A/A, *n* (%)	Age-adjusted OR (95 % CI) & *p* value
HPV-negative normal ($N = 111$)	75 (68)	36 (32)	1.00 (Ref.)
HPV-cleared normal ($N = 86$)	54 (63)	32 (37)	1.23 (0.66–2.33), $p = 0.58$
HPV16/18-positive CIN ($N = 41$)	25 (61)	16 (39)	1.33 (0.59–2.98), $p = 0.57$
HPV16/18-positive CaCx ($N = 107$)	48 (45)	59 (55)	*2.56 (1.42–4.62), p = 0.001*
Subject groups (*N*)[c]	G/G, *n* (%)	G/A + A/A, *n* (%)	Age-adjusted OR (95 % CI) & *p* value
HPV-cleared normal ($N = 86$)	54 (63)	32 (37)	1.00 (Ref.)
HPV16/18-positive CIN ($N = 41$)	25 (61)	16 (39)	1.08 (0.47–2.48), $p = 0.99$
HPV16/18-positive CaCx ($N = 107$)	48 (45)	59 (55)	*2.07 (1.12–3.87), p = 0.01*

The statistically significant changes in the allele/genotype frequencies with *p* values <0.05 are highlighted in italics

[a] Significant difference of G- and A-allele frequencies in human papillomavirus (HPV) type 16/18-positive cervical cancer (CaCx) cases compared to the HPV-negative control population. Distribution of genotype frequencies showed significant association of A-allele containing genotypes with CaCx cases compared to HPV-negative normal women

[b] HPV-cleared normal women

[c] *OR* odds ratio, *CI* confidence interval

Combined effect of HLA-DQB1 G > A and IL-1β −511 C/T polymorphisms on HPV16/18-mediated CaCx development

To determine whether the HLA-DQB1 and IL-1β −511 polymorphisms rendered the risk of disease development either singly or in combination, the analysis was further performed as shown in Table 4. It was evident that women having minor alleles at both of the loci were at significantly higher risk of the development of CIN [OR = 4.17 (1.00–19.99), $p = 0.02$ and OR = 3.96 (0.93–19.34), $p = 0.03$] and CaCx [OR = 3.54 (1.41–9.06), $p = 0.002$ and OR = 3.03 (1.15–8.08), $p = 0.01$] compared with the HPV-negative normal or HPV-cleared normal women. Even women with only minor alleles in IL-1β showed higher risk of the development of CIN [OR = 3.62 (0.85–17.95), $p = 0.04$] and CaCx [OR = 2.39 (0.91–6.37), $p = 0.05$] compared to HPV-cleared normal women. Interestingly, the risk association of the combined presence of the two minor alleles (i.e. HLA-DQB1 A-allele and IL-1β −511 T-allele) was also higher for CIN cases than for CaCx cases, as previously found in Table 3.

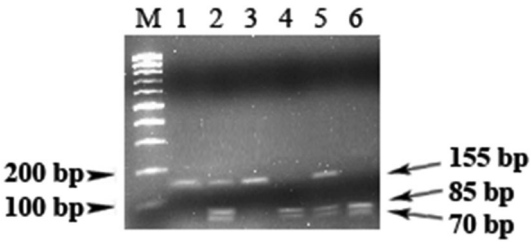

Fig. 2 Determination of IL-1β −511 (rs16944) genotypes by the PCR-restriction fragment length polymorphism (PCR–RFLP) method. Agarose gel electrophoresis photograph of IL-1β −511 PCR products after digestion with *Alu*I. M: 100-bp marker; lane 1–2: HPV16/18-positive cervical cancer (CaCx) samples; lane 3: HPV16/18-positive cervical swab from woman with cervical intraepithelial neoplasia (CIN); lane 4: cervical swab from HPV-cleared woman; lane 5–6: cervical swabs from HPV-negative women. In lanes 1 and 3, samples were homozygous for the minor T-allele; in lanes 2 and 5, samples were heterozygous, and the samples in lanes 4 and 6 were homozygous for the major C-allele

Discussion

In the present study, we have shown the risk effect of the HLA-DQB1 A-allele and the IL-1β −511 T-allele on the development of CaCx in HPV16/18-infected women. The statuses of the alleles in HPV-infected cervical swabs/lesions were compared with HPV-negative cervical swabs from normal women. It was evident that the HLA-DQB1 A-allele was associated with the development of CaCx. However, no such association was evident in HPV-cleared normal women and HPV16/18-positive women with CIN, indicating the importance of the A-allele in malignant transformation. Similarly, Wank et al. [11] showed that women expressing HLA DQw3 antigens are predisposed to CaCx. The chromosomal 6p21.31 region containing the HLA class I and II gene loci

Table 3 Distribution of IL-1β −511 C/T polymorphism in case and control populations

Subject groups (*N*)[a]	IL-1β –511 C allele	IL-1β –511 T allele	Age adjusted OR (95 % CI) & *p* value
HPV negative, normal ($N = 111$)	0.49	0.51	1.00 (Ref.)
HPV cleared, normal ($N = 86$)	0.47	0.53	1.08 (0.60–1.96), $p = 0.77$
HPV16/18 positive CIN ($N = 41$)	0.27	0.73	*2.60 (1.38–4.90), $p = 0.001$*
HPV16/18 positive CaCx ($N = 107$)	0.34	0.66	*1.87 (1.01–3.44), $p = 0.03$*
Subject groups (*N*)[b]	C/C, *n* (%)	C/T + T/T, *n* (%)	Age adjusted OR (95 % CI) & *p* value
HPV negative, normal ($N = 111$)	25 (23)	86 (77)	1.00 (Ref.)
HPV cleared, normal ($N = 86$)	19 (22)	67 (78)	1.03 (0.49–2.13), $p = 0.94$
HPV16/18 positive CIN ($N = 41$)	3 (7)	38 (93)	*3.68 (0.97–16.35), $p = 0.03$*
HPV16/18 positive CaCx ($N = 107$)	12 (11)	95 (89)	*2.03 (1.03–5.2), $p = 0.02$*
Subject groups (*N*)[c]	C/C, *n* (%)	C/T + T/T, *n* (%)	Age adjusted OR (95 % CI) & *p* value
HPV cleared, normal ($N = 86$)	19 (22)	67 (78)	1.00 (Ref.)
HPV16/18 positive CIN ($N = 41$)	3 (7)	38 (93)	*3.59 (0.92–16.38), $p = 0.03$*
HPV16/18 positive CaCx ($N = 107$)	12 (11)	95 (89)	*2.25* (0.96–5.31), *$p = 0.04$*

The statistically significant changes in the allele/genotype frequencies with *p* values <0.05 are highlighted in italics

[a] Significant difference of C and T-allele frequencies in human papillomavirus (HPV) type 16/18 positive cervical intraepithelial neoplasia (CIN) or cervical cancer (CaCx) cases compared to the HPV negative control population. Distribution of genotype frequencies showed significant association of T-allele containing genotypes with CIN and CaCx cases compared to HPV negative normal women

[b] HPV cleared normal women

[c] *OR* odds ratio, *CI* confidence interval

Table 4 Risk of human papillomavirus (HPV) type 16/18-mediated cervical intraepithelial neoplasia (CIN) or cervical cancer (CaCx) development because of the presence of HLA-DQB1 A-allele and IL-1β −511 T-allele, either singly or jointly

Groups	Presence or absence of HLA-DQB1 A-allele and IL-1β −511 T-alleles			
	00 (ref.)	10	01	11
HPV-negative normal (*N* = *111*)	25	0	50	36
HPV-cleared normal (*N* = *86*)	19	0	35	32
Age-adjusted OR (95 % CI), *p* value	1.00	0.00	0.92 (0.41–2.05), $p = 0.97$	1.17 (0.51–2.69), $p = 0.83$
HPV-negative normal (*N* = *111*)	25	0	50	36
HPV16/18-positive CIN (*N* = *41*)	3	0	20	18
Age-adjusted OR (95 % CI), *p* value	1.00	0.00	3.33 (0.82–15.63), $p = 0.06$	*4.17 (1.00–19.99), p = 0.02*
HPV-negative normal (*N* = *111*)	25	0	50	36
HPV16/18-positive CaCx (*N* = *107*)	10	2	44	51
Age-adjusted OR (95 % CI), *p* value	1.00	$p = 0.09$	2.2 (0.89-5.55), $p = 0.06$	*3.54 (1.41–9.06), p = 0.002*
HPV-cleared normal (*N* = *86*)	19	0	35	32
HPV16/18-positive CIN (*N* = *41*)	3	0	20	18
Age-adjusted OR (95 % CI), *p* value	1.00	0.00	*3.62 (0.85–17.95), p = 0.04*	*3.96 (0.93-19.34), p = 0.03*
HPV-cleared normal (*N* = *86*)	19	0	35	32
HPV16/18-positive CaCx (*N* = *107*)	10	2	44	51
Age-adjusted OR (95 % CI), *p* value	1.00	$p = 0.27$	*2.39 (0.91–6.37), p = 0.05*	*3.03 (1.15–8.08), p = 0.01*

The statistically significant changes in the allele/genotype frequencies with p values <0.05 are highlighted in italics

00 = HLA-DQB1 G-allele and IL-1β −511 C-allele; 10 = HLA-DQB1 A-allele and IL-1β −511 C-allele; 01 = HLA-DQB1 G-allele and IL-1β −511 T-allele; 11 = HLA-DQB1 A-allele and IL-1β −511 T-allele

OR odds ratio, *CI* confidence interval

is extremely polymorphic, and the polymorphism varies with the ethnicity of the population [6]. As a result, different studies among Japanese, Caucasian, African American and Brazilian populations showed contradictions in risk association of the HLA-DQB1 A-allele with CaCx development [11, 13, 27–32]. Similar to our findings, risk association of the HLA-DQB1 A-allele with CaCx development was reported in India by Bhattacharya et al. [25] However, the correlation with persistence of HPV was not considered. On the other hand, Maciag et al. [33] reported a lower risk of the DQB1*0301–DRB1*1102 haplogroup in a cohort study of HLA haplotype distribution in persistent HR-HPV infection in the cervix. Therefore, our findings on HPV persistence, the HLA-DQB1 G > A polymorphism and risk association of CaCx development are worthy of note.

The presence of the IL-1β −511 T-allele showed a significantly increased risk of CIN and CaCx development in HPV16/18-infected women compared with HPV-negative normal women. However, no significant association was found between HPV-negative and HPV-cleared normal women. Interestingly, when the HPV-cleared normal women were compared with the HPV16/18-positive CIN and CaCx women, 3.59- and 2.25-fold risk associations were seen for the presence of the T-allele, respectively. In previous studies, the association of the IL-1β −511C/T polymorphism with gastric, breast, lung and liver cancer, in addition to the cervix, has been well documented [18, 26, 34–37]. Similar to our data, a 2.42-fold (for C/T + T/T genotypes) and 2.8-fold (for T/T genotype) risk association was found with CaCx in Korean and Indian women, respectively [38, 39]. The −511 C/T polymorphism (rs16944) located in the IL-1β promoter region adjacent to the TATA box was associated with increased levels of IL-1β and higher risk of CaCx development [15, 18]. Moreover, Chen H et al. demonstrated that the two linked SNPs, the −511T and −31C alleles, could strongly enhance the transcription of IL-1β [17]. However, the −511 C- or T-allele alone did not show any allele-specific differences in transcription factor binding, suggesting that the combined interactions of the −511 C/T and −31T > C polymorphisms on the transcription of IL-1β influenced the binding of transcription factors to regulatory elements within this region [17]. Interestingly, apart from its pro-inflammatory function, IL-1β has also been suggested to be an important factor in tumourigenesis [40–42]. Thus, it seems that the presence of the −511 T-allele might lead to inflammation in the HPV16/18-infected cervix due to increased IL-1β levels, which in turn might lead to the development of CaCx.

In comparison with the HPV-negative and HPV-cleared women, it was evident that either single or synergistic presence of the HLA-DQB1 A-allele and the IL-1β T-allele in HPV16/18-infected women rendered an increased risk of CIN/CaCx development, validating these as predisposing alleles for the development of the disease. Previously,

polymorphism of HLA class II and IL-1 genes has been associated with susceptibility and the progression of biliary cirrhosis [43]. However, to the best of our knowledge, no such study associating these two risk alleles has been done in cancer. Thus, our data suggest that persistent HPV16/18 infection due to the presence of the HLA-DQB1 A-allele (rs6457617) and hyper-secretion of IL-1β due to the presence of the −511T-allele (rs16944) lead to chronic inflammation in the cervix for the initiation of carcinogenesis.

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Conflict of interest The authors declare that there are no conflicts of interest with regard to the work presented in this article.

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