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ORIGINAL ARTICLE

Impact of polymorphism in *IL-1RA* gene on the risk of cervical cancer

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Abstract
Introduction Cervical cancer, the second most common malignancy in women worldwide, is almost invariably associated with infection by human papillomavirus (HPV). However, although many women are infected with high-risk types of HPV, only a subset of infected women will ever develop cervical cancer. Therefore, host genetic factor may play a role in cervical carcinogenesis. Several studies suggested that immunological components play a key role in the development of cervical cancer. Polymorphism in the *IL-1RA* gene was associated with various malignant diseases. Data are lacking for cervical cancer.
Materials and methods In a case−control study we analyzed the polymorphism of *IL-1RA* in 150 women with cervical cancer and 209 healthy controls. Genomic DNA fragments were amplified by PCR.
Results There was a strong significantly protective association between heterozygous *AB* genotype and HPV 18 (OR = 0.11, 95% CI = 0.04–0.30, $p = 0.0000000$). Similarly this result was demonstrated, in combined *AB* + *BB* genotypes of *IL-1RA* with HPV 18 (OR = 0.12, 95% CI = 0.05–0.30, $p = 0.0000000$) and HPV type 16 + 18 (OR = 0.18,95% CI = 0.08–0.38, $p = 0.000005$). We found high protective significant association between heterozygous genotype *AB* with adenocarcinoma (OR = 0.19, 95% CI = 0.09–0.40, $p = 0.0000002$) as well.
Conclusion These findings therefore suggest that the *IL1-RA* polymorphism is associated with cervical cancer.

Keywords Interleukin-1 receptor antagonist · HPV · Cervical cancer · Polymorphism

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Introduction

Cervical cancer remains the second-most common cause of cancer-related deaths in women worldwide, with about 450,000 new cases diagnosed every year [5, 18]. Although it is the most common female gynaecological malignancy in India, accounting for 26% of female cancers, with 90,000 women developing the disease every year [27]. Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease in both men and women worldwide [8]. More than 200 types of HPV have been recognized on the basis of DNA sequence data showing genomic differences. Eighty-five HPV genotypes are well characterized [36]. Based on their association, HPVs can also be grouped to high-risk and low-risk HPV types. Low-risk HPV types include types 6, 11, 42, 43, and 44. High-risk HPV types include 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70. Incorporated in the high-risk group are some HPV types that are less frequently found in cancers but are often found in squamous intraepithelial lesions (SILs) [10]. Also, human papillomaviruses are well known oncogenic dsDNA viruses linked to cervical cancer, and associated with the high-risk group of viral oncogenes E6 and E7 [35]. Among the high-risk HPVs, HPV 16 and HPV 18 are associated with 70% of all cervical carcinomas, and HPV type 16 correlates to 50% of

cervical carcinomas [6, 13]. Among anti-inflammatory strategies, both at systemic and local level, the use of the IL-1 receptor antagonist (*IL-1RA*) has received vast attention. *IL-1RA* belongs to the IL-1 family, which consists of three linked genes mapping within a 430 kb region of the long arm of chromosome 2 in humans, encoding the secreted lycoproteins IL-1b, IL-1a, and IL-1Ra. All three molecules bind to IL-1 receptors [11]. IL-1Ra inhibits IL-1 by acting as a competitive receptor antagonist with no detectable agonistic activity, thus representing a natural powerful mechanism to control IL-1 dependent responses and avoid pathological derangement [21, 9]. The polymorphic gene that encodes *IL-1RA* seems to play an important role in the development of various diseases [33]. The *IL-1RA* gene is also polymorphic due to a variable number (2–6) of tandem repeat of 86 bp (VNTR) within its second intron [30]. Our basic objective of this study was to provide the relationship between polymorphism of *lL-lRA* (VNTR) gene and susceptibility to cervical cancer in North Indian population and tried to reveal the correlation between this genotype and HPV types 16 and 18 distribution.

Materials and methods

Patient and control

The case−control study involved collection of peripheral blood samples (2–5 ml) of 350 North Indian subjects. The 150 cases were newly diagnosed, previously untreated, and histologically confirmed cervix cancer patients. The samples were collected from Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, and Government Medical College (GMC), Chandigarh. The control peripheral blood samples ($n = 200$) were collected from these institutes with no history of cancer or pre-cancer.

Informed consent was obtained from all the cases and controls. Detailed data regarding age, use of oral contraceptive, education, menarche, menopausal status, number of children, age at marriage and birth of first child, cigarette smoking history, and spouse's smoking history were also obtained.

DNA extraction

Genomic DNA was extracted from EDTA anti-coagulated peripheral blood according to a standard proteinase K digestion and phenol chloroform extraction method [24].

IL-1RA polymorphism analysis

The oligonucleotide primers flanking the 86 bp repeat region in intron 2 of *IL-1RA* were used (Table 1). Amplification reactions were carried out in a volume of 25 µl containing 100 ng genomic DNA, 0.25 mmol/l dNTPs (MBI Fermentas, Burlington, Ontario, Canada), 2 mmol/l $MgCl_2$, 10 mM Tris–HCL (pH 8.3), 50 mM KCl, 1.5 U of Taq polymerase (MBI Fermentas), and 0.3 mmol/l of each primer (Sigma-Aldrich, Bangalore, India). PCR conditions included an initial denaturing step at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, and 70°C for 2 min; and a final extension at 70°C for 4 min. Using this PCR strategy, the common allele (allele 1) generated a 410 bp band (including four copies of an 86 bp repeat). The uncommon alleles generated a 240 bp band (two copies of the same repeat; allele 2) a 500 bp band (five copies of the same repeat; allele 3), and a 325 bp band (allele 4). PCR products were resolved on a 3% agarose gel and stained with ethidium bromide (Sigma-Aldrich, USA)

HPV detection and typing

For HPV detection and typing to identify the HPV genome, general primers GP5 and GP6 with β-globin were used (Table 1), as described by Papadakis et al. [23]. β-globin was used as an internal standard. A total of 100 ng DNA from each patient was amplified by PCR containing 10 pmol of each primer, 2.5 mM of $MgCl_2$, 0.2 mM each

Table 1 Primer sequence and length of PCR product

Primer pair	Sequence 5′−3′	PCR product size	References
β2-globin	TCCAACATCAACATCTTGGT	123	Papadakis et al. [23]
	TCCCCCAAATTCTAAGCAGA		
GP5	TTTGTTACTGTGGTAGATAC	139–145	Papadakis et al. [23]
GP6	GAAAAATAAACTGTAAATCA		
HPV 18	AAACTAACTAACACTGGGTTATACA	143	Papadakis et al. [23]
	ATGGCACTGGCCTCTATAGT		
HPV 16	CTGCAAGCAACAGTTACTGCGACG	315	Papadakis et al. [23]
	CATACATCGACCGGTCCACC		
IL-1RA (VNTR)	CTCAGCAACACTCCTAT	410, 240, 500, 325	Tarlow et al. [30]
	TCCTGGTCTGCAGGTAA		

dNTPs, 1 U Taq polymerase in final (50 µl). The mixture was heated at 94°C for 3 min and samples were amplified for 40 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s; followed by elongation at 72°C for 5 min. PCR products were expected to be 139–145 bp, depending on HPV type [28]. HPV typing of all HPV-positive samples was carried out using multiplex PCR. Specific pairs of primers were used to simultaneously amplify regions of HPV types 16 and 18 in the same reaction tube, giving different lengths of amplified DNA. To establish type specificity of primer-directed amplification, PCR products were analyzed by electrophoresis in a 2% agarose gel, stained with ethidium bromide and observed under UV light transilluminator.

Statistical analysis

Age, age at marriage and at birth of first child, age at menarche and menopause, HPV status (positive, negative), and genotype of *lL-lRA* gene were tabulated for cases and controls (Table 2). Cases were further categorized into histological and clinical subtypes for checking significance correlations. Under the hypothesis that *lL-lRA* genotype may be associated with the risk of cervix cancer, we tested combination of *lL-lRA* genotype in models for squamous cell carcinoma (SCC), adenocarcinoma (AC), and stages of cancer (stages IA, IIA, IIIA, IB, IIB, IIB, IV); and the interaction of this gene with HPV 16 and HPV 18 were tested. The association between polymorphism in *lL-lRA* gene with the risk of cervical cancer was estimated by computing odds ratio (OR) and 95% confidence intervals (95% CI) by using a multivariate logistic regression analysis that included several potential confounding variables. Statistical analysis was performed using SPSS, version 10.0 (SPSS, Chicago, IL, USA) Epical version 3.2.

Result

We have analyzed the genotype of the *lL-lRA* gene polymorphism in cervical cases and healthy controls derived from North Indian population.

Demographic variables for cases and controls are summarized in Table 2. Variables have also been categorized for squamous cell carcinoma (SCC) and adenocarcinoma

Table 2 Demographic characteristics of cervix cancer cases and controls

Variable	Cases (150)	Controls (209)	SCC	AC
Age	48.55 ± 9.43	48.81 ± 9.64	48.39 ± 9.42	49.68 ± 9.64
Age at menarche	14.87 ± 1.14	14.02 ± 1.09	14.90 ± 1.16	14.68 ± 1.00
Age at marriage	16.36 ± 3.03	20.31 ± 3.46	16.68 ± 2.97	14.74 ± 2.56
Age at first child birth	18.39 ± 3.39	22.31 ± 4.30	18.61 ± 3.16	16.84 ± 4.45
Children	4.11	2.50	4.09 ± 1.51	4.21 ± 1.84
Age at menopause	48.31 ± 3.56	48.26 ± 2.39	48.29 ± 3.62	48.44 ± 3.40
HPV status				
HPV absent	43	–	6	37
HPV 16	76	–	63	13
HPV 18	22	–	19	3
HPV 16 + 18	9	–	6	3
OR	3.6	–	3.6	7.08
P	0.001	–	0.001	0.001
Use of contraceptive				
Nonuser	94 (63.51)	83 (46.36)	79 (60.3)	16 (84.2)
User	54 (36.48)	96 (53.63)	52 (39.7)	3 (15.8)
OR	0.5	–	0.57	0.19
P	0.002	–	0.02	0.003
AB	59 (49.4)	91 (43.5)	7 (11.9)	52 (88.1)
CB	6 (4.3)	4 (1.9)	1 (19.7)	5 (83.3)
BB	11 (7.9)	13 (6.2)	3 (27.3)	8 (72.7)
AA	56 (40.3)	94 (45.0)	4 (7.1)	52 (92.9)
CA	6 (4.3)	5 (2.4)	1 (16.7)	5 (83.3)
DD	–	1 (0.5)	–	–
DA	–	1 (0.5)	–	–
DB	1 (0.7)	–	–	1 (100)

Data on 11 cases in genotype study are missing

ADC adenocarcinoma,*OR* odds ratio, and *SCC* squamous cell carcinoma

Significance set at < 0.05

Table 3 Allele frequency (%) of interleukin-1 receptor antagonist among women with cervical cancer and the healthy controls

IL-RA allele	Case (%)	Control (%)
A	69	77
B	26	22
C	2.9	1.43
D	0.48	0.79

(AC) cervix cancer. One hundred and twenty-nine were identified as SCC and 19 as AC. The average age in years was calculated as 48.55 ± 9.43 for cases and 48.81 ± 9.64 for controls. Compared to the controls, study group had age at the time of the marriage (16.36 ± 3.39; young), the birth of first child (18.39 ± 3.39) , and a greater mean number of children (4.11). Age at menarche and menopause were found to be comparable between cases and controls. Alleles frequencies *lL-lRA* are shown in Table 3.The frequency of allele A and B in cases is greater than control group. The association between the *lL-lRA* gene and cervical cancer is summarized in Table 4. The combination of *CB* and *CA* genotypes increased the risk of cervical cancer (OR = 2.24, 95% CI = 0.81–6.22). Table 5 shows interaction between *lL-lRA* and HPV type 16 and 18 in cervical cancer patients and healthy controls. There was a strong significantly protective association between heterozygous *AB* genotype and HPV 18 (OR = 0.11, 95% CI = 0.04–0.30, $p = 0.0000000$). Similarly this result was demonstrated, in combined *AB* + *BB* genotypes with HPV 18 (OR = 0.12, 95% CI=0.05–0.30, $p = 0.000$) and with HPV type 16 + 18 (OR = 0.18, 95% CI = 0.08–0.38, $p = 0.000005$).The association of *lL-lRA* genotype with type of cancer are briefed in Table 6. There was high protective significant association between heterozygous genotype *AB* with adenocarcinoma (OR = 0.19, 95% CI = 0.09–0.40, $p = 0.0000002$). The association between the *lL-lRA* genotype and the stages of cervical cancer are given in Table 7. We found a statistically highly significant protection between heterozygous *AB* genotype in stage IA (OR = 0.03, 95% CI = 0.00–0.21, $p = 0.0000000$), stage IIA (OR = 0.14, 95% CI = 0.06–0.34, $p = 0.0000000$), stage IIIA (OR = 0.38, 95% CI = 0.23–0.63, p = 0.00008) stage IB (OR = 0.11, 95% CI = 0.04–0.30, $p = 0.0000000$), stage IIB (OR = 0.37, 95% CI = 0.20–69, $p = 0.001$); and *AB* + *CB* + *BB* genotypes in stage IA (OR = 0.14, 95% CI = 0.06–0.31, $p = 0.000$), stage IIIA (OR = 0.28, 95% CI = 0.15–0.53, $p = 0.0000300$), stage IIB (OR = 0.16, 95% CI = 0.08–34, $p = 0.000$).

Table 4 Genotype *IL-1RA* gene in case and control

AAGenotype	Case 139 (%)	Control 209 (%)	OR (95% CI)	*P*
AA	56 (40.3)	94 (45.0)	1.0 (ref)	1.0 (ref)
AB	59 (42.4)	91 (43.5)	1.09 (0.67–1.78)	0.8
AB/BB	70 (50.3)	104 (49.7)	1.13 (0.70–1.81)	0.6
DD/DB	2 (0.7)	1 (0.5)	1.79 (0.78–4.08)	0.5
CB/CA	12 (8.6)	9 (4.3)	2.24 (0.81–6.22)	0.1
BB	11 (7.9)	13 (6.2)	1.42 (0.55–3.67)	0.5

Table 5 Assessments of interaction between *IL-1RA* and HPV in cervical cancer and control

AAGenotype *IL-1RA*	Type of HPV	n/c	OR (95% CI)	*P*
AA	Without HPV	56/94	1.00 (ref)	1.0 (ref)
BB	HPV Type 16	6/13	0.85 (0.42–1.69)	0.8
AB	HPV Type 16	33/91	0.61 (0.35–1.05)	0.07
AB	HPV Type 16 + 18	10/91	0.18 (0.08–0.40)	0.000002
AB/ BB	HPV Type 18	5/104	0.12 (0.05–0.30)	0.0000000
CB/A	HPV Type 18	2/4	0.89 (0.28–2.82)	1.0
BB	HPV Type 18	1/1	0.19 (0.03–1.28)	0.03
AB	HPV Type 18	4/91	0.11 (0.04–0.30)	0.0000000
AB/BB	HPV Type 16	39/104	0.63 (0.37–1.06)	0.08
AB/BB	HPV Type 16 + 18	11/104	0.18 (0.08–0.38)	0.0000005
CB	HPV Type 18	2/4	0.89 (0.28–2.82)	1.0
CB	HPV Type 16	4/4	1.34 (0.65–2.76)	0.4
CA	HPV Type 16	3/5	1.00 (0.40–2.52)	1.0
CB/A	HPV Type 16	7/9	1.31 (0.41–4.10)	0.8

Table 6 Determination of interaction between *IL-1RA* genotype and type of cervical cancer

Genotype *IL-1RA*	Stage of cancer	n/c	OR (95% CI)	*P*
AA intact	1.0 (ref)	56/94	1.00 (ref)	1.0 (ref)
AB	SCC	52/91	0.96 (0.58–1.59)	0.9594444
	AC	7/91	0.19 (0.09–0.40)	0.0000002
BB	SCC	8/13	1.03 (0.36–2.88)	0.8625293
	AC	3/13	0.50 (0.18–1.42)	0.2295430
CB	SCC	8/13	1.49 (0.80–2.77)	0.4598827
	AC	3/13	0.54 (0.09–3.13	0.6524898
AB/BB	AC	10/104	0.16 (0.07–0.35)	0.0000002
CB/CA	SCC	10/9	1.87 (0.65–5.37)	0.2992052
	AC	2/9	0.49 (0.14–1.74)	0.3301742
AB/CA	SCC	57/96	1.00 (0.61–1.63)	0.9166237
	AC	8/96	0.14 (0.06–0.32)	0.0000002
AB/CB	SCC	57/95	1.01 (0.62–1.65)	0.9290893
	AC	8/95	0.14 (0.06–0.33)	0.0000002
AB/CB/BB/CA	SCC	70/108	1.09 (0.68–1.74)	0.7982109
	AC	12/113	0.18 (0.08–0.37)	0.0000002

Table 7 Association between the *IL-1RA* genotypes and stage of cervical cancer

AAGenotype *IL-1RA*	Stage of cancer	n/c	OR (95% CI)	*P*
AA intact	1.0 (ref)	56/94	1.00 (ref)	1.0 (ref)
AB	Stage IA	1/91	0.03 (0.00–0.21)	0.0000000
	Stage IIA	5/91	0.14 (0.06–0.34)	0.0000000
	Stage IIIA	15/91	0.38 (0.23–0.63)	0.0000817
	Stage IB	4/91	0.11 (0.04–0.30)	0.0000000
	Stage IIB	20/91	0.37 (0.20–0.69)	0.0011222
	Stage IIIB	14/91	0.26 (0.13–0.52)	0.0000443
BB	Stage IIA	3/13	0.50 (0.18–0.42)	0.2295430
	Stage IIIA	1/13	0.19 (0.03–1.28)	0.0359755
	Stage IIB	4/13	0.63 (0.26–1.52)	0.3911350
CB	Stage IA	1/4	0.54 (0.09–3.13)	0.6524898
AB/BB	Stage IIA	8/104	0.13 (0.05–0.30)	0.0000000
	Stage IIIA	16/104	0.26 (0.13–0.50)	0.0000176
	Stage IB	4/104	0.10 (0.04–0.27)	0.0000000
	Stage IIIB	17/104	0.27 (0.14–0.52)	0.0000322
CA	Stage IIA	1/5	0.45 (0.07–2.70)	0.4165003
	Stage IIIA	2/5	0.77 (0.23–2.51)	1.0000000
AB/CB/BB	Stage IA	9/108	0.14 (0.06–0.31)	0.0000000
	Stage IIA	8/104	0.13 (6.05–0.30)	0.0000000
	Stage IIIA	18/108	0.28 (0.15–0.53)	0.0000306
	Stage IIB	7/108	0.16 (0.08–0.34)	0.0000000

Discussion

Identification of SNPs (single nucleotide polymorphisms) in human genome has great implications in the study of disease susceptibility. The SNPs in *lL-lRA* gene (VNTR) have been found to be associated with different immunological diseases [3]. Experimental data supported the role for *lL-lRA* cytokine as autocrine or paracrine stimuli in murine and human carcinogenesis [34]. Decades of studies have confirmed that cervical infection by high-risk HPV types is a precursor event to cervical cancer. The natural history of cervical cancer as a continuous single disease process progressing gradually from mild cervical intraepithelial neoplasia (CIN1), to more severe neoplasia and micro-invasive lesion (CIN2 or CIN3) and finally to invasive disease has been the basis for diagnosis, therapeutic measure, and

secondary preventive strategies [16]. The plasma level of *IL-RA* was influenced by the number of repeats in the *IL-RA* VNTR [1] and the allele with two repeats (designated as allele B in this study) has been reported to be associated with various diseases like, vestibulite, ulcerative colitis, alopecia areala, psoriasis, autoimmune conditions [2, 19], and idiopathic recurrent miscarriage [31]. El-Omar et al. [12] and Machado et al. [20] have reported an association of the IL-1 gene cluster polymorphism with enhanced production of IL-1b and gastric cancer, respectively. Their finding complements the most widely accepted multistage model of gastric carcinogenesis and underlines the fact that host genetic factors may determine why some people infected with *Helicobacter pylori* develop gastric cancer while others do not [12, 20]. This hypothesis can be transferred and applied to other solid tumor such as cervix cancer. Sehouli et al. [26] established that *lL-lRA* associated with cervical cancer and Allele 2 heterozygous has a greater risk in developing cervical cancer ($p = 0.0400000$). Mustea et al. [22] reported that allele 2 of the gene encoding for *IL1-RA* as a genetic determinant of cervical cancer. Our study confirmed these data ($p = 0.0000200$) in patient who were suffering from adenocarcinoma. The allele frequencies were different between cases and controls. Therefore, we did not find any controversy with pervious studies in North Indian population [3]. The polymorphism of *IL-1RA* seems to be involved in the induction of different solid tumors [26]. Bid et al. [4] suggested that *IL-1RA* intron 2 polymorphism plays a prominent role in bladder cancer in North India population. Grimm et al. [15] also demonstrated the same result with respect to vulvar carcinogenesis. Viet et al. [32] found that the *IL-1RA* polymorphism is associated with colorectal carcinogenesis. Hu et al. [17] showed that the allele 2 of *IL-1RN* (*IL-RN*×2) increased the risk of lung cancer in the Chinese population. Glas et al. [14] revealed that the homozygous genotype *IL-1RN*×2/2 of the *IL-RN* gene is strongly associated with early-stage gastric cancer ($p < 0.0001$)

So, *lL-lRA* mutation serum provided information about the prognosis of the patients. We observed association between specific alleles and clinical feature such as stage, and histological type in cervical cancer patient. Regarding other cancers such as ovarian cancer, no relation was found between specific allele and clinical feature [25]. Also, we demonstrated the influence of heterozygous *AB* genotype and *AB* + *BB* genotype *IL-RA* on risk of cervical cancer in patients showed high-risk of HPV 16 and 18 types. Our study showed that heterozygous *AB* genotype *lL-lRA* was increased in cervical cancer patients who were prone to high-risk of types 16 and 18. This is perhaps the first study until now that provided data regarding interaction between *lL-lRA* and HPV in cervical cancer and the first report on polymorphic changes in gene encoding IL-RA in patient with cervical cancer from North Indian population. The study concluded that cervical cancer is known to be a polygenic disease and genetic factors play an additional role in the indication of its malignancy, whereas only some of the genes were identified in cervical cancer patients. Some of them can mask potential influences of polymorphisms in the *lL-lRA* gene. These must be included in a panel of polymorphic genes which are associated with cervical cancer, similar to the gene regulating cell cycle [7] and MTHFR [29].

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