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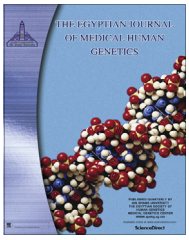

ORIGINAL ARTICLE

Cytokine gene polymorphisms and their association with cervical cancer: A North Indian study

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KEYWORDS

Cervical cancer;
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Abstract *Introduction:* The production of cytokines, growth factors and adhesion molecules promotes tumor progression and involves inflammation, angiogenesis and thrombosis, thus providing optimal conditions for cancer development.

Materials and methods: The present study was undertaken to evaluate association of cytokine gene polymorphisms with cervical cancer in a north Indian population. Genotyping of single nucleotide polymorphisms (SNPs) *viz*. *IL-6*-597G/A (rs1800797), *IL-1β*-511C/T (rs16944) and *TNF-α*-308G/A (rs1800629) was carried out in 100 each of cases and healthy age matched controls by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). Genotype and allele frequencies were calculated by SPSS (ver.16) and gene–gene interaction was analyzed using SHEsis (ver. Online).

Results: Epidemiological studies showed that women >40 years have higher risk of cervical cancer due to early pregnancies. *IL-6* and *TNF-α* promoter polymorphisms showed significant association ($P < 0.001$) while the SNP combinations G A T* and G G T* of *IL-6*-597A/G, *TNF-α*-308G/A and *IL-1β*-511C/T polymorphisms showed increased risk up to 9.0 and 3.30 times respectively.

Conclusion: Therefore, the promoter polymorphisms in cytokine genes can be used as biomarkers to predict cervical cancer susceptibility in a north Indian population. However, such studies need to be carried out in different ethnic populations in order to discover the specific risk alleles, genotypes and combinations for disease prediction.

1. Introduction

Cervical cancer is the second most common cancer in women worldwide with 5,30,000 new cases every year. A mortality of 2,70,000 cases and 5-year prevalence of 1,547,161 was reported in 2013 [1]. The highest incidence is found in developing countries out of which 25% is from India.

Epidemiological and clinical data show that the development of cervical cancer is a multifactorial process in which infection with human papillomavirus (HPV) takes a central place along with other risk factors such as smoking, immunosuppression, immunodeficiency, diet, parity, age at first full-term pregnancy and family history [2].

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In cancer development; inflammation, angiogenesis and thrombosis are involved which strongly correlate with immune cells residing in the microenvironment of cancerous tissues. Immune cells are involved in the production of cytokines (pro- and anti-inflammatory), growth factors and adhesion molecules which promote tumor progression by a signaling cascade and provide optimal growth conditions for cancerous cells. A number of previous reports suggested that chronic inflammation is associated with precancerous intraepithelial lesion and cervical cancer [3]. IL-6 acts as a defense mechanism at acute inflammatory levels but during chronic inflammation it behaves like a pro-inflammatory cytokine involved in immune regulation [4,5], hematopoiesis [6,7] and oncogenesis thereby inducing acute phase responses [8]. During chronic inflammation, IL-6 also favors mononuclear monocyte chemoattractant protein (MCP-1) secretion, angio-proliferation and anti-apoptotic functions on T cells. It is expressed by a wide variety of different cell types including keratinocytes of the uterine cervix [9]. A promoter SNP-597 (A/G) in *IL-6* gene located on chromosome 7p21 [10] is a susceptibility factor in many diseases like coronary heart disease, breast cancer, cervical cancer *etc* [11].

Cytokine family interleukin-1 (pro-inflammatory) consists of several members including interleukin-1beta (IL-1β) and interleukin-1receptor antagonist (IL-1RN) which are components of innate immune system as well as chronic inflammation. IL-1β is a pro-inflammatory cytokine produced by blood monocytes and tissue macrophages which regulate the expression of several molecules involved in inflammation. IL-1β acts synergistically with chemical carcinogens resulting in proliferation of mutated cells and further accumulation of genetic defects. IL-1RN inhibits the activities of IL-1β by competitively binding to IL-1β receptor and modulating a variety of interleukin-1 related immune and inflammatory responses [12–15].

Tumor necrosis factor alpha (TNF-α) is another potential pro-inflammatory cytokine and plays a role in inflammation and malignant diseases [16]. *TNF-α* gene located on chromosome 6 between HLA class I and II regions (within the major histocompatibility complex, MHC) activates the positive cell cycle regulator NF-jB resulting in proliferation of cells, invasion and finally metastasis [17,18]. A single nucleotide polymorphism (SNP) in the promoter region of *TNF-α* gene associated with its regulation and expression may contribute to the pathogenesis and promote malignant progression of cervical cancer. In the present study, the association of genetic polymorphisms in *IL-6*, *IL-1β* and *TNF-α* genes was studied in cervical cancer patients from north India.

2. Subjects and methods

2.1. Patient selection and sample collection

Our study included cervical cancer patients (n = 100) and normal control subjects (n = 100) enrolled in the outpatient unit of Department of Obstetrics and Gynecology, King George's Medical University, Lucknow, India. The study was conducted after due approval of Institutional Ethics Committee (No. 4135/R.Cell-13, dated 15/4/2013) and written consent from all subjects. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. After selection, subjects were counseled and cervical biopsy was conducted by expert gynecologists and sent for histopathological examination. Clinical details of patients and other risk factors *viz*. smoking status, parity, abortion, and use of contraception were precisely recorded. Blood samples (2 ml) from all study subjects were collected in EDTA vials and stored at −20 °C until further use. The inclusion/exclusion criteria for cases and controls are given below:

Inclusion criteria for cases:

- Histopathologically proven cases of squamous cell carcinoma (SCC) all stages and cervical intraepithelial neoplasia (CIN).
- Women between 40 and 70 year with cervical cancer symptoms such as vaginal discharge, pain in lower abdomen, menstrual irregularity and contact bleeding.
- Positive cervical biopsy.

Exclusion criteria for cases:

- Women > 70 years.
- Cases having double malignancy.
- Cases having any co-morbid conditions such as diabetes, tuberculosis *etc*.
- Negative cervical biopsy.
- Cases already on follow-up.
- Not willing to participate.

Inclusion criteria for control subjects:

- Healthy age matched.
- Histopathologically negative for all stages of squamous cell carcinoma (SCC) and cervical intraepithelial neoplasia (CIN).
- No previous history of any type of cancer.

2.2. DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using salting out method [19] with slight modifications [20]. Genotyping of three polymorphisms *IL-6*-597A/G (rs1800797), *IL-1β*-511C/T (rs16944) and *TNF-α*-308G/A (rs1800629) were performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR–RFLP). The primers designed by Primer 3.0 online software were F-5′-GGAGTCACACACTCCACCT-3′/R-5′-CTGATT GGAAACCTTATTAAG-3′;F-5′-AGGCAATAGGTTT TGAGGGCCAT-3′/R-5′-TTGGGGACACAAGCATCAAG G-3′ and F-5′-TGGCATTGATCTGGTTCATC-3′/R-5′-GTT TAGGAATCTTCCCACTT-3′ respectively. The 15 μl reaction mixture contained 100 ng of template DNA, buffer (100 mMTris, pH 9.0; 500 mMKCl; 15 mM $MgCl_2$; 0.1% gelatin), 200 μM dNTP, 10 pmol of each primer and 1.0 unit Taq DNA polymerase (Biosciences, India). The PCR products of *IL-6*, *TNF-α* and *IL-1β* were digested with FokI, NcoI and SacI restriction enzymes respectively (Thermo Fisher Scientific Inc., USA), electrophoresed on 12.5% polyacrylamide gels (Sisco Research Laboratories Pvt. Ltd., India), stained with EtBr (Sisco Research Laboratories Pvt. Ltd., India) and documented

in Geldoc system (Vilber Lourmat, France). The genotyping results were cross checked randomly in order to confirm our methodology.

2.3. Statistical analysis

Allele frequencies and carriage rates of alleles in all groups were compared in a 2 × 2 contingency table and genotype frequencies in a 2 × 3 contingency table using Chi square test (χ^2) and Fisher's exact *t*-test. Hardy–Weinberg equilibrium at individual locus was assessed by χ^2 statistics using Statistical Package for Social Science (SPSS) version 16.0. All P values were two-sided and differences were considered statistically significant for $P < 0.05$. Odds Ratio (OR) at 95% confidence intervals (CI) was determined to describe the strength of association by Logistic Regression Model. The associations of cervical cancer risk factors and gene polymorphisms with cervical cancer were evaluated by Logistic Regression Model (dominant model). All statistical analyses were carried out using SPSS software, version 16.0.

Table 1 Effect of socio demographic characteristics on cervical cancer.

Demographic Profile	Controls (n = 100) (%)	Cases (n = 100) (%)	χ^2	p-value
Age (in Years)				
⩽40	22(22.0)	20(20.0)	0.121	0.729
>40	78(78.0)	80(80.0)		
Place of residence				
Rural	80(80.0)	88(88.0)	2.381	0.123
Urban	20(20.0)	12(12.0)		
Educational status				
Illiterate	40(40.0)	56(56.0)	**5.128**	**0.024**#
Literate	60(60.0)	44(44.0)		
Socio-economic status				
Lower	40(40.0)	52(52.0)	4.520	0.104
Middle	50(50.0)	44(44.0)		
Upper	10(10.0)	04(04.0)		
Personal history				
(A) Parity				
None	08(08.0)	01(01.0)	**22.752**	**<0.001**#
⩽2	68(68.0)	44(44.0)		
>2	24(24.0)	55(55.0)		
(B) Age at first full term pregnancy				
⩾20	84(84.0)	60(60.0)	**14.286**	**<0.001**#
<20	16(16.0)	40(40.0)		
(C) Menstrual cycle				
Irregular	68(68.0)	42(42.0)	**13.657**	**<0.001**#
Regular	32(32.0)	58(58.0)		
(D) Menstrual hygiene				
Cloths	28(28.0)	59(59.0)	**19.551**	**<0.001**#
Napkins	72(72.0)	41(41.0)		
Reuse of cloths				
No	21(75.0)	22(37.3)	**10.803**	**<0.001**#
Yes	07(25.0)	37(62.7)		
(E) Use of contraception				
Oral contraceptive pills	19(19.0)	11(11.0)	**12.723**	**0.005**#
Condoms	23(23.0)	14(14.0)		
Others	16(16.0)	08(08.0)		
None	42(42.0)	67(67.0)		
Smoker				
Active smoker	21(21.0)	38(38.0)	**6.948**	**0.008**#
Passive smoker	79(79.0)	62(62.0)		
HR-HPV				
Positive	36(36.0)	86(86.0)	**52.543**	**<0.001**#
Negative	64(64.0)	14(14.0)		

χ^2 Chi-square, #implies significant at 5% level.
Bold denotes significant p values.

2.4. Gene–gene interaction analysis

Gene–gene interaction and pairwise linkage disequilibrium (LD) based on '*D*' statistics and correlation coefficient (r^2) of frequencies were analyzed using SHEsis [21].

3. Results

A large number of women in the age group >40 years showed a higher incidence of cervical cancer as compared to the lower age group (⩽40 year) which was evident from demographic data (Table 1). Risk factors viz. higher parity, lower age at first full term pregnancy, irregular menstrual cycle, menstrual hygiene, no contraception showed significant relation with cervical cancer. A significant correlation with smoking as well as high risk human papillomavirus (HR-HPV) incidence was observed in cases ($p < 0.001$) (Table 1).

Controls and cervical cancer cases (histopathologically proven) were successfully genotyped for *IL-6*-597A/G, *IL-1β*-511C/T and *TNF*-α-308G/A polymorphisms (Fig. 1). The allele and genotype frequency distributions as well as carriage rates are shown in Table 2. All allelic and genotypic frequencies were found to be in Hardy–Weinberg equilibrium (HWE). In *IL-6*-597A/G polymorphism, 'GG' (25%) and 'AG' (40%) genotype frequencies in cases were found to be higher in comparison to controls showing significant difference ($p < 0.001$) (Table 2). Moreover, the prevalence of -597*G allele was significantly higher in cases (45.0%, $p < 0.001$) with

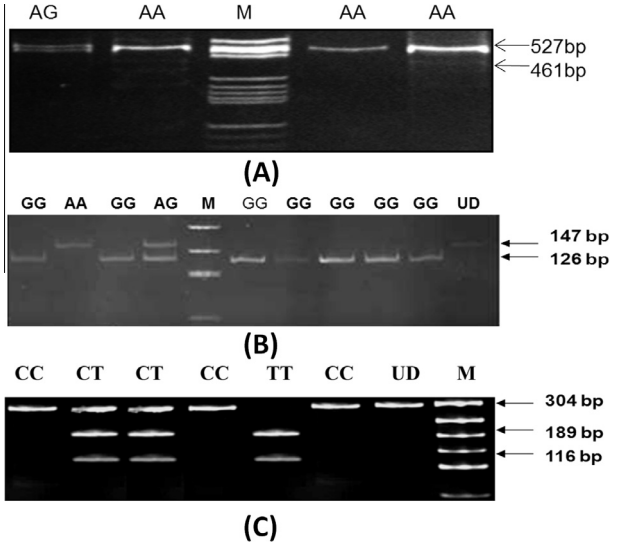


Figure 1 Polyacrylamide gels (12%) stained with EtBr showing genotypes of different gene polymorphisms. (A) *IL-6*-597A/G genotypes; M, pBR322/HaeIII. (B) *TNF*-α-308G/A genotypes; M, pUC19/MspI. (C) *IL-1β*-511C/T genotypes; M, pUC19/MspI. UD, Undigested. Markers obtained from Biosciences, India.

an Odds Ratio (OR) of 5.4. The carriage rate analysis also showed that the presence of -597*G allele of *IL-6*-597A/G increases the risk of cervical cancer in our population up to 6.2 times ($p < 0.001$) (Table 2). Although the frequency of cases with 'TT' genotype (57% vs 47%) and -511*T allele (88%) was higher in comparison to controls, the IL-1b-511C/T polymorphism did not show significant association (Table 2).

TNF-α-308G/A polymorphism showed a higher 'AA' genotype frequency in cases (30%) in comparison to controls (9%) and significant difference ($P < 0.001$). The prevalence of -308*A allele in cases was higher in comparison to controls (63% vs 53%) with an OR of 1.5 and showed significant association with cervical cancer ($p < 0.043$) (Table 2).

In order to examine the role of risk factors in cervical cancer, we stratified the analysis of effects of *IL-6*, *IL-1β* and *TNF*-α polymorphisms on cervical cancer according to various risk factors. The effect of *IL-6*-597A/G polymorphism on the risk of cervical cancer showing significant association included high parity ($p < 0.001$), lower age at full term pregnancy ($p < 0.001$), irregular menstrual cycle ($p < 0.001$), menstrual hygiene ($p < 0.001$) and smoking ($p < 0.001$) (Table 3). Similar result was also observed in the case of *IL-1β* gene polymorphism with positive HR-HPV infection status ($p < 0.001$) and active smoking ($p < 0.001$) (Table 4). The association of *TNF*-α with various risk factors such as high parity, lower age at of first full term pregnancy, irregular menstrual cycle and hygiene were found to be significant ($p < 0.001$) in cases of cervical carcinoma (Table 5).

Gene-gene interaction analysis showed that individuals with the SNP combinations G A T* and G G T* of *IL-6*-597A/G, *TNF*-α-308G/A and *IL-1β*-511C/T polymorphisms have an increased risk of cervical cancer up to 9.0 and 3.3 times respectively in the study population (Fig. 2).

Table 2 Genotypic, allelic and carriage rate frequencies of *IL-6*-597A/G, *TNF*-α-308G/A and *IL-1β*-511C/T gene polymorphisms in healthy controls ($n = 100$) and cervical cancer cases ($n = 100$).

Genotype frequency		Number (%frequency)			χ^2	*p*-value
	IL-6	AA	AG	GG	**39.702**	**< 0.001**#
	Controls	77 (77.0)	20 (20.0)	3 (3.0)		
	Cases	35 (35.0)	40 (40.0)	25 (25.0)		
	IL-1β	CC	CT	TT	2.166	0.339
	Controls	2 (2)	51 (51)	47 (47)		
	Cases	1 (1)	42 (42)	57 (57)		
	TNF-α	GG	GA	AA	**35.485**	**< 0.001**#
	Controls	3 (3)	88 (88)	9 (9)		
	Cases	4 (4)	66 (66)	30 (30)		
Allele frequency		Number (%frequency)		*p*-value	Odds Ratio (OR)	95% CI
	IL-6	A	G			
	Controls	174 (87.0)	26 (13.0)	**< 0.001**#	**5.476**	3.330-9.004
	Cases	110 (55.0)	90 (45.0)			
	IL-1β	C	T			
	Controls	55 (27.5)	145 (72.5)	0.203	1.345	0.852-2.123
	Cases	44 (22)	156 (88)			
	TNF-α	G	A			
	Controls	94 (47)	106 (53)	**0.043**#	**1.510**	1.013-2.251
	Cases	74 (37)	126 (63)			
Carriage rate	IL-6	A (+)	A (−)			
	Controls	97 (97.0)	3 (3.0)	**< 0.001**#	0.093	0.027-0.319
	Cases	75 (75.0)	25 (25.0)			
		G (+)	G (−)			
	Controls	23 (23.0)	77 (77.0)	**< 0.001**#	**6.217**	3.341-11.570
	Cases	65 (65.0)	35 (35.0)			
	IL-1β	C (+)	C (−)			
	Controls	53 (53)	47 (47)	0.158	0.669	0.383-1.168
	Cases	43 (43)	67 (67)			
		T (+)	T (−)			
	Controls	98 (98)	2 (2)	0.568	2.020	0.180-22.645
	Cases	99 (99)	1 (1)			
	TNF-α	G (+)	G (−)			
	Controls	91 (91)	9 (9)	**< 0.001**#	0.231	0.103-0.517
	Cases	70 (70)	30 (30)			
		A (+)	A (−)			
	Controls	97 (97)	3 (3)	0.701	0.742	0.162-3.405
	Cases	96 (96)	4 (4)			

χ^2 Chi-square, 95%CI = confidence interval, OR = Odds Ratio, #implies significant at 5% level.
Bold denotes significant *p* values.

Table 3 Association between cervical cancer risk factors and *IL-6*-597A/G gene polymorphism.

IL-6–597A/G	Controls (n = 100)		Cases (n = 100)		p-value	OR, (95% C.I)
	AA (n = 77)	AG (n = 20) + GG (n = 3)	AA (n = 35)	AG (n = 40) + GG (n = 25)		
Parity						
None	5	3	00	1	.999	5.3×10^8 (.00)
⩽2	55	13	20	24	**< 0.001**#	**5.07 (2.17–11.84)**
> 2	17	7	15	40	**0.001**#	**6.47 (2.24–18.72)**
Age at first full term pregnancy						
⩾20	45	15	27	57	**< 0.001**	**10.22 (4.95–21.10**
< 20	32	8	8	8	**0.030**#	4.0 (1.45–13.95)
Menstrual cycle						
Regular	52	15	18	24	**< 0.001**#	**4.62 (1.99–10.69)**
Irregular	9	7	17	41	0.051	3.10 (0.994–1.412)
Menstrual hygiene						
Cloths	23	5	17	42	**< 0.001**#	**11.36 (3.71–34.80)**
Napkins	54	18	18	23	**0.001**#	**3.83 (1.69–8.66)**
Reuse of cloths						
No	12	4	9	18	**0.011**#	6.0 (1.50–23.99)
Yes	11	1	8	24	**0.002**#	**33.0 (3.66–297.2)**
Use of contraception						
OCP	12	8	4	7	0.213	2.62 (0.574–11.99)
Condoms	16	7	0	8	0.998	$1.84 * 10^9$ (0.00)
Others	8	8	9	5	0.433	0.556 (0.128–2.41)
None	41	0	22	45	0.997	$3 * 10^9$ (0.00)
Smoking						
Smoker	12	9	4	34	**< 0.001**#	**11.33 (2.94–43.68)**
Non-smoker	65	14	31	31	**< 0.001**#	**4.64 (2.16–9.95)**
HR-HPV infection status						
Positive	20	16	28	58	0.433	0.55 (0.128–2.41)
Negative	37	7	7	7	**0.014**#	5.28 (1.40–19.84)

95% CI = confidence interval, OR = Odds Ratio, #implies significant at 5% level.
Bold denotes significant p values.

4. Discussion

Risk factors for cervical cancer showed that the development of cancer is a multifactorial process in which infection with high risk human papillomavirus (HR-HPV) takes a central place in the development of cervical cancer. However, HPV infection is not the only cause of cervical cancer but other risk cofactors have been identified including oral contraceptive use, smoking, immunosuppression, immunodeficiency, diet, parity, age at the first full-term pregnancy and family history of cervical cancer. In the present study, it was found that women of age > 40 years have higher incidence of cervical cancer as compared to the age group ⩽40 years. This might be due to the fact that during adolescence (15–19 years) cervical epithelium is more susceptible to HPV; therefore, early age (< 40 years) of sexual intercourse and subsequent pregnancy become risk factors for cervical cancer [22]. It has been reported that the disease progression takes 10–15 years to manifest. Therefore, cases with HPV infection and subjected to other risk factors show the disease beyond 40 years of age [23]. It was also found that females getting pregnant at < 20 years of age with more than two children are more susceptible to cervical cancer and showed significant correlation ($p < 0.001$). Poor menstrual hygiene such as use of homemade sanitary napkins and their reuse after washing were found to be independent risk predictors for cervical cancer in women. This factor was linked to low socio-economic conditions since the majority of the study population was illiterate and belonged to low economic strata in rural areas, and therefore showed higher frequency of cervical cancer.

Functional DNA polymorphisms which alter the expression of inflammatory molecules play a decisive role at different stages of tumor development including pathogenesis, invasion and metastasis. HPV infection and inflammation during cervical cancer are critical components in progression of the disease. During HPV infection, cytokine genes responsible for inflammation are regarded as a potential source of cervical cancer risk. Inflammation involves extensive tissue remodeling events which are orchestrated by complex networks of cytokines, chemokines and bio-active lipids working across multiple cellular compartments to maintain tissue homeostasis. Only 5–10% of all cancers are caused by inheritance of mutated genes and somatic mutations, whereas the remaining 90–95% have been linked to lifestyle factors and environment [24].

In neoplastic diseases, circulating levels of IL-6 increase markedly during development and progression of tumors. Polymorphisms in *IL-6* gene are reported as susceptibility factors in several diseases such as multiple myeloma [25], rheumatoid arthritis [4], Castleman's disease [26], AIDS [27,28], mesangial proliferative glomerulonephritis [29], psoriasis [30], Kaposi's sarcoma [31], sepsis [32], osteoporosis [33,28] and cervical cancer [11]. The association of *IL-6*-597A/G polymorphism with cervical cancer in the study population showed that the prevalence of *IL-6-597**G allele was significantly

Table 4 Association between cervical cancer risk factors and *IL-1β*-511C/T gene polymorphism.

IL-1β-511C/T	Controls (n = 100)		Cases (n = 100)		p-value	OR, 95%CI
	CC (n = 2) + CT (n = 51)	TT (n = 47)	CC (n = 1) + CT (n = 42)	TT (n = 57)		
Parity						
None	7	1	1	0	1.00	000(00)
⩽2	32	36	16	28	0.265	1.55 (0.715–3.384)
>2	14	10	26	29	0.367	1.562 (0.593–4.115)
Age at first full term pregnancy						
⩾20	34	26	36	48	0.103	1.744 (0.893–3.404)
<20	19	21	7	9	0.799	1.163 (0.362–3.735)
Menstrual cycle						
Regular	36	32	15	27	0.080	2.025 (0.918–4.465)
Irregular	17	15	28	30	0.660	1.214 (0.512–2.882)
Menstrual hygiene						
Cloths	14	31	15	27	0.649	0.813 (0.333–1.985)
Napkins	39	16	27	30	**0.012**$^{\#}$	2.708(1.241–5.910)
Reuse of cloths						
No	4	3	17	10	0.778	0.784 (0.145–4.244)
Yes	8	13	12	19	0.964	0.974 (0.312–3.044)
Use of contraception						
OCP	14	6	6	5	0.392	1.944 (0.423–8.928)
Condoms	16	7	3	5	0.120	3.810(0.707–20.533)
Others	10	6	4	10	0.069	4.167 (0.894–19.419)
None	13	28	30	37	0.180	0.573 (0.253–1.294)
Smoking						
Smoker	16	5	16	22	**0.015**$^{\#}$	**4.400(1.335–14.506)**
Non-smoker	37	42	27	35	0.697	1.142(0.585–2.229)
HR-HPV infection status						
Positive	27	9	37	49	**0.002**$^{\#}$	**3.973 (1.670–9.453)**
Negative	26	38	6	8	0.878	0.112 (0.283–2.940)

95% CI = confidence interval, OR = Odds Ratio, $^{\#}$implies significant at 5% level.
Bold denotes significant p values.

higher in cases (45.0%, $p < 0.0001$). Gene-gene analysis also showed that promoter polymorphism in *IL-6* gene in combination with -308*A allele of *TNF-α* and -511*T allele of *IL-1β* genetic variants increases the risk of cervical cancer up to 9.0 times in the present study (Fig. 2). The association between *IL-6* promoter polymorphism and cancer risk was evident among Asians and Africans but not Caucasians [34]. Other *IL-6* SNPs like -174G/C polymorphism has been associated with an increased risk of breast cancer [35,36], leukemia [37], colorectal cancer [38] and basal cell cancer [39].

The *IL-1β* polymorphism was not found to be associated with the risk of cervical cancer in the study population. There are previous reports which suggest that IL-1β may be involved in early steps of cervical carcinoma development and progression [40,14]. Increased levels of IL-1β reduce apoptosis by changing the ratio of BCL-2/BAX protein and increased p53 mutation which leads to DNA damage in epithelial cells of the cervix [41]. Various epidemiologic risk factors of cervical cancer as positive HR-HPV infection status and active smoking are associated with IL-1β in the present study. However, it can be validated further by increasing the sample size of the study.

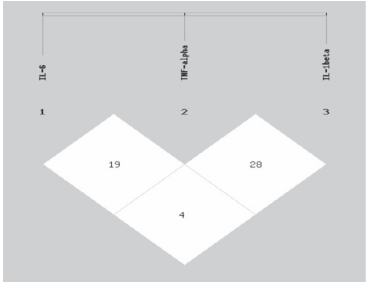
In our study, *TNF-α* polymorphism showed a significant association with various risk factors and cervical carcinomas while previous studies observed the same in Caucasian ethnic groups but not Asians [42]. In the present study -308*A allele was higher in cases (63%) with an OR of 1.5 which was earlier shown in Portuguese population with an OR of 1.8 [43]. The women carrying *TNF-α*-308*A allele were seen to have an increased disease risk up to 2 folds. In contrast, the South African population showed no association of *TNF-α* -308G/A polymorphism and risk of cervical cancer [44]. The difference between these polymorphisms may be due to the variation of ethnic groups, showed sample size, patient recruitment standards and geographical or environmental factors. The present study supports a potential role of genetic variations in the *TNF-α* promoter region in susceptibility to cervical cancer.

The results obtained from our study are consistent with several previous studies [15,45–47] but similar genotyping analysis in other ethnic populations are still necessary to validate the association between SNPs and susceptibility to cervical cancer. SNP studies show a considerable level of variation among various ethnic populations around the world. Therefore, it is essential to perform association studies/SNP analyses in individual populations so that they can be benefitted. Individuals at risk will be able to take prior precautionary measures and avoid or delay the onset of disease. The challenge for the future will be to understand the genotypic interactions between SNPs in the same gene or genes at different loci. Recent data implicate that cytokine gene polymorphisms are important in the pathogenesis of various neoplastic and non-neoplastic human diseases.

Table 5 Association between cervical cancer risk factors and *TNF*-α-308G/A gene polymorphism.

TNF-α-308G/A	Controls (n = 100)		Cases (n = 100)		p-value	OR, 95%CI
	GG (n = 3) + GA (n = 88)	AA (n = 9)	GG (n = 4) + GA (n = 66)	AA (n = 30)		
Parity						
None	6	2	1	0	0.999	00(00)
⩽2	62	6	30	14	**0.003**#	**4.822 (1.686–13.794)**
>2	23	1	39	16	**0.035**#	**9.436 (1.173–75.905)**
Age at first full term pregnancy						
⩾20	55	5	63	21	**0.014**#	3.667 (1.296–10.376)
<20	36	4	7	9	**0.001**#	**11.571 (2.771–48.316)**
Menstrual cycle						
Regular	65	3	28	14	**<0.001**#	**10.833 (2.884–40.684)**
Irregular	26	6	42	16	0.353	1.651 (0.573–4.756)
Menstrual hygiene						
Cloths	27	1	44	15	**0.036**#	**9.205 (1.150–73.691)**
Napkins	64	8	26	15	**0.002**#	**4.615 (1.747–12.195)**
Reuse of cloths						
No	19	1	18	5	0.146	5.278 (0.561–49.662)
Yes	8	0	26	10	0.999	4.971×10^8(00)
Use of contraception						
OCP	18	1	9	2	0.283	4.00 (0.319–50.220)
Condoms	18	5	7	1	0.574	0.514 (0.051–5.221)
Others	14	2	11	3	0.517	1.909 (0.270–13.490)
None	40	1	43	24	**0.003**#	**22.326 (2.885–172.763)**
Smoking						
Smoker	17	4	29	9	0.681	1.319 (0.352–4.943)
Non-smoker	74	5	41	21	**<0.001**#	**7.580 (2.660–21.604)**
HR-HPV						
Positive	31	5	63	23	0.130	2.263 (0.785–6.523)
Negative	60	4	7	7	**<0.001**#	**15.00 (3.495–64.376)**

95% CI = confidence interval, OR = Odds Ratio, #implies significant at 5% level.
Bold denotes significant p values.



	Case(freq)	Control(freq)	Chi2	Fisher's p	Pearson's p	Odds Ratio [95%CI]
A A C*	10.75(0.054)	33.90(0.170)	13.500	0.000241	0.000240	0.278 [0.136~0.570]
A A T*	64.46(0.322)	55.44(0.277)	0.977	0.322880	0.322820	1.241 [0.808~1.906]
A G C*	14.73(0.074)	10.38(0.052)	0.807	0.369182	0.369134	1.453 [0.640~3.298]
A G T*	21.06(0.105)	72.28(0.361)	36.625	1.54e-009	1.47e-009	0.208 [0.122~0.356]
G A C*	18.40(0.092)	11.72(0.059)	1.609	0.204752	0.204673	1.629 [0.762~3.486]
G A T*	37.39(0.187)	4.94(0.025)	27.839	1.37e-007	1.35e-007	9.084 [3.474~23.748]
G G T*	33.09(0.165)	11.34(0.057)	11.990	0.000539	0.000538	3.300 [1.629~6.684]

Figure 2 Haploview of SNPs *viz*. of *IL-6*-597A/G, *TNF*-α-308G/A and *IL-1β*-511C/T showing association with cervical cancer in a north Indian population. Pairwise linkage disequilibrium (LD) (SHEsis, ver. Online). 95%CI = confidence interval. * indicates allele combination of *IL-6*-597A/G, *TNF*-α-308G/A and *IL-1β*-511C/T gene polymorphisms.

Disclosure statement

No competing financial interests exist.

Conflict of interest

None declared.

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References

[1] GLOBOCAN, World Health Organization, 2013.
[2] Vaccarella S, Herrero R, Dai M, Snijders PJ, Meijer CJ, Thomas, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol Biomarkers Prev 2006;15:2148–53.
[3] Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. J Immunol 2005;175:3463–8.
[4] Hirano T, Matsuda T, Turner M, Miyasaka N, Buchan G, Tang B, et al. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur J Immunol 1988;18:1797–802.
[5] Smyth MJ, Ortaldo JR, Bere W, Yagita H, Okumura K, Young HA. IL-2 and IL-6 synergize to augment the pore-forming protein gene expression and cytotoxic potential of human peripheral blood T cells. J Immunol 1990;145:1159–66.
[6] Ogawa M, Clark SC. Synergistic interaction between interleukin-6 and interleukin-3 in support of stem cell proliferation in culture. Blood cells 1987;14:329–37.
[7] Bruno E, Hoffman R. Effect of interleukin 6 on in vitro human megakaryocytopoiesis: its interaction with other cytokines. Exp Hematol 1989;17:1038–43.
[8] Baumann H, Isseroff H, Latimer JJ, Jahreis GP. Phorbol ester modulates interleukin 6-and interleukin 1-regulated expression of acute phase plasma proteins in hepatoma cells. J Biol Chem 1988;263:17390–6.
[9] Woodworth CD, Simpson S. Comparative lymphokine secretion by cultured normal human cervical keratinocytes, papillomavirus-immortalized, and carcinoma cell lines. Am J Pathol 1993;142:1544–55.
[10] Sehgal PB, Zilberstein A, Ruggieri RM, May LT, Ferguson-Smith A, Slate DL, et al. Human chromosome 7 carries the beta 2 interferon gene. Proc Natl Acad Sci 1986;83:5219–22.
[11] Nogueira de Souza NC, Brenna SMF, Campos F, Syrjänen KJ, Baracat EC, Silva IDCG, et al. Interleukin-6 polymorphisms and the risk of cervical cancer. Int J Gynecol Cancer 2006;16:1278–82.
[12] Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis 2002;34:204–9.
[13] Engels EA, Wu X, Gu J, Dong Q, Liu J, Spitz MR, et al. Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. Cancer Res 2007;67:6520–7.
[14] Sobti RC, Tamandani DMK, Shekari M, Kaur P, Malekzadeh K, Suri V. Interleukin 1 beta gene polymorphism and risk of cervical cancer. Int J Gynaecol Obstet 2008;101:47–52.
[15] He B, Zhang Y, Pan Y, Xu Y, Gu L, Chen L, et al. Interleukin 1 beta (IL1B) promoter polymorphism and cancer risk: evidence from 47 published studies. Mutagenesis 2011;26:637–42.
[16] Beutler B, Bazzoni F. TNF, apoptosis and autoimmunity: a common thread? Blood Cells Mol Dis 1998;24:216–30.
[17] Aggarwal BB. Nuclear factor-κB: the enemy within. Cancer Cell 2004;6:203–8.
[18] Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res 2009;15:425–30.
[19] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
[20] Gautam S, Agrawal CG, Bid HK, Banerjee M. Preliminary studies on CD36 gene in type 2 diabetic patients from north India. Indian J Med Res 2011;134:107–12.
[21] Shi YY, He L. SHEsis is a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15:97–8.
[22] Mitra S. Study of the risk factors for cancer cervix in a speciality hospital in Kolkata. J Com Med 2009;5:1–5.
[23] Au WW, Abdou-Salama S, Sierra-Torres CH, Al-Hendy A. Environmental risk factors for prevention and molecular intervention of cervical cancer. Int J Hyg Environ Health 2007;210:671–8.
[24] Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, et al. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res 2008;25:2097–116.
[25] Kawano M, Hirano T, Matsuda T, Taga T, Horii Y, Iwato K, et al. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature 1988;332:83–5.
[26] Yoshizaki K, Matsuda T, Nishimoto N, Kuritani T, Taeho L, Aozasa K, et al. Pathological significance of interleukin 6 (IL-6/BSF-2) in Castleman's disease. Blood 1989;74:1360–7.
[27] Nakajima K, Yamanaka Y, Nakae K, Kojima H, Ichiba M, Kiuchi N, et al. A central role for Stat3 in IL-6-induced regulation of growth and differentiation in M1 leukemia cells. EMBO J 1996;15:3651–8.
[28] Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, et al. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J 1994;13:1189–96.
[29] Horii Y, Iwano M, Hirata E, Shiiki M, Fujii Y, Dohi K, et al. Role of interleukin-6 in the progression of mesangial proliferative glomerulonephritis. Kidney Int Suppl 1993;39:S71–5.
[30] Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, et al. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci 1989;86:6367–71.
[31] Miles SA, Rezai AR, Salazar-Gonzalez JF, Vander Meyden M, Stevens RH, Logan DM, et al. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. Proc Natl Acad Sci 1990;87:4068–72.
[32] Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Espevik T, et al. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. J Exp Med 1989;169:333–8.
[33] Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, et al. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 1992;257:88–91.
[34] Xu B, Niu XB, Wang ZD, Cheng W, Tong N, Mi YY, et al. IL-6-174G > C polymorphism and cancer risk: a meta-analysis involving 29,377 cases and 37,739 controls. Mol Biol Rep 2011;38:2589–96.
[35] Smith KC, Bateman AC, Fussell HM, Howell WM. Cytokine gene polymorphisms and breast cancer susceptibility and prognosis. Eur J Immunogenet 2004;31:167–73.
[36] Hefler LA, Grimm C, Lantzsch T, Lampe D, Leodolter S, Koelbl H, et al. Interleukin-1 and interleukin-6 gene polymorphisms and

the risk of breast cancer in caucasian women. Clin Cancer Res 2005;11:5718–21.
[37] Ennas MG, Moore PS, Zucca M, Angelucci E, Cabras MG, Melis M, et al. Interleukin-1B (IL1B) and interleukin-6 (IL6) gene polymorphisms are associated with risk of chronic lymphocytic leukaemia. Hematol Oncol 2008;26:98–103.
[38] Yu Y, Wang W, Zhai S, Dang S, Sun M. IL-6 gene polymorphisms and susceptibility to colorectal cancer: a meta-analysis and review. Mol Biol Rep 2012;39:8457–63.
[39] Rizzato C, Canzian F, Rudnai P, Gurzau E, Stein A, Koppova K, et al. Interaction between functional polymorphic variants in cytokine genes, established risk factors and susceptibility to basal cell carcinoma of skin. Carcinogenesis 2011;32:1849–54.
[40] Hall SK, Perregaux DG, Gabel CA, Woodworth T, Durham LK, Huizinga TWF, et al. Correlation of polymorphic variation in the promoter region of the interleukin-1β gene with secretion of interleukin-1β protein. Arthritis Rheum 2004;50:1976–83.
[41] Simonart T, Van Vooren JP. Interleukin-1β increases the Bcl-2/Bax ratio in Kaposi's sarcoma cells. Cytokine 2002;19:259–66.
[42] Pan F, Tian J, Ji CS, He YF, Han XH, Wang Y, et al. Association of TNF-α-308 and-238 polymorphisms with risk of cervical cancer: a meta-analysis. Asian Pac J Cancer Prev 2012;13:5777–83.
[43] Medeiros R, Prazeres H, Pinto D, Macedo-Pinto I, Lacerda M, Lopes C, et al. Characterization of HPV genotype profile in squamous cervical lesions in Portugal, a southern European population at high risk of cervical cancer. Eur J Cancer Prev 2005;14:467–71.
[44] Govan VA, Constant D, Hoffman M, Williamson AL. The allelic distribution of-308 tumor necrosis factor-alpha gene polymorphism in South African women with cervical cancer and control women. BMC Cancer 2006;6:24.
[45] Singh H, Sachan R, Goel H, Mittal B. Genetic variants of interleukin-1RN and interleukin-1β genes and risk of cervical cancer. BJOG 2008;115:633–8.
[46] Singh H, Jain M, Sachan R, Mittal B. Association of TNF-α (-308G > A) and IL-10 (-819C > T) promoter polymorphisms with risk of cervical cancer. Int J Gynecol Cancer 2009;19:1190–4.
[47] Wang Q, Zhang C, Walayat S, Chen HW, Wang Y. Association between cytokine gene polymorphisms and cervical cancer in a Chinese population. Eur J Obstet Gynecol Reprod Biol 2011;158:330–3.