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Cytokine gene variants and treatment outcome of cisplatin-based concomitant chemoradiotherapy in cervical cancer

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ABSTRACT

Background: Cervical cancer is the second most common cancer among women after breast cancer. Its standard treatment is cisplatin-based concomitant chemoradiotherapy. Chronic inflammation in uterine cervix triggers both pro- and anti-inflammatory pathways. The unpredictability in toxicity and efficacy of treatment is a major challenge. We hypothesized a link between IL-1, IL-6 and TNF gene variants and treatment response.

Material & Methods: We genotyped 246 cervical cancer cases and 246 controls by PCR, PCR-RFLP and ARMS-PCR. Treatment and response were evaluated by RECIST criteria. Chemotherapy and radiation doses were same for all patients, whilst 48 were followed-up for 36 months after treatment.

Results: SNPs in *IL-1RN, IL-1β, IL-6* and *TNFα* were linked with cervical cancer. Cases with certain allele combinations in *IL-1RN, IL-1β, IL-6*(-597A/G) and *TNF-α* showed odds ratios (95% CI) of up to 17.54 (2.7–24.08) for the presence of cervical cancer. Variant *IL-1β* (-511T/C) was linked to vital status but none were linked to overall survival.

Conclusion: Certain cytokine gene variants may help detect susceptibility to cervical cancer and predict response to chemoradiotherapy.

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KEYWORDS
Cytokine gene variants; cervical cancer; genotypic analysis; chemo radiation therapy (CRT); treatment outcome

Introduction

Cervical cancer is the second common cancer in women between 15 and 44 years of age worldwide, with a prevalence estimated to be around 2.5 million, with some 569,847 new cases and 311,365 deaths worldwide [1]. HPV types 16 and 18 are responsible for about 80% of all cervical cancer cases worldwide. Predisposing cofactors responsible for the development of cervical cancer are age, early marriage, number of abortions, young age at first delivery, parity, oral contraception, multiple sexual partners, smoking, low socio-economic status, menstrual hygiene and unhealthy living conditions [2,3].

HPV infection abrogates initial steps of the innate immune system, involving Toll-like receptor signalling and cytokine synthesis and secretion [4,5]. Peghini et al. (2012) [6] reported that immunostimulatory signals (Th1 cytokine profile) are hampered whereas proinflammatory and immunosuppressor signals (Th2 cytokine profile) are stimulated in cervical cancer. Several other studies also reported this shift of cytokine patterns in preneoplastic and cancer specimens [7]. Th1 cytokines *i.e.* IL-1 (IL1-RN and IL1β), TNF-α, interferon, etc., are potent activators of cell-mediated immunity that may precede HPV clearance, while Th2 cytokines *i.e.* IL-4, IL-6, IL-10 and TNF-β impair the immune response, leading to an inefficient virus elimination and chronic infection [8].

IL-1 (both IL-1α and -β) is present in high levels in cervical cancer tissue, being secreted by keratinocyte damage and by tumour-associated macrophages (TAM); which induce progression, metastasis, tumour growth and angiogenesis [9]. IL-6 is upregulated during cervical carcinogenesis [10], playing a role in HPV-immortalized and carcinoma-derived cervical cell line proliferation [11]. TNF-α creates an antitumoural milieu for virus elimination and supports activation of macrophages, dendritic, NK cells recruited at the tumour site. Moreover, it inhibits HPV oncogene transcription, proliferation [12] and causes apoptosis of cervical cancer cell lineage [13]. Many previous studies reported that genetic polymorphisms in cytokine genes contribute to variation in levels of cytokines produced, which may influence the severity of several infectious diseases [14]. Therefore, the modulation of cytokine expression is a key event for the induction of chronic infection and cancer development since last three decades. Treatment of cancer has been on the basis of targeted therapies.

A standard treatment for cervical cancer is cisplatin-based concomitant chemoradiotherapy (CRT), a combination shown to improve tumour control and overall survival [15]. However, CRT may bring widespread early or late toxicities, and genetic polymorphisms in candidate genes show association with treatment outcome [16,17].

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Therefore, genetic variants in cytokine genes might influence the treatment outcome of CRT. We hypothesized variants in *IL1-RN, IL1- β, IL6* and *TNF-α* are predictive biomarkers for clinical response and overall survival of cervical cancer patients to CRT.

Material & methods

The study included cervical cancer cases and normal healthy control subjects enrolled from 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, selected as per inclusion and exclusion criteria [3,18].

Demographic and clinical characteristics of patients were obtained from medical records while staging and clinical diagnosis of patients were performed by expert clinicians as per guidelines of International Federation of Gynecology and Obstetrics (FIGO) [3]. The patients with stages IIB-IIIB of cervix carcinoma belonged to same ethnicity with no associated co-morbid conditions and were exposed to first course of CRT.

Chemotherapy and radiation dose were same for all patients, that being pelvic external beam radiotherapy (for a total dose of 50 Gy in 25 fractions) with weekly concomitant cisplatin (40 mg/m^2) followed by three applications of high dose rate intracavitary brachytherapy 7 Gy/fraction at one-week intervals. The patients who did not complete the planned chemoradiation dose or violated the treatment protocol were excluded. The response to treatment was measured by Response evaluation criteria in solid tumours (RECIST) criteria version 1.0 after one month. The primary endpoint was overall survival (OS) from the date of diagnosis to the date of death from any cause. Women were followed-up after treatment and checked for survival those who were alive at the end of study were censored [17].

Five millilitre venous blood samples were obtained from all subjects at the start of treatment regimen. Genomic DNA was extracted by standard salting out method with slight modifications [3,19], checked on 1% agarose gel and quantified in a biophotometer (Eppendorf, Germany). *IL-1RN* (86bp VNTR) polymorphism was genotyped by conventional polymerase chain reaction (PCR). *IL-1b* -511C/T (rs16944), *IL-6*-597G/A (rs1800797) and *TNF-α*-308G/A (rs1800629) SNPs were genotyped in cases and healthy age matched controls by PCR–restriction fragment length polymorphism (PCR–RFLP) [3]. Gene–gene interactions were analysed using SHEsis software (ver. Online) http://analysis.bio-x.cn/myAnalysis.php.

Results

The genotypic and allelic frequency distributions of *ILRN* 86bp VNTR, *IL-1β* -511C/T (rs689466), *IL-6-5*97A/G (rs1800797) and *TNF-α-3*08G/A (rs1800629) polymorphisms were analysed. All genotype and allele frequencies of the variants were in Hardy–Weinberg equilibrium. Four different alleles of *ILRN* 86bp VNTR (I to IV) were observed in the study population. Both genotypic and allelic frequencies of *1RN* 86bp VNTR were linked to cervical cancer (Table 1). *IL-1β* variant -511C/T differences failed to reach significance. Genotype 'AG' and allele 'G' of *IL-6-5*97A/G and genotype 'GG' and allele 'G' of *TNF-α-3*08G/A were strongly linked to cervical cancer. *TNF-α-3*08G/A links with cervical cancer were modestly significant (Table 2).

Combinations of gene variants are shown in Table 3. In haplotype analysis of two SNPs in *IL-1*, *IL-1RN* 86bp VNTR and *IL-1β* -511C/T (rs689466), only the 'II T*' haplotype (Allele II of *IL-1RN* VNTR and 'T' of *IL-1β* -511C/T) was linked to cervical cancer. Similarly, the interaction analysis of *IL-6*-597A/G (rs1800797) and *TNF-α-3*08G/A (rs1800629) SNPs showed that the combinations 'G A*' and 'G G*' was linked to cervical cancer. Of the combinations of all four gene variants, four allelic combinations I-C-A-A, I-C-G-G, I-T-G-A and II-T-G-A were all very significantly ($p \leq 0.001$) linked, whereas combinations I-C-A-G, and II-T-G-G were strongly ($p < 0.01$) linked to cervical cancer.

Of 246 cervical cancer cases, 48 (mean [SD] age 48.4 [11.4]) were followed up for 36 months after completion of their chemoradiotherapy. Immediate responses were observed after the completion of treatment. According to response, 37 (77.0%) cases were responders (Complete Response, CR+ Partial Response, PR) and 11 (23.0%) were non-responders. Out of 37 responders, 25 (52.0%) showed complete response (CR), 12 (25.0%) showed partial response (PR), while among 11 (23.0%) non-responders, six (12.5%) had stable disease (SD) and

Table 1. Genotypic and allelic frequencies of *IL-1RN* 86 bp VNTR (Intron 2).

Genotype frequencies							
Genotype	I/I	II/II	I/II	I/III	II/III	IV/IV	P-value
Controls n = 190 (%)	132(69.5)	10(5.3)	41(21.6)	2(1.0)	3(1.6)	2(1.0)	0.04
Cases n = 190 (%)	105(55.2)	14(7.4)	54(28.4)	7(3.7)	9(4.7)	1(0.6)	
Allele frequencies							
Alleles	I	II	III	IV	P value	OR (95%CI)	
Controls n = 380 (%)	307(80.8)	64(16.8)	5(1.3)	4(1.1)	0.005	1.46 (1.12–1.91)	
Cases n = 380 (%)	271(71.4)	91(23.9)	16(4.2)	2(0.5)			

Table 2. Genotypic and allelic frequencies of *IL-1β* -511T/C, *IL-6*-597A/G and *TNF-α*-308G/A polymorphism.

IL-1β -511T/C				
Genotype frequencies				
Genotypes	TT	TC	CC	P- value
Controls[a] n (%)	86 (41.4)	98 (47.1)	24 (11.5)	0.053
Cases[a] n (%)	106 (50.9)	84 (40.4)	18 (8.7)	
Allele frequencies				
Alleles	T	C	P- value	OR (95% CI)
Controls[b] n (%)	270 (64.9)	146 (35.1)	0.054	1.33 (0.10-1.79)
Cases[b] n (%)	292 (71.2)	120 (28.8)		
IL-6-597A/G				
Genotype frequencies				
Genotype	AA	AG	GG	P- value
Controls[c] n (%)	182 (74.0)	47 (19.1)	17 (6.9)	0.001
Cases[c] n (%)	86 (35.0)	101 (41.0)	59 (24.0)	
Allele frequencies				
Alleles	A	G	P- value	OR (95% CI)
Controls[d] n (%)	411 (83.5)	81 (16.5)	0.001	4.36 (3.24-5.86)
Cases[d] n (%)	273 (55.4)	219 (44.6)		
TNF-α -308G/A				
Genotype frequencies				
Genotype	GG	GA	AA	P- value
Controls[e] n (%)	64 (29.1)	128 (58.2)	28 (12.7)	0.023
Cases[e] n (%)	92 (41.8)	103 (46.8)	25 (11.4)	
Allele frequencies				
Alleles	G	A	P- value	OR (95% CI)
Controls[f] n (%)	256 (58.2)	184 (41.8)	0.032	0.74 (0.57-0.97)
Cases[f] n (%)	287 (65.2)	153 (34.8)		

CI = Confidence Interval; OR = Odds Ratio. [a]n = 208, [b]n = 416, [c]n = 246, [d]n = 492, [e]n = 220, [f]n = 440.

5 (10.5%) had progressive disease (PD). Cytokine gene polymorphisms (*IL-1RN* 86 bp VNTR, *IL-1β* -511T/C, *IL-6*-597A/G and *TNF-α* 308G/A) and clinical response in cervical cancer cases were analysed. Cases with 'I/I' genotype of *IL-1RN* 86bp VNTR showed a decrease in response when compared to 'I/II' genotype (66.7 *vs* 82.3%, p = 0.282). However, individuals with 'I/III' genotype showed 90% response but did not show any significant association. Cases with 'TT' genotype of *IL-1β* -511T/C polymorphism showed decrease in response when compared to 'TC' genotype (72.2 *vs* 82.6%, p = 0.429). However, 'CC' genotype of *IL-1β* -511T/C polymorphism was seen in 71.4% responders (p = 0.968). Cases with 'AA' genotype of *IL-6*-597A/G polymorphism showed greater response when compared to 'AG' genotype (90.0 *vs* 76.9%, p = 0.424). However, 'GG' genotype was seen in 72.0% responders although this was not significant (p = 0.272). Cases with 'GG' genotype of *TNF-α*-308G/A polymorphism showed a decrease in response when compared to 'GA' genotype (84.0 *vs* 57.1%, p = 0.144). However, 'AA' genotype showed 75.0% response, but there was no significant association (p = 0.397).

Of the 48 cervical cancer cases, 37 (77.1%) were alive and 11 (22.9%) were dead at the end of follow-up. The number of cases with genotypes 'I/II+I/III' of *IL-1RN* 86bp VNTR polymorphism alive when compared to 'I/I' genotype (85.2 *vs* 66.7%), but this variant did not show any significant association (p = 0.130). A higher number of cases with genotypes 'TC+CC' of *IL-1β* -511T/C polymorphism were alive when compared to 'TT' genotype (93.3 *vs* 50.0%, p = 0.002). Unlike above, fewer cases with genotypes 'AG+GG' of *IL-6*-597A/G polymorphism were alive when compared to 'AA' genotype (73.7 *vs* 90.0%). Live cases with 'GA+AA' genotype of *TNF-α*-308G/A polymorphism were higher in number as compared to 'GG' genotype (80.5 *vs* 57.2%), but did not show any significant association (p = 0.189).

Table 4 shows details of the long-term follow-up. Only the TT or TC+CC genotype of *IL-1β* -511T/C had a marked influence on mortality, but this did not translate to longer survival (Figure 1(a–d)).

Discussion

We analysed various combinations of four SNPs in three cytokine genes to determine any link with cervical cancer [20]. Inflammatory cytokines *IL-1RN* and *IL-1β* are members of *IL-1* family and are mainly produced by activated macrophages, T and B lymphocytes [21,22]. We found that only 'II T*' haplotype (Allele II of *IL-1RN* VNTR and 'T' of *IL-1β* -511T/C) showed significant links with cervical cancer. Notably, this haplotype combination showed 6.5 times higher risk of gastric cancer in an Italian population [23]. Singh et al. [24] also reported that haplotypes 'I T' (Allele

Table 3. Haplotypic analysis and gene–gene interaction of cytokine genes and their allelic combinations in controls (n = 220) and cervical cancer cases (n = 220).

IL-1RN 86bp VNTR and *IL-1β* -511C/T (rs16944)				
Haplotype	Controls (Freq)	Cases (Freq)	P-value	OR (95%CI)
I C	88 (0.2)	115 (0.3)	0.045	0.72 (0.52–0.99)
I T	182 (0.5)	193 (0.5)	0.586	0.92 (0.691–1.23)
II C	18 (0.1)	19 (0.1)	0.990	0.10 (0.51–1.93)
II T	75 (0.2)	44 (0.1)	0.001	1.91 (1.28–2.87)
IL-6 -597A/G (rs1800797) and *TNF-α*-308G/A (rs1800629)				
Alleles combined	Controls (Freq)	Cases (Freq)	P-value	OR (95%CI)
A A	214 (0.49)	174 (0.40)	0.007	0.69 (0.53–0.90)
A G	140 (0.32)	75 (0.17)	<0.001	0.44 (0.32–0.61)
G A	49 (0.11)	106 (0.24)	<0.001	2.53 (1.75–3.65)
G G	37 (0.08)	85 (0.13)	<0.001	2.62 (1.73–3.95)
IL-1RN 86bp VNTR, *IL-1β* -511T/C (rs16944), *IL-6*-597A/G (rs1800797) and *TNF-α*-308G/A (rs1800629)				
Alleles combined	Controls (Freq)	Cases (Freq)	P-value	OR (95%CI)
I C A A	55 (0.14)	1 (0.07)	0.001	0.47 (0.29–0.76)
I C A G	43 (0.11)	10 (0.05)	0.002	0.43 (0.25–0.75)
I C G A	15 (0.04)	3 (0.04)	0.869	0.94 (0.44–2.00)
I C G G	0 (0.00)	8 (0.08)	0.001	23.40 (48.91–97.93)
I T A A	87 (0.23)	3 (0.19)	0.230	0.81 (0.57–1.15)
I T A G	69 (0.18)	22 (0.06)	<0.001	0.27 (0.17–0.46)
I T G A	16 (0.04)	55 (0.15)	<0.001	4.16 (2.32–7.46)
I T G G	23 (0.06)	32(0.08)	0.177	1.47(0.84–2.58)
II T A A	28 (0.07)	30 (0.08)	0.716	1.11 (0.64–1.90)
II T A G	14 (0.04)	13 (0.03)	0.920	0.96 (0.44–2.09)
II T G A	1 (0.01)	19 (0.05)	<0.001	17.54 (2.70–24.08)
II T G G	2 (0.01)	13 (0.03)	0.003	8.26 (1.62–14.15)

I, Allele I of *IL-1RN* 86bp VNTR; II, Allele II of *IL-1RN* 86bp VNTR; C, Allele C of *IL-1β* -511T/C; T, Allele T of *IL-1β* -511T/C; A, Allele A of *IL-6*-597A/G; G, Allele G of *IL-6*-597A/G; A, Allele A of *TNF-α*-308G/A; G, Allele G of *TNF-α*-308G/A.

Table 4. **C**ytokine gene polymorphisms (*IL-1RN* 86bp VNTR, *IL-1β* -511T/C, *IL-6*-597A/G, *TNF-α*-308G/A), vital status and overall survival in cervical cancer cases.

Genes	*IL-1RN* VNTR		*IL-1β* -511T/C		*IL-6* -597A/G		*TNF-α* -308G/A	
Genotype	I/I	I/II+ I/III	TT	TC+CC	AA	AG+GG	GG	GA+AA
Total n (%)	21 (43.8)	27 (56.2)	18 (37.5)	30 (62.5)	10 (20.8)	38 (79.2)	7 (14.5)	41 (85.5)
Mortality Status								
Dead (n = 11)	7 (33.3)	4 (14.8)	9 (50.0)	2 (6.67)	1 (10.0)	10 (26.3)	3 (42.8)	8 (19.5)
Alive (n = 37)	14 (66.7)	23 (85.2)	9 (50.0)	28 (93.3)	9 (90.0)	28 (73.7)	4 (57.2)	33 (80.5)
P-value	0.130		0.002		0.275		0.189	
Overall survival								
No. of cases (%)	21 (43.0)	27 (56.2)	18 (37.5)	30 (62.5)	10 (20.8)	38 (79.2)	7 (14.5)	41 (85.5)
Median survival (in months)	24	21	21	22	19	22	23	24
HR (95%CI)	0.66 (0.33–1.31)		1.04 (0.50–2.16)		1.27 (0.55–2.94)		3.28 (0.57–8.91)	
P-value	0.10		0.61		0.38		0.50	

CI = Confidence Interval; HR = Hazard Ratio.

I of *IL-1RN* VNTR and 'T' of *IL-1β* -511T/C) and 'II T' (Allele II of *IL-1RN* VNTR and 'T' of *IL-1β* -511T/C) were significantly associated with higher susceptibility to cervical cancer and its progression.

Gene–gene interaction results due to the effect of one gene being modified by several other genes. It provides a starting point for deciding an analytical approach for detecting interactions in epidemiological and genetic studies of common human diseases [25]. As cervical cancer is a complex disease, we tested the hypothesis related to gene–gene interactions. The combination of alleles 'G A*' and 'G G*' of *IL-6*-597A/G and *TNF-α*-308G/A showed significant association with cervical cancer: similar results have been reported in multiple myeloma [26] and type 2 diabetes [27]. Four allelic combinations 'I C G G*', 'I T G A*', 'II T G A*' and 'II T G G*' of *IL-1RN* 86bp VNTR, *IL-1β* -511T/C, *IL-6*-597A/G and *TNF-α*-308G/A showed significant links with cervical cancer, the strongest being 'I C G G*' followed by 'II T G A*'. In a study of gene–gene interaction analysis in oral squamous cell carcinoma, the allele 'G' of both *IL-6* and *TNF-α*, 'T' of *IL-1β*, 'I' and 'II' of *IL-1RN* increased the risk up to 18.7 and 7.3 times, respectively, [3].

Cervical cancer is a locally advanced cancer (unsuitable for surgery) where concurrent chemoradiotherapy is the standard treatment and its outcome is affected by many factors [16]. Individuals with a TC +CC genotype of *IL-1β* -511T/C variant were very likely to survive, although this failed to pass through to an improved survival time, indicating other important contributors that we are unable to identify. We have previously demonstrated that the *GSTP1* AG+GG

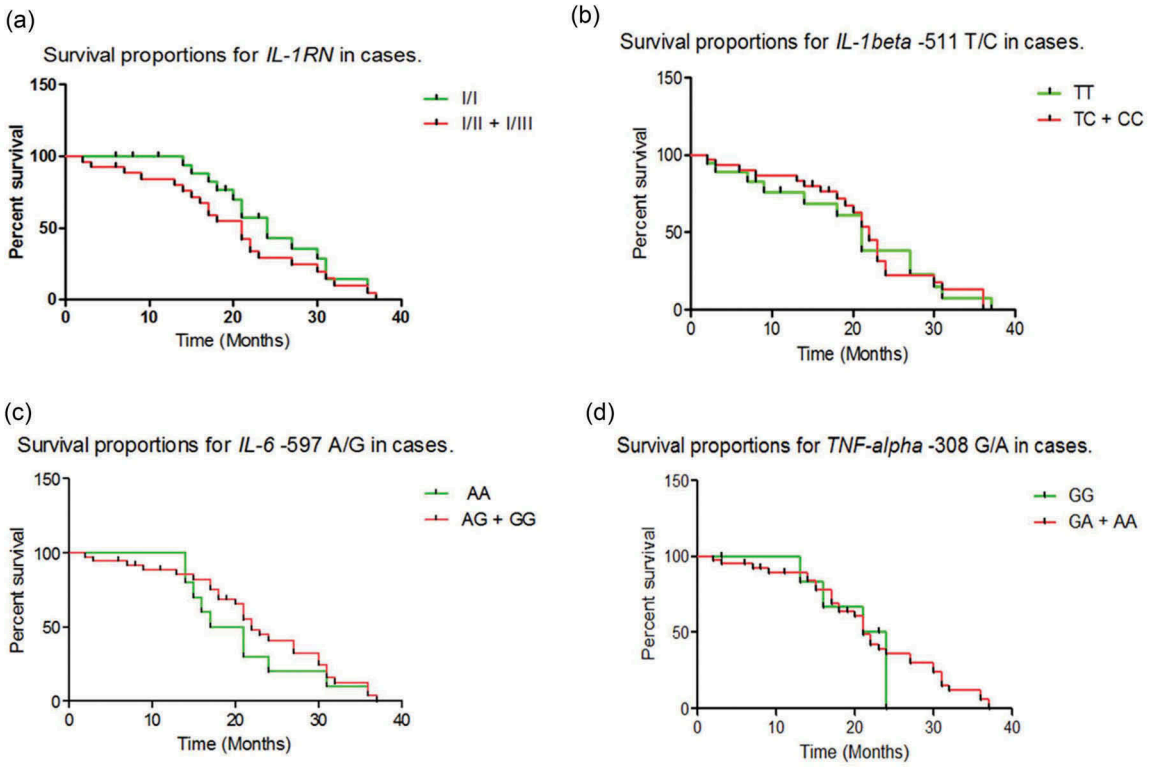

Figure 1. Cytokine gene polymorphisms (*IL-1RN* 86bp VNTR, *IL-1β* -511T/C, *IL-6*-597A/G, *TNF-α*-308G/A) and overall survival after CRT treatment among cervical cancer cases (a) *IL-1RN* VNTR, (b) *IL-1β* -511T/C, (c) *IL-6*-597A/G and (d) *TNF-α*-308G/A.

polymorphism is not associated with survival, while in combination with *GSTM1*null genotype (M1-/AG+GG) and *GSTM1, GSTT1*null (M1-/T1-/AG+GG), it showed reduced hazard ratio indicating better survival [17]. However, the small number of individuals in the treatment outcome study is the major limitation, but nonetheless, a larger study is justified.

Metastatic or recurrent cancer of the uterine cervix remains a major cause of death for women. Therefore, recognizing resistance or susceptibility to the current standard cisplatin-based treatment with genetic biomarkers may improve patient treatment outcomes. Moreover, identification of disease phenotype by the modifying effects of polymorphic variants of cytokine in cervical cancer will have clinical importance leading to the discovery of newer factors possibly contributing to the development of the disease.

This study represents an advance in biomedical science as it points to potential value of SNPs in IL-1, IL-6 and TNF genes as markers of cervical cancer and possible predictors of short-term response to chemoradiotherapy.

Summary table

What is known about this subject:
- Cervical cancer is associated with aberrant immunological activity.
- *IL-1β, IL-6* and *TNF*-α SNPs are present in certain cancers.

What this paper adds:
- The allele combination 'ICGG' of *IL-1RN* (86bp VNTR), *IL-1β* (-511T/C), *IL-6*(-597A/G) and *TNF-α*(−308G/A) is strongly linked to cervical cancer.
- The *IL-1β* -511T/C SNP is strongly linked to mortality.

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Disclosure statement

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