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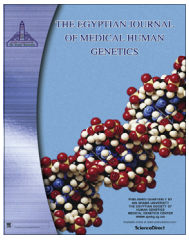

ORIGINAL ARTICLE

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