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IL-6 -572 G>C POLYMORPHISM AND PERIPHERAL INTERLEUKIN-6 LEVEL IN BREAST CANCER: REPORT FROM A TERTIARY CARE CENTRE FROM NORTH INDIA

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ABSTRACT Interleukin-6 is able to promote tumour growth by upregulating anti-apoptotic and angiogenic proteins in tumour cells. In murine models it has been demonstrated that antibodies against IL-6 diminish tumour growth. Several reports have highlighted the prognostic importance of IL-6 in several cancers. The aim of the study was to measure serum Level of IL-6 and its significance in breast cancer and to study the significance of -572G>c polymorphism and its association with breast cancer in patients in a tertiary care center in North India.

KEYWORDS : IL-6, Breast cancer, Genetic polymorphism

INTRODUCTION

Interleukin-6 is able to promote tumour growth by upregulating anti-apoptotic and angiogenic proteins in tumour cells. In murine models it has been demonstrated that antibodies against IL-6 diminish tumour growth. Several reports have highlighted the prognostic importance of IL-6 in e.g., prostate and colon cancer. Interleukin-6 (IL-6) is a 30 kDa glycoprotein characterized by functional pleiotropy[1-4]. Due to the involvement of IL-6 in a variety of physiological and patho physiological processes it was also named B cell stimulatory factor (BSF-2), β2-interferon (IFN-β2), hybridoma/plasmacytoma growth factor (HPGF) and cytotoxic T cell differentiation growth factor. Interleukin-6 is involved in the upregulation of acute phase response proteins associated with inflammation and injury, in hematopoietic stem cell differentiation, in the proliferation and differentiation of a variety of cell types, e.g., neuronal and myeloid cells and is being produced by B and T cells, endothelial cells, fibroblasts, macrophages and epithelial cells. Through low affinity binding with the 80 kDa α-subunit gp80 and subsequent association with the signal transducing β-subunit of the IL-6R complex, gp130, downstream IL-6 mediated effects are propagated. [4] Interleukin-6 is constitutively expressed by renal, bladder, prostate, cervical, glioblastoma and breast carcinoma cells and immunohistochemical studies have demonstrated IL-6 expression in the cytoplasm of colon, prostate and breast carcinoma cells. Breast, prostate, renal, myeloma and ovarian cancer cells also express the IL-6 receptor.[5-8] With respect to the role of IL-6 in mediating tumour growth, contradictory and conflicting results have been reported. Described effects of IL-6 on include the direct enhancement of auto- and paracrine-mediated tumour growth, or an anti-tumour effect by enhancement of the immune response and inhibition of tumour cell proliferation. In cancer cell culture experiments, depending on the cell type examined, IL-6 enhances, inhibits or has no effect on cell proliferation and differentiation.[9-13] The proliferation of the human breast carcinoma cell lines T47D, MCF-7 and ZR-75-1 are inhibited whereas MCF-10A cells are growth stimulated by exogenous IL-6. In vivo studies have also shown enhanced, unaltered and diminished growth of tumour cells transfected with the IL-6 gene, depending on the in vivo model used.[14-18]

The aim of the study was to measure serum Level of IL-6 and its significance in breast cancer and to study the significance of -572G>c polymorphism and its association with breast cancer in patients in a tertiary care centre in North India.

MATERIALS AND METHODS

A cross sectional study using 35 cases of breast cancer (Stage I and above) and 36 non breast cancer patients (controls) was done at General Surgery OPD clinic at CSMMU, Lucknow via consecutive sampling.Peripheral venous blood samples of the cases and controls were collected. 1.5 ml each was stored in two Eppendorf vials. Remaining 2 ml was transferred into a EDTA vials. Samples were stored at –20°C and were transferred to Endocrinology Laboratory, CDRI, Lucknow. Following quantification and purification of DNA the amplification and genotyping.

DNA extraction and purification

DNA was extracted from fresh blood samples by QIAamp DNA mini kit (QUIAGEN, Germany). The cells were lysed and protein degraded followed by phase separation. The DNA was precipitated and purified followed by re-suspension of same in distilled water. Purified DNA was quantified and tested for purity in Nanodrop spectrophotometer (Nanodrop, USA). The quantity and purity of DNA was checked spectrophotometrically by measuring optical density (OD) at 260 nm and 280 nm. The ratio of absorbance at 260 and 280 nm of DNA 1.7-1.9 was used for the study. The quality and purity was confirmed by 0.8% agarose gel electrophoresis in 1 x Tris-Borate-EDTA (TBE) buffer. DNA was stored at –20°C for longer duration.

Genotyping of Il-6 Gene Polymorphism

The primers used for PCR amplification of selected polymorphisms were designed using GeneTool software.

PCR reactions were performed under the following conditions: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds and polymerization at 72°C for 45 seconds, with a final polymerization step at 72°C for 7 minutes. The PCR products were directly sequenced using dideoxy cycle sequencing chain termination method (Big Dye V3.1, Applied Biosystems, Foster City, Ca, USA) on ABI 3730 DNA analyzer. Sequence editing and multiple alignments were carried out using AutoAssembler software (Applied Biosystems, USA).

Estimation of levels of peripheral expression of IL-6 in serum

Circulating peripheral levels of TNF-α were measured with commercially available ELISA kits according to the manufacturers' instructions. ELISA kits were procured from Diaclone (Gen-probe). For ELISA, 2 ml peripheral blood of the patients and controls was collected followed by isolation of serum. Samples with inadequate serum quantity or quality due to hemolysis were excluded from biochemical measurements.

RESULTS

The present study comprised of 35 breast cancer patients (mean age= 45.42+15.56 years) enrolled from the Department of Surgery, CSJMMU, Lucknow during the period of July 2011-2012. This study was conducted with prior clearance from the ethical committee of CSJMMU, Lucknow.

Control women (n=36, mean age=41+17.30 years) were recruited from hospital OPD and staff and confirmed that there was no detectable breast cancer at the time of sampling, and all had no personal history of breast cancer. Demographic details of patients and controls were listed in Table 1. 4 (11.50%) patients with breast cancer had a positive family history, whereas 31 (89.38%) patients of breast cancer had a negative family history. Tobacco intake was found in 4 (10.62%) patients of breast cancer and 1 (4.42%) subject in the control group. Polymorphism of IL-6 seen in the 35 cases of breast cancer were G>G = 28, G>C = 5 and C>C= 2 (Table 2). There was no significance in the incidence of G>C polymorphism in either patients or controls using Fisher's exact test (5 vs. 3, p>0.05). The concentration of IL-6 in cases (n=35) was 41.3pg/ml vs. controls (n=36) was 48.7pg/ml and we could find no significant difference in IL-6 level among cases and control (p>0.05) (Table 3).

Table 1 Demographic variables

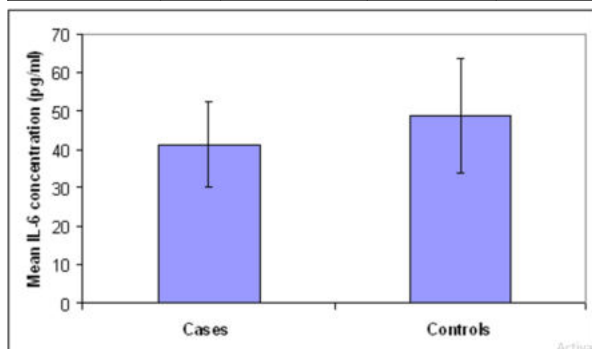
Variables	Cases (N = 35)	Controls (N = 36)
Age (mean±SD)	45.42±15.56	41±17.30
BMI (Kg/m^2)	21.64+6.21	23.19 ± 5.14
Age at menarche (years, mean ± SD)	14.15 ± 1.87	14.12 ± 2.10
Age at diagnosis for cases or at interview for controls		
≤ 30 years	5 (15.93%)	4 (10.62%)
31-45 years	11 (31.86%)	16 (42.48%)
46-60 years	11 (33.63%)	11 (29.20%)
61-75 years	5 (13.27%)	3 (12.39%)
76-90 years	3 (5.31%)	2 (5.31%)
Family history		
Positive	4 (11.50%)	0 (0%)
Negative	31 (88.50%)	36 (100%)
Tobacco chewing/smoking habit		
Yes	4 (10.62%)	1 (4.42%)
No	31 (89.38%)	35 (95.58%)

Table 2 Distribution of genotypes of IL-6 -572 (G>C) polymorphism among cases and controls

	GG	GC	CC	Fisher's exact test	OR	95%CI
Cases (n=35)	28	5	2	P = 0.315	2.4	0.57-10.03
Controls (n=36)	33	3	0	Ref		

Table 3 Comparative serum level IL-6 between cases and controls

IL-6	Mean	Std. deviation	95% C.I	P value
Cases (n=35)	41.3	11	-19.58 - 102.3	0.86
Controls (n=36)	48.7	15	-12.28 - 109.6	



DISCUSSION

Cytokines are factors that are known to have both tumor-promoting and inhibitory effects on breast cancer growth depending presumably on their relative concentrations and the presence of other modulating factors. Different cytokines play an important role in controlling the immune system. Interleukin-6 (IL-6) is a pleiotropic cytokine with obviously tumor-promoting and tumor-inhibitory effects.[19-24] Here, we review the role of IL-6 in in vitro experiments of breast tumor cells, in venous blood sample and assess its potential as a prognostic indicator in breast cancer patients. In summary, results regarding the effect of IL-6 on breast tumor cells are not unique indicating both tumor-promoting and inhibitory effects of IL-6. Concerning patients' serum IL-6 levels, data are surprisingly unique showing IL-6 to be a negative prognostic indicator in breast tumor patients . But in our study this relation was not established and the levels in both cases and controls were same .

It has been long established that the pathologic variables of tumour size, lymph node status, and histologic tumour grade are significant prognostic indicators in breast carcinoma [25-26]. More recently, biomarkers of prognosis have been identified and a radiological predictor of survival has been discovered, but the value of tumour size, lymph node status, and tumour grade as powerful predictors of survival remains [27-31]

Thus the levels of IL-6 correlates with all the aspects of breast cancer like tumour size lymph node involvement, distant metastasis and the final TNM staging of the disease. The overall survival of the patient also seems to be affected in patients with elevated levels of IL-6.[31-33]

Similar results were also seen where the serum level of IL-6 is an independent prognosis factor, which confirms results obtained by Zhang et al on a smaller series [34].

In other studies also IL-6 expression in early-stage breast carcinomas has been correlated with low grade, oestrogen receptor status and good prognosis [35, 36], several studies have shown that IL-6 may contribute to disease progression, particularly in advanced breast cancer patients.

As tumours evolve toward a metastatic phenotype and interfere with other endogenous or exogenous factors, IL-6 activity on cancer cells and their environment might actually shift from growth inhibition and differentiation to proliferation and antiapoptosis [37].

This could explain the favourable prognosis associated with the presence of tumour IL-6 in early-stage breast cancer [36] and the poor survival associated with high serum IL-6 levels in this series of metastatic breast cancer patients.

It is noteworthy that, as in early-stage breast cancer, IL-6 functions as an inhibitor of cancer cell growth in benign prostate hyperplasia (38)

In correlation with other studies , we have observed that some tumor cells within primary breast cancer tissue do not have the ability to produce IL-6. IL-6 could therefore be used as a predictive factor for the development of multidrug resistance and poor response to chemotherapy. Consistent with this hypothesis, elevated IL-6 serum levels have been found in ovarian cancer patients who do not respond to chemotherapy. Elevated levels of IL-6 in serum have also been correlated with prostate metastasis and morbidity. Additional studies need to be performed to ascertain whether a correlation exists between the presence of IL-6- producing breast cancer cells and the response of patients to chemotherapy.

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