Whites Science
A Scientific Erudition

THE INTERLEUKIN-6-174 G/C AND 572 G/C PROMOTER POLYMORPHISM ASSOCIATED WITH HIGH RISK OF ORAL PRE CANCER AND CANCER IN NORTH INDIAN POPULATION

Shalini Gupta[1]*,KumudNigam[1] ,M.L.B.Bhatt[2], Abbas Ali Mahdi[3]
[1]Department of Oral Pathology &Microbiology, King George's Medical University, Lucknow, India
[2]Department of Radiotherapy, King George's Medical University, Lucknow, India
[3]Department of Biochemistry, King George's Medical University, Lucknow, India

ABSTRACT

Introduction : Interleukin-6 (IL-6) encodes a cytokine protein, which causes inflammation, maintains immune homeostasis and plays an essential role in oral pathogenesis. The aim of this study was to evaluate the association between IL-6 (−174 and −572) G/C promoter gene polymorphismsand risk of Oral pre cancer and cancer among Indians.

Methods: Single nucleotide polymorphismin IL-6 genes was genotyped in Oral pre cancer and cancer patients and healthy controls by PCR-RFLP method. Genotype and allele frequencies were analyzed by chi-square test and strength of associations by odds ratio with 95% confidence intervals.

Results: There were significant differences in the GC genotype and C allele frequencies of the IL-6 (−174) G/C gene polymorphism between Oral pre cancer and cancer patients and controls. The difference between Oral pre cancer and cancer patients and controls was statistically significant in homozygous GG and heterozygous GC genotype (OR: 0.5824, CI: 0.3963–0.8559; p: 0.0077*) and homozygous GG and CC genotype (OR: 0.3983, CI: 0.1804–0.8791; p: 0.0316*). C allele was also significantly increased, as compared with the G allele [OR: 0.5894, CI: 0.4349−0.7987; p: 0.0008*]. The genotype GC and C allele frequencies of the IL-6 (−174) G/C gene polymorphism with oral submucous fibrosis and malignancy, was significantly associated with oral pre cancer and cancer. However, frequency of IL-6 (−572)G/C gene polymorphism was not significantly associated with Oral pre cancer and cancer patients (p N 0.05).

Conclusion: The genotype GC and allele C of IL-6 (−174) G/C gene polymorphism play a significant role in Oral pre cancer and cancer susceptibility but IL-6 (-572) G/C gene polymorphism was not significantly associated with oral pre cancer and cancer.

Keywords: Interlukin, PCR-RFLP, Oral pre cancer and cancer, Gene polymorphism.

INTRODUCTION

Interleukin-6 (IL-6)is a proinflammatory cytokine produced by a number of cell types including cancer cells[1]. Elevated levels of circulating IL-6 have been seen in many clinical situations characterized by tissue injury, such as trauma (including major surgery),ischemia,burns,malignant conditions,exposure to toxins and aseptic irritants,immune hypersensitivity reactions,inflammatory,infectious and autoimmune diseases[2]. IL-6, also known as B cell differentiation factor, is an immune regulatory cytokine, involved in the regulation of various cellular functions (angiogenesis, apoptosis, proliferation, differentiation and regulation of immune response). As a growth factor IL-6 plays a significant role in cell differentiation and is believed to be involved in tumor progression[3]. High IL-6 levels have been shown to correlate with poor prognosis and high mortality in prostate and colorectal cancer patients.[4,5]

*Corresponding author:
Email: sgmds2002@yahoo.co.in

http://dx.doi.org/10.20530/IJIBCS_8_13-24
ISSN 2047-9093 © 2016

Oral squamous cell carcinoma (OSCC) is a common malignant tumor of the oral cavity and has a high incidence with poor prognosis in India,[6,7] OSCC is one of the major cause of cancer-related deaths. In India over the world, It is predominant in Indian males[8,9]. OSCC is multistep progression which is influenced by several environmental factors, such as tobaccochewing, smoking and alcohol consumption and further alteration in genes, such as tumor suppressor genes and oncogenes[10,11]. Several studies have observed that the common polymorphisms in angiogenesis, inflammation and thrombosis-related genes, are associated with increased risk oforal cancer and other cancerous condition[8-12]. One such factor, related with both thrombosis and malignancy, is interleukin-6 (IL-6).[13-17]IL-6 may also have an essential role in the growth and differentiation of malignant tumors.[18,19]IL-6 is a multifunctional cytokine of Th2 type which has both, pro-inflammatory and anti-inflammatory cytokine activities[12]. It is a glycoprotein which consists of184 amino acids and has a molecular weight of 26 kDa[13]. It is involved in tumor growth,differentiation of malignant cancer cells and microenvironment immune-modulation.[20,14] These properties are the result of enhanced neo-angiogenesis, inhibition of cancercell apoptosis and acquired cell resistance[16]. IL-6 is secreted by T cells, which iscapable of stimulating immune responses, including responses to tumors. The IL-6 gene is located on chromosome 7p21 and the SNPs at the 5′ flanking region of the IL-6 promoter, to be identified as IL-6−174 and −572 (15). IL-6 (−174) G/C genepolymorphism is one of the most frequentlystudied polymorphisms. IL-6 (−174) G/C gene polymorphism alters the expression of IL-6 gene. The genotypes GC, CC and allele C of IL-6 (−174) G/C gene polymorphism are mutated and increased the risk of oral cancer[21]. The previous study shown that the IL-6 (−174) G/C gene polymorphism was significantly associated with some cancers other also such of breast, ovarian, prostatic, gastric, cervical and colorectal cancer[22-26]. The IL-6 (−572) G allele is also associated with increased level of IL-6 protein in serumthan C/C allele and is significantly associated with the risk of oral cancer[27,28]. On the contrary IL-6 (−174 and −572) C/G gene polymorphisms are not associatedwith the risk of gastric cancer, moreover they lower the risk of bladder cancer[29,30].Since the association between IL-6 (−174G/C and−572G/C) gene polymorphismwith OSCC in the Indian population has yet not been studied, we investigated the role of IL-6 promoter (−174) G/C and (−572) G/C gene polymorphisms in pathogenesis of OSCC in Indian population.

Our study have focus the association between IL-6 (174G/C and 572G/C). The study focus specifically the rate of IL-6 promoter with (-174 G/C and -572 G/C) genome polymorphism and their pathogensis in oral cancer patients. The previous study with IL-6 (-174 G/C and -572 G/C) focus in other cancer condition. Our study have also assess the relationship between IL-6 174 G/C promoter polymorphism and IL-6 572 G/C in North Indian population.

MATERIALS AND METHODS

Collection of samples

The study comprised with 250 patients sample. All the subject samples were histopathologically confirmed oral pre cancer and cancer who were registered in the department of Oral Pathology & Microbiology and department of Radiotherapy, King George's Medical University, Lucknow and 250 healthy controls. Informed written consent was obtained from all subjects. The research study is ethically approved by the Institutional ethics committee K.G.M.U, Lucknow, India.

Extraction of genomic DNA and molecular analyses

Venous blood samples were collected in EDTA tubes and stored at −80 °C, till DNA extraction. Genomic DNA extraction from blood samples was carried out by salting out method[31].

Genotyping of IL-6 (–174) G/C gene polymorphism

The IL-6 (-174) G/C polymorphism was analysed by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism. Genomic DNA was amplified using the standard PCR conditions: 94˚C for 4 min, 35 cycles at 94˚C for 30 s, 61˚C for 30 s, 72˚C for 45 s, and finally 72˚C for 10 min. The primers used for amplification of the IL-6 (-174) G/C gene polymorphisms were as follows: forward primer 5′-GGAGTCACACACTCCACCT-3′and reverse primer 5′-GTGGGGCTGATTGGAAACC- 3′. Amplification success of samples was monitored on 2% agarose gel by Gel electrophoresis. There after the PCR products were subjected to digestion by N1aIII enzyme to screen for the G174C polymorphism.

Genotyping of IL-6 (–572) G/C gene polymorphism

The IL-6 (-572) G/C polymorphism was analysed

by the polymerase chain reaction (PCR) followed by restriction fragment length polymorphism. Genomic DNA was amplified using the following PCR conditions: 94˚C for 4 min, 35 cycles at 94˚C for 30 s, 61˚C for 30 s, 72˚C for 45 s, and finally 72˚C for 10 min. The primers used for amplification of the IL-6 (-572) G/C gene polymorphisms were as follows: forward primer 5'-GGAGACGCCTTGAAGTAACTGC-3'and reverse primer 5'-GAGTTTCCTCTGACTCCATCGCAG- 3'. Amplification success of samples was monitored on 2% agarose gel by Gel electrophoresis. There after the PCR products were subjected to digestion by DdeI enzyme to screen for the G572C polymorphism.

STATISTICAL ANALYSIS: The statistical significance in this study was evaluated by Chi-square test. Odds ratio (OR) was calculated as an estimate of relative risk of having disease according to the relative frequency of different genotypes among the cases as well as the controls. ORs are given with 95% confidence interval (CI).

RESULTS

The demographic profile evaluated included age, gender, relative environmental risk factors and tumor staging which may contribute the progression of Oral pre cancer and cancer are shown in (Table 1).

The genotype and allele frequencies of the IL-6 (−174) G/C and IL-6 (−572) G/C gene polymorphisms among the controls and Oral pre cancer and cancer patients are shown in (Table 2). The frequencies of the GG, GC and CC genotypes of IL-6 (−174G/C) were 54.80%, 37.20% and 8.00% in the Oral pre cancer and cancer patients and 68.80%, 27.20% and 4.00% in controls, respectively. On the other hand the allele frequencies of theG and C were 73.40% and

Table 1: Demographic and risk factors in patient and controls and their association with risk of Oral pre cancer and cancer

Demographic Character	Cases (n=250)	Control (n=250)	P- value
Male	150 (60%)	175 (70%)	0.3131
Female	100 (40%)	75 (30%)	0.1236
Age distribution			
<20 – 40	160 (64.00%)	131 (52.40%)	0.2010
<40- 70	90 (36.00%)	119 (47.60%)	0.1088
Habitual risk			
Areca nut/ Pan masala	80 (32%)	165 (66%)	0.0011*
Alcohol consumption	40 (16%)	10 (4%)	0.0011*
Smoking	60 (24%)	30 (12%)	0.0051*
Tobacco chewing	70 (28%)	45 (18%)	0.0455*
TNM staging			-
Tumor Stage		-	-
I	76 (30.40%)		
II	68 (27.20%)		
III	56 22.40%)		
IV	50 (20.00%)		
Tumor T Status		-	-
≤T2	151 (60.40%)		
>T2	99 (39.60%)		
Lymph Node		-	-
N0	177 (70.80%)		
N1+N2	73 (29.20%)		
Metastasis		-	-
M0	202 (80.80%)		
M1	48 (19.20%)		
Cell differentiated grade		-	-
Grade 1	85 (34.00%)		
>Grade 1	165 (66.00%)		

*=significant value

Table: 2 Genotypes and allelic distribution of IL-6 (174G/C) andIL-6 (572G/C) gene in Oral pre cancer & cancer with controls.

IL-6 (G174C) genotypes	Cases (n=250)	Controls (n=250)	p- value	Odds Ratio	95% CI
GG	137 (54.80%)	172 (68.80%)	-	-	-
GC	93 (37.20%)	68 (27.20%)	0.0077*	0.5824	0.3963-0.8559
CC	20 (8.00%)	10 (4.00%)	0.0316*	0.3983	0.1804-0.8791
G	367 (73.40%)	412 (82.40%)	-	-	-
C	133 (26.60%)	88 (17.60%)	0.0008*	0.5894	0.4349-0.7987
IL-6 (G572C) genotypes					
GG	67 (26.80%)	75 (30.00%)	-	-	-
GC	160 (64.00%)	155 (62.00%)	0.5397	0.8654	0.5820-1.287
CC	23 (9.20%)	20 (8.00%)	0.5891	0.7768	0.3920-1.540
G	294 (58.80%)	305 (61.00%)	-	-	-
C	206 (41.20%)	195 (39.00%)	0.5188	0.9125	0.7085-1.175

*=significant value

Table 3: The frequency of distribution of polymorphism IL-6 (174G/C) andIL-6 (572G/C) genotypes in oral pre cancer & cancer (Oral submucous fibrosis, Lichenplanus, Leucoplakia, Malignancy) with controls.

Genotypes	Oral submucous fibrosis (n=100)	P- value	Lichenplanus (n=50)	P- value	Leucoplakia (n=50)	P- value	Malignancy (n=50)	P- value	Controls (n=250)
IL-6 (G174C) genotypes									
GG	88 (88.00%)	-	35 (70.00%)	-	41 (82.00%)	-	26 (52.00%)	-	172 (68.80%)
GC	11 (11.00%)	0.0011*	15 (30.00%)	0.9480	09 (18.00%)	0.1838	23 (46.00%)	0.0170*	68 (27.20%)
CC	01 (1.00%)	0.1662	00 (0.00%)	0.3272	00 (0.00%)	0.2635	01 (2.00%)	0.6974	10 (4.00%)
G	187 (93.50%)	-	85 (85.0%)	-	91 (91.00%)	-	75 (75.00%)	-	412 (82.40%)
C	13 (6.50%)	0.0003*	15 (15.00%)	0.6283	9 (9.00%)	0.0473*	25 (25.00%)	0.1124	88 (17.60%)
IL-6 (G572C) genotypes									
GG	63 (63.00%)	-	18 (36.00%)	-	29 (58.00%)	-	14 (28.00%)	-	75 (30.00%)
GC	31 (31.00%)	<0.0001*	28 (56.00%)	0.4943	18 (36.00%)	0.0003*	31 (62.00%)	0.9823	155 (62.00%)
CC	06 (6.00%)	0.0546	04 (8.00%)	0.9940	03 (6.00%)	0.2231	05 (10.00%)	0.8396	20 (8.00%)
G	169 (84.50%)	-	61 (61.00%)	-	79 (79.00%)	-	47 (47.00%)	-	305 (61.00%)
C	31 (15.50%)	<0.0001*	39 (39.00%)	1.0000	21 (21.00%)	0.0009*	53 (53.00%)	0.0130*	195 (39.00%)

*= significant value

26.60% in the Oral pre cancer and cancerpatients, and 82.40% and 17.60% in controls, respectively (Table 2).Therewere significant differences in the GC genotype and C allele frequencies of the IL-6 (−174)

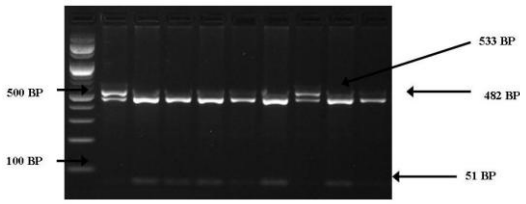

Figure 1: 2% Agarose gel analysis of IL-6 (G174C) polymorphism. Lane 1 100 bp Ladder, Lane 8 GG genotype 533 bp, Lane 2 GC genotype 533,482,51 bp, Lane 3,4,5,6,7 CC genotype 482,51 bp.

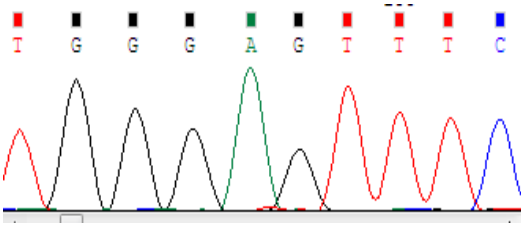

Figure 2:A Forward sequence G to C was observed in blood sample at nucleotide position 174 in gene IL-6 (G174C)

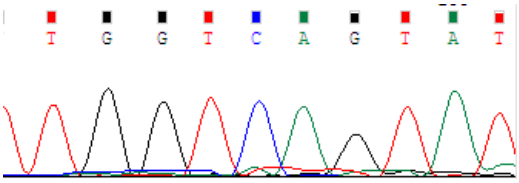

Figure 3:A Reverse sequence G to C was observed in blood sample at nucleotide position 174 in gene IL-6 (G174C)

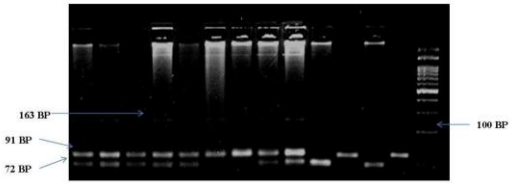

Figure 4: 3% Agarose gel analysis of IL-6 (G572C) polymorphism. Lane 1 100bp Ladder, Lane 6,9,11 GC genotype 163,91,72 bp, Lane 10,12,13,14 CC genotype 91,72 bp.

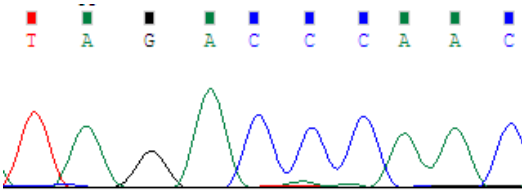

Figure 5:A Forward sequence G to C was observed in blood sample at nucleotide position 572 in gene IL-6 (G572C)

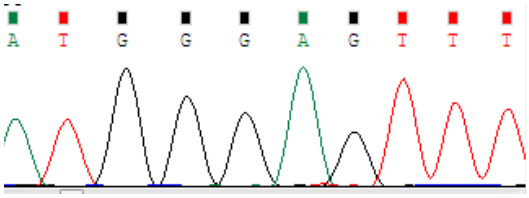

Figure 6:A Reverse sequence G to C was observed in blood sample at nucleotide position 572 in gene IL-6 (G572C)

G/C genepolymorphism between Oral pre cancer and cancer patients and controls. The difference between Oral pre cancer and cancer patients and controls wasstatistically significant in homozygous GG and heterozygous GC genotype (OR: 0.5824, CI: 0.3963–0.8559; p:0.0077*) and homozygous GG and CC genotype (OR: 0.3983, CI: 0.1804–0.8791; p: 0.0316*). C allele was also significantlyincreased, as compared with the G allele [OR: 0.5894, CI: 0.4349−0.7987; p: 0.0008*] (Table 2).

The genotypeGC and C allele frequencies of the IL-6 (−174)G/C gene polymorphismwith oral submucous fibrosis and malignancy, were significantly associated with oral pre cancer and cancer(Table 3).

The frequencies of the GG, GC and CC genotypes of IL-6 (−572) G/C were 26.80%, 64.00% and 9.20% in theOral pre cancer and cancer patients and 30.00%, 62.00% and 8.00% in controls, respectively. On the other hand the allele frequenciesof the G and C were 58.80% and 41.20% in the Oral pre cancer and cancer patients and 61.00% and 39.00% in controls, respectively(Table 2). The genotypes and allele frequencies of the IL-6 (−572) G/C were not significantly different betweencontrols and Oral pre cancer and cancer patients.

The correlation between genotypes and allele frequencies of IL-6 (−174) G/C and (−572) G/C gene polymorphisms with oral submucous fibrosis, lichen planus, leukoplakia, malignancy susceptibility, is shown in (Table 3).

The genotype frequencies of GG, GC and CC of the IL-6 (−174) G/C gene polymorphisms were significantly associated with the development oforal pre cancer and cancer as compared with controls (Table 2). However the GC, GC, CC genotype of the

Table 4: Analysis of IL-6 (174G/C) andIL-6 (572G/C) gene polymorphism in oral pre cancer & cancer and controls with individuals under smoking, masala tobacco chewing and alcohol consumption criteria.

Genotypes	Cases	Controls	P-value	Odds Ratio	95% CI
Smoking	**(n=60)**	**(n=30)**			
		IL-6 (G174C)			
GG	32 (53.33%)	19 (63.33%)	-	-	-
GC	28 (46.67%)	08 (26.67%)	0.2086	2.078	0.78- 5.48
CC	00 (00.00%)	03 (10.00%)	-	-	-
G	92 (76.67%)	46 (76.67%)	-	-	-
C	28 (23.33%)	14 (23.33%)	1.0000	1.000	0.48- 2.08
		IL-6 (G572C)			
GG	28 (46.67%)	19 (63.34%)	-	-	-
GC	31 (51.67%)	11 (36.66%)	0.2326	1.912	0.77- 4.71
CC	01 (1.66%)	00 (00.00%)	-	-	-
G	73 (60.83%)	36 (60.00%)	-	-	-
C	47 (39.16)	24 (40.00%)	0.9141	1.035	0.54- 1.95
Tobacco Chewing	**(n=70)**	**(n=45)**			
		IL-6 (G174C)			
GG	41 (58.57%)	26 (57.78%)	-	-	-
GC	28 (40.00%)	17 (37.78%)	0.9127	1.044	0.47- 2.27
CC	01 (1.43%)	02 (4.44%)	0.7178	3.154	0.27- 36.5
G	110 (78.57%)	69 (76.67%)	-	-	-
C	30 (21.43%)	21 (23.33%)	0.8597	1.116	0.59- 2.10
		IL-6 (G572C)			
GG	25 (35.72%)	24 (53.34%)	-	-	-
GC	43 (61.42%)	20 (44.44%)	0.0746	2.160	1.00- 4.66
CC	02 (2.86%)	01 (2.22%)	0.5985	1.920	0.16- 22.5
G	84 (60.00%)	57 (63.34%)	-	-	-
C	56 (40.00%)	33 (36.66%)	0.7130	1.152	0.66- 1.98
Alcohol Consumption	**(n=40)**	**(n=10)**			
		IL-6 (G174C)			
GG	26 (65.00%)	06 (60.00%)	-	-	-
GC	14 (35.00%)	04 (40.00%)	0.7683	1.238	0.29- 5.13
CC	00 (00.00%)	00 (00.00%)	-	-	-
G	66 (82.50%)	16 (80.00%)	-	-	-
C	14 (17.50%)	04 (20.00%)	0.7946	1.179	0.34- 4.06
		IL-6 (G572C)			
GG	17 (42.50%)	06 (60.00%)	-	-	-
GC	22 (55.00%)	04 (40.00%)	0.5670	1.941	0.47- 7.99
CC	01 (2.50%)	00 (00.00%)	-	-	-
G	46 (57.50%)	11 (55.00%)	-	-	-
C	34 (42.50%)	09 (45.00%)	0.8399.	1.107	0.41- 2.96
Areca nut/Pan masala	**(n=80)**	**(n=165)**			
		IL-6 (G174C)			
GG	41 (51.25%)	54 (32.72%)	-	-	-
GC	26 (32.50%)	87 (52.73%)	0.0032*	2.541	1.398-4.617
CC	13 (16.25%)	24 (14.55%)	-	-	-
G	108 (67.50%)	195 (59.10%)	-	-	-
C	52 (32.50%)	135 (40.90%)	0.0896	1.438	0.9666-2.139
		IL-6 (G572C)			
GG	39 (48.75%)	49 (29.69%)	-	-	-
GC	27 (33.75%)	91 (55.15%)	0.0019*	2.683	1.470-4.894
CC	14 (17.50%)	25 (15.15%)	0.4885	1.421	0.6528-3.094
G	105 (65.62%)	189 (57.27%)	-	-	-
C	55 (34.37%)	141 (42.72%)	0.0003*	0.4141	0.2589-0.6624

*= significant value

IL-6 (−572) G/C gene polymorphism were not significantly associated with

the development of oral pre cancer and cancer (Tables 2).In this study, the frequency of heterozygous genotype GC of IL-6 (−174) G/C and heterozygous genotype GC & C allele of IL-6 (−572) G/C was highly associated with risk for areca nut/

pan masala in OSCC patients (p=0.0032 and p= 0.0019, p=0.0003) (Table 4).

Further, the genotype frequency of CYP1A1(C4887A) in tumourstage (I/II and III/IV), tumour T status, lymph node status, cell-differentiated grade and metastasis (M0 and M1) was significant in between early and late stages of OSCC patients (Table 5).

Table 5: Analysis of genotype and allele frequencies in IL-6 (174G/C) andIL-6 (572G/C) gene polymorphism with tumor stage, tumor T Status, lymph node, metastasis and cell differentiated grade in oral pre cancer & cancer.

	IL-6 (174G/C) andIL-6 (572G/C) Genotypes/Alleles				
Tumor Stage	**I+II (n=144)**	**III+IV (n=106)**	**P-value**	**Odds Ratio**	**95% CI**
		IL-6 (G572C)			
GG	109 (75.70%)	81 (76.41%)	-	-	-
GC	34 (23.61%)	24 (22.65%)	0.9863	1.053	0.57-1.91
CC	01 (0.69%)	01 (0.94%)	0.8340	1.346	0.08-21.8
G	252 (87.50%)	186 (87.74%)	-	-	-
C	36 (12.50%)	26 (12.26%)	0.9370	1.022	0.59-1.75
		IL-6 (G174C)			
GG	81 (56.25%)	43 (40.56%)	-	-	-
GC	52 (36.11%)	56 (52.83%)	0.0122*	2.029	1.19-3.44
CC	11 (7.63%)	7 (6.60%)	0.9318	1.199	0.43-3.31
G	212 (73.61%)	144 (67.92%)	-	-	-
C	76 (26.38%)	68 (32.07%)	0.1978	1.317	0.89-1.94
Tumor T Status	**< T2 (n=151)**	**> T2 (n=99)**			
		IL-6 (G572C)			
GG	115 (76.16%)	75 (75.76%)	-	-	-
GC	36 (23.84%)	22 (22.22%)	0.9545	1.067	0.58-1.95
CC	00 (00.00%)	02 (2.02%)	-	-	-
G	266 (88.08%)	172 (86.87%)	-	-	-
C	36 (11.92%)	26 (13.13%)	0.7925	1.117	0.65-1.91
		IL-6 (G174C)			
GG	86 (56.95%)	38 (38.38%)	-	-	-
GC	57 (37.75%)	51 (51.52%)	0.0141*	2.025	1.18-3.46
CC	8 (5.30%)	10 (10.10%)	0.0686	2.829	1.03-7.73
G	226 (74.84%)	130 (65.66%)	-	-	-
C	76 (25.16%)	68 (34.34%)	0.0344*	1.555	1.05-2.30
Lymph Node	**N0 (n=177)**	**N1+N2 (n=73)**			
		IL-6 (G572C)			
GG	135 (76.27%)	55 (75.34%)	-	-	-
GC	42 (23.72%)	16 (21.91%)	0.9722	1.069	0.55-2.06
CC	00 (00.00%)	02 (2.73%)	-	-	-
G	312 (88.14%)	126 (86.30%)	-	-	-
C	42 (11.86%)	20 (13.70%)	0.6770	1.179	0.66-2.08
		IL-6 (G174C)			
GG	89 (50.28%)	35 (47.94%)	-	-	-
GC	81 (45.76%)	27 (36.98%)	0.6854	1.180	0.65-2.11
CC	7 (3.96%)	11 (15.08%)	0.0119*	3.99	1.43-11.1
G	244 (68.92%)	112 (76.72%)	-	-	-
C	110 (31.08%)	34 (23.28%)	0.1011	1.485	0.95-2.31
Metastasis	**M0 (n=202)**	**M1 (n=48)**			
		IL-6 (G572C)			
GG	154 (76.24%)	36 (75.00%)	-	-	-
GC	47 (23.26%)	11 (22.92%)	0.9975	1.001	0.47-2.12
CC	01 (0.50%)	01 (2.08%)	0.8364	4.278	0.26-70.70
G	355 (87.87%)	83 (86.46%)	-	-	-
C	49 (12.13%)	13 (13.54%)	0.8373	1.135	0.58-2.18
		IL-6 (G174C)			
GG	106 (52.48%)	18 (37.5%)	-	-	-
GC	87 (43.06%)	21 (43.75%)	0.4092	1.421	0.71-2.83

CC	09 (4.46%)	09 (18.75%)	0.0101*	5.889	2.05-16.8
G	268 (66.33%)	88 (91.66%)	-	-	-
C	136 (33.67%)	8 (8.34%)	0.0010*	5.582	2.62-11.8
Cell differentiated grade	**Grade 1(n=85)**	**> Grade 1(n=165)**			
		IL-6 (G572C)			
GG	65 (76.47%)	125 (75.76%)	-	-	-
GC	20 (23.53%)	38 (23.03%)	0.9695	1.012	0.54-1.88
CC	00 (00.00%)	02 (1.21%)	-	-	-
G	150 (88.23%)	288 (87.28%)	-	-	-
C	20 (11.77%)	42 (12.72%)	0.9353	1.068	0.60-1.88
		IL-6 (G174C)			
GG	34 (40.00%)	90 (54.54%)	-	-	-
GC	43 (50.58%)	65 (39.39%)	0.0629	1.751	1.00-3.04
CC	8 (9.42%)	10 (6.07%)	0.2291	2.118	0.77-5.81
G	105 (61.76%)	251 (76.07%)	-	-	-
C	65 (38.24%)	79 (23.93%)	0.0012*	1.967	1.31-2.93

*=significant value

In the case of G174C polymorphism, an undigested 533 bp band showed wild-type GG genotype, while two bands of 482 and 51 bp confirmed mutant CC genotype and three bands of 533, 482 and 51 bp were detected in the heterozygous GC genotype (Figure 1).

Followed by this forward and reverse sequence of IL-6(-174G/C) were also observed and confirmed the nucleotide sequence in given sample.

In the case of G572C polymorphism, an undigested 163 bp band showed wild-type GG genotype, while two bands of 91 and 72 bp confirmed mutant CC genotype and three bands of 163, 91 and 72 bp were detected in the heterozygous GC genotype (Figure 4).

Followed by this forward and reverse sequence of IL-6(-572G/C) were also observed and confirmed the nucleotide sequence in given sample.

DISCUSSION

In the present study, we found that the promoter (−174) GC genotype is significantly associated with increased risk of oral pre cancer and cancer. Moreover, the −174 C allele was also associated with a high risk for development of Oral pre cancer and cancer. The genotype and allele frequencies of IL-6 (−572) G/C were not significantly associated with the risk of Oral pre cancer and cancer. The reported observations of various studies, about the association of IL-6 gene polymorphism with various cancers, are variable. A previous study by [21]reported that the IL-6 gene polymorphism is strongly correlatedwith increased risk for oral cancer by affecting IL-6 gene expression.The GC heterozygotes and CC homozygoteswere significantly associatedwith the development and progression of oral cancer[21]. Our finding are also supported by De Michael et al [23]and Landi et al [24], who reported that the genotypes (GC, CC) and allele C frequencies of IL-6 (−174) G/C were significantly associated with increased risk for breast and colorectal cancers[23,24]. Several previous studies have shown that the elevated level of IL-6 protein in serum is present in many cancers (OSCC, breast, prostate, ovarian, gastric, colorectal and cervical cancers), in which the C allele of promoter (−174G/C) gene polymorphismwas found to be associated with increased production of IL-6 protein and increase the risk of cancers [32, 22, 33, 34, 35, 36]. On the contrary, several studies reported that the IL-6 (−174) G/C gene polymorphism was not significant associatedwith many cancers (breast, prostate, colorectal, lung and gastric cancer) lymphoma, melanoma and multiple myeloma [29, 37]. Individuals with the C allele of IL-6 (−572) G/C gene polymorphism and the GG genotype for the IL-6 (−174) G/C gene polymorphismwere associatedwith decreased risk of colon cancer, but with an increased risk of rectal cancer [25]. Liang et al. (2013) reported that the C allele at (−572) locus of IL-6 genepolymorphism was significantly associated with lung cancer [38]. Yin et al. (2012) suggested that the IL-6 (−174 C/G and−572 C/G) gene polymorphisms were not associated with the risk of gastric cancer [30].

The immune response to inflammationfrequently plays an important role in the pathogenesis of a tumor [39]. Genetic alterationalso plays a significant role in the inflammatory response, which may be associated with the risk ofdevelopment of various cancers, including the oral cancer [39]. IL-6 is an immuneregulatorycytokine with biological functions of pro-inflammation, anti-inflammation and angiogenesis.These responses are triggered by activation of the JAK tyrosine kinase family and then further stimulated bythe MAPK, PI3K, or STAT

signaling pathways [40, 41, 34, 42]. It has been suggested that polymorphisms in genes, encoding inflammatory mediators,may influence the plasma level and the biological activity of the corresponding proteins [43]. IL-6 (−174G/C) promoter SNP is a crucial mediator of the Th2 response, that has been associatedwithincreased IL-6 production [39]. During the inflammation, the C allele of IL-6 (−174) G/C genepolymorphism elevates the level of IL-6 protein [44,45]. Inaddition, several studies have reported that IL-6 protein level increase with the development and progression of tumors, including oral cancer [23, 46] and this increased level of IL-6 proteinwas associatedwith poor prognosis and highmortalityin various cancers [5, 47].

In our study the genotypes and allele frequencies of IL-6 (−174 and−572) G/C gene polymorphism were not associated with environmental factors (tobacco chewing, smoking and alcohol consumption) and tumor progression. These results are similar to that of [48],who showed that IL-6 (−174) G/C gene polymorphism was not associated with either breast cancer risk or severity and prognosis, as assessed by tumor grade and lymph node status Balasubramaniam et al [48]. In contrast, VairakAtaris et al [21]demonstrated that the CC and GC genotypes of IL-6 (−174) G/C polymorphism were significantly associated with the development and progression of cancer, in alcohol consuming patients. Furthermore, C allele carriers have twice as much greater relative risk for developing oral cancer at stages III & IV than stage I & II [21].

It can be concluded that the present study provides evidence of the correlation between IL-6 (−174) G/C gene polymorphism with risk of OSCC. The GC genotype and C allele are significantly associated with the risk of OSCC, whereas IL-6 (−572) G/C polymorphism is not associated with OSCC. Moreover related environmental factors and gene interactions are not associated with pathogenesis of OSCC. IL-6(−174 and −572) promoter gene polymorphisms are not significantly associated with progression of OSCC. Genotyping in the our study showed that unlike (-174G/C and -572G/C)polymorphism was significantly associated in our study samples. The gene IL-6 174G/C genotypes showed in support at the meta genomic study in our sample and prevalence of GC genotype “c” allele and GC genotype of IL-6 174G/C were found significantly associated for oral cancer patients as frequently is higher in controls. There were no significant association of IL-572G/C genotype allele were observed in IL-6-572G/C polymorphism. Our statically data suggest that IL-6 gene polymorphism play an important role to determine the progression and susceptibility of oral pre cancer and oral cancer. This study given a prominent lead to contribution of IL-6 gene with the development of OSCC patients in North Indian population.This finding suggests that IL-6 may be used as a diagnostic marker for effective management of OSCCin future, though further studies with larger sample sizes will be necessary to confirm this.

ACKNOWLEDGEMENTS

This study was supported by Council of Science & Technology U.P. (UPCST). We are grateful to UPCST for their financial support to carry out this research project.

REFERENCES

1. Conze D, Weiss L, Regen PS, et al. Autocrine production of interleukin 6 causes multidrug resistance in breast cancer cells. Cancer Res. 2001 Dec15;61(24):8851-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11751408.

2. Papanicolaou DA. The Pathophysiologic Roles of Interleukin-6 in Human Disease. Ann Intern Med. 1998 Jan 15;128(2):127. Available from: http://dx.doi.org/10.7326/0003-4819-128-2-199801150-00009.

3. Hirano T. Interleukin 6 and its Receptor: Ten Years Later. International Reviews of Immunology. 1998 Jan;16(3-4):249–84. Available from: http://dx.doi.org/10.3109/08830189809042997.

4. Nakashima J, Tachibana M, Horiguchi Y, et al. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res. 2000 Jul;6(7):2702-6. Available form: http://www.ncbi.nlm.nih.gov/pubmed/10914713.

5. Chung Y-C, Chang Y-F. Serum interleukin-6 levels reflect the disease status of colorectal cancer. J SurgOncol. 2003 Jul 21;83(4):222–6. Available from: http://dx.doi.org/10.1002/jso.10269.

6. Álvarez Marcos CA, FernándezEspina H, LlorentePendás JL, Franco Gutiérrez V, Hermsen MAJA, Cuesta Albalad MP, et al. Segundostumoresprimarios en el cáncerescamoso de cabeza y cuello. ActaOtorrinolaringológica Española. 2006 Jan;57(10):462–6. Available from: http://dx.doi.org/10.1016/s0001-6519(06)78749-0.

7. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer.The Lancet. 2008

May;371(9625):1695–709. Available from: http://dx.doi.org/10.1016/s0140-6736(08)60728-x.

8. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, et al. Cancer mortality in India: a nationally representative survey. The Lancet. 2012 May;379(9828):1807–16. Available from: http://dx.doi.org/10.1016/s0140-6736(12)60358-4.

9. Kuruvilla J. Utilizing dental colleges for the eradication of oral cancer in India. Indian J Dent Res. 2008;19(4):349. Available from: http://dx.doi.org/10.4103/0970-9290.44541.

10. Ko Y-C, Huang Y-L, Lee C-H, Chen M-J, Lin L-M, Tsai C-C. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. Journal of Oral Pathology and Medicine. 1995 Nov;24(10):450–3. Available from: http://dx.doi.org/10.1111/j.1600-0714.1995.tb01132.x.

11. Williams HK. Molecular pathogenesis of oral squamous carcinoma.Molecular Pathology. 2000 Aug 1;53(4):165–72. Available from: http://dx.doi.org/10.1136/mp.53.4.165.

12. Sansone P, Bromberg J. Targeting the Interleukin-6/Jak/Stat Pathway in Human Malignancies. Journal of Clinical Oncology. 2012 Feb 21;30(9):1005–14. Available from: http://dx.doi.org/10.1200/jco.2010.31.8907.

13. Guo Y, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treatment Reviews. 2012 Nov;38(7):904–10. Available from: http://dx.doi.org/10.1016/j.ctrv.2012.04.007.

14. Seike T, Fujita K, Yamakawa Y, Kido MA, Takiguchi S, Teramoto N, et al. Interaction between lung cancer cells and astrocytes via specific inflammatory cytokines in the microenvironment of brain metastasis. Clinical & Experimental Metastasis. 2010 Oct 17;28(1):13–25. Available from: http://dx.doi.org/10.1007/s10585-010-9354-8.

15. Terry CF. Cooperative Influence of Genetic Polymorphisms on Interleukin 6 Transcriptional Regulation. Journal of Biological Chemistry. 2000 Mar 22;275(24):18138–44. Available from: http://dx.doi.org/10.1074/jbc.m000379200.

16. Wojcik, E., Jakubowicz, J., Skotnicki, P., Sas-Korczyńska, B., Kulpa, J.K. IL-6 and VEGF in small cell lung cancer patients. Anticancer Res. 2010; 30: 1773–1778. Available from: http://www.jourlib.org/references/10493289.

17. Culig Z, Steiner H, Bartsch G, Hobisch A. Interleukin-6 regulation of prostate cancer cell growth. J Cell Biochem. 2005;95(3):497–505. Available from: http://dx.doi.org/10.1002/jcb.20477.

18. Mandal S, Abebe F, Chaudhary J. -174G/C polymorphism in the interleukin-6 promoter is differently associated with prostate cancer incidence depending on race. Genet Mol Res. 2014;13(1):139–51. Available from: http://dx.doi.org/10.4238/2014.january.10.5.

19. Zarogoulidis, P., Yarmus, L., Darwiche, K.,Walter, R., Huang, H., Li, Z., Zaric, B., Tsakiridis, K., Zarogoulidis, K. Interleukin-6 cytokine: a multifunctional glycoprotein for cancer. Immunome Res. 2013 Aug 12;9(62):16535. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24078831.

20. Lagmay JP, London WB, Gross TG, Termuhlen A, Sullivan N, Axel A, et al. Prognostic Significance of Interleukin-6 Single Nucleotide Polymorphism Genotypes in Neuroblastoma: rs1800795 (Promoter) and rs8192284 (Receptor). Clinical Cancer Research. 2009 Aug 11;15(16):5234–9. Available from: http://dx.doi.org/10.1158/1078-0432.ccr-08-2953.

21. Vairaktaris, E., Yiannopoulos, A., Vylliotis, A., Yapijakis, C., Derka, S., Vassiliou, S., Nkenke, E., Serefoglou, Z., Ragos, V., Tsigris, C., Vorris, E., Critselis, E., Avgoustidis, D., Neukam, F.W., Patsouris, E. Strong association of interleukin-6–174G>C promoter polymorphism with increased risk of oral cancer. Int. J. Biol. Markers. 2006 Oct-Dec;21(4):246-50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17177164.

22. Berek JS, Chung C, Kaldi K, Watson JM, Knox RM, Martínez-Maza O. Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer. American Journal of Obstetrics and Gynecology. 1991 Apr;164(4):1038–43. Available from: http://dx.doi.org/10.1016/0002-9378(91)90582-c.

23. DeMichele, A., Martin, A.M., Mick, R., Gor, P.,Wray, L., Klein-Cabral, M., Athanasiadis, G., Colligan, T., Stadtmauer, E.,Weber, B. Interleukin- 6–174G N C polymorphism is associated with improved outcome in high-risk breast cancer. Cancer Res. 2003 Nov 15;63(22):8051-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14633738.

24. Landi, S., Moreno, V., Gioia-Patricola, L., Guino, E., Navarro, M., de Oca, J., Capella, G., Canzian, F. Bellvitge Colorectal Cancer Study Group. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. Cancer Res. 2003 Jul 1;63(13):3560-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12839942.

25. Slattery ML, Curtin K, Baumgartner R, Sweeney C, Byers T, Giuliano AR, et al. IL6, Aspirin, Nonsteroidal Anti-inflammatory Drugs, and Breast Cancer Risk in Women Living in the Southwestern United States. Cancer Epidemiology Biomarkers & Prevention. 2007 Apr 1;16(4):747–55. Available from: http://dx.doi.org/10.1158/1055-9965.epi-06-0667.

26. Yeh K-Y, Li Y-Y, Hsieh L-L, Chen J-R, Tang R-P. The −174 G/C Polymorphism in Interleukin-6 (IL-6) Promoter Region is Associated with Serum IL-6 and Carcinoembryonic Antigen Levels in Patients with Colorectal Cancers in Taiwan. J ClinImmunol. 2009 Aug 29;30(1):53–9. Available from: http://dx.doi.org/10.1007/s10875-009-9324-6.

27. Jerrard-Dunne P, Sitzer M, Risley P, Buehler A, von Kegler S, Markus HS. Inflammatory Gene Load Is Associated With Enhanced Inflammation and Early Carotid Atherosclerosis in Smokers. Stroke. 2004 Oct 7;35(11):2438–44. Available from: http://dx.doi.org/10.1161/01.str.0000144681.46696.b3.

28. Seow A. Joint effect of asthma/atopy and an IL-6 gene polymorphism on lung cancer risk among lifetime non-smoking Chinese women. Carcinogenesis. 2006 Jan 16;27(6):1240–4. Available from: http://dx.doi.org/10.1093/carcin/bgi309.

29. Xu B, Niu X-B, Wang Z-D, Cheng W, Tong N, Mi Y-Y, et al. IL-6 −174G>C polymorphism and cancer risk: a meta-analysis involving 29,377 cases and 37,739 controls. MolBiol Rep. 2010 Nov 23;38(4):2589–96. Available from: http://dx.doi.org/10.1007/s11033-010-0399-1.

30. Yin Y-W, Sun Q-Q, Hu A-M, Wang Q, Liu H-L, Hou Z-Z, et al. Associations between interleukin-6 gene −174 C/G and −572 C/G polymorphisms and the risk of gastric cancer: A meta-analysis. J SurgOncol. 2012 Jun 18;106(8):987–93. Available from: http://dx.doi.org/10.1002/jso.23199.

31. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells.Nucleic Acids Research. 1988;16(3):1215–1215. Available from: http://dx.doi.org/10.1093/nar/16.3.1215.

32. Bachelot T, Ray-Coquard I, Menetrier-Caux C, Rastkha M, Duc A, Blay J-Y. Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. British Journal of Cancer. 2003 Jun 2;88(11):1721–6. Available from: http://dx.doi.org/10.1038/sj.bjc.6600956.

33. Hefler, L.A., Grimm, C., Lantzsch, T., Lampe, D., Leodolter, S., Koelbl, H., Heinze, G., Reinthaller, A., Tong-Cacsire, D., Tempfer, C., Zeillinger, R. Interleukin-1 and interleukin-6 gene polymorphisms and the risk of breast cancer in Caucasian women. Clin. Cancer Res. 2005 Aug 15;11(16):5718-21. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16115908.

34. Kishimoto, T., 1989. The biology of interleukin-6.Blood.1989 Jul;74(1):1-10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2473791.

35. Slattery ML, Curtin K, Baumgartner R, Sweeney C, Byers T, Giuliano AR, et al. IL6, Aspirin, Nonsteroidal Anti-inflammatory Drugs, and Breast Cancer Risk in Women Living in the Southwestern United States. Cancer Epidemiology Biomarkers & Prevention. 2007 Apr 1;16(4):747–55. Available from: http://dx.doi.org/10.1158/1055-9965.epi-06-0667.

36. Zhang, G.J., Adachi, I. Serum interleukin-6 levels correlate to tumor progression and prognosis in metastatic breast carcinoma. Anticancer Res. 1999 Mar-Apr;19(2B):1427-32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10365118.

37. Yu K-D, Di G-H, Fan L, Chen A-X, Yang C, Shao Z-M. Lack of an association between a functional polymorphism in the interleukin-6 gene promoter and breast cancer risk: a meta-analysis involving 25,703 subjects. Breast Cancer Research and Treatment. 2009 Dec 31;122(2):483–8. Available from: http://dx.doi.org/10.1007/s10549-009-0706-5.

38. Liang J, Liu X, Bi Z, Yin B, Xiao J, Liu H, et al. Relationship between gene polymorphisms of two cytokine genes (TNF-α and IL-6) and occurring of lung cancers in the ethnic group Han of China. MolBiol Rep. 2012 Oct 26;40(2):1541–6. Available from: http://dx.doi.org/10.1007/s11033-012-2199-2.

39. Savage, S.A., Abnet, C.C., Haque, K., Mark, S.D., Qiao, Y.L., Dong, Z.W., Dawsey, S.M., Taylor, P.R., Chanock, S.J. Polymorphisms in interleukin −2, −6, and −10 are not associated with gastric cardia or esophageal cancer in a high-risk Chinese population. Cancer Epidemiol. Biomarkers Prev. 2004 Sep;13(9):1547-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15342460.

40. Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and cancer: How hot is the link? Biochemical Pharmacology. 2006 Nov;72(11):1605–21. Available from: http://dx.doi.org/10.1016/j.bcp.2006.06.029.

41. Chen C-J, Sung W-W, Lin Y-M, Chen M-K, Lee C-H, Lee H, et al. Gender Difference in the Prognostic Role of Interleukin 6 in Oral Squamous Cell Carcinoma. Culig Z, editor. PLoS ONE. 2012 Nov 21;7(11):e50104. Available from: http://dx.doi.org/10.1371/journal.pone.0050104.

42. Schafer ZT, Brugge JS. IL-6 involvement in epithelial cancers. J Clin Invest. 2007 Dec 3;117(12):3660–3. Available from: http://dx.doi.org/10.1172/jci34237.

43. Jibiki, T., Terai, M., Shima, M., Ogawa, A., Hamada, H., Kanazawa, M., Yamamoto, S., Oana, S., Kohno, Y. Monocyte chemoattractant protein 1 gene regulatory region polymorphism and serum levels of monocyte chemoattractant protein 1 in Japanese patients with Kawasaki disease. Arthritis Rheum.2001 Sep;44(9):2211-2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11592387.

44. Belluco, C., Olivieri, F., Bonafe, M., Giovagnetti, S., Mammano, E., Scalerta, R., Ambrosi, A., Franceschi, C., Nitti, D., Lise, M. 174G N C polymorphism of interleukin 6 gene promoter affects interleukin 6 serum levels in patients with colorectal cancer. Clin. Cancer Res. 2003 Jun;9(6):2173-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12796383.

45. Berger FG. The interleukin-6 gene: a susceptibility factor that may contribute to racial and ethnic disparities in breast cancer mortality. Breast Cancer Research and Treatment. 2004 Dec;88(3):281–5. Available from: http://dx.doi.org/10.1007/s10549-004-0726-0.

46. Vucicević Boras, V., Cikes, N., Lukać, J., Virag, M., Cekić-Arambasin, A. Salivary and serum interleukin 6 and basic fibroblast growth factor levels in patients with oral squamous cell carcinoma. Minerva Stomatol. 2005 Oct;54(10):569-73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16224376.

47. Michalaki, V., Syrigos, K., Charles, P.,Waxman, J. Serumlevels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br. J. Cancer. 2004 Jun 14;90(12):2312-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15150588.

48. Balasubramanian SP, Azmy IA, Higham SE, Wilson AG, Cross SS, Cox A, et al. BMC Cancer. 2006;6(1):188. Available from: http://dx.doi.org/10.1186/1471-2407-6-188.