ejpmr, 2016,3(8), 550-558

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CYTOKINE GENE POLYMORPHISMS AND THEIR ASSOCIATION WITH ORAL SQUAMOUS CELL CARCINOMA (OSCC): A NORTH INDIAN STUDY

Maneesh Kumar Gupta, Nibha Sagar, Rajeev Pant[1] and Monisha Banerjee*

*Molecular & Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow-226007, India.
[1]Department of Radiotherapy, Lucknow Cancer Institute, Lucknow- 226001, India.

***Corresponding Author: Prof. Dr. Monisha Banerjee**

Molecular & Human Genetics Laboratory, Department of Zoology, University of Lucknow, Lucknow-226007, India

Article Received on 12/06/2016 **Article Revised on 03/07/2016** **Article Accepted on 24/07/2016**

ABSTRACT

Oral Squamous Cell Carcinoma (OSCC), the eighth most common cancer worldwide has several risk factors such as alcohol, tobacco and smoking. Oral cancer involves production of cytokines, growth factors and adhesion molecules which promote optimal growth conditions for cancerous cells. The present study was undertaken to evaluate association of cytokine gene polymorphisms with oral cancer. Genotyping of SNPs *viz. IL-6*-597G/A (rs1800797), *TNF-α*-308G/A (rs1800629), *IL-1β*-511C/T (rs16944) and *IL-1RN* Variable Number of Tandem Repeats (VNTR) in intron 2 was carried out in 140 healthy age/sex matched control subjects and 130 oral cancer patients by PCR-RFLP. Genotype and allele frequencies were calculated and statistically analyzed by chi-square (χ^2) using SPSS (ver.21.0). Gene-gene interaction, pairwise linkage disequilibrium (LD) based on 'D' statistics and correlation coefficients (r2) of frequencies were analyzed using SHEsis (ver. Online). Genotypic frequencies of *IL-6*, *IL-1β* and *IL-1RN* and allelic frequency of *IL-6* showed significant association with oral cancer in north Indian population (p<0.001). Therefore, genetic polymorphisms in associated genes can be used as markers to predict oral cancer susceptibility. Gene-gene interaction analysis showed that individuals with SNP combinations T G G I* and T G G II* of *IL-1β*-511 C/T, *IL-6*-597A/G, *TNF-α*-308G/A and *IL-1RN* polymorphisms increase the risk of OSCC upto 18.7 and 7.3 folds respectively in the study population. This is probably the first report from India showing the combinatorial effect of these gene polymorphisms and OSCC susceptibility. The study will help to predict individuals at risk of developing OSCC and will provide leads for other cancers as well.

KEYWORDS: Oral Squamous Cell Carcinoma; OSCC; IL-6; TNF-α; IL-1β; IL-1RN; North Indian population.

INTRODUCTION

The eighth most common type of cancer worldwide is Oral Squamous Cell Carcinoma (OSCC), it is more frequent in men with a history of tobacco, smoking, heavy alcohol use and those infected with human papillomavirus (HPV).[1, 2] According to Indian Council of Medical Research there is a sharp increase in the number of oral cancer cases and is expected to increase by 2020. Development of OSCC is multistep process resulting due to chronic inflammation and genetic factors such as alterations in oncogenes and tumor supressor genes. Immune cells produce cytokines (pro- and anti-inflammatory), growth factors and adhesion molecules which promote tumor progression by signaling cascade and provide optimal cell growth conditions for cancer.[3] Cytokines are produced by tumor cells, macrophages, NK cells and other phagocytic cells[4, 5] which play an important role in progression and regulation of cellular/humoral immune responses during malignancies. They act by activating transcription factors such as NF-kB, AP-1/AP-2 and STAT3 thus stimulating immune cell proliferation and survival. The involvement of inflammation, angiogenesis and thrombosis during OSCC development strongly correlate with microenvironment of immune cells residing in the cancerous tissues. This inflammatory microenvironment increase the DNA mutation rate and enhance proliferation of mutated cells.[6] The onset of neoplastic initiation is closely related to chronic cytokine production. Cancerous cells either directly secrete *IL-6*, *TNF-α*, *IL-1* or induce cells within the tumor microenvironment to do so.[7]

In neoplastic disease, IL-6 circulating levels increase markedly during development and progression of tumors. It is a multifunctional inflammatory cytokine which acts as growth promoting and anti-apoptotic factor produced by T-cells and other tumor cells.[8] Transcription factor AP-2 gets enhanced in the presence of *IL-6*, a potent cell cycle regulator that activates oncogenes Ras and cerB2 which are directly involved in carcinogenesis. *IL-6* also influences P^{53} tumor suppressor gene by supporting hypermethylation of its promoter that leads to suppression of apoptosis and uncontrolled cell growth.[9, 10]

T-cells, macrophages and NK cells produce *TNF-α* which mediate the expression of genes such as growth factors, cytokines, inflammatory mediators and acute phase proteins.[11] Positive cell cycle regulator NF-κB activated by *TNF-α*, results in evasion of apoptosis and enhanced cell proliferation. It is also a potent endogenous mutagen causing direct damage to DNA through the induction of reactive oxygen species (ROS).[12]

IL-1 gene family contains three proteins, IL-1α, IL-1β and their naturally occurring inhibitor IL-1RN. The sources of IL-1β in oral cavity are monocytes/macrophages, fibroblasts and mucosal epithelial cells which are involved in secretion of endogenous pyrogens resulting in both inflammatory response as acute and chronic.[13] IL-1β enhances carcinogenesis by increasing the action of chemical carcinogens resulting in proliferation of mutated cells and accumulation of genetic damage.[14, 15, 16]

Interleukin-1 receptor antagonist (*IL-1RN*) gene (at 2q14.2) has a 86-bp variable number of tandem repeat (VNTR) polymorphism within intron 2.[17] IL1RN, the anti-inflammatory molecule is a naturally occurring antagonist of IL-1 but share 70% sequence homology.[18, 19] The balance between IL-1 and IL-1RN in local tissues plays an important role in the susceptibility and severity of many diseases including cancer.[20, 21]

In the present study, single nucleotide polymorphisms (SNPs), upstream of the transcription start site in *IL-6*, *TNF-α*, *IL-1β* and 86bp VNTR in *IL-RN* gene were studied in a North Indian population.

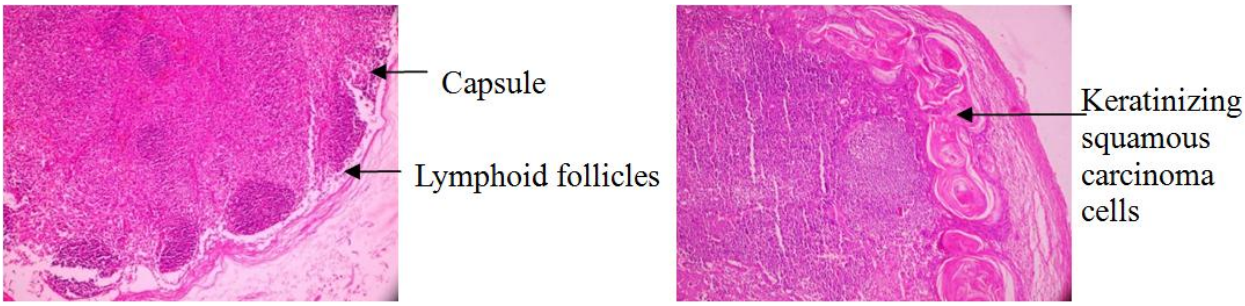


Fig 1: Hematoxylin and eosin stained Lymph nodes. (A) Normal and (B) Tumor metastasis

MATERIALS AND METHODS

Patient Selection and Sample Collection

Oral squamous cell carcinoma cases (n=130) and age/sex matched normal control subjects (n=140) were enrolled after due approval of Institutional Ethics Committee and written consent from all subjects. Control subjects with previous history of cancer were excluded from the study. Clinical details of patients' addiction *viz*. smoking, tobacco, alcohol, *etc.* were precisely recorded. The patient selection criterion was high staged locoregional nodal metastasis. Blood samples (2ml) were collected in EDTA vials from all individuals and stored at -20^{0}C until further use. The comparative histology of normal and cancerous lymph nodes of OSCC is shown in Figure 1.

DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using salting out method[22] with slight modifications.[23] Genotyping of four polymorphisms *IL-6*-597A/G (rs1800797), *TNF-α*-308G/A (rs1800629), *IL-1β*-511C/T (rs16944) and *IL-1RN* (VNTR in intron 2) was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR–RFLP) and Variable Number of Tandem Repeat (VNTR) analysis. The 15 µl reaction mixture contained 100 ng of template DNA, buffer (100mM Tris, pH 9.0; 500mMKCl; 15mM $MgCl_2$; 0.1% gelatin), 200 µM dNTP, 10 pmol of each primer and 1.0 unit Taq DNA polymerase (Biosciences, India). The primers designed by Primer 3.0 online software were F-5'- GGAGTCACACACTCCACCT-3' and R-5'-CTGATTGGAAACCTTATTAAG-3';F-5'-AGGCAATAGGTTTTGAGGGCCAT-3' and R-5'-TTGGGGACACAAGCATCAAGG-3'; F-5'-TGGCATTGATCTGGTTCATC-3' and R-5'-GTTTAGGAATCTTCCCACTT3' and F-5'-CTCAGCAACACTCCTAT- 3' and R-5'-TCCTGGTCTGCAGGTAA3' respectively. The PCR products of *IL-6*, *TNF-α* and *IL-1β* were digested with FokI, NcoI and SacI restriction enzymes (Thermo Fisher Scientific Inc., USA) respectively and electrophoresed on 12.5 % polyacrylamide gels while PCR products of *IL-1RN* VNTR were electrophoresed on 2% agarose gels, stained with EtBr and documented in Geldoc system (Vilber Lourmat, France).

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 ($\chi 2$) and Fisher's exact t-test. Hardy–Weinberg equilibrium at individual locus was assessed by $\chi 2$ statistics using Statistical Package for Social Science (SPSS ver 21.0). 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.

Gene-gene interaction, pairwise linkage disequilibrium (LD) based on 'D' statistics and correlation coefficient (r2) of frequencies was analyzed using SHEsis.[24]

RESULTS

The *IL-6*-597A/G, *TNF-α*-308G/A, *IL-1β*-511C/T and *IL-1RN* gene polymorphisms were successfully genotyped in 140 controls and 130 OSCC cases (Figure 2). The allele and genotype frequency distributions as well as carriage rates are shown in Tables 1 and 2. All allele and genotype frequencies were found to be in Hardy-Weinberg equilibrium (HWE).

IL-6-597A/G (rs1800797) polymorphism showed significant genotypic and allelic associations (p<0.001) with 'AA' (22.3%) genotype was rare while 'GG' (36.2%) and 'AG' (41.5%) were most prevalent in OSCC. Allele -597*G of *IL-6*-597A/G also show significant relation with OSCC (p<0.001). The carriage rate analysis also showed that presence of -597*A allele of *IL-6*-597A/G increase the risk of OSCC in our population upto 19.5 times (p<0.001) (Table 1).

The *TNF-α*-308G/A (rs1800629) polymorphism showed 'AA' genotype in 9.3% of cases which was higher in comparison to controls (2.9%) with no significant association. However, the prevalence of -308*A allele of *TNF-α* was higher in OSCC (18.8%) and showed significant association (p=0.02) (Table 1).

Table 1: 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 = 140) and OSCC cases (n = 130).

		Number (%frequency)				**P value**
	IL-6	**AA**	**AG**	**GG**		
	Controls	108 (77.1)	28 (20.0)	4 (2.9)		**<0.0001#**
	Cases	29 (22.3)	54 (41.5)	47 (36.2)		
Genotype frequency	***TNF-α***	**GG**	**GA**	**AA**		
	Controls	111 (79.3)	25 (17.9)	4 (2.9)		0.73
	Cases	93 (71.5)	25 (19.2)	12 (9.3)		
	IL-1β	**CC**	**CT**	**TT**		
	Controls	17 (12.1)	29 (20.7)	94 (67.2)		**0.004#**
	Cases	4 (3.1)	43 (33.1)	83 (63.8)		
		Number (%frequency)		**P value**	**Odd's Ratio (OR)**	**%95 CI**
	IL-6	**A**	**G**			
	Controls	244 (87.1)	36 (12.9)	**<0.0001#**	**8.956**	5.841-13.733
	Cases	112 (43.1)	148 (56.9)			
Allele frequency	***TNF-α***	**G**	**A**			
	Controls	247 (88.2)	33 (11.8)	**0.023#**	**1.738**	1.078-2.804
	Cases	211 (81.2)	49 (18.8)			
	IL-1β	**C**	**T**			
	Controls	63 (22.5)	217 (77.5)	0.412	1.190	0.785-1.802
	Cases	51 (19.6)	209 (80.4)			
	IL-6	**A (+)**	**A (-)**			
	Controls	136 (97.1)	4 (2.9)	**<0.0001#**	**19.523**	6.692-55.389
	Cases	83 (63.8)	47 (36.2)			
		G (+)	**G (-)**			
	Controls	32 (22.9)	108 (77.1)	**0.001#**	**0.085**	0.48-0.151
	Cases	101 (77.7)	29 (22.3)			
	TNF-α	**G (+)**	**G (-)**			
	Controls	136 (97.1)	4 (2.9)	**0.036#**	**3.458**	1.086-11.009
Carriage rate	**Cases**	118 (90.8)	12 (9.2)			
		A (+)	**A (-)**			
	Controls	29 (20.7)	111 (79.3)	0.140	0.657	0.376-1.148
	Cases	37 (28.5)	93 (71.5)			
	IL-1β	**C (+)**	**C (-)**			
	Controls	46 (32.9)	94 (67.1)	0.569	0.864	0.523-1.428
	Cases	47(36.2)	83(63.8)			
		T (+)	**T (-)**			
	Controls	123 (87.9)	17 (12.1)	**0.010#**	**0.230**	0.075-0.702
	Cases	126 (97.0)	4 (3.0)			

χ 2 Chi-square, 95% CI= Confidence Interval, OR= Odds Ratio, #implies significant at 5% level

IL-1β-511C/T (rs16944) polymorphism showed higher 'CT' genotype frequency in OSCC (33.1%) which was significantly associated with the disease (p=0.004). -511*T allele frequency was higher in OSCC (80.4%) as compared to controls (77.5%) but no significant association was observed. Carriage rate analysis showed significant association of -511*T allele and OSCC (p=0.010) (Table 1).

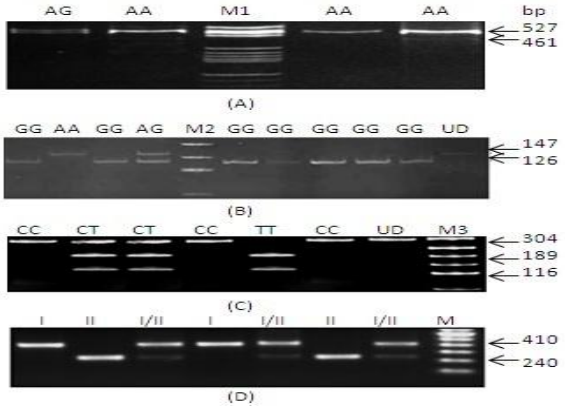


Fig 2: Polyacrylamide gels (12.5 %) showing genotypes of different gene polymorphisms.

(A) *IL-6*-597A/G genotypes; M1, pBR322/HaeIII (B) *TNF-α*-308G/A genotypes; M2, pUC19/MspI. (C) *IL-1β*-511C/T genotypes; M, pUC19/MspI. (D) Agarose gel (2.0%) showing VNTRs of *IL-1RN*; M: 100 bp Ladder. UD: Undigested.

Different combinations of three alleles (I, II and III) of 86bp VNTR polymorphism in intron 2 of *IL-1RN* gene were observed in study population. Out of 140 controls 73 were I/I (52.1 %), 33 were II/II (23.6 %), 32 were I/II (22.9 %) and 2 were I/III (1.4%) while in OSCC, 44 were I/I (33.8 %), 21 were II/II (16.2 %), 61 were I/II (46.9 %) and 4 were I/III (3.1%) respectively. The percentage of I/II OSCC individuals were higher than controls (46.9 vs. 22.9%) and genotype frequency showed highly significant association (p<0.0001). In carriage rate analysis, II* allele of *IL-1RN* was found to be significantly associated with OSCC (p=0.006) (Table 2).

Table 2: Genotypic, allelic and carriage rate frequencies of *IL-1RN* gene polymorphisms in healthy controls (n=140) and OSCC cases (n=130).

Genotype frequency		**Number (%frequency)**				**P value**		
	IL-1RN	**I/I**	**II/II**	**I/II**	**I/III**			
	Controls	73 (52.1)	33 (23.6)	32 (22.9)	2 (1.4)	**<0.0001**		
	Cases	44 (33.8)	21 (16.2)	61 (46.9)	4 (3.1)			
Allele frequency		**Number (%frequency)**			**P value**	**Odd's Ratio (OR)**	**%95 CI**	
	IL-1RN	**I**	**II**	**III**				
	Controls	180 (64.3)	98 (35.0)	2 (0.7)	0.155	1.271	0.913-1.771	
	Cases	153 (58.8)	103 (39.6)	4 (1.6)				
Carriage rate	***IL-1RN***	**I (+)**	**I (-)**					
	Controls	107 (76.4)	33 (23.6)		0.130	0.625	0.340-1.148	
	Cases	109(83.8)	21 (16.2)					
		II (+)	**II (-)**					
	Controls	65 (46.4)	75 (53.6)		**0.006**[#]	**0.457**	0.820-2.535	
	Cases	82 (63.1)	48 (36.9)					
		III (+)	**III (-)**					
	Controls	2 (1.4)	138 (98.6)		0.370	0.457	0.820-2.535	
	Cases	4 (3.1)	126 (96.9)					

χ 2 Chi-square, 95% CI= Confidence Interval, OR= Odds Ratio, #implies significant at 5% level

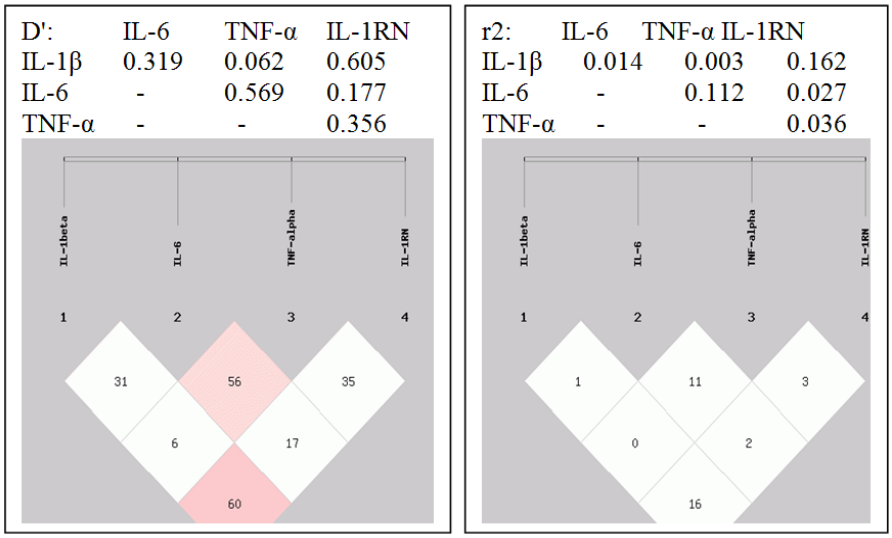
The genotypic frequency of gene polymorphisms was studied in OSCC individuals using tobacco and alcohol, *IL-6* genotypes alone showed significant association in OSCC with both tobacco addiction (p=0.010) and alcohol abuse (p=0.007) (2.3 and 1.9 times higher risk respectively) (Table 3). *TNF-α* genotypes also showed significant association and increased the risk of OSCC upto 4.4 times with tobacco addiction (Table 3).

Table 3: Genotypic frequencies of *IL-6*-597A/G, *TNF-α*-308G/A, *IL-1β*-511C/T and *IL-RN* VNTR polymorphisms in OSCC cases with and without tobacco and alcohol addiction.

Genotypes	Tobacco		p-value	OR (CI 95%)	Alcohol		p-value	OR (CI 95%)
	With	Without			With	Without		
IL-6								
AA	27	2	**0.010**[#]	**2.340 (1.227-4.466)**	20	9	**0.007**[#]	**1.958 (1.206-3.178)**
AG	44	10			20	34		
GG	32	15			16	31		
TNF-α								
GG	89	4	**<0.0001**[#]	**4.402 (2.188-8.857)**	44	49	0.945	0.982 (0.577-1.669)
GA	16	9			14	11		
AA	7	5			5	7		
IL-1β								
CC	3	1	0.199	0.646 (0.331-1.259)	1	3	0.111	1.718 (0.882-3.344)
CT	26	17			32	11		
TT	62	21			41	42		
IL-1RN								
I/I	31	13	0.154	0.742 (0.493-1.118)	24	20	0.212	1.263 (0.875-1.824)
II/II	10	11			9	12		
I/II	50	11			27	34		
I/III	3	1			1	3		

χ 2 Chi-square, 95% CI= Confidence Interval, OR= Odds Ratio, #implies significant at 5% level

Gene-gene interaction analysis showed that individuals with SNP combinations T G G I* and T G G II* of *IL-1β*-511 C/T, *IL-6*-597A/G, *TNF-α*-308G/A and *IL-1RN* polymorphisms increase the risk of OSCC upto 18.7 and 7.3 folds respectively in the study population (Figure 3).



	Case(freq)	Control(freq)	Chi2	Fisher's p	Pearson's p	OddsRatio[95%CI]
C A G I*	6.78(0.026)	12.00(0.043)	0.996	0.318212	0.318151	0.617[0.237~1.607]
C A G II*	12.69(0.049)	42.00(0.150)	14.371	0.000152	0.000151	0.299[0.155~0.575]
T A G I*	52.49(0.202)	163.52(0.584)	79.830	4.88e-015	4.32e-019	0.179[0.121~0.265]
T A G II*	21.69(0.083)	17.48(0.062)	1.081	0.298503	0.298437	1.414[0.734~2.723]
T G A II*	8.28(0.032)	24.00(0.086)	6.538	0.010586	0.010576	0.361[0.161~0.811]
T G G I*	58.36(0.224)	4.48(0.016)	59.384	2.30e-014	1.36e-014	18.717[7.028~49.844]
T G G II*	37.57(0.145)	6.52(0.023)	27.652	1.51e-007	1.48e-007	7.375[3.149~17.274]

Fig 3: Haploview of SNPs *viz.IL-6*-597A/G, *TNF-α*-308G/A, *IL-1β*-511C/T and *IL-1RN*showing association with OSCC in North Indian population. Pairwise linkage disequilibrium (LD). (D') in subjects is represented as pink squares for little LD and red for high LD. (SHEsis Software, ver. online). 95%CI= confidence interval.

* indicate allele combination of *IL-6*-597A/G, *TNF-α*-308G/A and *IL-1β*-511C/T gene polymorphisms.

DISCUSSION

The association of *IL-6*-597A/G, *TNF-α*-308 G/A, *IL-1β*-511C/T and *IL-1RN* gene polymorphisms with OSCC has been studied in north Indian population. It has been reported that functional DNA polymorphisms that affect gene expression of inflammatory molecules may confer susceptibility to disease, its progression and severity.[25, 26] IL-6 expression was reported to be higher in serum, saliva and tumor biopsies obtained from patients with OSCC.[27] IL-6 also showed association with cardiovascular disease in patients with rheumatoid arthritis and ovarian cancer.[28] In the present study, OSCC patients with tobacco and alcohol addiction showed significant association with *IL-6*-597A/G genotypes with an increased risk upto 2.3 and 1.19 respectively. Gene-gene interaction analysis also showed that *IL-6* genotypes in combination with other genetic variants increased the risk of OSCC. Thus, it can be concluded that SNPs in the promoter region of *IL-6* gene might be risk factors for OSCC development. Other *IL-6* SNPs like -174G/C polymorphism has been associated with increased risk of breast cancer[29, 30], cervical cancer[31], prostate cancer[32], leukaemia[33], colorectal cancer[12] and basal cell cancer. However, *IL-6* was found to have a protective role in colorectal and gastric cancers. The association between *IL-6* polymorphisms and cancer risk was evident among Asians and Africans but not Caucasians.[34] OSCC cases with alcohol and tobacco addiction were more likely to carry the 'G' allele of *IL-6*-597A/G and *TNF-α*-308G/A in the study population.

Several studies have evaluated the association of *TNF-α* promoter SNPs with risk of several types of cancer, including those of cervix, stomach, colon, rectum and non-Hodgkin lymphoma.[35, 36, 37, 38] The results of genotyping of *TNF-α* promoter polymorphism (-308G/A) did not show any association with development of OSCC in our population. However, previous studies showed a significant increase in risk associated with allele 'A'[39, 40, 41], while two other studies reported a decreased risk in the east Asian region where smoking and use of alcohol were predominant risk factors for OSCC.[38, 42] Gene-gene analysis showed that promoter polymorphism in *TNF-α* of -308*G allele in combination with -511*T allele of *IL-1β*, -597*G of *IL-6* and II* of *IL-1RN* increases the risk of OSCC upto 18 times. A meta-analysis based study comparing individuals carrying GA/AA genotypes with GG genotype of TNF-α-308G/A polymorphism also showed that the risk remained significant among both Caucasians and Asians.[43] OSCC with tobacco addiction showed a 4.4 times higher risk in the present study.

Cytokine IL-1β has proven to be a multi-effect mediator of many physiological functions such as angiogenesis and posttraumatic inflammatory reaction.[44, 45, 46] As a result, any alteration in the IL-1β serum level may affect its biological activity. IL-1β and TNF-α have the ability to regulate immune response and simultaneously induce the release of secondary cytokines such as IL-6. Interestingly, high levels of TNF-α and IL-6 have already been strongly associated with increased risk of OSCC.[47, 48] In addition, IL-6 and TNF-α stimulate oral cancer cells to increase secretion of matrix metalloproteinases (MMPs) and at least one of them, MMP-1, has also been associated with increased risk for oral cancer by promoting angiogenesis and invasion.[49, 48]. Moreover, increased levels of IL-6 may inhibit IL-1β.[50], therefore the role of IL-1β in oral oncogenesis may be minimal in comparison with that of other factors. The *IL-1β* -511*T allele frequency and carriage rate were found to be associated with risk of OSCC in the present study, thus play a crucial role in tumor progression and metastasis.

In the present study, *IL-1RN* VNTR polymorphism showed highly significant association with OSCC, II* allele being most significantly associated. An earlier study in Korean women has reported that *IL-1RN* allele decreased the risk of breast cancer with a marginal significance.[51] IL-1RN expression was higher in OSCC when compared to normal tissue, this was associated with active tumor development in OSCCs occurring in buccal mucosa, oral floor *etc*.[21] *IL-1RN* polymorphism was reported to be significantly associated as a risk marker in Portuguese population in nasopharyngeal carcinoma.[17] OSCC is more common in men than women, among those with a history of tobacco or heavy alcohol use and individuals infected with HPV.[52] The use of tobacco (including smokeless tobacco) and excessive consumption of alcohol showed prominent significance with -597*G allele of *IL-6* and -308*G allele of *TNF-α* polymorphism in the present study.

Moreover, in gene-gene interaction analysis allele 'G' of both *IL-6* and *TNF-α*, 'T' of *IL-1β*, 'I' and 'II' of *IL-1RN* increased the risk of OSCC upto 18.7 and 7.3 times respectively. The results showed that genotypes and alleles are positively associated with OSCC manifestation individually as well as in combination. The power for analyzing binary traits of *IL-6*, *TNF-α*, *IL-1β* and *IL-1RN* polymorphisms associated with OSCC in case-control design was limitation of our study. Further investigations are warranted to validate ethnic differences in the effect of such polymorphisms on cancer risk.

CONCLUSION

The main objective of the current report was to evaluate the combinatorial effect of *IL-6*-597A/G, *TNF-α*-308G/A, *IL-1β*-511C/T and *IL-1RN* gene polymorphisms for determining susceptibility to OSCC. Genotypic frequencies of *IL-6*, *IL-1β*, and *IL-RN* except *TNF-α* and allelic frequency of *IL-6* ($p<0.001$) and *TNFα* ($p=0.023$) showed significant association with OSCC. *IL-6* genotypes showed significant association in patients with tobacco and alcohol addiction while *TNF-α* increases the risk of OSCC upto 4.4 times in subjects consuming tobacco. Promoter polymorphisms affect gene expression and these different levels of gene expression involved in

inflammation results in development of OSCC. This study will help to predict individuals at risk of developing OSCC and provide leads for other cancers as well. The knowledge of risk alleles will enable individuals to take precautionary measures before hand and prevent or delay the onset of disease. Larger cohorts are needed to confirm these results and more importantly to investigate the complex interactions among the genetic variants in DNA repair, inflammation and other non-genetic susceptibility genes.

ACKNOWLEDGEMENTS

The work was supported by research grants from Indian Council of Medical Research (ICMR) (IRIS ID: 2009-07210) and Department of Science and Technology, New-Delhi, India. MKG is thankful to ICMR for Junior and Senior Research Fellowships. NS is grateful to Council of Scientific and Industrial Research, New Delhi for Research Associateship. The departmental equipment facility provided by DST-FIST-PURSE is duly acknowledged.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Chang F, Syrjanen S, Kellokoski J, Syrjanen K. Human papillomavirus (HPV) infections and their associations with oral disease. J Oral Pathol Med, 1991; 20: 305-317.
2. Byakodi R, Byakodi S, Hiremath S, Byakodi J, Adaki S, Marathe K, et al,. Oral cancer in India: an epidemiologic and clinical review. J Community Health, 2012; 37: 316-319.
3. Mantovani A, Muzio M, Garlanda C, Sozzani S, Allavena P. Macrophage control of inflammation: negative pathways of regulation of inflammatory cytokines. Novartis Found Symp, 2001; 234: 120-31.
4. Kurzrock R. Cytokine deregulation in cancer. Biomed Pharmacother, 2001; 55: 543-547.
5. Jin P, Panelli MC, Marincola FM, Wang E. Cytokine polymorphism and its possible impact on cancer. Immunol Res, 2004; 30: 181-190.
6. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell, 2010; 140: 883-899.
7. Portier M, Zhang XG, Ursule E, Lees D, Jourdan M, Bataille R, et al,. Cytokine gene expression in human multiple myeloma. Br J Haematol, 1993; 85: 514-520.
8. Ishihara K, Hirano T. Molecular basis of the cell specificity of cytokine action. Biochimica Biophysica Acta (BBA)-Molecular Cell Research, 2002; 1592: 281-296.
9. Salgado R, Junius S, Benoy I, Van Dam P, Vermeulen P, Van Marck E, et al,. Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. Int J Cancer, 2003; 103: 642–646.
10. Xiao W, Hodge DR, Wang L, Yang X, Zhang X, Farrar WL. NF-kappa B activates IL-6 expression through cooperation with c-Jun and IL6-AP1 site, but is independent of its IL6-NFkappaB regulatory site in autocrine human multiple myeloma cells. Cancer Biol Ther, 2004; 3: 1007-1017.
11. Lytvyn OI, Kopitsa MP, Petyunina OV. Interaction between inflammation and thrombosis in acute coronary syndrome. Kardiol Pol, 2004; 61: 110-116.
12. Landi S, Moreno V, Gioia-Patricola L, Guino E, Narvaro M, de Oca J, et al,. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB 1 and peroxisome proliferator activated receptor gamma with colorector cancer. Cancer Res, 2003; 63: 3560-3566.
13. Bird S, Zou J, Wang T, Munday B, Cunningham C, et al,. Evolution of interleukin-1beta. Cytokine Growth Factor Rev, 2002; 13: 483-502.
14. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood, 1996; 87: 2095-2147.
15. Saijo Y, Tanaka M, Miki M, Usui K, Suzuki T, et al,. Proinflammatory cytokine IL-1 beta promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: in vivo analysis of tumor-stromal interaction. J Immunol, 2002; 169: 469-475.
16. Song X, Voronov E, Dvorkin T, Fima E, Cagnano E, et al,. Differential effects of IL-1 alpha and IL-1 beta on tumorigenicity patterns and invasiveness. J Immunol, 2003; 171: 6448-6456.
17. Sousa H, Breda E, Santos AM, Catarino R, Pinto D, Canedo P, et al,. IL-1RN VNTR polymorphism as a susceptibility marker for nasopharyngeal carcinoma in Portugal. Arch Oral Biol, 2013; 58: 1040-1046.
18. Dinarello CA. The IL-1 family and inflammatory diseases. Clin Exp Rheumatol, 2002; 20: S1-S13.
19. Arend WP. Interleukin 1 receptor antagonist: A new member of the interleukin 1 family. J Clin Invest, 1991; 88: 1445-1451.
20. Arend WP. The balance between IL-1 and IL-1Ra in disease. Cytokine Growth Factor Rev, 2002; 13: 323-340.
21. Shiiba M, Saito K, Yamagami H, Nakashima D, Higo M, Kasamatsu A, et al,. Interleukin-1 receptor antagonist (IL1RN) is associated with suppression of early carcinogenic events in human oral malignancies. Int J Oncol, 2015; 46: 1978-1984.
22. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res, 1988; 16: 1215.
23. Gautam S, Agrawal CG, Bid HK, Banrejee M. Preliminary studies on CD36 gene in type 2 diabetic patients from north India. Ind J Med Res, 2011; 134: 107-112.
24. Shi YY, He L. SHEsis is a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Research, 2005; 15: 97-98.
25. Xu J, Lowey J, Wiklund F, Sun J, Lindmark F, Hsu FC, et al,. The interaction of four genes in the

inflammation pathway significantly predicts prostate cancer risk. Cancer Epidemiol Biomarkers Prev, 2005; 14: 2563-2568.
26. Vairaktaris EC, Yapijakis Z, Serefoglou S, Derka S, Vassiliou E, Nkenke A, et al,. The interleukin-8 (−251A/T) polymorphism is associated with increased risk for oral squamous cell carcinoma. Eur J Surg Oncol, 2007; 33: 504-507.
27. Wang YF, Chang SY, Tai SK, Li WY, Wang LS. Clinical significance of interleukin-6 and interleukin-6 receptor expressions in oral squamous cell carcinoma. Head Neck, 2002; 24: 850-858.
28. Bushley AW, Ferrell R, McDuffie K, Terada KY, Carney ME, Thompson PJ, et al,. Polymorphisms of interleukin (IL)-1α, IL-1β, IL-6, IL-10, and IL-18 and the risk of ovarian cancer. Gynecologic Oncology, 2004; 95: 672-679.
29. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Chouchane L. Genetic variation in pro-inflammatory cytokines (interleukin-1 beta, interleukin-1 alpha and interleukin-6) associated with the aggressive forms, survival and relapse prediction of breast carcinoma. Eur Cytokine Netw, 2005; 16: 253-260.
30. 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: 5718-5721.
31. Srivani R, Nagarajan B. A prognostic insight on in vivo expression of interleukin-6 in uterine cervical cancer. Int J Gynecol Cancer, 2003; 13: 331-339.
32. Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer, 2004; 90: 2312-2316.
33. Nearman ZP, Wlodarski M, Jankowska AM, Howe E, Narvaez Y, Ball E, et al,. Immunogenetic factors determining the evolution of T-cell large granular lymphocyte leukaemia and associated cytopenias. Br J Haematol, 2007; 136: 237-248.
34. Xu B, Niu XB, Wang ZD, Cheng W, Na Tong, Mi YY, et al,. IL-6 2174G>C polymorphism and cancer risk: a meta-analysis involving 29,377 cases and 37,739 controls. Mol Biol Rep, 2011; 38: 2589-2596.
35. Deshpande A, Nolan JP, White PS, Valdez YE, Hunt WC, Peyton CL, et al,. TNF-alpha promoter polymorphisms and susceptibility to human papillomavirus 16-associated cervical cancer. J Infect Dis, 2005; 191: 969-976.
36. Garrity-Park MM, Loftus EV Jr, Bryant SC, Sandborn WJ, Smyrk TC. Tumornecrosis factor-alpha polymorphisms in ulcerative colitis-associatedcolorectal cancer. Am J Gastroenterol, 2008; 03: 407-415.
37. Rothman N, Skibola CF, Wang SS, Morgan G, Lan Q, Smith MT, et al,. Genetic variation in TNF and IL10and risk of non-Hodgkin lymphoma: a report from the Inter Lymph Consortium. Lancet Oncol, 2006; 7: 27-38.
38. Yang CM, Hou YY, Chiu YT, Chen HC, Chu ST, Chi CC, et al,. Interaction between tumor necrosisfactor-alphagene polymorphisms and substance use on risk of betel quid-relatedoral and pharyngeal squamous cell carcinoma in Taiwan. Arch Oral Biol, 2011; 56: 1162-1169.
39. Gupta R, Sharma SC, Das SN, Association of TNF-alpha and TNFR1 promoters and 3′ UTR region of TNFR2 gene polymorphisms with genetic susceptibility to tobacco-related oral carcinoma in Asian Indians. Oral Oncol, 2008; 44: 455-463.
40. Vairaktaris E, Yapijakis C, Serefoglou Z, Avgoustidis D, Critselis E, Spyridonidou S, et al,. Gene expression polymorphisms of interleukins-1 beta, -4, -6, -8, -10, and tumor necrosis factors-alpha, -beta: regression analysis of their effect upon oral squamous cell carcinoma. J Cancer Res Clin Oncol, 2008; 134: 821-832.
41. Yapijakis C, Serefoglou Z, Vylliotis A, Nkenke E, Derka S, Vassiliou S, et al,. Association of polymorphisms in Tumor Necrosis Factor Alpha and Beta genes with increased risk for oral cancer. Anticancer Res, 2009; 29: 2379-2386.
42. Liu CJ, Wong YK, Chang KW, Chang HC, Liu HF, Lee YJ. Tumor necrosis factor-alpha promoter polymorphism is associated with susceptibility to oral squamous cell carcinoma. J Oral Pathol Med, 2005; 34: 608-612.
43. Ding B, Fu S, Wang M, Yue C, Wang W, Zhou D, et al,. Tumornecrosis factor alpha −308 G > A polymorphisms and cervical cancer risk:a meta-analysis. Int J Gynecol Cancer, 2012; 22: 213-219.
44. Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone MA. Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in humanvascular endothelial cells. J Exp Med, 1984; 160: 618-623.
45. Osnes LT, Westvik AB, Joo GB, Okkenhaug C, Kierulf P. Inhibition of IL-I induced tissue factor (TF) synthesis and procoagulant activity (PCA) in purified human monocytes byIL-4, IL- 10, and IL-13. Cytokine, 1996; 8: 822-827.
46. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nature Med, 2000; 6: 389-395.
47. Vairaktaris E, Yapijakis C, Serefoglou Z, Derka S, Vassiliou S, Nkenke E, et al,. The interleukin-8 (–251A/T) polymorphismis associated with increased risk for oral squamous cell carcinoma. Eur J Surg Oncol, 2007; 33: 504-507.
48. Yapijakis C, Serefoglou Z, Vylliotis A, Nkenke E, Derka S, Vassiliou S, et al,. Association of polymorphisms in Tumor Necrosis Factor Alpha and Beta genes with increased risk for oral cancer. Anticancer research, 2009; 29: 2379-2386.
49. Sundelin K, Roberg K, Grenman R, Hakansson L. Effects of cytokines on matrix metalloproteinase expression in oral squamous cell carcinoma *in vitro*. Acta Otolaryngol, 2005; 125: 765-773.

50. Kishimoto T, Akira S, Narazaki M, Taga T, Interleukin-6 family of cytokines and gp130. Blood, 1995; 86: 1243-1254.
51. Hefler LA, Grimm C, Lantzsch T, Lampe D, Leodolter S, Koelbl H. Interleukin-1 and Interleukin-6 Gene Polymorphisms and the Risk of Breast Cancer in Caucasian Women. Clin. Cancer Res, 2005; 11: 5718-5721.
52. National Cancer Institute, NIH, Surveillance, Epidemiology, and End Results Program, 2012.