RESEARCH ARTICLE

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Single Nucleotide Polymorphisms in Cytokine Genes are Associated with the Susceptibility to Oral Squamous Cell Carcinoma

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Abstract

Oral squamous cell carcinoma (OSCC) is the most common type of cancer among men in the Indian subcontinent. Cytokines regulate inflammation and angiogenesis in a variety of cancers. Genetic variability in the cytokine genes can potentially influence the predisposition to oral carcinogenesis. The aim of the current study was to investigate the associations of SNPs in cytokine genes with the susceptibility of oral squamous cell carcinoma. In the present study, we have analyzed the allelic frequency of 32 single nucleotide polymorphisms (SNPs) using MassArray-based iPLEX assay in 16 cytokine genes in 166 OSCC patients and 151 healthy subjects from central India. Out of 32 SNPs analyzed, five SNPs were significantly associated with the risk of OSCC. AA and GG genotypes of IL-1β +3953 were associated with an increased and decreased risk of OSCC, respectively. In several genetic models, GG genotype and G allele in IL-12A 3'UTR G>A were found to be associated with an increased risk of OSCC. Similarly, the GG genotype of IL-12B +1188 T>G was associated with increased susceptibility to OSCC. We conclude that SNPs in the genes coding for IL-1β, IL-12A and IL-12B are associated with increased genetic susceptibility to OSCC in the central Indian population.

Keywords: Interleukin-1β- Interleukin-12A- Interleukin-12B- SNPs- oral cancer- genetic susceptibility

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Introduction

Oral cancer, a subdivision of Head and Neck Cancers, includes cancers of the lips, tongue, buccal mucosa, gingiva, retromolar trigone, and hard palate (Ferlay et al., 2021). It is the most common type of cancer among men in the Indian subcontinent. According to GLOBOCAN 2020, more than 375,000 new cases of cancer of the lip, tongue, and mouth, and 177,000 deaths have been reported worldwide (Ferlay et al., 2021). South-Central Asia and parts of Oceania contribute to half of the total cases of cancer of the lip, tongue, and mouth; and Papua New Guinea, Pakistan, and India have the highest incidence (Miranda-Filho and Bray, 2020). More than 90% of oral cancers are oral squamous cell carcinoma (OSCC) in origin (Miranda-Filho and Bray, 2020). In India, more than 135, 000 new cases were reported, and over 75, 000 people died due to OSCC in 2020 (Ferlay et al., 2021; Miranda-Filho and Bray, 2020).

There is up to a 20-fold geographical variation in the incidence rates of oral cancer. In India and large parts of southern Asia, the incidence of oral cancer is highest, owing to the consumption of smokeless tobacco, with or without betel quid (Miranda-Filho and Bray, 2020). Compared to the global average, age-standardized rates (ASR) are more than twice (14.8 per 100,000) in Indian men (Miranda-Filho and Bray, 2020). In addition, genetic predisposition also plays an important role in the etiology of oral cancer (Liao et al., 2014). It is possible that inter-individual and inter-population differences in risk could be partially explained by different distributions of genetic variants, including single nucleotide polymorphisms (SNPs) (Sun et al., 2015). Consequently, SNPs may cause variation in the ability to metabolize carcinogens and/or effective repair of the damage caused by them and immune response to tumor cells (Sun et al., 2015). By using a candidate gene approach, a number of genetic association studies have

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investigated the putative correlation between functional DNA polymorphisms in cytokine genes and head and neck cancer (Serefoglou et al., 2008). In the event of OSCC, an individual's genetic susceptibility most likely comes from a combination of several unfavorable but rather common genetic polymorphisms (Dong et al., 2016). Genetic polymorphisms in biotransformation enzymes, DNA repair pathway, apoptotic pathway, human papillomavirus-related pathways, mitochondrial polymorphisms, and immune system pathways are associated with the risk of OSCC (Lacko et al., 2014; Lesseur et al., 2016).

Cytokines regulate and mediate inflammation and angiogenesis. New evidence suggests that up to 25% of all cancers are due to chronic infection or other types of chronic inflammation (Greten and Grivennikov, 2019). Over the past decade, many studies have suggested that leukocytes and their relevant cytokines may play a central role in inflammatory infiltration and malignant transformation (Coussens and Werb, 2002). Variability in the coding and non-coding sequences of cytokine genes can strongly affect immune system activity and its capacity to monitor and clear cancerous cells (Gholamalizadeh et al., 2021). Genetic variants in the immune system influence the oral carcinogenesis and metastasis potential of oral cancer (Erdei et al., 2013). SNPs in a few cytokines have been associated with the risk of oral carcinogenesis (Singh et al., 2015, 2016). In OSCC, elevated levels of TNF-α, IL-8 and IL-6 contribute to pro-angiogenic and pro-inflammation. Identification of polymorphisms in inflammatory genes may help understanding inter-individual differences in susceptibility to oral cancer. However, the role of SNPs in cytokine genes with the susceptibility to OSCC in Indian population has not been fully understood.

In the present article, we have analyzed the allelic frequency of 32 SNPs in 16 cytokine genes in OSCC patients and healthy subjects using the high throughput iPlex platform of Agena MassARRAY. We found that out of 32, three SNPs are significantly associated with the risk of OSCC. Among these, two SNPs in the coding regions of interleukin (IL) 1-β, IL-12B, and one SNP in IL-12 3'UTR were found to be significantly associated with the risk of oral cancer.

Materials and Methods

Study Setting and Subjects

This study was conducted in the Department of Biochemistry and Department of Otorhinolaryngology and Head & Neck Surgery, AIIMS Bhopal, and Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal between the time period from 2015-2019. Patients with oral cavity cancer were enrolled in the study after obtaining informed consent according to the selection criteria. Both newly diagnosed and follow-up OSCC patients (N=166) who had not undergone surgery, chemotherapy, or radiation therapy were included in the study. A disease summary questionnaire was administered to record the patient's demographic details, such as name, age, gender, smoking, and tobacco chewing habit. Tumor Node Metastasis (TNM) staging was recorded from their medical history file. Sex- and exposure-matched, apparently cancer-free healthy participants (N = 151) with or without the habit of tobacco/supari chewing (Gutkhas, Jarda, Supari, etc.) but no visible lesions in the oral cavity were enrolled as control subjects.

Ethical Approval

Ethical approval was obtained from Institutional Human Ethics Committee, AIIMS Bhopal, before any participants were enrolled in the study. All participants were requested to sign a written informed consent, and only eligible and consenting participants were included in the study.

Blood Collection and DNA Extraction

Venous blood (4 ml) was collected from cases and controls in EDTA-containing vacutainer vials. Samples were transported to the laboratory within 1 hour of collection. Genomic DNA was isolated by phenol: chloroform method (Barker, 1998). DNA was quantitated using a nano-spectrophotometer, and absorbance at 260/280 nm and 260/230 nm was recorded. DNA samples with the values of A260/280 in the range of 1.75-1.85 were used for genotyping.

SNP Genotyping

Based on a literature search, we identified 32 potential SNPs (Supplementary Table S1) in 16 cytokine genes that may be associated with the susceptibility of OSCC in Indian patients. The unique ID of each SNP was verified with dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/). SNP genotyping was done using a high throughput method using MassARRAY system (Agena Bioscience, San Diego, CA, USA). The MassARRAY System combined with iPLEX® chemistry enables analysis for single nucleotide variations/polymorphisms (SNPs), insertions and deletions (indels), and copy number variation (CNV). Genotyping was performed without knowledge of the case/control status of the subjects, and reproducibility was confirmed by repeat analysis of a randomly chosen subgroup of 5% of study participants.

Primers for single base extension and PCR were designed by using Assay Design Suite V2.0 online (Agena Bioscience, San Diego) (Supplementary Table S2). SNP genotyping was performed by using Agena MassARRAY system using matrix-assisted laser desorption ionization-time of flight mass spectrometry method (MALDI-TOF) according to the manufacturer's instructions. Briefly, each DNA sample was diluted to 20ng/µl, and 1 µl of DNA was mixed with 0.95 µl of DNase-free water, 0.625 µl of PCR buffer, 0.325 µl of 25mM MgCl2, 1 µl of PCR primers and 0.1 µl of 5 units/l HotStar Taq (Qiagen). The reaction was incubated in a thermal cycler at 94°C for 15 min followed by 45 cycles at 94°C for 20 s, 56°C for 30 s, and 72°C for 1min. After PCR amplification, the remaining dNTPs were dephosphorylated by adding 1.53 µl of water, 0.17mL of shrimp alkaline phosphatase (SAP) buffer, and 0.3 units of SAP (Agena Biosciences). Thereafter, the single primer extension (SPE) over the SNP was carried out by mixing 0.755 µl of water, 0.2 µl of iPLEX buffer, 0.2 µl

of termination mix, 0.041 µl of iPLEX enzyme (Agena), and 0.804 µl of 10 µM extension primer. The SPE reaction was performed at 94°C for 30 s, followed by 40 cycles at 94°C for 5 s, 5 cycles of 52°C for 5 s and 80°C for 5 s. The PCR product was desalted by adding 6 µg of cation exchange resin (Agena). The completed genotyping reactions were analyzed onto a 384 well spectroCHIP using a MassARRAY Nanodispenser (Agena) and mass was determined by the MALDI-TOF. Genotype calling was performed using the MassARRAY Typer software version 4.0 (Agena).

Sample size and statistical Analysis

Sample size was calculated by assuming 5% minor allele frequency, 5% type I error, and statistical power of 80%, case: control ratio (1:1), and odds ratio of 1.5; the minimum sample size comes to 159. Statistical analyses were performed using SPSS software (version 21; IBM, New York, USA). Categorical variables were presented as n (%) of subjects and analyzed using the χ^2-test. The Hardy–Weinberg equilibrium and between-group comparison of genotype distribution was analyzed using the χ^2-test. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were used to assess the effect of each SNP on OSCC risk. P value < 0.05 was considered statistically significant.

Results

Demographic Characteristics of the participants

This is a case-control study that includes 166 OSCC cases and 151 healthy controls belonging to the central region of India. The mean age for cases was 46.35 (23-74) years, and for control subjects, it was 38.1 (21-78) years. A total of 123 males and 43 females (cases) and 122 males and 29 females (controls) were recruited for the study. The subjects were well-matched for the tobacco chewing status (Table 1). Buccal mucosa was the most common site of the tumor followed by tongue (Table 1). Majority of OSCC patients (69%) presented in advanced stage (Stage III and IV) and most of the patients had well differentiated squamous cell carcinoma (Table 1).

Allele and genotype frequency distribution

The genotype and allele frequencies of 32 SNPs in 16 cytokine genes were analyzed in patients with OSCC, and cancer-free controls are summarized in Table 2. The genotype call rate for 28 SNP assays was more than 98%, and for three assays call rate was more than 96%. However, for one of the SNPs, TNF-α -863 C>A (rs1800630) call rate was less than 95%; hence it was excluded from further analysis. Allele distributions of the 30 tested SNPs were in Hardy–Weinberg equilibrium in the control subjects. However, one of the SNP, IL-18 -137 G>C (rs1872238), deviated from the Hardy–Weinberg equilibrium. Allelic frequency for most of the SNPs in cytokine genes tested in this study was in concordance with the aggregate reference frequency reported for the South Asian population in the ALFA project (Home - SNP - NCBI, n.d.) (https://www.ncbi.nlm.nih.gov/snp/).

AG genotype of IL-1β +3953 is associated with the susceptibility of OSCC

The GG genotype of IL-1β +3953 was the major allele in both control subjects (79.6%) and OSCC patients (68.75%) (Tables 2-3). A 13.6% decrease in the genotype frequency of GG was noted in OSCC cases, compared to controls. Furthermore, a statistically significant difference was found in the frequency distribution of GG, AA and GA genotypes, between cases and healthy controls (Heterozygous model, GA vs GG, OR=2.17, Confidence Interval (CI)-1.22-3.85, p = 0.005; Dominant model, GA +AA vs GG, OR=1.77, CI-1.05-2.98, p = 0.02; and Recessive model, GG vs AA+GA, OR=0.56, CI-0.33-0.95, p = 0.02). GA genotype was associated with the increased susceptibility to OSCC.

AA genotype of IL-12A 3'UTR G>A (rs568408) is protective against OSCC

A statistically significant difference was observed in the genotype frequency of IL-12 3'UTR (Table 2 and Table 4) in OSCC patients and control subjects. In several assessment models, A allele and AA genotype was significantly associated with reduced likelihood of

Table 1. Distribution of Selected Demographic Variables of OSCC Patients and Healthy Subjects

Factors	Healthy N = 151[1]	OSCC Patients N = 166[1]
Age (years)	38.1 (21-78)	46.35 (23-75)
Gender		
Female	19.2	25.8 (%)
Male	80.8	74.2 (%)
Lesion site		
Alveolus	NA	4.08 (%)
Buccal Mucosa	NA	57.14 (%)
Gingivobuccal sulcus	NA	4.08 (%)
Hard palate	NA	2.05 (%)
Tongue	NA	28.57 (%)
Lip		4.08 (%)
Histopathology		
Poorly differentiated	NA	23.2 (%)
Moderately differentiated	NA	16.5 (%)
Well differentiated	NA	60.3 (%)
TNM Staging		
Stage I	NA	3.84 (%)
Stage II	NA	26.92 (%)
Stage III	NA	11.53 (%)
Stage IV	NA	57.69 (%)
Somkeless Tobacco chewing		
No	19.8	22.36 (%)
Yes	80.2	77.63 (%)
Smoking		
No	31.2	36.8 (%)
Yes	68.8	63.20 (%)

[1], Mean (Standard deviation) or Frequency (%); *, OSCC-Oral Squamous cell carcinoma

Table 2. Genotype Distribution of SNPs

S. No.	Polymorphism	Genotype	Patients (Cases)		Controls	
			No.	%	No.	%
1	IL-1a +4845G>T (rs17561) C>T	CC	72	44.7	64	43.20
		TT	12	7.40	17	11.48
		CT	77	47.80	67	45.20
2	IL-1b -511C>T (rs16944) G>A	AA	57	35.18	46	31.94
		GG	28	17.28	21	14.58
		AG	77	47.53	77	53.40
3	IL-1b -31G>A (rs1143627)	GG	59	36.64	47	32.19
		GA	78	48.44	78	53.42
		AA	24	14.90	21	14.38
4	IL-1b +3953 (rs1143634)	AA	5	3.125	8	5.44
		GG	110	68.75	117	79.60
		AG	45	28.125	22	14.66
5	IL-2 -330T>G (rs2069762) A>C	CC	44	27.33	32	21.77
		AA	35	21.74	43	29.25
		CA	82	50.31	72	48.97
6	IL-2 +114T/G (rs2069763) C>A	CC	75	46.01	69	47.26
		AA	12	7.36	15	10.27
		CA	76	46.62	62	42.46
7	IL-4 -33C>T (rs2070874)	CC	121	73.78	98	65.77
		TT	8	4.87	7	4.69
		CT	35	21.34	44	29.53
8	IL-4 -590C>T (rs2243250)	CC	119	73.00	98	65.77
		TT	7	4.29	7	4.69
		CT	37	22.70	44	29.53
9	IL-6 -572G/C (rs1800796)	GG	66	40.50	54	36.00
		CC	27	16.56	30	20.00
		GC	70	42.94	66	44.00
10	IL-6 6331T>C (rs10499563)	CC	9	5.69	8	5.48
		TT	89	56.33	95	65.06
		CT	60	37.97	43	29.45
11	IL-6 -174G>C (rs1800795)	GG	121	75.15	122	82.43
		CC	0	0.00	3	2.02
		GC	40	24.84	23	15.54
12	IL-8 -251T>A (rs4073)	TT	59	36.87	57	39.58
		AA	24	15.00	25	17.36
		TA	77	48.12	62	42.46
13	IL-8 -781C>T (rs2227306)	CC	83	52.86	87	60.84
		TT	11	7.00	8	5.59
		CT	63	40.13	48	33.56
14	IL-10 -592A>C (rs1800872)	GG	41	25.30	32	21.62
		TT	38	23.45	28	18.92
		GT	83	51.23	88	59.45
15	IL-10 -1082A>G (rs1800896)	CC	14	8.70	8	5.36
		TT	92	57.14	91	61.07
		CT	55	34.16	50	33.55
16	IL-10 -819C>T (rs1800871) G>A	GG	39	23.92	30	19.86
		AA	39	23.92	30	19.86
		GA	85	50.30	91	60.26

Table 2. Continued

S. No.	Polymorphism	Genotype	Patients (Cases)		Controls	
			No.	%	No.	%
17	IL-12A 3'UTR G>A (rs568408)	GG	72	44.72	52	35.37
		AA	15	9.31	24	16.32
		GA	74	45.96	71	48.30
18	IL-12B +1188A>C (rs3212227) T>G	GG	27	17.08	13	9.80
		TT	50	31.64	46	34.58
		GT	81	51.26	74	55.64
19	IL-13 -1112C>T (rs1800925)	CC	98	60.49	86	61.00
		TT	10	6.17	3	2.12
		CT	54	33.33	52	36.36
20	IL-16 -295T>C rs4778889	TT	123	75.92	109	73.64
		CC	1	6.17	3	2.02
		TC	38	23.45	36	24.32
21	IL-16 3441T>G (rs11556218)	TT	147	89.63	128	87.07
		GG	0	0.00	1	0.68
		TG	17	10.36	18	12.24
22	IL-17A 197G>A (rs2275913)	AA	26	15.95	23	15.33
		GG	49	30.06	46	30.66
		GA	88	54.00	81	54.00
23	IL-17A 1249C>T (rs3748067)	CC	131	79.39	114	76.00
		TT	6	3.63	6	4.00
		CT	28	16.97	30	20.00
24	IL-17F 7383A>G (rs2397084) C>T	TT	141	85.97	127	85.81
		CC	0	0.00	1	0.675
		TC	23	14.02	20	13.51
25	IL-17F 7488A>G (rs763780) T>C	TT	139	84.24	127	85.23
		CC	0	0.00	1	0.671
		TC	26	15.75	21	14.09
26	IL-18 -607C>A (rs1946518) G>T	GG	85	53.46	75	50.33
		TT	19	11.95	16	10.74
		GT	55	34.59	58	38.92
27	IL-18 -137G>C rs187238	GG	17	10.69	18	12.2
		CC	49	30.81	35	23.8
		GC	93	58.49	94	63.94
28	IL-27 2905T>G	AA	115	70.55	102	68.91
	rs181206 A>G	GG	4	2.45	10	6.75
		AG	44	26.99	36	24.32
29	TNF-a -238 G/A rs361525	GG	146	90.68	133	89.26
		AA	1	0.621	1	0.671
		GA	14	8.69	15	10.06
30	TNF-a -308 G/A (rs1800629)	GG	143	89.02	126	84.56
		AA	3	1.83	3	2.01
		GA	18	10.97	20	13.42
31	TNF-a -863C>A (rs1800630)	CC	29	22.65	26	20.96
		AA	56	43.75	69	55.64
		CA	43	33.59	29	23.38
32	TNF-a -857C/T rs1799724	CC	118	71.08	108	72.48
		TT	11	6.62	4	2.68
		CT	37	22.29	37	24.83

Table 3. Association of IL-1B +3953 (rs1143634) with OSCC

Model Type	Genotypes	Corrected χ^2	Odds Ratio (95% CI) Cases vs Control	Fisher Exact P value
Dominant Model	(AA+GA) vs GG	4.12	1.77 (1.05-2.98)	0.02
Heterozygote Model	GA vs GG	6.50	2.17 (1.22-3.85)	0.005
Recessive Model	GG (AA+GA)	4.12	0.56 (0.33-0.95)	0.02

OSCC, Oral Squamous cell carcinoma; CI, Confidence of interval; χ^2, Chi -Square

Table 4. Association of IL-12A 3'UTR G>A (rs568408) with OSCC

Model Type	Genotypes	Corrected χ^2	Odds Ratio (95% CI) Cases vs Control	Fisher Exact P value
Dominant Model	(GG+GA) vs AA	2.8	1.89 (0.95-3.77)	0.04
Recessive Model	AA (GG+GA)	2.8	0.52 (0.26-1.04)	0.04
Homozygotes Model	AA vs GG	3.82	0.45 (0.21-0.94)	0.025
	GG vs AA		2.21 (1.06-4.63)	

OSCC (Recessive model, AA vs GG+GA, OR=0.52, CI -0.26-1.04 p = 0.04; and Homozygotes model, AA vs GG, OR=0.45, CI-0.21-0.94 p = 0.025). In contrast, G allele and GG genotype was associated with increased susceptibility to OSCC (Dominant model, GG+GA vs AA, OR=1.89, CI-0.95-3.77 p = 0.04; Allelic model, G vs A, OR=1.42, CI-1.02-1.98 p = 0.021; Homozygotes model, GG vs AA, OR=2.21, CI-1.06-4.63 p = 0.025).

GG genotype of IL-12B +1188 T>G (rs3212227) is associated with increased susceptibility to OSCC

Heterozygous TG genotype of IL-12B +1188 T>G (rs3212227) was the major genotype in both control subjects (55.64%) and OSCC patients (51.26%), and there was a 75% increase in the frequency of GG genotype in OSCC (17.08% vs 9.8%) (Table 2, Table 5). In recessive and homozygous, GG genotype was significantly associated with increased risk of susceptibility to OSCC (Recessive model, GG vs TT+TG, OR=1.90, CI-0.93-3.85 p = 0.05; Allelic model, G vs T, OR=1.91, CI-0.88-4.14 p = 0.07; Homozygotes model, GG vs AA, OR=2.21, CI-1.06-4.63 p = 0.025).

Discussion

Several genetic association studies have indicated that common DNA polymorphisms in low-penetrance genes may affect an individual's susceptibility to malignancy (Lacko et al., 2014). OSCC is a multifactorial disease, with the exception of rare familial cases. Interactions between exposure to carcinogens and a particular risk-bearing genotype cause this. In OSCC, an individual's genetic susceptibility most likely comes from a combination of several unfavorable but common genetic polymorphisms.

IL-1β is a key modulator and key pro-inflammatory cytokines of survival pathways and has been used in in vivo numerous times to demonstrate its role in tumor progression (Rébé and Ghiringhelli, 2020; Yuan et al., 2022). Evidence supports a pro-tumorigenic role for IL-1β polymorphism across all cancer types and is associated with poor prognosis (Rébé and Ghiringhelli, 2020; Wang et al., 2019). Increased serum levels of IL-1β were associated with various malignancies (Rébé and Ghiringhelli, 2020). Inflammation in oral cancer may be modulated by host genetic factors such as polymorphisms in inflammatory genes. Functional IL-1β +3953 C/T or G/A is associated with alteration in serum IL-1β levels (Pociot et al., 1992). In our study, a significant decrease in G allele was noted in the OSCC patients, indicating it as a protective allele. Consistently, a similar decrease in G allele has been shown in OSCC patients from Europe (Vairaktaris et al., 2007). In north Indian population, a second SNP -511 C/T in IL-1β suggested that the presence of T allele increases the risk of OSCC (Lakhanpal et al., 2014). Further functional characterization of IL-1β +3953 will give a clearer picture of how SNPs affect the predisposition of oral cancer.

IL-12 is a pro-inflammatory cytokine secreted by dendritic cells and activates phagocytic cells. IL-12 is a 70 kDa heterodimer consisting of two subunits, a heavy (p40) and a light (p35) chain subunit, encoded by two genes, IL-12B and IL-12A, respectively (Tugues et al., 2015). IL-12 can stimulate the growth and function of T-cells along with stimulating the production of interferon-gamma and TNF-α from T and NK cells. It is also known to induce anti-tumor activity (Tugues et al.,

Table 5. Association of IL-12B +1188 T>G (rs3212227) with OSCC

Model Type	Genotypes	Corrected χ2	Odds Ratio (95% CI) Cases vs Control	Fisher Exact 2 Tailed P value
Dominant Model	(TT+TG) vs GG	2.67	0.52 (0.26-1.06)	0.05
Recessive Model	GG (TT+TG)	2.6	1.90 (0.93-3.85)	0.05

2015). Mice deficient in IL-12A developed an increased number of papilloma and were more susceptible for developing lymphomas in mice (Meeran et al., 2006). Moreover, mice engineered to express IL-12 in B16 melanoma tumors were shown to regulate the tumor vasculature either by upregulating adhesion molecules that may facilitate leukocyte recruitment (Eisenring et al., 2010). Our study provided the first evidence suggesting the relationship between IL-12 gene polymorphism and the development of oral cancer. A significant marginal risk of oral cancer was observed for the GG genotype of IL-12β. Polymorphism in the 3′-UTR of IL-12A was associated with decreased IL-12 production, which corresponded with increased susceptibility to developing glioblastomas (Zhao et al., 2009). Various studies showed a significant association between of IL-12β SNP with breast cancer (Núñez-Marrero et al., 2020), colorectal cancer (Jelassi et al., 2022), and many other cancers. IL-12B rs3212227 polymorphism is crucial for the development of cancer because the A > C allele can decrease cytokine levels (do Carmo Vasconcelos de Carvalho et al., 2012). Consistent with a previous meta-analysis study on hepatocellular carcinoma (Kong et al., 2020), in our study, haplotype analysis of GG+GG in IL-12A and IL-12B yielded an elevated risk for oral cancer.

In conclusion, in the central Indian population, SNPs in IL-12β (+1188 A/C), IL-12A (3' UTR >A) and IL-1β (+3953 G/A)) are significantly associated with an increased risk of oral cancer. This relationship suggests that polymorphism of inflammatory response genes may be responsible for host-genetic susceptibility to oral cancer. IL-1β, IL-12A and IL-12B SNPs could be potential candidate genetic factors in future studies to elucidate the risk of oral cancer.

Author Contribution Statement

Ritu Pandey: Execution, Data collection, Interpretation or analysis of data, Writing of the manuscript; Rajeev Nema: Conception and Interpretation of data; Supriya Vishwakarma: Data Collection, Execution and Interpretation of data; Ajay Pal Singh: Data Collection, Execution; Sruthy Mohan: Data Collection; Priti Patel: Data Collection, Execution; Subhojit Halder: Data Collection, Execution; Anupam Halder: Data Collection, Execution; Renu Singh: Clinical Diagnosis and Enrollment of subjects; Rahul Agarwal: Clinical Diagnosis and Enrollment of subjects; Vikas Gupta: Clinical Diagnosis and Enrollment of subjects, revision of manuscript; Ashok Kumar: Conception, data interpretation, manuscript editing, Supervision. .

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