RESEARCH PAPER

Association between CCL2 gene polymorphism with susceptibility to Oral Squamous Cell Carcinoma among South Indian Populations

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Abstract:

Introduction

Numerous inflammation-mediated molecular pathways, including the cyclooxygenase (COX)-2, epidermal growth factor receptor (EGFR), p38a MAP kinase, TNF, NF-kB, STAT, RhoC, and PPARy pathways, have been investigated and evaluated as significant players in OSCC carcinogenesis, Such gene mutations can further cause cancer development near the pharynx and oral cavity and more than 90% of such cancer cases are OSCCs (Oral Squamous Cell Carcinoma). Till date, there is minimal study that has analyzed allele, genotype, or haplotype frequencies of CCL-2 polymorphisms in patients with OSCC.

Objective

We hypothesized that CCL-2 polymorphisms might modify the relative risk for the development of OSCC. On the basis of these findings, we evaluated SNP in CCL-2 gene in groups of non-OSCC controls and OSCC participants.

Materials and Methods

This study employed a cross-sectional design involving individuals from Chennai, Tamil Nadu, India. A total of 50 individuals who reported to Saveetha Dental College, Chennai, were included in this study. The subjects were divided into a control group A (N = 25) and OSCC group B (N = 25) based on clinical examination. Sample collection and DNA extraction, A volume of 5 mL of venous blood was collected and DNA isolation was performed according to the modified Miller et al 1998 protocol. CCL2 gene (OSCC) polymorphisms were assessed by PCR amplification and restriction digestion.statistical analysis was performed using the Statistical Package for the Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, IL). The distribution of genotypes and allele frequencies in the CP and control groups was compared using the Chi- square test.

Results

Genotype frequencies of *CCL2* gene polymorphism *(rs1024611)* among the cases and controls, The genotype frequency of cases and controls do not differ significantly χ2df (**P =** 0.8353). Agarose gel electrophoretogram showed partial amplification of *CCL2* gene spanning polymorphic site *(rs1024611)* run along with standard DNA ladder. Agarose gel electrophoretogram showed *PvuII* digested amplicon of *CCL2* spanning *rs1024611* site (Homozygous TT - 333 bp; Heterozygous CT - 333 + 250 + 73 bp; Homozygous CC - 250 + 73 bp).

Conclusion: It has been concluded that there is no significant association between CCL2 gene polymorphism with susceptibility to OSCC among South Indian Populations.

Keywords: Alleles, Genetic polymorphism, South Indian Population, OSC, *CCL2* gene polymorphism *(rs1024611)*

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INTRODUCTION:

Head and neck cancer is one of the most prevalent types of cancer (1).Its prevalence varies around the world; in less developed nations like India, it is the disease most frequently diagnosed in male patients, whereas in the West, it accounts for just 1% to 4% of all cancer cases (2). In 2018, the total incidence of lip, oral cavity, and oropharynx cancer was estimated at 4,47,751 new cases

and 2,28,389 fatalities, accounting for 2.4% of all cancer deaths (3). The Asian continent has the highest incidence and mortality rates of oral cavity and oropharynx cancers among all other countries (4). Head and neck cancer is fourteenth in terms of incidence but thirteenth in terms of mortality.Several factors including usage of tobacco products and poor prosteses have been attributed to oral squamous cell carcinoma.(5–7)

Although improvements in preoperative care and surgical technique have increased survival rates to some extent, the overall 5-year survival rate for OSCC patients has remained at 50% over the past three decades (8). Cervical node and distant metastases are the main factors in OSCC-related fatalities (9).

The complicated process of OSCC metastasis includes cell separation from tumour tissue, cell motility control, invasion, proliferation, and evasion through the lymphatic or blood capillaries . Patients with metastatic OSCC disease currently have few treatment options. Through bacterial infection, exposure to allergens and toxic chemicals, obesity, and autoimmune illness, the inflammatory response is triggered (10,11). Cancer-related inflammation is regarded as the seventh cancer characteristic (12). White blood cell infiltration, the predominance of tumor-associated macrophages, the presence of polypeptide messengers of inflammatory cytokines like tumour necrosis factor (TNF), interleukin (IL)-1, and IL-6, chemokines like CCL2, and the occurrence of tissue remodelling and angiogenesis are the main features of cancer-related inflammation (13).

A growing body of research has demonstrated that inflammation associated with cancer affects hormone and chemotherapeutic drug responses, angiogenesis, metastasis, modification of adaptive immune responses, and malignant cell proliferation and survival (13,14). Numerous inflammation-mediated molecular pathways, including the cyclooxygenase (COX)-2, epidermal growth factor receptor (EGFR), p38a MAP kinase, TNF, NF-kB, STAT, RhoC, and PPARy pathways, have been investigated and evaluated as significant players in OSCC carcinogenesis (15). Cancer-related inflammation is a therapeutic target, just like other cancers.

Such gene mutations can further cause cancer development near the pharynx and oral cavity and more than 90% of such cancer cases are OSCCs (Oral Squamous Cell Carcinoma). OSCC occurs when a group of neoplasms affects any region of the oral cavity, pharyngeal regions or salivary glands. It is present in high numbers, particularly in South Asia, in unindustrialised countries like India. However, no specific gene has been identified to have been causing OSCC. (16)

Till date, there is minimal study that has analyzed allele, genotype, or haplotype frequencies of CCL-2 polymorphisms in patients with OSCC.

We hypothesized that CCL-2 polymorphisms might modify the relative risk for the development of OSCC. On the basis of these findings, we evaluated SNP in CCL-2 gene in groups of non-OSCC controls and OSCC participants.

MATERIALS AND METHODS

This study employed a cross-sectional design involving individuals from Chennai, Tamil Nadu, India. A total of 50 individuals who reported to Saveetha Dental College, Chennai, were included in this study. The subjects were divided into a control group A (N = 25) and OSCC group B (N = 25) based on clinical examination. The OSCC group contained 26 patients (17 male, 8 female) between 40 to 60 years. The control group contained 25 healthy subjects (17 male, 8 female) between 40 to 60 years.

A detailed history of dental treatment, family history of OSCC, smoking habits as well as general health concerns were obtained from the subjects. Except for the presence of OSCC, the patients included in this study were systemically healthy. Pregnant or lactating mothers, immunocompromised individuals and subjects who had undergone surgery within the past 6 months were excluded from this study. The study was approved by the institutional ethics committee.

Sample collection and DNA extraction

A volume of 5 mL of venous blood was collected from the antecubital fossa and dispersed in a sterile tube containing a pinch of ethylenediaminetetraacetic acid. It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified Miller et al 1998 protocol.

Polymerase Chain Reaction and Restriction Endonuclease Digestion

CCL2 gene (OSCC) polymorphisms were assessed by PCR amplification and restriction digestion. The following primers, forward primer: 5’-CTGTTGAAGGCCTAGAGCTGCTGCTCC-3’ and

reverse primer: 5'-GATCTTCTCTTCAAACTCT ACCC-3', were used for amplification of DNA spanning the SfcI polymorphic site, of the MMP8 gene. The amplification of DNA was performed in 20µl volumes using 10ng of genomic DNA and 5 pmol/µl each of forward and reverse primers along with PCR master mix (Takara, Japan). The cycling conditions were as follows: Initial denaturation at 94oC for 5 min, denaturation at 94oC for 35 s, annealing at 60oC for 35 s, extension at 72oC for 35 s, and a final extension at 72oC for 5 min. 5 µl of PCR product was checked on a 1% agarose gel. 15 µl of PCR product was digested using SfcI restriction enzyme procured from New England Biolabs, England. Digestion was carried out at 37oC for 2 h. The digested product was visualized on a 2% agarose gel, and the results were documented.

Statistical Analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, IL). The distribution of genotypes and allele frequencies in the CP and control groups was compared using the Chi- square test. The risk associated with individual alleles or genotypes was calculated as the odds ratio (OR) with 95% confidence intervals. Statistical significance in all tests was determined at $P < 0.05$.

Results:

Table 1: Genotype frequencies of *CCL2* gene polymorphism *(rs1024611)* among the cases and controls

Groups	**AA**	**AG**	**GG**	**A**	**G**	**HWE (p value)***
Case (N=25)	11	8	6	0.6	0.4	0.0955
Control (N=25)	10	10	5	0.6	0.4	0.4046

*For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly χ2df (**P =** 0.8353).

Table 2: Overall genotype distribution of the *CCL2* gene polymorphism *(rs1024611)* in cases and controls

Dominant				
Genotypes	**Case**	**Control**	**Unadjusted OR [95% CI]**	**P value**
AA	11	10	1.1786 [0.3829 - 3.6275]	0.7745
AG + GG	14	15		
Recessive				
AG + AA	19	20	0.7917 [0.2067 - 3.0316]	0.7331
GG	6	5		
Allele				
A	30	30	1.0000 [0.4493 - 2.2259]	1.0000
G	20	20		

Figure 1: Agarose gel electrophoretogram showing partial amplification of *CCL2* gene spanning polymorphic site *(rs1024611)* run along with standard DNA ladder [Lane M = 100 bp DNA marker]

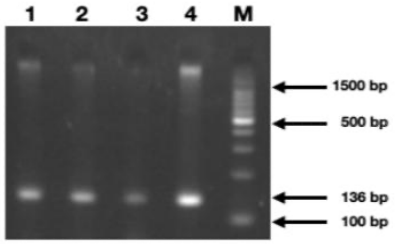
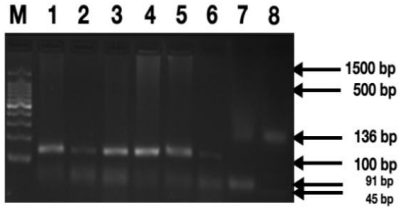
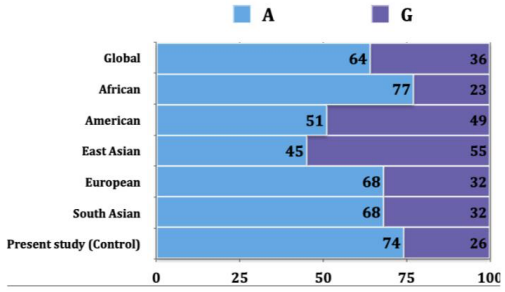


Figure 2: Agarose gel electrophoretogram showing *PvuII* digested amplicon of *CCL2* spanning *rs1024611* site (Homozygous TT - 333 bp; Heterozygous CT - 333 + 250 + 73 bp; Homozygous CC - 250 + 73 bp) [Lane M = 100 bp DNA marker]



Graph 1: The graph depicts the allele frequency of *CCL2* gene polymrophism *(rs1024611)* in different population [Data acquired from Ensembl database]



PCR information:

Primer sequence:

Forward: 5'-GGGAGGGCATCTTTTCTTGA-3'

Reverse: 5'-AAAGTGACTTGGCCTTTGCAT-3'

Amplicon size: 136 bp, **Annealing temperature:** 58 degree C for 30 seconds

Discussion

Genetic polymorphisms, like SNPs, may influence disease in multiple complex ways acting with other genetic variants and environmental factors to influence disease susceptibility and progression. Many studies have revealed that SNPs may be as degree celsius iated with susceptibility to OSCC (17)

Our study results showed that the genotype frequency of CCL-2 polymorphism did not differ significantly. The prevalence of homozygous and heterozygous mutant genotypes had no significant difference between periodontitis and healthy control groups. The detected frequency of AG and AA genotypes had no significant difference between periodontitis group and healthy controls. There was no significant difference in A allele and G allele between periodontitis and the healthy control group.

In cancer metastasis, tumor cells express chemokine receptors, and the metastatic sites secrete specific chemokines to attract the tumor cells (18)(19). Among the various chemokines associated with cancer progression, CCL2/CCR2 signaling may be considered a key player in promoting tumorigenesis and metastasis (20,21). During cancer metastasis, CCL2 facilitates intravascular invasion of tumor cells into circulation, possibly by recruiting myeloid host cells to facilitate this process. Furthermore, tumor cell entrapment in small capillaries may initiate CCR2+ myeloid and CCR2+ endothelium-associated tumor cell extravasation. CCL2 promotes tumor growth and colonization at metastatic sites by recruiting additional myeloid and endothelial cells [39]

In a previous study done by (22) it stated that the CCL2-2518A/G (rs1024611) polymorphism is significantly associated with risk of gynecological cancer, and the association differs by ethnicity. And in another study done by (23) it stated that CCL2 gene polymorphisms (rs1024611 and rs4586) confer susceptibility to OA and may be potential markers for early diagnosis of OA. But in our present study CCL2 did not show any association with OSCC.

Since the present study was subjected to one particular ethnicity, the future studies need to evolve such that multicenter studies are conducted to have better understanding of CCL-2 gene polymorphism among various populations. The strength of the present study is that all the confounding factors such as smoking and systemic disorders were excluded during the recruitment of the sample. Further studies are required to explore the interaction of genes with microbial and environmental factors in the etiopathogenesis of OSCC.

Conclusion:

From the study, it has been concluded that there is no significant association between CCL2 gene polymorphism with susceptibility to OSCC among South Indian Populations. This study can be experimented in various types of oral malignancy with increased sample size in different geographical locations.

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