

# The Role of Interleukin-1 and Tumor Necrosis Factor in the Progression of Oral Squamous Cell Carcinoma: A Biomarker Analysis

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## ABSTRACT

**Background:** Oral squamous cell carcinoma (OSCC), a prevalent malignancy in the head and neck region, is significantly influenced by its tumour microenvironment. Cytokines such as Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) play critical roles in regulating inflammatory responses, impacting tumour progression and patient outcomes. **Objective:** This study aimed to evaluate the levels of TNF and IL-1 in saliva and their association with OSCC progression, tumour differentiation, and TNM staging, using salivary biomarkers to provide insights into disease monitoring. **Methods:** A case-control study was conducted involving 40 OSCC patients and 40 healthy controls. Salivary cytokine levels were quantified using enzyme-linked immunosorbent assay (ELISA). The relationship between cytokine levels, tumour differentiation, and TNM staging was statistically analyzed. **Results:** TNF and IL-1 levels were significantly elevated in OSCC patients compared to controls ( $p < 0.05$ ). Higher IL-1 levels were observed in poorly differentiated tumours, while both TNF and IL-1 showed increased levels in advanced TNM stages, correlating with a more robust inflammatory response as the disease progressed. **Conclusion:** Salivary levels of TNF and IL-1 are closely associated with OSCC progression, tumour differentiation, and staging, highlighting their potential as non-invasive biomarkers for monitoring disease progression and response to therapy. These findings support the utility of cytokine profiling in saliva for improving diagnostic accuracy, developing targeted therapies, and enhancing patient management strategies in OSCC.

**Keywords:** Oral Squamous Cell Carcinomas, TNF, IL-1.

## INTRODUCTION

Oral squamous cell carcinomas (OSCC) are the most common mucosal malignancies in the head and neck region [1]. Projections indicate an anticipated increase in OSCC cases, reaching 856,000 by 2035, aligning with projected global demographic trends [2]. It is a tumour with squamous characteristics originating from the mucosal epithelium of the oral cavity [3], constituting 75–90% of malignancies in this anatomical area [4]. Its impact on morbidity and mortality is substantial due to the critical role of oral tissues in functions like swallowing, chewing, speaking, and facial aesthetics [5]. In the tumour microenvironment, inflammatory and stromal cells are integral components that impact the initiation, growth, survival, invasion, and metastasis of OSCC [6]. Cytokines within this microenvironment play a crucial role in cancer progression, influencing processes like invasion and metastasis [7]. Particularly, inflammatory cytokines, notably those released by tumour cells, play a vital role in shaping the tumour microenvironment [8]. Patients with oral cancer exhibit elevated levels of pro-inflammatory mediators such as IL-6, IL-8, and TNF- $\alpha$ . TNF- $\alpha$  in the tumor microenvironment promotes invasion by boosting pro-inflammatory and pro-invasive characteristics in OSCC cells and facilitating recruitment and activation of inflammatory cells via paracrine pathways [9]. However, the extensive and diverse role of TNF- $\alpha$  in various inflammatory conditions poses a potential complicating factor, especially given its increased levels in prevalent oral disease states [1]. The presence and levels of Interleukin-1 (IL-1) in the saliva of individuals with OSCC are investigated to understand its potential as a biomarker or indicator of inflammatory processes associated with oral cancer. Monitoring IL-1 in saliva may offer insights into the local inflammatory environment

and its connection to OSCC. This study examined IL-1 and TNF levels in the saliva of individuals with OSCC across various tumour sites, stages, and histopathological grades (Table 1-5).

## MATERIALS AND METHOD

A case-control study completed from the first of December (2022) till the first of March (2023). Forty patients (27 female, and 17 male) and different age ranges (20–31 years) with oral squamous cell carcinoma and without any systemic diseases were who reserved from the specialised dental center in the Ba'aquba Diyala governorate, then classified according to the site of oral squamous cell carcinoma, histopathological grade, and TNM stage for the patients' group. Control group included forty subjects without oral squamous cell carcinoma. The University of Diyala granted ethical approval; the study protocol accepted by the ethics committee, and informed consent obtained by each participant. A single examiner examined each patient in a uniform setting, utilising a chair light and mirror to examine the oral cavity. The medical history, site of oral squamous cell carcinoma, histopathological grade, and TNM stage of the patients noted. Not agitated Using a ten-minute timer, saliva samples will be taken by spitting into a test tube in accordance with 17 prior to clinical evaluation between 9 and 11 Am. All patients instructed not to smoke or engage in strenuous physical activity following the saliva collection, to have no medication, to be free of acute or chronic illnesses, and to consume only water one hour prior to collection. The saliva samples centrifuged at 2000–3000 rpm for about twenty minutes. Following this, the samples were all stored at roughly 18–20°C until the time of the enzyme-linked immune sorbent assay (ELISA) for the

measurement of IL-1 and TNF. A statistical tool for social science used for data description, analysis, and presentation (SPSS version 22, Chicago, Illinois, USA). The quantitative variable mean measured using standard error (SE). For the qualitative variable, frequency %

utilised. When comparing two groups, the independent sample T-test utilised, and when comparing more than two groups, an ANOVA was employed. Not the significant  $P > 0.05$ , Significant  $P < 0.05$  are the levels of significance.

## Results

**Table 1:** Characterisation of Study Subjects and Clinical Features of OSCC

| Groups                               | Total Number | Female     | Male     | Age Range     |
|--------------------------------------|--------------|------------|----------|---------------|
| Control                              | 40           | 17 (43%)   | 23 (57%) | (24-76) Years |
| Patients                             | 40           | 23 (57%)   | 17 (43%) | (33-90) Years |
| Site of Oral Squamous Cell Carcinoma | Total Number | Percentage |          | Age Range     |
| Lip                                  | 10           | 25 %       |          | (46-87) Years |
| Border of Tongue                     | 14           | 35 %       |          | (33-90) Years |
| Hard Palate                          | 1            | 2.5 %      |          | (49) Years    |
| Soft Palate                          | 3            | 7.5 %      |          | (38-56) Years |
| Tip of Tongue                        | 3            | 7.5 %      |          | (55-76) Years |
| Floor of the Mouth                   | 1            | 2.5 %      |          | (83) Years    |
| Retromolar Area                      | 1            | 2.5 %      |          | (77) Years    |
| Buccal Mucosa                        | 7            | 17 %       |          | (39-80) Years |
| Histopathological Grade              | Total Number | Percentage |          | Age Range     |
| Well Differentiated                  | 14           | 35 %       |          | (33-90) Years |
| Moderately Differentiated            | 20           | 50 %       |          | (39-87) Years |
| Poorly Differentiated                | 6            | 15 %       |          | (38-83) Years |
| TNM Stage                            | Total Number | Percentage |          | Age Range     |
| Stage I                              | 24           | 60 %       |          | (33-90) Years |
| Stage II                             | 13           | 32.5 %     |          | (38-83) Years |
| Stage III                            | 3            | 7.5 %      |          | (63-80) Years |

*Symbols and Abbreviations: N/A*

*Note: This table provides a comprehensive overview of the total number of study subjects, distinguishing between control and patient groups, along with detailing the general characteristics (age and sex), the site of oral squamous cell carcinoma, histopathological grade, and TNM stage for the patient group. Percentages represent the proportion of individuals within each category relative to the total number of patients.*

**Table 2:** Changes in IL-1 and TNF Levels Between Control and Patients' Groups

| Parameters   | Mean $\pm$ Standard Error |                    | T-Test  |
|--------------|---------------------------|--------------------|---------|
|              | Control                   | Patients           | P-Value |
| IL-1 (pg/ml) | 375.72 $\pm$ 16.59        | 575.12 $\pm$ 26.42 | 0.000   |
| TNF (pg/ml)  | 26.18 $\pm$ 1.50          | 54.71 $\pm$ 2.37   | 0.000   |

\*Significant at  $P < 0.05$ .

*Symbols and abbreviations: IL: Interleukin, TNF: Tumour Necrosis Factor, OSCC: Oral Squamous Cell Carcinoma.*

*Note: The table shows significant increases in IL-1 and TNF levels in the patients' group compared to the control group, indicating a strong association with OSCC. Statistical significance is marked at  $P < 0.05$ .*

**Table 3:** Changes in IL-1 and TNF Levels Among Histopathological Grades of OSCC

| Parameters   | Mean $\pm$ Standard Error |                           |                       | ANOVA P-Value     |               |                     |
|--------------|---------------------------|---------------------------|-----------------------|-------------------|---------------|---------------------|
|              | Well Differentiated       | Moderately Differentiated | Poorly Differentiated | Well & Moderately | Well & poorly | Moderately & poorly |
| IL-1 (Pg/Ml) | 534.71 $\pm$ 31.84        | 534.84 $\pm$ 38.42        | 765.28 $\pm$ 47.14    | 0.998             | 0.002         | 0.001               |
| TNF (Pg/Ml)  | 52.50 $\pm$ 3.62          | 53.11 $\pm$ 3.68          | 63.50 $\pm$ 5.10      | 0.908             | 0.117         | 0.121               |

\*Significant At  $P < 0.05$ .

*Symbols and abbreviations: IL: Interleukin, TNF: Tumour Necrosis Factor, OSCC: Oral Squamous Cell Carcinoma.*

*Note: This table demonstrates the changes in IL-1 and TNF levels among OSCC patients classified by histopathological grade. IL-1 levels were significantly higher in patients with poorly differentiated tumours compared to those with well and moderately differentiated tumours. No significant difference in TNF levels was observed across the groups.*

**Table 4:** Changes in IL-1 and TNF Levels Among TNM Stages of OSCC

| Parameters   | Mean $\pm$ Standard error |                     |                    | ANOVA P-value |             |              |
|--------------|---------------------------|---------------------|--------------------|---------------|-------------|--------------|
|              | Stage I                   | Stage II            | Stage III          | Stage I&II    | Stage I&III | Stage II&III |
| IL-1 (pg/ml) | 486.08 $\pm$ 34.19        | 602.00 $\pm$ 137.88 | 614.64 $\pm$ 33.78 | 0.028         | 0.034       | 0.271        |
| TNF (pg/ml)  | 48.59 $\pm$ 2.63          | 63.87 $\pm$ 55.50   | 69.08 $\pm$ 4.88   | 0.002         | 0.014       | 0.538        |

\*Significant at  $P < 0.05$ .

*Symbols and abbreviations: IL: Interleukin, TNF: Tumour Necrosis Factor.*

*Note: This table outlines significant increases in IL-1 and TNF levels in patients with TNM II and III stages compared to the I stage, indicating a correlation with the progression of OSCC. No significant difference in IL-1 and TNF levels was observed between stages II and III.*

**Table 5:** Changes in IL-1 and TNF Levels Among Sites of OSCC

| Site of OSCC                             | IL-1               | TNF              |
|------------------------------------------|--------------------|------------------|
|                                          | Mean $\pm$ SE      | Mean $\pm$ SE    |
| Lip                                      | 553.90 $\pm$ 48.50 | 52.82 $\pm$ 4.70 |
| Border Of Tongue                         | 576.64 $\pm$ 54.61 | 56.48 $\pm$ 4.26 |
| Soft Palate                              | 558.33 $\pm$ 67.97 | 63.66 $\pm$ 8.69 |
| Tip Of Tongue                            | 724.00 $\pm$ 32.88 | 62.76 $\pm$ 8.78 |
| Buccal Mucosa                            | 582.42 $\pm$ 61.55 | 48.13 $\pm$ 4.95 |
| ANOVA P-Value                            | 0.669              | 0.477            |
| *Significant at P < 0.05.                |                    |                  |
| ANOVA P-value: IL-1 = 0.669, TNF = 0.477 |                    |                  |

Symbols and abbreviations: IL: Interleukin, TNF: Tumour Necrosis Factor, OSCC: Oral Squamous Cell Carcinoma.

Note: No significant differences in IL-1 and TNF levels were found among the five groups representing different sites of OSCC, indicating consistent inflammatory response levels across varied tumour locations.

## Discussion

Examining of TNF and IL-1 in OSCC involves studying the roles and levels of these inflammatory mediators in the situation of oral cancer. Elevated TNF and IL-1 concentrations associated with OSCC, and their investigation helps understand their potential contributions to the pathogenesis, progression and microenvironment of OSCC. we noted a rise in IL-1 and TNF levels in the patient group compared to the control group. Within the patient group, IL-1 levels rose significantly in those with poorly differentiated tumours compared to well, moderately differentiated ones. When considering TNM stages, IL-1 and TNF levels were obviously higher in patients with TNM II and III stages compared to stage I. However, no significant differences in IL-1 and TNF levels noted among the five groups categorised by the site of OSCC, including the lip. Immunosuppression in cancer patients is a well-recognised phenomenon, involving cytokines alterations and the immune cell balance [10]. Goertzen et al. [11] discovered a notable elevation in pro-inflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and TNF $\alpha$  in saliva samples of OSCC patients. The above elevation aligns with previous findings by Jou et al., linking similar salivary biomarkers to the OSCC tumour stage and illustrating their diagnostic potential for human oral cancers [12]. A study in Taiwan replicated these findings for IL1 $\beta$  and IL8 [13]. Additionally, investigations within Indian study cohorts identified salivary IL1 $\beta$ , IL6, and IL8 as noteworthy biomarkers for OSCC and OPMD [14-17]. Meanwhile, studies in the USA highlighted salivary IL6 and IL8 as significant biomarkers for tongue OSCC [18] and for monitoring the progression of oral lichen planus [19]. Elevated levels of IL-1 and TNF in saliva in oral squamous cell carcinoma patients, indicating their potential significance as biomarkers or indicators of inflammatory processes within the oral cavity.

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## Conclusion

The role of IL-1 and TNF in patients with oral squamous cell carcinoma (OSCC) is complex. Elevated levels of these cytokines associated with inflammation and tumour progression. However, further research is needed to fully understand their specific contributions and potential as therapeutic targets in managing OSCC.

## Data Availability Statement

Access to the datasets will be granted for academically rigorous, peer-reviewed scientific research, subject to approval by the principal investigator. Access request directed to Dr. Mustafa Ghani Taher, with a response timeframe of 30-35 days. All data usage will be subject to a data use agreement that specifies conditions of use and restrictions, including no further sharing of the data and strictly using it for the agreed purposes. The datasets generated during and analysed for the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author upon reasonable request. The shared data will include de-identified participant information, saliva analysis results, and clinical evaluation outcomes.

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