# Salivary concentration of TNFα, IL1α, IL6, and IL8 in oral squamous cell carcinoma

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## Abstract

Objectives: The aim of this study was to compare the concentration of tumor necrosis factor α, interleukin 1α, 6, and 8 in the saliva of oral squamous cell carcinoma patients with control group.

Study design: In this study 18 subjects were involved, nine patients with oral squamous cell carcinomas and nine age-sex-matched healthy individuals that were matched for gingival conditions too. Active dental abcesses, collagen vascular diseases, and infectious diseases during one month before saliva sampling were considered as exclusion criteria. Unstimulated whole saliva was collected and after processing the samples were analyzed by Enzyme Linked Immune Assay.

Results: The concentration of salivary interleukin 6 in oral squamous cell carcinoma patients was higher than control group and it was statistically significant ($p < 0.05$). The concentration of salivary tumor necrosis factor α, interleukin 1α and 8 in case group was higher than control group but it was not statistically significant ($p > 0.05$).

Conclusions: These results shows that more studies are needed to accept the utility of these cytokines in predicting or diagnosis of oral squamous cell carcinoma or evaluation of treatment.

***Key words:*** *Oral squamous cell carcinoma, saliva, TNF-α, IL-1α, IL-6, IL-8.*

## Introduction

Cancer of the mouth and pharynx is the sixth most common cancer worldwide. Over 90% of these oral–pharyngeal cancers are squamous cell carcinomas (SCC) (1). Despite advances in surgery, radiation, and chemotherapy, the five-year survival rate for oral cancer has not improved significantly over the past several decades and it remains at about 50 to 55 percent (1-3). The absence of definite early warning signs for most head and neck cancers suggests that sensitive and specific biomarkers are likely to be important in screening high-risk patients and target tumors with a propensity for metastatic spread. The identification of molecular markers in body fluids that would predict the development of cancer in its earlier stage or in precancerous stage would constitute such a tool (4). A number of molecular markers have been used to detect these tumors with varying degrees of specificity and sensitivity. To date, no reliable or clinically applicable marker has been shown to universally identify oral cavity and oropharyngeal squamous cell carcinoma or tumor aggressiveness (5, 6).

Previous studies of in vitro human cell lines as well as OSCC tumors have demonstrated that concentration of certain proinflammatory, proangiogenic cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, and IL-8 are increased. There is evidence that the-

se cytokines are produced in a dysregulated fashion in oropharyngeal SCC and that they have roles in cell growth, invasion, interruption of tumor suppression, immune status and even survival (1).

The aim of this study was to compare the concentration of TNF-α, IL-1α, IL-6, and IL-8 in the saliva of oral squamous cell carcinoma patients with control group.

## Material and Methods

Patients with oral squamous cell carcinoma referred to oral disease clinic, faculty of dentistry of Tehran University of Medical Science and Meraj institute enrolled in the study. For each case an age-sex-matched healthy individual, that was matched for gingival conditions too, was selected from individuals visited oral disease clinic as control.

The trial was performed in accordance with the Declaration of Helsinki and subsequent revisions (7) and approved by ethics committee at Tehran University of Medical Science. The details of the study were explained to all participants and written informed consents were obtained before entering into the study.

Complete histories were obtained. Participants with infectious diseases during one month before saliva sampling, active dental abcesses, and collagen vascular diseases were excluded from the study. None of the lesions had been treated in any manner prior to sample collection. None of the control participants had oral lesions.

Modified Gingival Index (MGI) was used to assess gingival condition of participants (Table-1). MGI eliminates the use of probing or pressure to establish the presence or absence of bleeding. It is also makes it possible to detect and record earlier, more subtle visual changes in gingival inflammation, permits the intra- and intercalibration of examiners, and is noninvasive upon repeated evaluations (8).

**Table 1.** The modified gingival index.

| Score | Definition |
|---|---|
| 0 | Absence of inflammation |
| 1 | Mild inflammation; slight change in color, little change in texture of any portion of but not the entire marginal or papillary gingival unit |
| 2 | Mild inflammation; criteria as above but involving the entire marginal or papillary gingival unit |
| 3 | Moderate inflammation; glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit |
| 4 | Severe inflammation; marked redness, edema and/or hypertrophy of the marginal or papillary gingival unit, spontaneous bleeding, congestion, or ulceration |

Participants were refrained from eating, drinking, using chewing gum, mint, etc for at least one hour before evaluation. Unstimulated whole saliva (UWS) was collected between 9:00 and 11:00 a.m. using standard technique (9). Samples were obtained by requesting subjects to swallow first, tilt their head forward, and expectorate all saliva into a 50 ml centrifuge tubes for 5 min without swallowing. The saliva samples were frozen at_70 °C, until analysis.

A standard tissue biopsy was taken from each patient. The diagnosis was confirmed by two pathologists independently.

All samples were centrifuged at 4500 _ g for 20 min. Supernatants were drawn off and used in the ELISA cytokine assays. TNF-alpha, IL-1-alpha, IL-6, and IL-8 concentrations were determined by the quantitative sandwich ELISA technique as previously described by Ondrey (10) using commercially available kits (Bender MedSystems, Vienna, Austria) according to manufacturer's recommendation.

## Statistical analysis

Spss (Version 13, spss INC) software was used to analyze the data. Results were expressed as mean ± standard deviation. One-Sample Kolmogorov-Smirnov Test was used for determining the distribution of data. Mann-Whitney U was used for data analysis. $P<0.05$ was considered to be statistically significant.

## Results

Nine patients with oral squamous cell carcinoma were enrolled in this study. The demographic data of participants was shown in table-2. The mean age of case group was 71.33 years slightly higher than control group (67.33 years).

The concentration of salivary TNF-α, IL-1α, IL-6, and IL-8 in case and control group was measured by ELISA tests. The mean and standard deviation of concentrations of these cytokines in case and control group was analyzed.

ELISA analysis of whole unstimulated saliva in this case control study showed that the elevation of IL-6 in oral squamous cell carcinoma patients was statically significant ($p<0.05$). The concentration of salivary TNF-α, IL-1α and IL-8 of oral squamous cell carcinoma patients was higher than control group but this difference was not statistically significant:

(1) TNF-α: (mean ± Std: TNF-αOSCC = 35.2 ± 51.8 pg/ml versus controls = 4.1 ± 2.1 pg/ml; $p > 0.05$)

(2) IL-1α: (mean ± Std: IL-1α-OSCC = 201.7 ± 178.8 pg/ml versus controls = 178.2 ± 170.7 pg/ml; $p > 0.05$)

(3) IL-6: (mean ± Std: IL-6-OSCC = 40.9 ± 79.5 pg/ml versus controls = 2.5 ± 1.3 pg/ml; $p < 0.05$)

(4) IL-8: (mean ± Std: IL-8-OSCC = 1093.7 ± 1089.0 pg/ml versus controls = 700.7 ± 1031.5 pg/ml; $p > 0.05$)

**Table 2**. The demographic data of participants.

| Participants | Group | Sex | Age | MGI |
|---|---|---|---|---|
| 1 | Case | Female | 60 | Edentulous |
| 2 | Case | Male | 70 | 3 |
| 3 | Case | Male | 80 | Edentulous |
| 4 | Case | Female | 62 | Edentulous |
| 5 | Case | Male | 64 | 3 |
| 6 | Case | Male | 81 | Edentulous |
| 7 | Case | Female | 76 | Edentulous |
| 8 | Case | Female | 74 | Edentulous |
| 9 | Case | Female | 75 | Edentulous |
| 10 | Control | Female | 60 | Edentulous |
| 11 | Control | Male | 64 | 3 |
| 12 | Control | Male | 60 | Edentulous |
| 13 | Control | Female | 67 | Edentulous |
| 14 | Control | Male | 57 | 3 |
| 15 | Control | Male | 86 | Edentulous |
| 16 | Control | Female | 73 | Edentulous |
| 17 | Control | Female | 69 | Edentulous |
| 18 | Control | Female | 70 | Edentulous |

MGI: Modified Gingival Index

## Discussion

A vast number of molecular markers have been correlated with OSCC outcome, illustrating the complex events leading to carcinogenesis and cancer progression. Some of the proposed markers are frequently debated and sometimes results seem to contradict each other. Several factors may explain this situation, such as the small number of individuals included in each study or the heterogeneity of selected patients, which frequently differ in various features, notably tumor location (11).

The present study was conducted to test the hypothesis that the elevation of salivary TNF-α, IL-1α, IL-6, and IL-8 are informative biomarkers for oral squamous cell carcinoma.

The results showed the elevation of IL-6 in unstimulated whole saliva of subjects with oral squamous cell carcinoma compared with controls was statistically significant ($p < 0.05$). Although concentration of TNF-α, IL-1α, and IL-8 was higher in patients with oral squamous cell carcinoma but this difference was not statistically significant ($p > 0.05$).

It is known that salivary and serum concentration of these pro-inflammatory cytokines may be increased as a result of various oral cavity inflammatory conditions (eg, gingivitis) (12, 13). In this study the case and control group were matched for gingival condition using MGI.

Investigators found that serum concentration of IL-1α, IL-6, TNF-α, soluble TNF receptor I (sTNF-RI), and C-reactive protein (CRP) were higher in patients with oral squamous cell carcinoma than in controls and the increased serum levels appeared to be related to the clinical stage of disease. They have suggested that IL-6 and sTNF-RI to be the most sensitive parameters in early stages and may be used as additional markers in oral cancer (14).

St John et al (5) found that IL-8 was detected at higher concentrations in the saliva and IL-6 was detected at higher concentrations in the serum of patients with oral squamous cell carcinoma. They did not consider the gingival or periodontal condition but from the fact that their results were so significant for IL-8 in saliva and not for IL-6, they suggested that the oral squamous cell carcinoma contribution to the elevation of IL-8 in saliva outweighs any potential background contribution by the host's potential inflammatory conditions.

Rhodus et al (1) showed that TNF-α, IL-1α, IL-6, and IL-8 were elevated in the whole unstimulated saliva of subjects with oral squamous cell carcinoma (n=13) compared with oral premalignant lesions (n=13) and controls (n=13).

In another study they analyzed and compared the level of TNFα, IL-1α, IL-6, and IL-8 in whole unstimulated saliva among oral lichen planus patients with dysplasia and individuals of control and oral squamous cell carcinoma. In moderate and severe dysplasia, the level of each cytokine was significantly higher than in control. In moderate dysplasia, TNF-α and IL-1α were significantly increased at a level without difference from oral squamous cell carcinoma, but IL-6 and IL-8 was detected at a concentration significantly lower than oral squamous cell carcinoma. In severe dysplasia, the level of TNF-α was also not significantly different from that of oral squamous cell carcinoma, and the level of IL-1α, IL-6, and IL-8 was still significantly lower than that of oral squamous cell carcinoma. The level of four cytokines between smokers and non-smokers in each group did not

show a significant difference. The control individuals in the study had no detectable gingival and/or periodontal inflammation (15).

There is controversy between the results of investigations, therefore, the result of these investigations could not express definitely about usefulness of these biomarkers. Further studies with larger sample size are needed to accept or reject the utility of these cytokines in predicting or diagnosis of oral squamous cell carcinoma or evaluation of treatment.

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