



## Comparison of the Concentration of Salivary IL-8 in Patients with Oral Squamous Cell Carcinoma and Healthy Subjects

Azizi A<sup>1\*</sup>, Dabirmoghadam P<sup>2</sup>, Keykha F<sup>3</sup>

<sup>1</sup>Prof, Oral Medicine Dept and Member of Cranio Maxillofacial Research Center, Dental Branch of Tehran, Islamic Azad University, Tehran, Iran.

<sup>2</sup>Assistant Prof, Otolaryngology Research center, Amiralam Hospital, Tehran University of Medical Sciences, Tehran, Iran.

<sup>3</sup>Dentist

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### ABSTRACT

**Background and Aim:** Oral cancer is one of the most common cancers of the head and neck. Considering the high prevalence and mortality percentage of oral cancer and the significant advances in immunology and identification of different cytokines, the aim of the present study was to compare the concentration of IL8 in saliva of patients with oral squamous cell carcinoma (OSCC) and healthy subjects.

**Methods and Materials:** In this case-control study, 20 individuals with a definite histopathology diagnosis of OSCC were selected as the case group and 20 healthy subjects were selected as the control group. The two groups were matched according to age, gender, cigarette smoking and alcohol consumption, medication consumption, periodontal disease (average CAL in first molars) and absence of systemic diseases. 5 ml of unstimulated whole saliva sample was collected with spitting method from each subject and the concentrations of salivary IL-8 were compared between the two groups. Linear regression and t-test were used for statistical analysis.

**Results:** The concentration of IL-8 equaled  $741.82 \pm 122.81$  pg/ml in the case group and  $341.25 \pm 83.52$  pg/ml in the control group and t- test showed that the concentration of IL-8 in patients with OSCC is higher than that in the control subjects and this difference is significant. ( $p=0.049$ ) According to regression equation, IL-8 has a significant correlation with tumor size index (T). ( $p=0.011$ )

**Conclusion:** The results of this study showed that the concentrations of salivary IL-8 were significantly different between the patients with OSCC and healthy subjects ( $p=0.049$ ) and the concentration of IL-8 has a significant correlation with tumor size (T).

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\*Corresponding author:

Arash Azizi

Email: drarashazizi@yahoo.com

## Introduction:

Oral cancer is one of the most common head and neck cancers. Different factors are involved in the development of these cancers including alcohol, tobacco, chronic inflammation, viral infections and genetic predisposition. <sup>(1)</sup> Since the prevalence and mortality percentage of oral cancer have increased during the last 30 years and considering the irreversible damages and complications caused by this disease in patients and consequently in the society, screening methods which reveal the premalignant and malignant stages before the incidence of the visible lesion are of special importance. <sup>(1, 2)</sup>

Nowadays, with significant advances in immunology field and identification of different cytokines and other mediators of immune reactions, diagnostic tests such as Enzyme Linked Immune Assay (ELISA) have extremely high sensitivity and accuracy for tracing the cytokine in a very small sample surface. <sup>(3)</sup>

Saliva is a suitable and accessible sample and most importantly, the sampling process doesn't cause any stress or inconvenience for subjects. On the other hand, saliva contains measurable concentrations of cytokines and has more advantages than hematologic tests. Interleukin 8 (IL-8) is a mediator from the cytokine family with inflammatory and angiogenic characteristics which is secreted by monocytes and macrophages and induces chemotaxis in lymphocytes and neutrophils. <sup>(4)</sup> A study by Sahebamee and colleagues showed that the concentration of IL-8 is not significantly different in OSCC patients and healthy subjects. <sup>(5)</sup> Osman performed a research about the use of salivary and serum IL-8 cytokines for SCC screening and concluded that salivary IL-8 has high sensitivity and specificity for prediction of OSCC. <sup>(6)</sup> Considering the controversial results regarding the concentration of IL-8 in oral cancer, the present study aimed to assess the concentration of IL-8 in cancer patients and healthy subjects.

## Materials and Methods:

In this case-control study, patients with OSCC that referred to the oral medicine depart-

ment and Amiralam hospital from 2013 to 2014 were selected.

Considering the results of the study by Osman et al and with the use of the submenu of comparison of two averages from the volume and sample determination menu of Minitab software and by considering  $\alpha=0.05$  and  $\beta=0.2$ , average standard deviation of 1.24 and minimum significant difference equal to one, <sup>(1)</sup> the minimum sample size required in each group (case and control) was calculated to be 20 samples. <sup>(6)</sup>

Patients in the case group were individuals with a definite clinical and histopathology diagnosis of oral cancer. The control group was comprised of 20 healthy subjects without oral cancer that were matched with the case group according to age and gender. In oral cancer patients, TNM was assessed as described below and was registered in patient files. <sup>(7)</sup>

T0: No evidence of primary tumor

Tis: Carcinoma in situ

T1: Tumor 2cm or less in greatest dimension

T2: Tumor more than 2cm but not more than 4cm in greatest dimension

T3: Tumor more than 4cm in greatest dimension

T4: Massive tumor with deep invasion into bone, muscle, skin, etc.

N0: No regional lymph node metastasis

N1: Single, homolateral palpable node <3cm in diameter

N2: Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension

N3: Metastasis in a lymph node more than 6 cm in greatest dimension; or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension; or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension

M0: No known distant metastasis

M1: Distant metastasis

The two groups were matched according to age, gender, cigarette smoking and alcohol consumption, medication consumption, periodontal disease (average CAL in first molars) and absence of systemic diseases. The subjects of the case group had not received any treatments in-

cluding radiotherapy, chemotherapy, surgery or alternative treatments. <sup>(8)</sup> After explaining the research to the patients, written consent forms were received. Moreover, the study protocol has been approved by the medical ethics committee of dental branch of Tehran Islamic Azad University of Medical Sciences. (Code :p/320/d/1) 5 ml of unstimulated whole saliva sample was collected with spitting method from each subject.

All subjects were asked to refrain from eating, drinking, and smoking and oral hygiene methods for at least one hour before saliva collection. ELISA test and Bender Medsystems kit (Vienna, Austria) were used for measurement of IL-8 concentration. The standard range of IL-8 in the mentioned kit is 30-1500 pg/ml. T-test was used for comparing the concentration of IL-8 and linear regression equation was applied for assessing the effect of different factors on the concentration of IL-8.

### Result:

40 individuals were selected for the present study. 20 patients had OSCC and 20 healthy subjects were placed in the control group and the subjects were matched according to the mentioned indices. Based on the performed analyses, the two groups were not significantly different regarding the indices. ( $p>0.05$ )

The concentration of IL-8 equaled  $741.82\pm 122.81$  pg/ml in the case group and  $341.25\pm 83.52$  pg/ml in the control group and t-test showed that the concentration of IL-8 in patients with OSCC is higher than that in the control subjects and this difference is significant.

( $p=0.049$ ) According to regression equation, IL-8 has a significant correlation with tumor size index (T) ( $p=0.011$ ), which means that concentration of IL-8 is higher in larger tumors and shows that the concentration of IL-8 is correlated not only with tumor but also with tumor size. IL-8 was not significantly correlated with factors such as age ( $p=0.902$ ), gender ( $p=0.959$ ), smoking ( $p=0.939$ ), alcohol consumption ( $p=0.964$ ), systemic disease ( $p=0.752$ ), medication consumption ( $p=0.849$ ), periodontal disease ( $p=0.957$ ), radiotherapy ( $p=0.984$ ), metastasis ( $p=0.97$ ) and lymph node involvement ( $p=0.98$ ).

### Discussion:

The present study showed that the difference in the concentration of IL-8 in the two assessed groups (case and control) was statistically significant, in a way that the concentration of IL-8 in the case group with OSCC was higher than that in the control group. ( $p=0.049$ ) Moreover, the concentration of IL-8 was directly correlated with tumor size.

Oral cancers especially OSCC comprise 80 to 90% of head and neck cancers and in universal statistics, the 5-year survival rate of head and neck cancers has reached below 50% during the last two decades.<sup>(9)</sup> SCC is a multistage disease and some authors have related multiple genetic and environmental changes to its incidence. Genetic changes which occur in OSCC can be used as a biomarker for early diagnosis. Most of these changes can be assessed in detached epithelial cells, saliva and serum.<sup>(10)</sup>

IL-8 is a cytokine produced by macrophages and epithelial cells etc. and has a role in angiogenesis.<sup>(11)</sup>

IL-8 secretion has been detected in correlation with different cancers such as Hodgkin syndrome and prostate cancer and also oral cancer.<sup>(12)</sup>

**Table 1- Status of individuals in the assessed groups based on the age and OSCC size**

T					Age (Mean±SD)	Index OSCC
0	1	2	3	4		
20	0	0	0	0	60.95±19.51	Control N1=20
100%	0%	0%	0%	0%		
0	6	13	0	1	62.95±18.38	Case N2=20
0%	30%	65%	0%	5%		

Multiple mechanisms have been proposed for the increased concentration of this IL in different cancers including oral cancer, which are described below:

Epidemiologic studies show that chronic inflammation is correlated with different types of cancer.<sup>(13)</sup> Inflammation has a major role in the initiation and also development of tumor, inflammatory and autoimmune diseases.<sup>(14, 15)</sup> Although acute inflammation is a defense mechanism for body, chronic inflammation can be correlated with many diseases such as cancer. Many genetic products of cells have been identified in chronic inflammatory conditions which can have major roles in the suppression of apoptosis and also in metastasis, invasion and angiogenesis. Some of these products include cytokines such as TNF, IL1, COX2, MMP, IL-8, and VEGF.<sup>(16)</sup> When epithelial cells transform to cancer cells, they continue to grow with suppressing the apoptosis process. Also, due to inflammatory pathways, concentration of IL-8 will increase which will initiate the development and metastasis of the cancer.<sup>(17)</sup>

Moreover, many studies have confirmed the production of cytokines like IL-8 by cancer cells and the increase of their receptors in premalignant lesions such as Lichen planus<sup>(18)</sup> and SCC.<sup>(19,20)</sup>

IL-8 and VEGF which are expressed simultaneously in head and neck carcinoma cells (HNSCCs) and a number of other cells, induce tumoral growth, angiogenesis and metastasis.

On the other hand, in lung cancer CD14 causes the secretion of IL6 and IL-8 by inducing alveolar monocytes and macrophages.<sup>(20)</sup>

In a study by Punyani et al in 2013, IL-8 was assessed in saliva of patients with premalignant lesions, oral cancer and healthy subjects and they concluded that the concentration of IL-8 was considerably higher in saliva of cancer patients. (p 0.0001) But the difference between healthy subjects and patients with premalignant lesions was not significant. (p=0.738)<sup>(21)</sup>

In another study by Sahebamee et al, the concentrations of IL-8, IL6, IL1 and TNF were compared between OSCC patients and healthy subjects.

They concluded that although the concentration of IL-8 was higher in the case group compared with the control group, but this difference was not significant. (p>0.05).<sup>(5)</sup> This conclusion is not in line with the results of the present study. This difference can be attributed to the fact that Sahebamee et al did not eliminate the confounding factors that can influence IL-8. While some of the advantages of our study include the elimination of other confounding factors that could increase IL8, and the homogenization of factors like age, gender, smoking, alcohol consumption, periodontal disease and absence of systemic diseases between the individuals of the two groups and also assessing the relation of IL-8 with TNM. One of the limitations of the present study was the small sample size.

### Conclusion:

The results of this study showed that the concentration of salivary IL8 was significantly higher in patients with OSCC compared with healthy subjects and the concentration of IL8 is directly correlated with tumor size.

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