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RESEARCH ARTICLE

SALIVARY EXPRESSION OF INTERLEUKIN-6 IN ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL SQUAMOUS CELL CARCINOMA PATIENTS

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ABSTRACT

Oral Squamous Cell Carcinoma is a common malignant tumor occurring with increasing frequency among individuals. The survival rate of oral cancer is 60-80% if detected during its early stages; however this number drops to 30-40% when the cancer is diagnosed during advanced stages. Direct contact between saliva and the oral lesion makes measurement of tumor markers in saliva an attractive, non-invasive, chair-side diagnostic/prognostic aid and alternative to serum testing. The connection between chronic inflammation and carcinogenesis is well known and it is believed that cytokines and mediators of inflammation has role in suppression of apoptosis and tumor progression.

Aim of the present study is to evaluate the change in levels of Interleukin-6 in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma patients and to develop a possible prognostic marker. To evaluate the reliability of salivary IL-6 as diagnostic/ prognostic marker in potentially malignant oral disorders (OPMD). And to develop a cost-effective chair side salivary prognostic marker of oral squamous cell carcinoma (OSCC).

Material and method: The salivary level of Interleukin-6 in (n=25) patients in each group of Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma patients and (n=25) healthy individuals (n=25) of control groups was measured using ELISA.

Result: Salivary IL-6 was detected at higher concentration in patients with OSCC and OPMD as compared to control group (P<0.001).

Conclusion: From the results of present study, it can be concluded that IL-6 is an important proinflammatory cytokine, detectable at higher concentration in saliva of patients of OPMD and OSCC and prove to be useful as biomarker for diagnosis and predicting malignant transformation.

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INTRODUCTION

Oral cancer is the 6th most prevalent cancer with the age standardized incidence rate of 3.9 per 100,000 population worldwide.(1) Oral squamous cell carcinoma(OSCC) accounts for more than 90% of oral cancers worldwide.(2) In India, the age standardized incidence rate of oral cancer is 12.6 per 100,000 population and a sharp increase in the incidence rate of this cancer has been reported in recent years.(3)early detection of malignancy, soon after transitioning from premalignancy, ensures effective treatment and consequently increased survival rate upto 80%.(4) Thus, early diagnosis and referral is a cornerstone to improve survival. The concept of two-step process of cancer development in the oral mucosa, that is, initial presence of a precursor lesion- oral potentially malignant disorders (OPMD) such as- leukoplakia, erythroplakia, oral lichen planus, oral submucous fibrosis, which has the potential to develop subsequently into oral Squamous Cell Carcinoma (OSCC) is well established.(5)The connection between chronic inflammation and carcinogenesis is well known and it is believed that cytokines and other mediators of inflammation play an important role in carcinogenesis(2,6). Interleukin-6 (IL-6) is a multifunctional cytokine and an important mediator of inflammation, as in acute inflammatory conditions, it drives differentiation of B-lymphocytes into antibody producing plasma cells(3).Direct contact between saliva and the oral lesion makes measurement of tumor markers in saliva an attractive, non-invasive, cost-effective, chair-side diagnostic/prognostic aid, alternative to serum testing.(7,8) Therefore, present study was undertaken on molecular markers which can be used to predict the risk of malignant transformation in oral potentially malignant disorders.

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MATERIALS AND METHODS

The study was conducted at Department of Oral Medicine and Radiology, Maulana Azad Institute of Dental Sciences and Department of Biochemistry,Govind Ballabh Pant Institute of Postgraduate Medical Education And Research. The study

protocol was approved by the Institutional Ethical Committee.Totally 75 cases were included under the study. The cases were divided into three groups consisting of 25 cases each. Group Acomprised of patients with clinically diagnosed andhistopathologically proven OSCC. Group B included the patients with clinically diagnosed and histopathologically proven OPMD (oral submucousfibrosis (n=10), oral lichen planus (n=10), leukoplakia (n=4), discoid lupus erythematosus (n=1)). Clinically proven cases of OPMD and OSCC with additional confirmation by biopsy of age group ranging from 20 to 70 years both males and females were included in the study. Group Ccomprised of age and sex matched healthy individuals with matched periodontal conditions. Patients who are already undergoing some treatment for existing oral potentially malignant disorders and patients with severe systemic diseases, pregnant and lactating females were excluded**.** Informed written consent was obtained from all the patients selected for the study. The clinically suspected cases of OPMD includes leukoplakia, oral submucous fibrosis, oral lichen planus, discoid lupus erythematosis and the patients with non-healing ulcer/ ulcero-proliferative growth with or without lymphadenopathy as OSCC. Incisional biopsy was done for all the suspected cases and diagnosis was established based on clinical and histopathological findings, except for healthy controls. All the cases selected under the three groups were age and sex matched. The periodontal status of all the cases (n=75) was matched using community periodontal index (CPI) as per WHO guidelines.[9]

Saliva samples were collected by simple drooling method. The saliva samples were collected before the biopsy procedure as biopsy might alter the level of IL-6 in the saliva sample. The saliva samples were used for the study only when the histopathological results confirmed the presence of either OPMD or OSCC.The participants were refrained from eating and drinking for at least one hour before sample collection. Unstimulated whole saliva was collected by requesting the subject to swallow first, tilt their head forward and expectorate all the saliva into a sterile wide mouthed vaccuntainer for 5 minutes without swallowing. There, the saliva samples were centrifuged at 3000 rpm for 10 minutes. Then the supernatants were carefully drawn using micropipettes and transferred to eppendorf tubes. The supernatants were then stored at --80˚C in the deep freezer until analysis.

Elisa Method

The concentration of Interleukin-6 present in the saliva samples was determined by using quantitative ELISA technique. Standard human IL-6 ELISA kit (Diaclone, France) was used for analysis with the help of ELISA reader (TECON). And Based on data information and collected, results were analysed using SPSS software (14th version) and Mann-Whitney statistical tests was applied.

RESULTS

Following observations were made

Mean Distribution of IL-6 According To Sex- The mean value for IL-6 was found highest (36.29) in males and lowest (20.18) in females (Table 1 and Figure 1). *Mean Distribution of IL-6 According To Habit*- Mean value for IL-6 was found highest (50.89) in patients with habit of smokeless form of tobacco intake, followed by patients with habit of smoking (47.19), and lowest in patients with no habit (5.96).

Table 1. Salivary il-6 levels (pg/ml) in patients and control group

	Group-a (n=25)	Group-b (n=25)	Group-c (n=25)
Mean	130.9	44.7	4.9
Standard deviation	80.93	70.90	6.80
Median	195.0	17.0	2.8
(min-max)	(4-200)	(0-200)	(0-25)

Table 2. Mann-whitney test for salivary il-6

Comparison groups	IL-6 p-Value
Group-a and Group-C (OSCC and Control)	0.001
Group-a and Group-C (OSCC and Control)	0.02
Group-a and Group-C (OSCC and Control)	0.001

p-value<0.05 is significant

AS shown in table 1, the mean value for IL-6 was highest(130.90±80.92) in group-A, followed by group-B (44.70±70.90) and lowest (4.96±6.80) in group-C. With MANN-WHITNEY test, the difference in mean values between groups was found statistically significant with p-value <0.05 (Table 2).

salivary IL-6 levels (pg/ml) in patients and control group

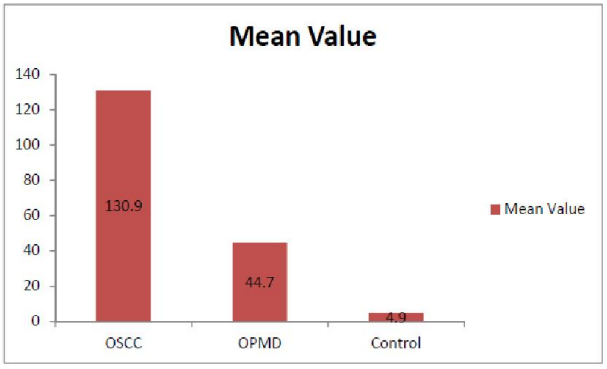
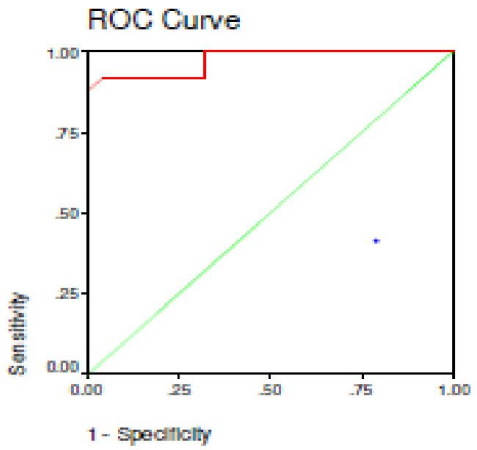


Figure 1.

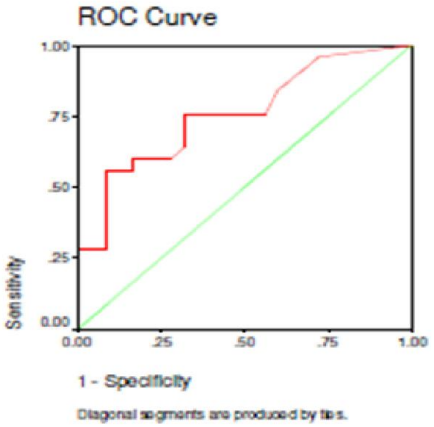


Graph 1. Group A and C: ROC Curve for IL 6

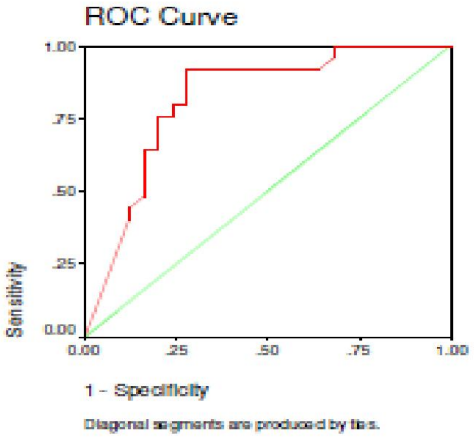
Roc curves and cut-off values for IL-6

Group A (OSCC) and group-C (CONTROL): As shown in graph1, the area under curve was 0.974 and mean value of IL-6

in OSCC patients and control were 130.9 and 4.9 respectively. The possible cut-off value derived from the ROC curve for IL-6 was 19pg/ml. At this value, sensitivity was 92% and specificity was 92%.If this cut-off value is lowered to 5.35pg/ml (close to mean value of control group i.e 4.9pg/ml), the sensitivity remains same but specificity reduces to 72%. Group A (OSCC) and group B (OPMD): As shown in graph 3, the area under curve was 0.819 and mean value of IL-6 in OSCC patients and OPMD patients were 130.9 and 44.7pg/ml respectively. The possible cut-off value derived from the ROC curve for IL-6 was 43.5pg/ml. (this cut-off value is close to mean value of GM-CSF in OPMD patients). At this value, sensitivity was 76% and specificity was 80%.



Graph 2. Group B and C: ROC Curve for IL 6



Graph 3. Group A and B: ROC Curve for IL 6

DISCUSSION

A number of tumour markers in serum for OSCC have been investigated in various studies and showed relatively moderate sensitivity and specificityvalues in relation to diagnosis, prognosis predicting or treatment monitoring.[10] Saliva as a sample has many advantages over serum and tissue. Measurement of molecular markers in saliva could potentially aid in development of a practical screening tool.[11] It is well recognized that numerous cytokines have various roles in the diseases of oral cavity. One such cytokine is IL-6. Furthermore, elevated levels of IL-6 have been found in patients with oral cancer as well as in patients with OPMD such as oral leukoplakia, oral lichen planus and oral submucous fibrosis discoid lupus erythematosus.[11] Thisstudy was aimed at comparing the level of salivary IL-6 in patients with OSCC and OPMD and healthy control subjects and to explore the possibility of using salivary IL-6 asa molecular marker for diagnosingOSCC and OPMD. And it was been found that IL-6 level was elevated in patients with OSCC and OPMD as compared to controls. Also it was been found that as age increases, there is increase in the concentration of IL-6.[14] Sex difference is also found in the expression of IL-6.[13] With regard to the age, 16(64%) patients of OSCC were above 40 years of age. This shows OSCC is more prevalent in older age group. This was consistent with observations of Warnakalsuriya, 2009[6] and Silverman *et al*, 1990[15]. In OPMD, 17(68%) patients were of younger age group, 20-40years, as compared to OSCC, which is more prevalent in older age group, above 40years of age.[15] This was in consistence with observations of Warnakalsuriya *et al*, 2009[5] and Silverman *et al*,1990.[15] With respect to gender distribution, number of male was more than the female in each of the three groups. This demonstrates that OPMD and OSCC are more common among male than female.

This finding was also consistent with that of Warnakulasuriya *et al*, 2009.[15] With respect to distribution of IL-6 according to gender, IL-6 levels were found more in males than females. This finding was also found consistent with observations of Prabhu *et al*.[16] In our study, we noted a significant increase in the concentration of salivary IL-6among patients with OPMD than the healthy controls. Also, it was found to be significantly lower when compared with OSCC patients. The possible cut off value derived from ROC curve comparing OPMD with control group was 3.85pg/ml, means patients having salivary IL-6 level greater than this value is suspecting of having OPMD. Various other studies by Rhodus *et al*[17] , Brailo *et al*[12] and Sharma *et al*[9] also reported increase in salivary IL-6 concentration than healthy controls. The increase in salivary IL-6 level in OPMD might be the result of local production, which could be secreted from two sources. One possibility is lesional epithelium itself and alternatively lymphocytes from discrete chronic inflammatory infiltrate which is present in tissue affected with OPMD.[12]

In our study, the concentration of salivary IL-6 was observed to be higher inOSCC patients than OPMD patients and healthy controls. This was in accordance with the studies of VucicevićBoras *et al*[19] , Rhodus *et al*[17], Katakura *et al*[20], SahebJamee *et al*[21], Sato *et al*[22], Brailo *et al*[12]. Direct contact between saliva and the oral cancer lesion makes measurement of tumormarkers in saliva an attractive alternative to serum testing.[12] The studies by VucicevićBoras *et al*[19], Brailo V *et al*[12] demonstrated that there is no significant difference in serum IL-6 among oral cancer and controls. However, several studies reported significantly higher concentration of serum IL-6 in oral cancer patients compared to healthy controls.Thus it can be concluded that altered cytokine production and responsiveness in oral cancer takes place primarily in the oral cavity and does not reflect on serum cytokine concentration.[12]In our study, OSCC patients have significantly higher concentration of salivary IL-6compared to patients with OPMD and healthy controls. One might think that higherconcentration of IL-6 might be the result of a lesion with epithelial discontinuity andsurrounding inflammation, not directly related to cancer. However, in the study by Rhodus NL *et al*[17], salivary IL-6 in oral cancer patients were significantly higher than in patients with dysplastic oral lichen planus, an inflammatory lesion that is often accompanied with epithelial discontinuity. This indicates that the increase in salivary IL-6 is due to the local production of this cytokine in cancer tissue.

This is in agreement with Pries *et al*[18] who stated that oral cancer cells and tumour infiltrating lymphocytes are capable of producing IL- 6. In the present study, there is significant increase in the concentration of salivary IL-6 in both OSCC and OPMD patients than the healthy controls. Also, there is significant increase in salivary IL-6 in OSCC than in OPMD patients. This clearly demonstrates than salivary IL-6 can be used as a biomarker to diagnose potentially malignant disorders and oral cancer. These results suggest that IL-6 asproangiogenic, proinflammatory cytokines is elevated in the saliva of patients with OSCC and OPMD as compared to controls which may have diagnostic and/or prognostic significance.

Conclusion

Salivary IL-6 can serve as a biomarker for oral squamous cellcarcinoma and oral potentially malignant disorders.Increase in the concentration of IL-6 in saliva might help to identifypotential individuals who are at increased risk of transformation to malignancy.Saliva as a sample has many advantages over serum and tissue. It is non-invasivemethod with easy chair-side collection of sample in sufficient quantities for analysis.Measurement of molecular markers in saliva could potentially aid in development of achair-side practical screening tool. With this biomarker, early detection of oral cancer can become much more feasible which can drastically increase the survival rate. Further longitudinal studies with increased sample size are needed to substantiate the utility of salivary IL-6 in predicting or diagnosing oral potentially malignant disorders and oral squamous cell carcinoma.

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