Non invasive Assessment of Interleukin 6 and its Clinico-pathologic correlation in patients with Oral Squamous cell carcinoma.

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Abstract

Background: Interleukin-6 (IL-6) is a pro inflammatory cytokine that promotes inflammation, development and progression of cancer. Quantitative assessment of IL6 in saliva will help in the early diagnosis of oral cancer. Whole saliva as an alternative laboratory tool to blood comprises a non-invasive, easy, rapid to collect, easy to handle and cost-effective sample convenient both for patient and the health personnel during screening of larger population. Hence the study aimed to estimate the concentration of salivary IL6 and clinically correlate these levels in patients with oral Squamous cell carcinoma.

Methods: A total of 72 subjects aged between 31-60 yrs were included in the study. Group I: Thirty six histological proven cases of oral Squamous cell carcinoma. Group 2: Thirty-six healthy controls. Unstimulated whole saliva sample was collected and the samples were analysed for interleukin-6 using ELISA kits. Data was analyzed using SPSS software version 22.

Results: The study showed a statistically significant elevation ofinterleukin-6 in saliva of patients with oral cancer 214.29±19.64 pg/mL as compared to the healthy control group 17.11±0.83 pg/mL with a p value of 0.001. The Salivary IL6 levels did not show any correlation with gender of patients both in OSCC and control subjects. The median Salivary IL6 levels were significantly higher in stage (I-II) compared to stage(III -IV).

Conclusion: Estimation of IL6 in saliva can be considered as a non invasive alternative laboratory tool to blood for oral cancer for screening among high risk subjects.

Key words: Interleukin-6,Saliva, Oral Squamous cell carcinoma.

Introduction

Head and neck cancers constitute 30-35% of all cancers in India and majority of them are oral cancers[1]. Oral Squamous cell carcinoma (OSCC) is a common malignant tumor of the oral cavity with poor prognosis[2]. The worldwide 5 year survival rate of head and neck cancers are consistently less than 50% despite the tremendous advances in the treatment modalities, one of the reasons being the late presentation and detection of these cases.Hence early detection through screening of high risk groups is an important approach towards better treatment outcome and survival rate. The various biomarkers in blood identified for screening oral cancers include IL-1β, IL-6, IL-8 and TNF-α[3].

The gold standard for the diagnosis of oral cancer is the histopathological examination of biopsy specimen from a suspected lesion, the procedure is laborious, time consuming and requires an experienced pathologist. Further the histopathology report will be reliable only when the specimen is representative, considering field cancerization and condemned mucosa. The procedure is also quiet unpleasant for the patient and unsuitable for community screening of high risk population for early diagnosis during the asymptomatic phase[4].

Many biochemical parameters in blood and saliva have been claimed as biomarkers for oral cancer, like lactate dehydrogenase, metalloproteinase 9, 8 Oxo guanine, p16 protein, Sialic acid etc[5,6]. Quiet

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recently estimation of IL6 levels has attracted a great interest as tumor marker in oral cancer and for its early diagnosis. Interleukin-6 is a Pro-inflammatory cytokine expressed by T and B cells, macrophages, epithelial cells and fibroblasts in response to infection, neoplastic changes in the cells. The physiological functions of IL6 include induction of acute phase proteins, T and B cell growth and differentiation and osteoclast activation[7,8]. This study aimed to estimate the concentration of salivary IL6 and clinically correlate these levels in patients with oral Squamous cell carcinoma.

Materials and Methods:

Source of data:

A Hospital based cross sectional analytical study done at a Tertiary care hospital & Research Center. The study was approved by the Central Ethics Committee of the Institution. The study group consisted of participants from the outpatient department of Otorhinolaryngology and Head and Neck Surgery. Subjects aged between 31- 60 years were included in the study. Group 1 included thirty six patients with histopathological confirmed, newly diagnosed and untreated oral Squamous cell carcinoma cases. Group 2 included thirty six healthy subjects as Controls. Patients who had undergone Radiotherapy, Oncosurgery or Neo adjuvant chemotherapy and patients with Immunodeficiency, preexisting systemic diseases like diabetes, hypertension and periodontitis that may affect the IL6 levels were excluded from the study. After obtaining informed written consent, the subjects were evaluated by complete medical history and clinical examination.

Sample collection and Preparation

Whole Saliva: 10 mL of unstimulated whole saliva sample was collected according to the method of Navazesh[9]. The sample was collected from OSCC and from healthy controls between 9 am - 12 noon. The subjects were asked to rinse the mouth thoroughly to remove any food debris and then after ten minutes, were asked to spit into sterile plastic containers, avoiding forcible spitting. The collected samples were centrifuged at 3000 rpm for 10 minutes and supernatants collected. This was done within one hour following collection to remove any debris or cellular matter, the supernatant separated was assayed forinterleukin-6.

Immunological Assay: Determination of Salivary Interleukin 6 Levels

Interleukin-6 quantification was done using standard ELISA kits by Krishgen BioSystems. The Human interleukin-6 in the micro titer wells interacts with Human interleukin-6 biotin conjugated antibody and the enzyme which was washed using wash buffer following which substrate was added to the reaction mixture, the reaction was stopped on addition of stop solution. Intensity of the yellow color formed in the micro titer wells was read at 450nm using ELISA Micro plate Reader. The absorbance read was compared with the standard curve to obtain the concentration of interleukin-6 in saliva and expressed in pg/ml.

Statistical analysis :

Data was analyzed using SPSS 22 version software. Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two groups.

p value <0.05 was considered as statistically significant.

Results

The demographic data and the clinic-pathological details of the study participants are represented in (Table 1). All the patients in our study had well differentiated squamous cell carcinoma. The study showed a statistically significant elevation ofinterleukin-6 in saliva of patients with oral cancer as compared to the healthy control group with a p value of 0.001 (Table 2, Fig 1). The salivary IL6 levels did not show any correlation with gender of patients both in OSCC and control subjects (Table 3). The median salivary IL6 levels were significantly higher in stage (I-II) compared to stage (III -IV).(Fig 2)

Table 1:Demographic and clinic pathological details of the study participants

Parameters	Patients (n= 36)	Healthy controls (n= 36)
Male	15	12
Female	21	24
Age Groups		
51-60 years	24	16
41-50 years	9	9
31-40 years	3	16
Habits		
Tobacco chewing	22	-
Smoking	8	-
Smoking and chewing	6	-

Site of the Primary tumor	17	-
Buccal mucosa	2	-
Vestibule	15	-
Buccal mucosa and Vestibule	1	-
Alveolar mucosa	1	-
Palate		
Staging of Oral Cancer		
Stage I	4	-
Stage II	11	-
Stage III	4	-
Stage IV	17	-

Table 2: Shows the Mean ± Standard Deviation(SD) levels of salivary IL6 in OSCC and Controls

	Group	N	Mean ±SD	t value	p value
Salivary IL6	OSCC	36	221.14 ± 110.6	11.02	<0.001***
	Controls	36	17.81 ± 4.5		

*** p value is highly statistically significant

Fig 1: Shows the Mean ± SD levels of Salivary IL6 in OSCC and Control subjects

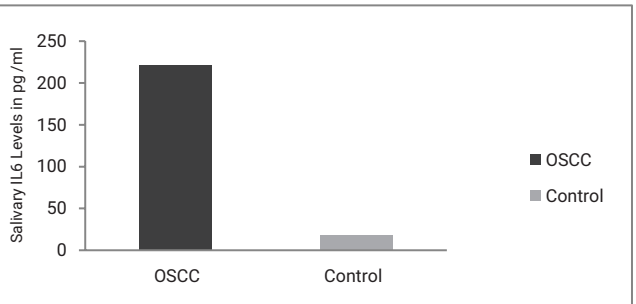

Fig 1 : Mean ±SD Salivary IL6 levels in OSCC and Control subjects

Table (2) & Fig (1) Shows that the mean differences Salivary IL6 in OSCC and Control subjects was found to be statistically significant (t=11.02, P<0.001). The increase in salivary IL6 levels in cases is significant compared to the control group.

Table 3: Comparison of Mean differences in salivary IL6 in female and male subjects with OSCC and Control group.

Gender	Females	Males	p Value
Group	Mean ± Std. Deviation	Mean± Std. Deviation	0.551
OSCC	231.44±108.91	206.68±115.26	
Control	18.17±4.54	15.32±3.62	

The Salivary IL6 levels patients having OSCC and in controls does not show any association with gender p value 0.551

Fig (2): Comparison of Salivary IL6 levels based on Clinical Staging in OSCC.

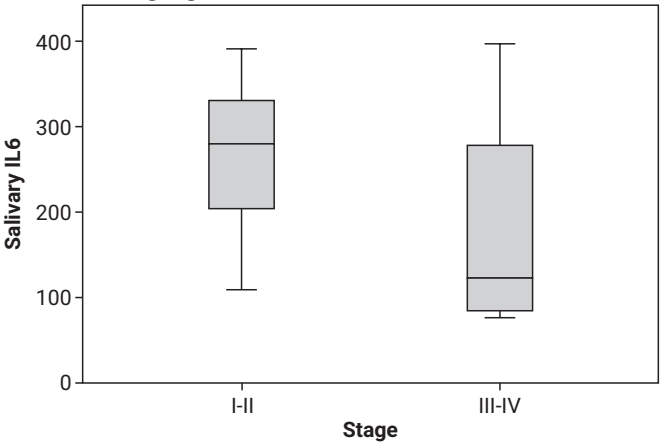

Fig 3 Box plot indicates that the median salivary IL6 Levels are higher in stage (I & II) compared to stage (III & IV) whereas in stage(I & II) has narrow IQR compared to stage (III & IV) which has a wider IQR indicating lot of variation in IL6 Salivary levels in higher grades. In stage (III & IV) though median levels in the group was low but may case have registered abnormal variations in higher grades.

Discussion

In the present study the concentrations of IL6 in unstimulated whole saliva in Oral Squamous cell carcinoma patients was significantly higher compared to the Control subjects. Suggesting that the contribution of oral Squamous cell carcinoma to the salivary IL6 elevation outweighs any potential background contribution by the host's inflammatory condition. Our findings are similar to the observation made by Rhodus et al who reported that IL6 levels were significantly elevated in OSCC patients compared to the pre malignant and control subjects[10].

This finding may also suggest that there is increased release of IL6 into the saliva by the OSCC patients that further contributes to the carcinogenesis and cancer progression in the oral mucosa.

There are various mechanisms suggested by which IL6 facilitates carcinogenesis. It brings about the inactivation of p53 tumor suppressor gene by hypermethylation of its promoter region thereby decreasing apoptosis and increasing cell growth[11] IL6 also causes immunomodulation to alter the local and systemic immune response to the tumor cells or direct anti-proliferative effective on the tumor[12,13-15].

The results of the present study are similar to the earlier observation by St. John et al. of the elevated

levels of IL-6 at both the m RNA and the protein level in the serum in OSCC patients. The elevation of IL-6 was shown to promote immune unresponsiveness and induction of wasting, cachexia, and hypercalcemia, all of which occurred in OSCC patients with poor prognosis.[16] In this study we also observed that the concentration of salivary IL6 in patients with stage (I & II) was higher as compared to the Stage (III&IV) OSCC patients.

This study did not find any variation in concentrations of Salivary IL6 with respect to gender. This is in contrast to the Immunochemistry study of IL6 in patients with OSCC by Chih-Jung Chen etalin which they mentioned that the high expression of cytosolic IL6 in OSCC tumors was correlated with female gender[17].

Saliva is an ultra filtrate of plasma, and it does reflect the changes taking place in the blood.

Analytes from blood are transferred to saliva through capillary walls, interstitial space, and acini or duct cells into the lumen of the salivary gland duct.[8] Thus saliva can be an alternative laboratory tool to blood for evaluation of the levels of IL6 in oral Squamous cell carcinoma patients as a biomarker for early diagnosis and particularly while screening the high risk population for this disease.

Conclusion

Salivary IL6 is significantly elevated in oral squamous cell carcinoma patients compared to controls. The concentration of Salivary IL-6 showed significantly higher levels in Stage (I & II). Thus the estimation of IL6 in saliva can be considered as a non invasive alternative laboratory tool for early diagnosis of oral cancer and screening among high risk subjects for OSCC. However further longitudinal studies with increased sample size needs to be done to validate the use of salivary IL6 as a screening tool.

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