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To compare the levels of salivary Interleukin 6 between oral potentially malignant disorders and oral squamous cell carcinoma

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***Abstract*---**To compare the levels of salivary Interleukin 6 between oral potentially malignant disorders and oral squamous cell carcinoma. After ethical clearance and informed consent, the saliva samples were collected from 20 patients of OPMD'S, 20 patients of OSCC and 10 controls. Salivary IL-6 levels were detected by enzyme linked Immunosorbent essay and appropriate statistical analysis was done. Significant differences in IL-6 concentration between OSCC, OPMD'S and controls were noted. Salivary IL-6 levels were higher in OSCC. The results of present study concluded that the level of salivary IL-6 in OSCC is significantly higher than oral potentially malignant disorders like OSMF and oral epithelial dysplasia and controls. This

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biomarker can be utilized as a major asset for early detection of cancer and can open a new horizon for treatment plans of targeted therapy.

***Keywords*---**OSCC, OSMF, IL-6.

Introduction

Oral cancer was previously rated as the sixth most common cancer worldwide.[1] It accounts for approximately 4% of all the malignancies.[1] Oral squamous cell carcinoma develops through a multistep process of genetic, epigenetic and metabolic changes resulting from exposure to carcinogens[5.] The initial presence of a precursor cell subsequently developing into cancer is well established in oral cancer. Oral leukoplakia, oral sub mucous fibrosis and lichen planus are major known precursor lesions[2.]

The early detection of cancer is of clinical importance because survival rates markedly improve when the oral lesion is identified at an early stage.[1] Ability to clinically predict malignant transformation of precancerous lesions is difficult and routine histopathological diagnosis has limited prognostic value.[2] Different techniques are being implemented to predict the development of oral cancer. These include vital staining, brush biopsy, auto-fluorescence spectroscopy, ELISA, chemiluminescent illumination, narrow band imaging and confocal microscopy.[3]

ELISA stands for Enzyme Linked Immunosorbent Essay. It is a biochemical procedure designed for detecting and quantifying peptides, proteins, antibodies and hormones in body fluids.[4] Amongst these fluids, saliva is now being assessed as a predictive, diagnostic and prognostic tool for carcinomatous, inflammatory and genetic diseases. The diagnostic potential of saliva is not limited to the diseases of the oral cavity, but covers systemic conditions as well. Saliva acts as a reservoir of steroids, amines, peptides, melatonin, insulin, leptin, ghrelin, secretory IgA enzymes, other enzymes and drugs. Human saliva represents whole body image sand is also known as the “mirror of the body”.[3] With the extensive research on oral cancer, emphasis has been laid on predictive biomarkers found in saliva.

Studies on salivary levels of biomarkers have proved to be of value in these cases, and research is looking at Saliva as a possible future “non-invasive, easy to collect, ethically sound, and economical” tool for screening and monitoring, in addition to diagnosis. Therefore, the present study was conducted to compare the salivary IL-6 levels in Potentially Malignant Disorders and Oral Squamous Cell Carcinoma.

Materials & Methods

The present study was conducted in the department of Oral Pathology and Microbiology, Swami Devi Dyal Hospital and Dental College, Barwala after approval from the constitutional ethical committee. The study included patients

aged between 21-90 years, without any chronic/acute illness, no acute or sub-acute inflammation or infection, with clinically and histopathologically diagnosed cases of Oral Potentially Malignant Disorders or squamous cell carcinoma and not undergoing any form of treatment for these lesions. Patients who did not consent, or had systemic illness and in which we were unable to collect sufficient amount of saliva were excluded from the study. The study samples included were categorized as follows:

GROUP 1: 20 samples from patients of Oral Potentially Malignant Disorders
GROUP 2: 20 samples from patients of Oral Squamous Cell Carcinoma

10ml saliva samples were taken from age and gender matched individuals. (To determine the baseline levels of IL-6). The procedure was performed with a 96-well ELISA plate reader, and 96-well microtiter plate (Diaclone Elisa Kit, France).

The data was compiled in MS excel and SPSS version 22 (SPSS Pty Ltd, Chicago, IL, USA) was used for the statistical analysis. Univariate and bivariate frequency tables were generated with percentages for comparison of various categories between Groups. Descriptive statistics were computed (Range, Mean, Median and Standard Deviation) for continuous variables studied. Comparison of mean values along with respective variances cross the 4 groups was carried out using one way Analysis of Variance (ANOVA) and F statistic with appropriate degree of freedom was computed. Any p - value < 0.05 was considered to be statistically significant.

Results

40 subjects were selected for the study (n=40). Cases were divided into two groups Group 1 & Group 2. Group 1(n=20) consists of potentially malignant disorders that is 10 cases of OSMF (n=10) & 10 cases of Oral Epithelial Dysplasia (n=10). Group 2 consists of 20 cases of Oral Squamous Cell Carcinoma (n=20). 10 healthy individuals were taken as controls.(figure 1). The mean age of three groups are given in figure 2. Majority of the lesions were present on the buccal mucosa. In OSMF the lesions were present on buccal mucosa 10(25%). In oral epithelial dysplasia the lesions were present on buccal mucosa 08(20%) followed by commissures 03(7.5%). In OSCC, lesions were present on tongue 09(22.5%) followed by buccal mucosa 07(17.5%), alveolus 03(7.5%), palate 02(5%) and gingiva 01(2.5%)(figure 3). The mean ELISA salivary IL-6 values for OSMF, OED and OSCC are 41.92 pg/ml, 58.39 pg/ml and 143.70 pg/ml respectively. There was no statistically significant difference among the mean ELISA value of salivary IL-6 in oral epithelial dysplasia and OSMF (p = 0.221). A statistically significant difference was present among the mean ELISA value of salivary IL-6 in oral epithelial dysplasia and OSCC (p = 0.000) as well as among OSMF and OSCC (p= 0.000). A statistically significant difference was present among the mean ELISA value of salivary IL-6 in mild dysplasia and severe dysplasia (p = 0.05). The comparison of mean ELISA value of salivary IL-6 in well differentiated OSCC and moderately differentiated OSCC was statistically significant (p=0.003), comparison of well differentiated OSCC and poorly differentiated OSCC was also statistically significant (p=0.000) and comparison of moderately differentiated OSCC and poorly differentiated OSCC was also statistically significant (p=0.000).

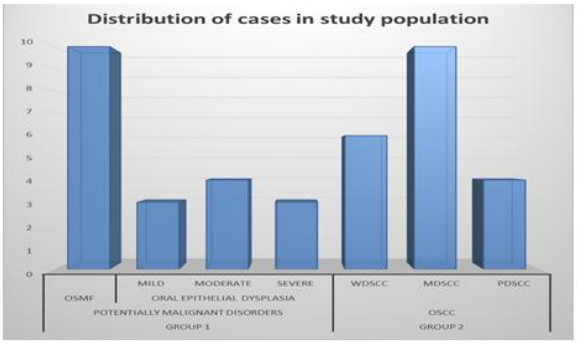

Figure 1: Distribution of subject

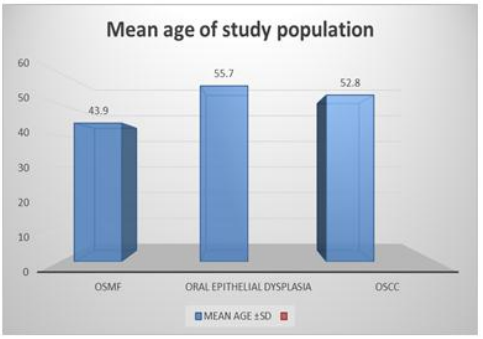

Figure 2: Demographic data

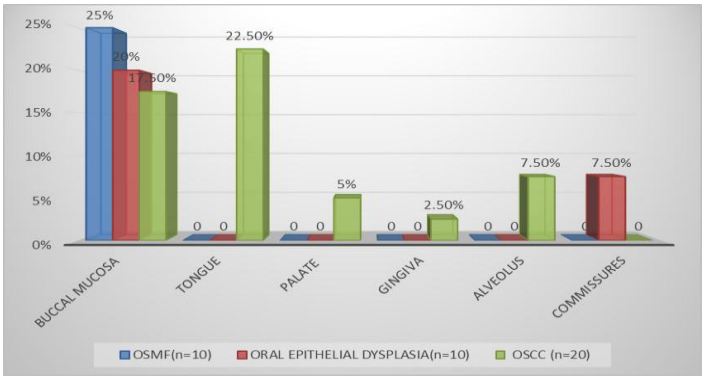

Figure 3: Site of lesion

Table 1
Comparison of IL-6 levels in saliva amongst various groups

Lesions	Mean± SD	P-value
OSMF	41.92±21.32	0.22
Oral Epithelial Dysplasia	58.39±20.90	
Oral Epithelial Dysplasia	58.39±20.90	<.001
OSCC	143.70±44.69	
OSMF	41.92±21.32	<.001
OSCC	143.70±44.69	

Table 2
Comparison of IL-6 levels in saliva amongst various sub-groups of oral epithelial dysplasia

Subgroups	Mean ±SD	P value
Mild dysplasia	33.5 ± 10.06	0.65
Moderate dysplasia	60.25 ± 13.08	

Mild dysplasia Severe dysplasia	33.5 ± 10.06 80.8 ± 0.86	0.05
Moderate dysplasia Severe dysplasia	60.25 ± 13.08 80.8 ± 0.86	0.175

Table 3
Comparison of il-6 levels in saliva amongst various sub-groups of oscc

Subgroups	Mean ±SD	P value
WDSCC MDSCC	98.3 ± 5.94 142.7 ± 24.9	0.003
WDSCC PDSCC	98.3 ± 5.94 214.0 ± 18.1	.000
MDSCC PDSCC	142.7 ± 24.9 214.0 ± 18.1	.000

Discussion

Various studies have been done in the past reflecting the use of interleukin 6 as a biomarker in serum. However, in the present study salivary samples have been taken owing to have an advantage over serum samples in being non- invasive, cost effective and lesser chances of infection in already immunocompromised patients.

The present study was planned to assess the diagnostic utility of salivary IL- 6 levels in potentially malignant disorders and OSCC. In our study, the mean age of patients of oral submucous fibrosis is 43.9 ± 54.8 years, oral epithelial dysplasia is 55.7 ± 68.5 years and oral squamous cell carcinoma is 52.8 ± 64.1 years**.** The age criteria were accordance with the previous study done by Kapila S.N et al (2017)[5]. We have found in our study that in both the groups, patients were more than 40 years of age. Similar results were found in the previous studies done by Kapila S.N et al (2017) and Abdulla R et al (2018)[6].

Oral cancers mainly found in males due to a high prevalence of tobacco use, particularly chewing, smoking, and alcohol drinking in the male population[7]. In our study male predominance is seen in both the study groups having 80% male patients and 20 % female patients. 08(20%) males and 02(5%) females in OSMF, 09(22.5%) males and 01(2.5%) females in oral epithelial dysplasia and 15(37.5%) males and 05(12.5%) female patients are observed in oral squamous cell carcinoma**.** The incidence of potentially malignant disorders and oral squamous cell carcinomas (OSCCs) varies in different parts of the world and this difference is largely attributed to the exposure to risk factors specific to the area. Oral cancer is related to the use of tobacco chewing in the form of betel quid, tobacco smoking and reverse smoking as well as other factors such as alcohol consumption, low socioeconomic status, poor hygiene, poor diet and viral infections, ill-fitting dentures, and chronic irritation from rough or fractured teeth[7].

In our study, the most common site of occurrence was the buccal mucosa 25(62.5%) in both the groups followed by tongue 09(22.5%), alveolus and commissures 03(7.5%), palate 02(5%) and gingiva 01(2.5%). This is probably because majority of the lesions correspond to the site of maximum exposure to betel quid and also to other related habits**.** Salian V et al (2016), Dineshkumar T et al (2016)[8] and Kapila S N et al (2017)[5] in their analysis also found buccal mucosa to be the most common site as seen in our study. Thus, our results are in accordance with previous findings. In contrast to our study, the study done by Shenoi R et al (2012)[9] in their study, found the mandibular alveolus to be the most common site.

Analysis of salivary IL-6 in oral potentially malignant disorders and oral squamous cell carcinoma patients was done by Enzyme Linked Immunosorbent Essay. The mean ELISA salivary IL 6 value in oral submucous fibrosis is 41.92±21.32pg/ml, in oral epithelial dysplasia is 58.39±20.90pg/ml and in oral squamous cell carcinoma is 143.70±44.69pg/ml. in our study; this was in accordance with the study done by Dineshkumar T et al (2016)[8]. Analysis of saliva revealed markedly higher levels of IL-6 in OSCC patients in comparison with patients of oral submucous fibrosis and oral epithelial dysplasia.

On comparing, the mean ELISA values were found to be statistical significant between oral epithelial dysplasia, OSMF and oral squamous cell carcinoma ($p < 0.001$). Juretic M et al (2013)[10] in their study stated that the IL 6 levels in whole saliva were significantly elevated in patients with oral premalignant and malignant disorders. Kaur J et al (2015)[2] also reported that the salivary and serum cytokines IL 6 were elevated in oral precancerous lesions, Oral epithelial dysplasia, oral submucous fibrosis and oral lichen planus. Dineshkumar T et al (2016)[8] also evaluated high salivary and serum Interleukin-6 Levels in Oral Premalignant Disorders and Squamous Cell Carcinoma. Similar results were observed in the studies of Babiuch K. et al (2020)[11] and Brailo V. et al (2012)[12]. These studies also observed that Salivary IL-6 levels were significantly higher in oral cancer patients than in patients with potentially malignant disorders.

In present study, on comparing the IL-6 levels in saliva in subgroups of oral epithelial dysplasia a statistically significant difference was observed only between mild epithelial dysplasia and severe epithelial dysplasia ($p = 0.05$). Similar results were observed in the study of Dineshkumar T et al (2016)[8] and Panneer selvam N et al (2015)[13]. On comparing the mean ELISA value of salivary IL-6 between subgroups of oral squamous cell carcinoma a highly significant statistical difference was observed between well differentiated OSCC and moderately differentiated OSCC ($P = 0.003$), well differentiated OSCC and poorly differentiated OSCC ($P = 0.000$) and moderately differentiated OSCC and poorly differentiated OSCC ($p = 0.001$). This data imply an oncogenic role for IL-6. It is possible that cytokines with pro-inflammatory and pro-angiogenic activity are produced by squamous cell carcinomas and could contribute to the progression of oral cancer[14]. The results observed in the study done by Juretic M et al. (2013)[10] also showed statistically significant difference between oral squamous cell carcinoma subgroup. The results observed in the study of Panneer selvam N et al (2015)[13] were not statistically significant.

From our study we have come to an inference that IL-6 is implicated in various cancers in suppressing apoptosis and accelerating uncontrolled cell growth via activating growth factor and related signaling pathways. The increase in salivary IL-6 in oral epithelial dysplasia, oral submucous fibrosis and OSCC might point out its local production by the tumor cells. Our study strongly suggests a positive correlation between salivary IL-6 and cancer development by observing much higher levels of salivary IL-6 in oral cancer patients than in oral potentially malignant disorders, suggesting a possible role of IL-6 in oral carcinogenesis and thus a reliable marker for early detection of malignant transformation with prognostic significance. The existence of difference in salivary interleukin 6 levels between these lesions might also indicate the progression of precancer to cancer. In our study, we suggest that IL-6 is not only a good biomarker in terms of diagnostic accuracy but also a good prognostic factor with high predictability for OSCC.

Conclusion

A vast number of molecular markers have been correlated with diagnosis of OSCC, illustrating the complex events leading to carcinogenesis and cancer progression. Proinflammatory cytokine interleukin 6 (IL-6) has various biological functions apart from regulating inflammatory response, this cytokine plays significant role in the development of cancer by enhancing proliferation and hindering apoptosis. It down regulates p53 expression by enhancing ribosome biogenesis, thus we hypothesized that IL-6 may cause similar changes in inflamed tissues, thus activating a mechanism that favours neoplastic transformation. In the past decade, saliva has emerged as a medium for disease analysis, including local and systemic conditions. It is more sensitive, non-invasive, cost-effective, and patient-friendly method that is easily collectable and transferable for various analysis or tests.

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