Interleukin-6 as a Diagnostic Salivary Biomarker in Oral Squamous Cell Carcinoma and Oral Potentially Premalignant Disorders: A Cross-sectional Pilot Study

Pathology Section

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ABSTRACT

Introduction: Worldwide, Oral Squamous Cell Carcinoma (OSCC) is the 6th major cancer and 2nd major in India. This high prevalence is linked to carcinogens, particularly smoking tobacco and chewing betel nuts and due to lack of a quick, accurate, and cost-effective screening diagnostic tool. Immunological biomarkers have the potential to identify and offer a prognostic assessment of this condition. Interleukin-6 (IL-6) is a significant salivary marker among the many other inflammatory markers.

Aim: To assess the level of IL-6 in OSCC and Oral Premalignant Diseases (OPMD)

Materials and Methods: The present cross-sectional pilot study was conducted at Pathology department of Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India for detection of IL-6 level in OSCC and OPMD saliva samples. A total of 30 patients from each group diagnosed with OPMD and OSCC were recruited consecutively over the period of two years (June 2022-May 2024). Demographic features such as age, gender, habit of tobacco chewing and site of lesion on buccal mucosa were recorded. The level of IL-6 was determined from saliva sample in each group and was compared with healthy control by using Enzyme-Linked Immunosorbent Assay. Kruskal Wallis test was applied for comparison between medians of two groups. IBM Statistical Package for Social Sciences (SPSS) version 25 was used for statistical analysis.

Results: The clinicodemographic features revealed that tobacco chewing was the most common aetiology in 80% of OSCC diagnosed cases and 90% in OPMD cases. The median and Interquartile Range (IQR) of salivary IL-6 in OSCC was 138.28 pg/mL (IQR 44.21-254.69), OPMD's 24.81 pg/mL (IQR 13.69-53.38) in comparison to healthy controls, 8.71 pg/mL (IQR 5.10-13.08).

Conclusion: The findings of this study demonstrate that salivary IL-6 level is higher in OSCC cases than OPMD cases and both have potential in early diagnosis. Due to wide array of functional characterisation saliva biomarkers, it can be a potential non invasive biomarker to differentiate the OSCC and OPMD cases.

Keywords: Betel nuts, Oral lesions, Tobacco, Enzyme linked immunoabsorbent assay

INTRODUCTION

Worldwide OSCC is 6th and 2nd most common oral cancer in India. Tobacco especially betel quid chewing is one of the region specific epidemiological factors [1]. Tissue biopsy and histopathological analysis are the gold standard method for diagnosis. Patient's willingness to undergo biopsies for histopathological diagnosis is poor, due to which the early detection is difficult. Moreover, differentiating OPMDs like leucoplakia, erythroplakia Oral Sub Mucous Fibrosis (OSMF) and OSCC is difficult because the sign and symptoms of OMPD and OSCC are near to similar. So, exploration of simple, reliable and cost-effective non-invasive diagnostic methods is essential to differentiate malignant and benign lesions.

Non-invasive sample collection and cost effective sampling methods derived from saliva and serum have been explored a lot for the analysis of varieties of tumour markers for positive diagnosis, stage prediction and disease prognosis [2]. Saliva has raised considerable interest for OSCC diagnosis for Circulating Tumour Cells (CTCs), circulating tumour DNA (ctDNA), miRNA and extracellular vesicles [3-6]. Pro-inflammatory cytokines like IL-6, IL-1, IL-8, Tumour Necrosis Factor (TNF) alpha and Interferon gamma favour the growth of the tumour. Cell proliferation, Epithelial- Mesenchymal Transition (EMT) and angiogenesis are the major factors for tumour growth and regulated by cytokines. Among the various reported markers, IL-6 is a prominent salivary marker for OSCC [7]. So, IL-6 levels in saliva could be used as a biomarker for diagnosis of OSCC and to differentiate it from OPMDs in high-risk cancer areas. Additionally,

IL-6 might be important as a potential therapeutic target in OSCC treatment. Objective of the study was to estimate the expression level of IL-6 in the OSCC and OPMD and to compare with healthy control for early diagnosis and therapeutic action.

MATERIALS AND METHODS

The present cross-sectional pilot study was conducted at pathology Department of Kalinga institute of Medical Sciences, Bhubaneswar, Odisha, India to determine the IL-6 level in both OSCC and OPMD diagnosed patients after the approval of Institutional Ethics Committee. (IEC No: KIIT/KIMS/IEC/1025/2022).

Sample size selection: For this pilot study minimum sample size was kept 30 for each group in equal ratio (1:1:1) among the groups as convenient sample. The sample collection from both OSCC and OPMD diagnosed patients were done from surgical and onco-surgical outpatient and inpatient over the period of two years (June 2022-May 2024). Saliva sample was collected in a sterile tube from each patient after signed consent.

Inclusion criteria: Inclusion criteria were, age more than 18 years, clinically diagnosed OSCC and OPMD cases who were not treated for any form of definitive therapy such as surgery, radiation, chemotherapy, or any other adjunctive treatments.

Exclusion criteria: Patients were excluded those having systemic illness, oral inflammatory conditions such as gingivitis, periodontitis and OSMF like difficulty in opening of mouth, palpable fibrotic bands etc.

Study Procedure

For comparison age sex-matched healthy individuals were included in the study population in a 1: 1: 1 ratio. For cases, before confirming with tissue histopathological analysis, 5 mL of saliva samples were collected. Samples were taken into analysis after the diagnosis of OSCC and OPMD cases by histopathological confirmation. For healthy control, age-sex-matched volunteers (n=30) with no current use of prescribed or non-prescribed medication, no chronic/ acute illnesses, no oral lesions no acute or sub-acute inflammation or infection of the oral cavity were included in the saliva sample collection.

Saliva samples (5 mL) were collected between 9 and 11 A.M under non-stimulatory conditions and immediately after collection, cell debris was removed from the sample by centrifugation. Supernatants were collected and then stored at -80ºC for further use. IL-6 quantification was done by using commercially used sandwitch precoated antibody plate (E-EL-H0149, E-EL-H0102 and E-EL-H0048 kits) (Elabscience; Wuhan, Hubei, China). IL-6 values were measured at 450 nm absorbance and the at the detection range of 7.8-500 pg/mL. As per the KIT manufacturer, Eight-point standard curve was used for calculation and interpolation of data, using the Graph Pad Prism (version 6 software)

STATISTICAL ANALYSIS

All statistical analyses were carried out using IBM SPSS version 25 with the Kruskal Wallis test method. Data has been expressed in mean±SD and p-value <0.05 was considered to be statistically significant.

RESULTS

Demographic features of three groups (OSCC, OPMD & healthy control) revealed the median age in each group was ranged between 21-90 years. OPMDs consisted of 16 cases of leucoplakia, three cases of lichen planus, five erythroplakia, and six cases of OSMF [Table/Fig-1]. Majority of patients were male in each group. The most common site of OSCC and OPMD cases is Buccal mucosa followed by the tongue. Tobacco chewing was the most common addictive habit in both the OSCC and OPMDs study groups. In both OSCC and OPMD cases, the range of age group was 41-50 years (43.3-36.6 %). In OSCC cases the male: female ratio is 6.5:1 and OPMDs cases the ratio is 5:1. Among the addictive habits Tobacco chewing remains the most important addictive habit in both OSCC and OPMDs cases which is 80% and 90% in both study groups respectively. In the majority of cases, the location was buccal mucosa (56.6% in OSCC and 76.6% in OPMD).

Category	OSCC (n=30)	OPMD (n=30)	Control (n=30)
Age (in years) Median (Range)	47 (31-90)	49 (21-70)	34 (31-70)
Sex			
Male	26 (86.66%)	25 (83.3%)	14 (46.6%)
Female	04 (13.3 %)	05 (16.66%)	16 (53. 3%)
Habits			
Tobacco Chewing	24 (80%)	27 (90%)	-
Smoking	04 (13.3%)	-	-
Alcohol	02 (6.66%)	-	-
None	-	03 (10%)	
Site of Lesion			
Buccal Mucosa	17 (56.66%)	23 (76.66%)	-
Gingiva	03 (10%)	0	-
Tongue	06 (20%)	05 (16.6%)	-
Palate	02 (6.66%)	0	-
Alveolus	01 (3.33%)	0	-
RMT	01 (3.33%)	02 (6.6%)	-

[Table/Fig-1]: Comparative demographic features between OSCC, OPMD cases and healthy control.

[Table/Fig-2] shows the level of IL-6 in saliva sample of cases and healthy control. In case of OSCC and OMPD, IL-6 is highly significant in OSCC followed by OMPDs as compared to healthy controls (p-value <0.00001) [Table/Fig-3]. According to the box plot analysis, the level of IL-6 is widely distributed with median: 138.28 pg/mL among OSCC patients distribution in comparison to OMPD (median: 24.81 pg/mL). While comparing with healthy control, both OSCC and OMPD cases were showing elevated level of IL-6 than healthy control (median: 8.71 pg/mL).

IL-6	Median	Q1	Q3	Kruskal Wallis test
OSCC (n=30)	138.28	44.21	254.69	H value: 48.58 p-value:<0.00001 (S)
OPMDs (n=30)	24.81	13.69	53.38	
Healthy control (n=30)	8.71	5.10	13.08	

[Table/Fig-2]: IL-6 levels in saliva sample of OSCC, OPMD and healthy controls.
p-value <0.05 is significant
S: Significant
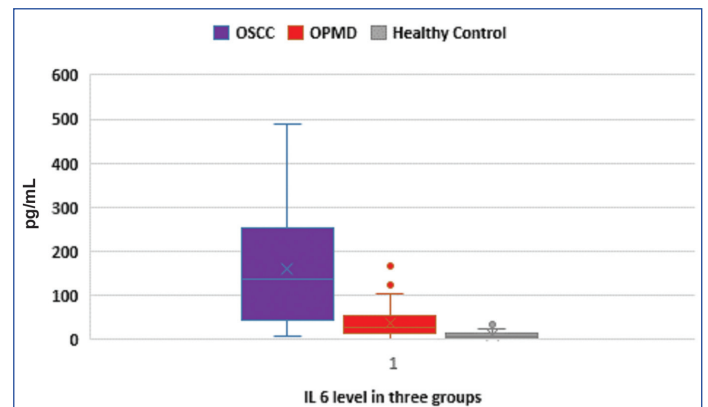
[Table/Fig-3]: Comparative IL-6 level among three groups in the distributed population of Oral Squamous Cell Carcinoma (OSCC), Oral Premalignant Disease (OPMD) and healthy control.

DISCUSSION

In the present study, median age of OSCC and OPMDs cases was 47 and 49 years respectively, which is supporting other authors. Shah Jigna S and Dubey Jaya reported that 45 years age is the most common age for OSCC and OPMDs [8]. Early and prompt diagnosis is the cornerstone for increased survival and longevity of the patients. Although histopathological diagnosis is the gold standard for diagnosis it requires skilled manpower and is time-consuming. So, there is a dire need for biomarkers that can help in aiding histopathological diagnosis for early detection of OSCC. Research is being conducted worldwide for the establishment of relatively simple and reliable non-invasive and cost-effective diagnostic methods that can differentiate malignant from benign lesions quickly with a concentration on analysing several biomarkers that can be obtained from oral tissue, saliva, and serum to know the prognosis of patients with OSCC and facilitate its early detection. In this study, evaluation of the IL-6, a biomarker in OSCC and OPMDs was done.

Complex events occur in carcinogenesis and progression of oral squamous cell cancer. A vast number of molecular markers have been correlated with the diagnosis of OSCC. Despite regulating the inflammatory process, pro-inflammatory cytokines like IL-6, have various biological functions. By enhancing proliferation and hindering apoptosis, IL-6 plays a significant role in the development of cancers [7]. IL-6 has been considered as a selective serum biomarker in various diseases. However, in the present study, salivary samples have been taken because of non-invasive, cost-effective, and lesser chance of infections in immune-compromised patients. Elevated level of salivary IL-6 in OSCC and OPMD indicates that the increase in IL 6 level is due to local production of the cytokine in oral cancer tissue. This is in agreement with Pries R and Wollenberg B who stated that oral cancer cells and tumour-infiltrating lymphocytes are capable of producing IL-6 [9,10].

Salivary IL-6 can be used as a biomarker for diagnosing potentially malignant disorders as well as oral cancers and it has potential role for therapeutic action. This suggests that pro-inflammatory cytokines are elevated in the saliva of patients with OSCC and OPMD as compared to controls which may have diagnostic significance.

Limitation(s)
Limitation of the study includes low sample size and inclusion of only one cytokine.

CONCLUSION(S)
The present study, suggests that IL-6 can be a good biomarker for a diagnosis and also a reliable marker for early detection of OCSS. It also emphasises the malignant transformation of OPMDs to OSCC. It is a most sensitive, non-invasive, cost-effective, and patient-friendly method that is easily collectible and transferable for various analyses. Further longitudinal studies with increased sample size are needed to substantiate the salivary IL-6 biomarker for diagnosis of OPMDS and OSCC.

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AUTHOR DECLARATION:
• Financial or Other Competing Interests: None
• Was Ethics Committee Approval obtained for this study?   Yes
• Was informed consent obtained from the subjects involved in the study?   Yes
• For any images presented appropriate consent has been obtained from the subjects.   NA