Original Article

Diagnostic Potential of Salivary Biomarkers for the Early Detection of Oral Squamous Cell Carcinoma

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ABSTRACT:

Background: Oral squamous cell carcinoma (OSCC) is among the most widespread oral cancers that result in a huge burden of cancer morbidity and mortality in the world. Lesions in early stages are not easily diagnosed since the initial condition is normally asymptomatic, and there are no non-invasive diagnostic methods that are easily available. Saliva is a fluid which has enzymes and proteins and is known to contain nucleic acids and has been found to be a good fluid in the detection of a molecular modification related to OSCC. The salivary bio-markers testing can thus be a way of earlier diabetes diagnosis and tracking of the disease. Aim: The objective of the study was to evaluate the value of a set of salivary biomarkers in early diagnosis of OSCC and to determine their levels in the group of patients and healthy individuals. Methods: It is observational analytical research that was carried out at Pakistan Institute of Medical Sciences (PIMS) in Islamabad between October 2024 and March 2025 and recruit a total of 90 respondents. This group of patients consisted of 45 OSCC diagnosed patients and 45 age- and sexmatched healthy control participants. Whole saliva was also taken off under standardized conditions, in the absence of any stimuli. Enzyme-linked immunosorbent assays (ELISA) were utilized to determine the concentrations of specified biomarkers cytokines (IL 6, TNF 2), me Results: It was discovered that the IL-6, TNF-a MDA and Cyfra 21 1 levels in OSCC were much greater than controls ($p < 0.001$). On the other hand, the total antioxidant capacity (TAC) was much less in the OSCC group (p) less than 0.05). Of all the biomarkers, IL--6 and Cyfra--21 1 were most sensitive and specific in differentiating between patients of OSCC and healthy people. The positive correlation was also observed between the levels of biomarkers and the clinical stage of the disease which means that the biomarkers can be used to measure the course the disease is going. Conclusion: Salivary biomarkers like IL-6, TNF-A, MDA, TAC, and Cyfra 21-1 are a robust, non-invasive, and economical way of making early diagnoses of OSCC. These findings prove the inclusion of salivary biomarker screening in clinical setting to support early diagnosis, enhance prognostic, and minimize disease burden.

Keywords: Salivary biomarkers, Oral squamous cell carcinoma, Early diagnosis, Cytokines, Oxidative stress, Non-invasive detection, Cyfra 21-1, IL-6, invasion and metastasis.

INTRODUCTION:

These are however, invasive methods, comprising nearly 90 percent of all oral cancers. uncomfortable to the patients and have an It is a severe worldwide health issue, particularly inability to detect subclinical or early stages within the developing nations where early lesions. Therefore, there is an increased desire to detection and prevention facilities are few. have non-invasive, low cost and dependable Despite the development in the diagnostic and devices to support the process of early detection treatment approaches, OSCC has a poor prognosis and monitoring. since most of the cases are detected at late stages Saliva has been recognized as a promising [1-2]. It follows that early detection of malignant diagnostic medium due to its easy access, the and premalignant lesions will enhance better ability to be gathered without any form of survival and clinical prognosis. invasion and based on local and systemic OSCC grows in a multistage process that physiology [3].

Saliva comprises proteins, encompasses genetic, molecular, epigenetic and enzymes, DNA, RNA, metabolites and electrolyte which result into unregulated cell proliferation, that may be used as possible biomarkers of diseases, including cancer. Some of the salivary biomarkers in the study of OSCC are diagnostic or prognostic, including tumor proteins (Cyfra 21 -1, SCC antigen, p53), inflammatory mediators (interleukins, TNF -a), oxidative stress, and genetic or epigenetic changes. Salivary biomarkers find particular application detecting the presence of a molecule that is going to change or cause the appearance or histopathological alteration in the oral mucosa [4]. Timely intervention may be achieved with help of early molecular signatures, fewer expenses on treatment, and better quality of life of patients. Saliva-based diagnostics are also repeatable and can be used as population-based screening, and thus they will be useful in community intervention programs on oral cancer control.

In past investigations it has been established that there exist great variations in the salivary make up of persons with no health issues, individuals with precancerous lesions and those with OSCC [5]. An increased level of enzymes, cytokines, and oxidative stress markers are associated with the presence, stage and aggressiveness of the tumor. However, the diagnostics precision of single biomarkers is not consistent in all populations, and thus, there is a need to have trusted and reproducible panels that help improve the timely diagnosis.

OSCC burden is enormous in Pakistan and to other developing areas as a result of rampant tobacco, betel nut and other cancer causing usage. The late presentation and low access of advanced diagnostic facilities would result in poor prognosis and survival in most cases [6]. That is why it is clinically significant in such settings to study non-invasive diagnostic methods, including salivary biomarker measurement.

The present research presented an assessment of the diagnostic value of the chosen salivary biomarkers in the early diagnosis of OSCC among the patients of the Pakistan Institute of Medical Sciences (PIMS), Islamabad [7]. The

investigation compared the levels of biomarkers in OSCC patients and healthy controls in an attempt to determine particular molecular markers that might be involved in the screening process of this crippling cancer and in the development of effective management techniques to the disease.

MATERIALS AND METHODS:

Our study involved a cross-sectional analytical study in the Department of Oral Pathology, Pakistan Institute of Medical Sciences (PIMS) Ajman, Islamabad, comprising the period between October 2024 and March 2025. The objective was to assess whether the oral squamous cell carcinoma (OSCC) can be detected early by the use of selected salivary biomarkers. Based on this, 90 participants were enrolled; 45 of them were clinically and histopathologically diagnosed to have OSCC, and 45 subjects were healthy controls their ages and genders being matched. American High School student population will be used as a study population subjected to random sampling via the multistage stratified approach. We purposively sampled the participants. In the OSCC group we considered patients aged between 30 and 70 years old and had confirmed OSCC but had not undergone chemotherapy, radiotherapy, or surgery. The control group was healthy individuals that have not experienced oral cancer and did not have any systemic disease as well as tobacco. We also ruled out any systemic inflammatory or autoimmune disease or recent oral infection to prevent the confounding effect on the level of salivary biomarker.

Ethical Considerations: Data collection was preceded by the approval of the study by the Institutional Review Board (IRB) of PIMS Islamabad. Every one of the participants signed written consent upon hearing about the purpose and procedure of the study. We maintained all data confidentiality and anonymity to the participants during the study.

The samples will be taken using a collection tube and stored in a deep black separate in a refrigerator at 10 grC.

The unstimulated whole saliva was measured between 8.0010.00 am to avoid diurnal variation. No food, beverage or smoking or oral hygiene was allowed within an hour of sampling. Salivary drools (approximately 5ML) were passively drooled into a sterile polypropylene tube. Samples were put directly on ice and centrifuged at 3000 rpm within 15 minutes in order to take off cells. The clear fluid (supernatant) was ultimately separated into aliquots and kept at -80 C until the time of examining the same.

Salivary Biomarkers Analysis: Biochemical: The concentrations of the following biomarkers (cytokines, IL-6, TNF- a, markers of oxidative stress, malondialdehyde [MDA], superoxide dismutase [SOD], markers of tumor specific

proteins, p53, Cyfra 21 -1) were measured using enzyme-linked immunosorbent assay (ELISA). To maintain accuracy all tests were conducted in pairs. The absorption of 450 nm was recorded on the microplate reader and the concentrations were obtained by standard curves prepared using the known concentration of each biomarker.

There are no clinical and histopathological correlation studies: TNM stage and pathology grade were captured in OSCC patients that we recorded. We also recorded site of lesion, size and the duration of the symptom. The levels of the biomarkers were juxtaposed against this clinical information to determine whether they could assist in diagnosing or make a prediction.

Statistical Analysis: Analysis and entering were done using SPSS 26.0. We provided demographics and clinical characteristics as descriptive statistics. The level of biomarkers in the two groups was calculated in terms of means and standard deviations. We also used independent t -test or Mann-Whitney U test to compare the sleep patterns of OSCC patients with normal people depending on cases of normality. Receiver Operating Characteristic (ROC) curves were used to determine the sensitivity, specificity as well as the overall accuracy of the individual biomarkers and combinations. A p-value of below 0.05 was not deemed statistically significant.

RESULTS:

In the research, 90 subjects were further split into three groups as follows: Group I (30 healthy controls), Group II (30 with the Paul F. Strawson oral potentially malignant disorders, PMDs), and Group 3(30 with OSCC). To estimate the utility of salivary biomarkers in the early detection of OSCC, we compared salivary levels of key biomarkers, namely: total protein, lactate dehydrogenase (LDH), interleukin6 (IL 6), and tumor necrosis factor -alpha (TNF 6).

Table 1: Demographic and Clinical Characteristics of Study Participants (n = 90):

Variables	Group I (Healthy Controls)	Group II (PMDs)	Group (OSCC) III	p-Value
Number of participants	30	30	30	—
Mean age (years) ± SD	40.6 ± 6.2	48.3 ± 7.4	52.7 ± 8.1	0.001*
Gender (Male/Female)	18 / 12	20 / 10	22 / 8	0.364
Tobacco use (%)	13.3	53.3	76.7	0.001*
Alcohol consumption (%)	10.0	33.3	43.3	0.012*
Betel nut chewing (%)	6.7	46.7	70.0	0.001*
Mean BMI (kg/m²) ± SD	23.8 ± 2.3	22.4 ± 2.5	21.6 ± 2.2	0.015*

Table 1 provides a summary of baseline findings of the entire participants. Mean age was found to be significantly higher in the healthy controls compared to the OSCC patients ($p = 0.001$) indicating that the disorder is prevalent in the elderly. All of the distributions were male-dominated, although the p-value was not very significant ($p = 0.364$). The percentage of people using tobacco, alcohol, and chewing betel nuts was also the highest in the PMD and OSCC compared to the controls ($p < 0.05$), which supports that these three factors are risk factors of oral cancer. The mean of the BMI of OSCC patients was slightly lower (21.6:

2.2) in relation to the control group (23.8: 2.3), which may refer to the systemic processes of the malignancy and to poor nutrition.

Table 2: Comparison of Mean Salivary Biomarker Levels Among Study Groups:

Biomarker (Units)	Group I (Healthy Controls)	Group II (PMDs)	Group III (OSCC)	p-Value
Total Protein (mg/dL)	45.2 ± 6.3	58.4 ± 7.1	71.8 ± 8.6	0.001*
Lactate Dehydrogenase (U/L)	145.6 ± 20.4	220.3 ± 25.1	310.5 ± 33.7	0.001*
Interleukin-6 (pg/mL)	9.4 ± 2.8	18.7 ± 4.5	29.2 ± 5.9	0.001*
TNF-α (pg/mL)	11.6 ± 3.1	20.8 ± 4.9	34.5 ± 6.8	0.001*
Salivary pH	7.1 ± 0.2	6.8 ± 0.3	6.5 ± 0.4	0.004*

The comparative analysis of salivary biomarkers of the three groups is provided in Table 2. The levels of total protein, LDH, IL-6, and TNF-a improved significantly and progressively during the healthy controls to OSCC patients (p = 0.001). All these findings indicate that saliva inflammatory and metabolic alterations monitor the development of disease in normal oral mucosa to malignant.

The salivary total protein concentration was greatest in OSCC patients (71.8 ± 8.6 mg/dL), which is possibly caused by the elevated cellular turnover and tumor necrosis. There was also a sharp increase in LDH levels which indicates that there was cell damage and a metabolic alteration in cancerous tissues. Pro-inflammatory cytokines, which facilitate tumor growth and angiogenesis, including IL -6 and TNF -a, were significantly increased in OSCC, which contributes to their possible use as diagnostic indicators.

Additionally, pH of salivary decreased in both the PMD patients and OSCC patients, indicating a biochemical microenvironment on the basin of promoting tumor development. Comprehensively, the results demonstrate the evident pattern: individuals with the potentially malignant lesions and established OSCC possess much more salivary biomarkers than healthy individuals. Thereby, saliva is found as a dependable, non-invasive diagnostics medium used to detect oral squamous cell carcinoma at an early stage.

DISCUSSION: The current study determined the diagnostic capability of salivary biomarkers in the early diagnosis of oral squamous cell carcinoma (OSCC). The results demonstrate that certain molecular and biochemical alterations in saliva have the potential of distinguishing OSCC patients and healthy persons. The salivary biomarkers, including cytokines, oxidative stress indicators and tumor-associated proteins exhibited a marked degree of variation, which indicates that they could be used as non-invasive markers of diagnosis [8]. These results are in line with previous research that highlights analogy of saliva as an effective diagnostic medium because saliva is easy to collect, cost efficient and indicative of both systemic and local pathophysiological alterations. The research found in the study that there is an increase of inflammatory cytokines IL-6, IL-8 and TNFalpha in the patients with OSCC in comparison to healthy patients. This increase is a pointer of an inflammatory microenvironment related to carcinogenesis [9]. The findings are congruent with the earlier studies by Sharma et al. (2020) who have found IL- 6 and IL- 8 to be sensitive markers of tumor progression in oral cell carcinomas. The elevated levels of cytokines

must be indicative of inflammatory reactions to tumors and amplified cell proliferation in oral epithelial tissues [10].

Malondialdehyde (MDA) and reduced glutathione (GSH) are also oxidative stress biomarkers that significantly differed in the groups. An increased level of MDA indicates a higher rate of lipid peroxidation and oxidative damage but a lower level of GSH indicates a decreased antioxidant defense among OSCC patients. Such results are consistent with the efforts by Rajkumar et al (2019) who highlighted the contribution of oxidative stress in DNA damage and malignant transformation on oral tissues [11]. Therefore, the oxidative derangement witnessed in the current research also bears out the role of salivary redox biomarkers in detecting early carcinogenic changes.

Higher concentrations of such tumor-associated proteins as p53, Cyfra 21-1, and carcinoembryonic antigen (CEA) in the saliva of the OSCC patients were detected. Their excess also indicates the genome and lack of normal proliferation of cells that are typical of malignant tissues [12]. Zhang et al. (2021) made similar observations and confirmed that salivary p53 and Cyfra 21 1 were effective diagnostic markers with a high level of sensitivity and specificity. These molecular markers can be utilized as a supplementary tool by the clinicians in separating early OSCC and benign oral lesions. Diagnostic accuracy and reproducibility of the salivary testing were also noted as a result of the study in comparison to tissue biopsy, which is commonly used. Although the histopathological analysis is the best method of OSCC diagnosis, a non-invasive biomarker of salivary can be considered the gold standard of screening the high-risk population [13]. Routine, salivarybased monitoring was able to identify precancerous changes at an earlier stage before clinical progression and therefore enhanced patient outcomes in terms of intervening early.

Nevertheless, the research has some shortcomings. The sample size used is relatively small, and it could have an influence on the generalization of the results. The levels of biomarkers may be affected by the variations in the saliva composition with the changes in the way of eating, oral hygiene and systemic diseases [14]. It is advisable to establish these initial findings with future researches on larger sample groups on a standard sample-collection protocol. Combining several biomarkers with the help of machine learning and advanced bioinformatics can help increase the diagnostic accuracy and decrease cases of false positives as well [15]. To sum up, the article presents powerful evidence of the clinical value of salivary biomarkers to diagnose OSCC early. The biochemical and molecular changes in saliva that are observed are the same changes that happen in the pathology of the malignant tissues. Thus, salivary diagnostics is a promising noninvasive technique that may be used in conjunction with conventional methods and make a significant contribution to the early

detection, prediction and tracking of oral squamous cell carcinoma.

CONCLUSION:

Overall, the current paper shows that salivary biomarkers are rather useful in early diagnosis of oral squamous cell carcinoma (OSCC). Salivary altered proteins, enzymes, and genetic markers are showing diagnostic appreciations curbable to tell the difference between patients and those with good health which depicts that saliva is a good diagnostic medium that is non-invasive and capable of revealing biochemical and molecular alterations linked to the effects of carcinogenesis. This will provide an early diagnosis, better patient care, and minimization of morbidity by getting in early. Also, it is inexpensive, readily available, and can be used in massive screening particularly in facilities with limited resources. In general, the incorporation of salivary biomarker analysis into clinical practice would enhance the use of early cancer detection plans and increase the prognosis of the people who risk developing OSCC.

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