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ORIGINAL ARTICLE

Altered Salivary Enzyme Activity and Its Physiological Implications in Oral Squamous Cell Carcinoma. A Clinical Study

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and remains a major cause of morbidity and mortality, largely due to delayed diagnosis. Saliva has gained increasing attention as a non-invasive diagnostic medium because it reflects pathological and physiological alterations occurring within the oral microenvironment. Changes in salivary enzyme activity may serve as potential diagnostic and prognostic indicators of OSCC.

Objective: To evaluate alterations in salivary enzyme activity and to assess their physiological significance in patients with oral squamous cell carcinoma.

Methods: This clinical observational study included 59 patients with a histopathologically confirmed diagnosis of OSCC. Unstimulated whole saliva samples were collected under standardized conditions prior to the initiation of any treatment. Salivary enzyme activities, including salivary amylase, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP), were measured using standard spectrophotometric methods. Enzyme levels were analyzed in relation to demographic characteristics and clinical stage of the disease. Statistical analysis was performed using appropriate tests, and a p-value < 0.05 was considered statistically significant.

Results: Significant alterations in salivary enzyme activity were observed among the study participants. Salivary LDH and ALP levels were markedly elevated, particularly in patients with advanced-stage disease, while salivary amylase activity was significantly reduced. A strong correlation was noted between enzyme activity levels and clinical stage, indicating a progressive biochemical abnormality with disease advancement.

Conclusion: Oral squamous cell carcinoma is significantly associated with altered salivary enzyme activity, which correlates with disease severity. Elevated LDH and ALP levels, along with reduced salivary amylase activity, reflect tumor-related metabolic and physiological disturbances. Salivary enzyme analysis shows promise as a non-invasive adjunctive tool for disease assessment and monitoring in patients with OSCC.

Keywords: Oral squamous cell carcinoma; Saliva; Enzyme activity; Lactate dehydrogenase; Alkaline phosphatase; Salivary amylase

INTRODUCTION

The most prevalent neoplasm of the oral cavity is oral squamous cell carcinoma (OSCC) which is a major health issue of great concern of the people, especially in South Asian nations where tobacco, betel quid, areca nut use, and alcohol consumption is very common [1]. OSCC is received as the malignant change of the oral epithelial cells and is marked with local invasion aggressiveness, early shift of the lymphatics, and high morbidity and mortality [2]. Although there are further improvements in diagnostic and treatment modalities, the late-stage manifestation is established and leads to low survival rates. As such, there is an increasing demand to have effective non-invasive biomarkers that are reliable and can lead to early diagnosis and further assessment of prognosis [3].

The use of saliva as a diagnostic biofluid has developed as an appealing diagnostic biofluid because of the ease with which it can be collected, non-invasive method of collection, and abundance of biologically active molecules in the saliva [4]. The salivary enzymes are important in ensuring oral homeostasis since they help in digestion, antimicrobial defense, antioxidant defense, and tissue integrity. The salivary enzyme activity can change as an indicator of underlying pathological events, such as oxidative stress, tissue inflammation, metabolic dysregulation and neoplastic transformation [5]. Alterations to the oral microenvironment due to tumor in OSCC have the potential to alter the normal characteristics of salivary gland functions and enzyme secretion [6].

There are various enzymes reported to be altered in their activity in oral malignancies including amylase, alkaline phosphatase, lactate dehydrogenase and antioxidant enzymes. Such changes could be explained by the presence of an enhanced cellular turnover, hypoxia, membrane damage and reprogramming of metabolism with the progression of cancer [7].

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The interpretation of the physiological implications of the modulated salivary enzyme activity can be valuable and can be used to aid in the likeness of tumor biology as well as assist in development of saliva based diagnostic and monitoring system [8]. The current clinical trial was aimed to assess the changes in the salivary enzyme activities in patients of oral squamous cell carcinoma and to investigate their physiological importance in continuation to the pathology of the diseases [9]. The proposed study will help extend the current literature on the potential of saliva as a diagnostic medium in oral oncology because the study is targeted at a specific patient population.

MATERIALS AND METHODS

This clinical observational study was conducted in the Department of Oral Biology, Margalla Institute of Health Sciences, Rawalpindi, Pakistan from June 2022 till June 2023. The study included 59 patients with a confirmed histopathological diagnosis of oral squamous cell carcinoma (OSCC) who presented to the affiliated tertiary care hospital during the study period. Only newly diagnosed, untreated cases were enrolled; patients who had previously received surgery, chemotherapy, or radiotherapy were excluded to avoid treatment-related biochemical alterations.

Adult patients aged 18 years and above, of either gender, were recruited after obtaining written informed consent. Patients with systemic conditions known to affect salivary composition or enzyme activity such as diabetes mellitus, hepatic disease, renal disorders, autoimmune diseases, or active oral inflammatory and infectious conditions were excluded. Individuals taking medications that could influence salivary flow or biochemical parameters were also excluded to minimize confounding effects.

The study protocol was conducted in accordance with the Declaration of Helsinki, and ethical approval was obtained from the Institutional Ethical Review Committee. Demographic data and

relevant clinical information, including tumor site and clinical stage, were recorded using a structured proforma.

Unstimulated whole saliva samples were collected from all participants between 9:00 AM and 11:00 AM to reduce circadian variation. Participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for at least one hour prior to sample collection. Saliva was collected using the passive drooling technique into sterile containers while participants were seated comfortably in a relaxed position.

Collected samples were immediately placed on ice and transported to the laboratory. Saliva samples were centrifuged at 3000 rpm for 10 minutes to remove cellular debris, and the clear supernatant was separated and stored at −20°C until analysis. The activities of salivary amylase, lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) were determined using standard spectrophotometric methods according to manufacturer and laboratory protocols. Enzyme activities were expressed in international units per liter (U/L).

Statistical analysis was performed using SPSS software (version specified). Continuous variables were expressed as mean ± standard deviation. Comparisons between early-stage and advanced-stage disease groups were carried out using appropriate statistical tests. A p-value < 0.05 was considered statistically significant.

RESULTS

The study involved fifty-nine patients with oral squamous cell carcinoma that had a histopathology diagnosis. Table 1 summarizes the demographic and clinical characteristics of the population of the study. Most of the patients were men, and most of them were found in the middle-older age groups. The most frequently anatomically affected sites were buccal mucosa and tongue. A large percentage of the patients put forward would have advanced-stage disease.

The Enzyme activity was significantly changed as shown by salivary biochemical examination in patients with oral squamous cell carcinoma. Table 2 shows mean salivary levels of enzymes. Salivary lactate dehydrogenase and alkaline phosphatase enzyme levels significantly increased because the levels were higher than normal indicating high cellular turnover and tissue damage due to the malignant change. Conversely, the salivary amylase activity was also relatively reduced, which implies that there is a lack of functioning salivary glands and a change in physiological patterns of glandular secretions in the patient of OSCC.

The additional analysis demonstrated that there was a strong correlation between environmental factors stemming from salivary enzyme activity and clinical stage of disease. Table 3 demonstrates a significant difference between the mean lactate dehydrogenase and alkaline phosphatase levels of patients with early- and advanced-stage oral squamous cell carcinoma. This implies that the dysregulation of the salivary enzymes increases as the disease progresses.

In general, the findings demonstrate a great change in salivary enzyme activity in oral squamous cell carcinoma patients, and enzyme levels have an inverse relationship with the severity of the disease. High levels of lactate dehydrogenase and alkaline phosphatase are indicators of increased metabolic rate, tissue damage, and tumor mass, whereas low levels of amylase are indicators of poor salivary physiology in malignant diseases.

Table 1: Demographic and Clinical Characteristics of OSCC Patients (n = 59)

Variable	Category	Frequency (n)	Percentage (%)
Gender	Male	38	64.4
	Female	21	35.6
Age Group (years)	≤40	12	20.3
	41–60	29	49.2
	>60	18	30.5
Tumor Site	Buccal mucosa	24	40.7
	Tongue	18	30.5
	Gingiva	9	15.3
	Floor of mouth	8	13.5
Clinical Stage	Stage I–II	21	35.6
	Stage III–IV	38	64.4

Table 2: Salivary Enzyme Activity Levels in OSCC Patients

Salivary Enzyme	Mean ± Standard Deviation	Reference Trend
Salivary Amylase (U/L)	82.4 ± 21.6	Decreased
Lactate Dehydrogenase (U/L)	412.7 ± 96.3	Increased
Alkaline Phosphatase (U/L)	186.9 ± 44.8	Increased

Table 3: Comparison of Salivary Enzyme Levels According to Clinical Stage

Enzyme	Stage I–II (Mean ± SD)	Stage III–IV (Mean ± SD)	p-value
Salivary Amylase (U/L)	94.6 ± 18.3	75.1 ± 19.8	<0.05
Lactate Dehydrogenase (U/L)	348.2 ± 72.5	447.9 ± 88.4	<0.001
Alkaline Phosphatase (U/L)	158.3 ± 36.7	202.6 ± 41.2	<0.01

DISCUSSION

The current clinical trial compared changes in salivary enzyme activity of patients with oral squamous cell carcinoma, and investigated its physiological and clinical consequences [10]. The results indicate that there is a considerable disproportion of major salivary enzymes, and thus in favor of the notion that deviant transformation in the oral cavity has a far-reaching influence on the biochemical make-up of saliva [11]. These alterations do not merely represent local tissue damages but also the entire metabolic and inflammatory alterations related to the development of tumors. There was a significant rise in the salivary lactate dehydrogenase levels of the study population, especially among the patients at the advanced stage of the disease [12]. Lactate dehydrogenase is a cytoplasmic enzyme that is released when cells are damaged and necrotic and whose high concentrations in saliva are indicative of an increased turnover in the epithelial cells, hypoxia in tumors, and degradation of the basement membrane in the oral squamous cell carcinoma. The gradual increase in the level of lactate dehydrogenase over the clinical stage is an indication that it could be useful as an indicator of tumor weight and severity of the disease. These results match the past accounts of high levels of lactate dehydrogenase activities in the presence of oral malignancy because of even higher levels of glycolytic metabolism and tissue damage [13].

Patients with advanced disease also showed a significant increase in the level of alkaline phosphatase activity. Alkaline phosphatase may also be used in membrane transport and cellular differentiation, and its rise can potentially be due to augmentation in osteoblastic functions, inflammatory reactions and tumor-enforced tissue modeling. High level of alkaline phosphatase in the case of oral squamous cell carcinoma has been linked to invasive growth patterns and potential bone involvement hence further supporting its use as an indicator of disease progression [13]. Conversely, there was a significantly low activity of salivary amylase especially in patients who had an advanced tumor. The salivary glands mainly secrete salivary amylase which is important in digestion of carbohydrates and homeostasis of the oral environment [14]. A decrease in amylase activity can be an indicator of impaired work of salivary glands because of tumor foci, plugging of the ducts, inflammatory processes, or disrupted autonomic regulation. Reductions in salivary amylase can be a potential cause of poor oral physiological performance, predisposition to infections, as well as weakened oral mucosal protection systems in the patients with oral squamous cell carcinoma [15].

The variations in the salivary enzyme activity significantly point to the dynamic interrelationship between the tumor biology and the oral microenvironment [16]. This is because the tumor is directly

exposed to saliva, and so it is used to reflect the pathological changes that are taking place in the oral tissues. The fact that enzyme activity and the clinical stage correlate, confirms the possibility of using salivary biomarkers in diseases monitoring [17]. The clinical exigence of saliva collection is also boosted by the fact that saliva collection does not involve invasive procedures and thus can be used especially in screening high-risk groups or in disease progress or response to treatment [18]. In spite of the strengths, it has some limitations too. The lack of a healthy control group restricts the ability of a direct comparison with the level of the baseline enzyme of a non-diseased patient [19]. The sample size and single-center nature of the study is also a limitation to the generalization of the results. Further research using cohort studies with control groups and longitudinal studies on salivary enzyme diagnostic and prognostic value in oral squamous cell carcinoma is justified [20].

CONCLUSION

This study illustrated great changes in the activity of salivary enzymes in patients with oral squamous cell carcinoma that indicate the presence of pathological and physiological disruption that is linked to malignancy. A high level of salivary lactate dehydrogenase and alkaline phosphatase with decreased salivary amylase activity was closely related to the severity of the disease and clinical stage. This evidence supports the possibility that one can use salivary enzymes as useful, noninvasive biochemical signs in determining the progression of disease in oral squamous cell carcinoma. Salivary enzyme analysis should be incorporated into clinical assessment routine to develop more effective strategies of early detection and supportive data on disease surveillance, especially in resource-constrained environments. More extensive longitudinal and cross-sectional research is proposed to provide a set of standard values of references and identify their clinical relevance.

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Authors' Contributions:

A.Y. conceived and designed the study, supervised data collection, and drafted the manuscript.

S.N. contributed to clinical assessment, patient recruitment, and critical revision of the manuscript.

M.S.S. performed laboratory analysis, data interpretation, and statistical evaluation.

All authors read and approved the final manuscript.

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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