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Analysis of Salivary LDH (Lactate Dehydrogenase) as a Screening Tool for OSCC (Oral Squamous Cell Carcinoma): A Cross-sectional Pilot Study

Fakhrul Imam[1], Jachmen Sultana [2], Fazlay Rabbani [3], Amrin Sultana [4]

1 Department of Dental Surgery, BIRDEM General Hospital, Shahbagh, Dhaka, Bangladesh.
2 Department of Oral and Maxillofacial Surgery, Dhaka Dental College and Hospital, Mirpur -14, Dhaka, Bangladesh.
3 Department of Oral and Maxillofacial Surgery, Dental Unit, Dhaka National Medical College, Dhaka, Bangladesh.
4 Upazila Health Complex, Monohargonj, Cumilla, Bangladesh.

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Correspondence:
Fakhrul Imam,
Department of Dental Surgery, BIRDEM General Hospital, Shahbagh, Dhaka, Bangladesh,
fakhrulimam2014@gmail.com

ABSTRACT

Objectives: This study aimed to evaluate the feasibility of salivary lactate dehydrogenase (sLDH) as a potential screening biomarker for oral squamous cell carcinoma (OSCC).

Materials and Methods: This cross-sectional, comparative observational pilot study was conducted in the Department of Oral and Maxillofacial Surgery, Dhaka Dental College and Hospital, Bangladesh. A total of 60 participants were enrolled, comprising 30 histopathologically confirmed OSCC patients and 30 age- and sex-matched healthy controls. Unstimulated whole saliva samples were collected in the morning to minimize diurnal variation. Samples were obtained in sterile containers, transported to the laboratory within 15 minutes, centrifuged, and analyzed spectrophotometrically for LDH concentration. Demographic data and clinical information were also recorded. Statistical analyses were performed using independent t-tests, and a p-value of <0.05 was considered statistically significant.

Results: The mean sLDH concentration was significantly higher in OSCC patients (1449.17 U/L; SD ± 774.8) compared to healthy controls (408.7 U/L; SD ± 401.6). The difference was statistically significant ($p = 0.00001$).

Conclusions: Salivary LDH levels were significantly increased in OSCC patients compared with healthy individuals, supporting its potential as a simple, non-invasive, and cost-effective adjunctive screening tool. Within comprehensive head and neck oncology programs, sLDH estimation may serve as an initial stratifier to prioritize patients for definitive diagnostic evaluation. Larger, multi-centric studies are recommended to validate these findings and establish diagnostic thresholds for clinical application.

Keywords: LDH, OSCC, Biomarker, Liquid biopsy.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the major global public health challenges. According to the most recent Global Cancer Observatory (GLOBOCAN) data, cancers of the lip and oral cavity collectively account for over 377,000 new cases and more than 177,000 deaths each year. The incidence and mortality are disproportionately high in South and Southeast Asia, where the use of tobacco, areca nut/betel quid, and alcohol is widespread (1). From an epidemiological perspective, OSCC continues to be a disease of major concern. According to the latest GLOBOCAN 2022 report, approximately 389,846 new cases of cancers of the lip and oral cavity were recorded globally (16th most common site), highlighting the urgent requirement for low-cost screening tools that can be effectively utilized in primary care and community-based settings (2,3).

In Bangladesh, cancers of head, neck and

maxillofacial region constitute a large share of the national cancer burden, highlighting the urgent need for early detection strategies that are practical and can be applied even in primary and secondary care facilities (4). Although advances in surgery, radiotherapy, and systemic treatment have improved outcomes, survival in OSCC remains heavily dependent on the stage at diagnosis. Late presentation, when curative resection is often no longer feasible, results in significant avoidable mortality (1,5). The updated fifth edition of the WHO classification (2022-2024) further emphasizes the prognostic importance of depth of invasion, perineural and lympho-vascular invasion, and nodal spread features that are commonly present at the time most symptomatic patients seek medical attention (5,6). These realities underline the necessity of low-cost, non-invasive, and accessible screening modalities in resource-limited environments.

Saliva is increasingly recognized as a practical "liquid biopsy" for oral diseases. It comes into direct contact with dysplastic and malignant oral mucosa, can be collected repeatedly without invasive procedures, and is suitable for point-of-care application (7,8). Unlike blood samples, saliva offers logistical advantages, because it does not require venipuncture, carries minimal risk of biohazard, and easier handling under ambient conditions. Furthermore, it reflects locally released cellular and metabolic products with better specificity for oral lesions (7,8). Multiple systematic reviews have demonstrated promising diagnostic accuracy for saliva-based biomarkers-including nucleic acids, proteins, cytokines, and metabolites-in OSCC. Several panels have reported pooled sensitivities and specificities exceeding 80%, although variability in assay techniques and pre-analytical handling remains a limitation (9,10). Within this context, sLDH appears particularly promising as a single-analyte biomarker, because the test is inexpensive, rapid, available in routine diagnostic laboratories, and biologically relevant to tumor metabolism.

LDH is a cytoplasmic enzyme that catalyzes the interconversion of pyruvate and lactate with concurrent NADH/NAD^+ cycling. Malignant cells rely on aerobic glycolysis (the "Warburg effect"), leading to lactate accumulation and upregulation of LDH activity to sustain biosynthetic pathways and redox balance under hypoxic and inflammatory conditions (11-13). Increased cellular turnover, membrane disruption, and necrosis in dysplastic and cancerous oral tissues can release LDH into saliva. Additionally, inflammatory changes in potentially malignant disorders, such as leukoplakia and oral submucous fibrosis (OSF), may cause elevated sLDH levels even before carcinoma develops (13, 14). Clinical studies consistently report higher sLDH levels in OSCC compared to healthy controls, and frequently higher than in oral potentially malignant disorders (OPMDs), suggesting good discriminatory potential (13-15). A recent systematic review and meta-analysis (2025) identified sLDH as one of the most promising single biomarkers for oral cancer detection, particularly due to its simplicity, reproducibility, and feasibility compared to multi-omic panels requiring advanced platforms (9). Comparative studies also indicated that salivary LDH performs better than serum LDH in distinguishing oral lesions, reinforcing saliva as the preferred diagnostic medium (15).

For sLDH to be effectively utilized as a screening tool, three aspects must be considered: analytic validity, clinical validity, and clinical utility. Pre-analytical standardization is associated with vital factors, such as time of collection, fasting status, oral rinsing, recent dental procedures or bleeding, storage temperature, and prompt centrifugation, significantly influencing LDH activity (8,16). Analytical concerns include isoenzyme distribution (LDH-A/LDH-5 predominance in glycolytic tumors), assay linearity at higher values, and inter-laboratory comparability. From a clinical standpoint, a threshold-based strategy should prioritize high sensitivity to minimize missed cases, followed by confirmatory pathways, such as clinical oral examination, adjunctive visualization, or biopsy for screen-positive individuals. As inflammatory oral conditions and OPMDs may also elevate sLDH, its role is best conceptualized as a triage tool in high-risk populations-such as tobacco or areca nut users, patients with chronic oral ulcerations, erythroplakia, or leukoplakia-rather than a definitive diagnostic test.

In comprehensive head and neck oncology programs, salivary lactate dehydrogenase (sLDH) may function as an adjunctive risk stratification biomarker, complementing established clinical and cytological tools. By reflecting tissue breakdown, metabolic reprogramming, and cellular turnover associated with dysplasia and malignancy, elevated sLDH levels can help categorize patients into higher or lower risk groups for oral squamous cell carcinoma (OSCC). This

biochemical stratification enables clinicians to prioritize high-risk individuals for urgent diagnostic evaluation or biopsy, thereby facilitating earlier detection and optimizing resource utilization in multidisciplinary care pathways.

Considering the high prevalence of OSCC in South Asia and the feasibility of near-patient salivary testing, the evaluation of sLDH as a screening modality is both relevant and timely for Bangladesh and similar settings. By employing standardized collection protocols and routine chemistry platforms, this research aims to generate evidence readily applicable to clinical practice, with the broader objective of integrating sLDH into pragmatic screening frameworks for early OSCC detection.

2. Materials and Methods

This cross-sectional comparative observational study was conducted at Dhaka Dental College hospital, involving 30 OSCC patients and 30 healthy controls. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants. The **s**ample size has been calculated using the formula:

$$n = \frac{Z^2 pq}{d^2}$$

where, n=sample size, Z=1.96 (Z value of standard normal distribution at 5% level of significance), p=5 % (p=0.05) of OSCC patients, q= (1-p) = (1-0.05) =0.95, and d=0.05 (acceptable error in the estimate of d is set at 10% of p). Using the above formula, the estimated sample size was 73, but due to limitations of time, 30 cases were included in this pilot study.

2.1 Saliva Collection and LDH Measurement

Saliva samples were collected from 30 patients with histologically confirmed OSCC and 30 healthy controls, following a standardized protocol. Unstimulated whole saliva samples were collected in a sterile, disposable plastic container by spitting method. Morning samples were preferred to avoid diurnal variations in salivary flow and changes in the sialo-chemistry. Patients were advised to avoid intake of water or food one hour prior to sample collection to avoid interference of food and water with the enzyme levels. Samples were transported immediately to the nearby laboratory within 15 minutes and immediately centrifuged at 3000 rpm for 15 minutes at 4 °C to remove cellular debris. The clear supernatant was separated and stored at -20 °C until biochemical analysis. Salivary LDH activity was estimated using a standard spectrophotometric method based on the conversion of pyruvate into lactate, measured at 340 nm using a semi-automated biochemistry analyzer.

All assays were performed in duplicate using commercially available LDH reagent kits according to the manufacturer's protocol. Calibration and internal quality control procedures were conducted daily to ensure assay accuracy, precision, and reproducibility.

All samples were analyzed with the same calibrated LDH analyzer, with laboratory personnel blinded to clinical status. Data integrity was maintained through careful entry and cleaning, ensuring objective, reproducible results.

2.2 Statistical Analysis

Descriptive statistics were used to summarize demographic data. Data analysis was performed using SPSS, version 22. Comparisons between groups were carried out using the Student's t-test. A p-value <0.05 was considered statistically significant.

3. Results

A total of 60 participants were included in the study, comprising 30 histopathologically confirmed OSCC patients (case group) and 30 healthy individuals (control group). The demographic details are presented in Table 1. Both groups were comparable in terms of age and gender distribution, minimizing potential demographic bias.

Table 1: Demographic characteristics of study participants

Parameters	OSCC Group (n=30)	Control Group (n=30)
Mean age (years)	52.4 ± 8.3	50.9 ± 7.6
Gender (male/female)	12/18	17/13

The mean salivary LDH level among the control group was 408.7 ± 401.6 U/L, while the OSCC group showed a markedly higher mean value of 1449.17 ± 774.8 U/L. The difference between groups was highly statistically significant ($p = 0.00001$).

The absence of significant variation in age and gender distribution indicates that the elevated LDH levels are primarily associated with disease status rather than with demographic factors. The marked increase in LDH among OSCC patients reflects enhanced cellular turnover and tissue necrosis characteristic of malignancy, consistent with its established role as a marker of tissue damage and anaerobic metabolism.

4. Discussion

This study substantiates the biological and clinical concept that sLDH levels rise progressively with epithelial dysplasia and malignant transformation in the oral cavity, mirroring enhanced glycolysis, membrane damage, and increased cellular turnover characteristic of OSCC (13). The findings from this pilot study, conducted within the Bangladeshi population, are particularly noteworthy, as they represent one of the first local datasets to explore this biomarker in OSCC. Despite the relatively small sample size, the study demonstrated a strong and consistent effect size, affirming the robustness of the underlying biological signal.

Our results are in agreement with contemporary evidence demonstrating consistently elevated sLDH levels in both OSCC and oral OPMDs when compared to healthy individuals. Several case-control studies and meta-analyses have confirmed increased sLDH in OSCC and OPMDs (e.g. leukoplakia, oral lichen planus), supporting its potential role as a biomarker for risk stratification in surveillance clinics (17-19). Furthermore, a recent systematic review and meta-analysis (2025) identified LDH-especially as part of panels including MMP-9 and chemerin-as one of the most promising salivary biomarkers for oral cancer, with significantly higher levels in OSCC compared with controls, and elevated levels in OPMDs relative to controls, thereby reflecting a continuum from premalignant change to invasive carcinoma (17,20).

From a screening perspective, sLDH offers several practical advantages: it is inexpensive, non-invasive, easy to administer, rapid, and suitable for repeated use over time (21-24). These attributes are particularly relevant for high-risk populations, where early lesions may be clinically inconspicuous and access to specialist facilities is often limited. However, variations in diagnostic performance across published studies are notable, largely due to differences in sample collection protocols (unstimulated *versus* stimulated saliva, time of collection), laboratory methodologies (colorimetric activity assays *versus* isoenzyme profiling), and patient-related confounders (periodontal disease, oral trauma or systemic illness). Systematic reviews of salivary biomarkers for OSCC detection have recorded wide variations in sensitivity and specificity, indicating the necessity of standardized pre-analytical and analytical protocols before large-scale clinical implementation (17,25).

In South Asian countries, such as Bangladesh and India, where betel quid and smokeless tobacco consumption remains highly prevalent, the need for practical and affordable screening strategies is especially pressing (26). The feasibility demonstrated by this pilot study confirms that sLDH assessment can be successfully integrated into local clinical workflows, even in resource-constrained settings. National data confirms a substantial share of new oral cancer cases linked directly to such exposures (2,13). These findings provide a compelling rationale for incorporating sLDH into screening programs for habitual users, particularly where visual inspection alone may overlook early mucosal changes or patients are reluctant to undergo biopsy.

A structured clinical application pathway may be proposed. sLDH should be employed as an adjunctive tool, rather than a stand-alone test, in community screening camps and outpatient dental or ENT clinics. The process should begin with a risk assessment, including evaluation of tobacco, betel quid, and alcohol use, as well as nutritional status, followed by an opportunistic oral examination with photographic documentation. SLDH should then be measured using a standardized kit under defined conditions (21). Patients should be referred based on a combination of clinical findings and sLDH levels; those with clinically suspicious lesions and/or persistently elevated sLDH on repeat testing after 2-4 weeks should be directed for specialist evaluation and histopathological examination.

For OPMD surveillance, sLDH may serve as a useful monitoring tool. Higher levels have been reported in leukoplakia and other potentially malignant lesions

compared with normal mucosa, and progressive increases could signal risk of malignant transformation and prompt early biopsy, while stable or declining values might justify conservative follow-up with risk-factor cessation counselling (17,18,25). Since periodontal disease and mucosal inflammation can influence sLDH levels, concurrent assessment and management of oral hygiene is advisable to improve specificity (17).

An additional promising approach is integration of sLDH with multi-marker panels. Recent studies emphasized the value of combining biochemical markers, such as matrix metalloproteinase-9 (MMP-9), cytokines, and nucleic acid signatures with sLDH to enhance diagnostic accuracy. Development of a low-cost composite biomarker index-incorporating sLDH as a measure of tissue breakdown/glycolytic activity together with structural and genetic markers-should be a priority under harmonized protocols (17,27).

This pilot study also highlights several limitations, including possible spectrum bias due to hospital-based sampling, inadequate adjustment for oral inflammatory conditions, and the absence of isoenzyme-specific profiling, which may improve specificity by identifying tumor-associated LDH isoforms. Another major limitation is the lack of ROC (Receiver Operating Characteristic) analysis to determine diagnostic performance parameters, such as area under the curve (AUC), sensitivity, specificity, and optimal cut-off value for sLDH, which would have strengthened the assessment of its screening potential. Nonetheless, the feasibility, reproducibility, and significant group differences observed support the scientific justification for a larger, multi-center validation study. Future research priorities include: (i) community-based prospective studies with pre-defined sLDH thresholds and blinded histopathological verification; (ii) comparative evaluations of activity assays *vs*. isoenzyme panels; (iii) external validation of composite biomarker models; and (iv) pragmatic trials assessing turnaround time, cost-effectiveness, and patient-oriented outcomes (17,25).

In summary, this pilot study provides early, locally relevant evidence supporting the feasibility of sLDH measurement as a screening adjunct in Bangladesh. sLDH is a biologically credible and practically feasible marker for OSCC screening and OPMD surveillance, particularly in resource-limited, high-incidence regions. When integrated into risk-based algorithms, supported by standardized protocols, and ideally combined with additional salivary biomarkers, sLDH has the potential to facilitate earlier detection across the disease spectrum and reduce diagnostic delays leading to timely intervention.

5. Conclusions

The present study highlights the potential of sLDH as a simple, non-invasive, and cost-effective biomarker for the early detection and screening of OSCC. Elevated sLDH levels were consistently associated with the presence of OSCC, reflecting enhanced glycolytic activity and cellular turnover within malignant tissues. These findings suggest that sLDH estimation may serve as a valuable adjunctive non-invasive potential screening tool in routine clinical practice, particularly in high-risk populations where early diagnosis is crucial for improving prognosis and survival outcomes.

However, the pilot nature of this study represents a major limitation, as the small sample size and single-center, hospital-based design may restrict generalizability and introduce potential selection bias. Therefore, further large-scale, multi-centric studies with standardized methodologies are warranted to validate its diagnostic accuracy, establish reference values, and integrate it effectively into population-based screening programs for OSCC.

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Conflict of Interests

The authors declare no conflict of interests.

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