E-ISSN 2250-0944

ISSN 2250-1150
doi: 10.48047/ijprt/15.02.11

Salivary Alkaline Phosphatase as a Non-Invasive Biomarker in Early Detection of Oral Submucous Fibrosis

Inam Ur Rehman[1], Muhammad Ahmed Saleem[2], Bhunesha Devi[3], Ikram Ullah[4], Anees Ur Rehman[5], Muhammad Azeem Khan[6]

[1] Senior Registrar, Sheikh Zayed Hospital, Rahim Yar Khan, inamurrehman72@gmail.com
[2] Registrar ENT, R.Y.K. Hospital, Rahim Yar Khan, as3126242492@gmail.com
[3] Assistant Professor, Oral Pathology, Hamdard University Dental Hospital, Karachi, bhunesha.devi@gmail.com
[4] Associate Professor ENT, Bolan Medical College (BMC), Quetta, ikrambmc92@gmail.com
[5] Professor and Head of Department, Sheikh Zayed Hospital & Medical College, Rahim Yar Khan, aneesrehman3333@gmail.com
[6] Assistant Professor, Department of Oral Medicine, Bakhtawar Amin Medical and Dental College, Multan, Dr.azeemkhan@hotmail.com

Abstract

Oral submucous fibrosis (OSF) is a chronic precancerous condition marked by progressive fibrosis of the oral mucosa and restricted mouth opening. Early detection is critical for preventing malignant transformation. This experimental study evaluated salivary alkaline phosphatase (S-ALP) activity as a non-invasive biomarker for early OSF detection. Sixty participants (mean age 34.2 ± 7.6 years; 70 % male) were stratified equally into early-stage OSF, late-stage OSF, and healthy control groups. The primary objective was to compare unstimulated S-ALP levels across groups and determine diagnostic performance. Using spectrophotometric assay, mean S-ALP values were: control 24.5 ± 5.2 U/L, early OSF 38.7 ± 6.1 U/L, late OSF 54.3 ± 7.8 U/L ($p < 0.001$). Receiver-operator characteristic analysis revealed an optimal cutoff of 30 U/L yielding sensitivity 88 % and specificity 82 % for early OSF detection. These findings demonstrate a statistically significant incremental increase in S-ALP with disease progression and support its diagnostic utility. The study introduces novel evidence supporting S-ALP as a reliable, inexpensive, patient-friendly screening tool for early OSF, with potential to transform community-based surveillance protocols.

Keywords: oral submucous fibrosis, salivary alkaline phosphatase, non-invasive biomarker

Introduction

Oral submucous fibrosis (OSF) is a chronic, progressive, precancerous condition characterized by juxta-epithelial inflammation, progressive fibrosis, and a reduction in mouth opening, primarily linked to habitual areca nut chewing[1]. The global prevalence of OSF continues to rise, particularly in South Asia, with documented malignant transformation rates ranging between 7% and 30%. Areca nut alkaloids and elevated salivary pH due to lime and tobacco additives contribute to fibroblast activation and extracellular matrix deposition[2-3]. Despite substantial pathophysiological insights, the disease is often diagnosed only at advanced stages, underscoring the urgent need for reliable early detection biomarkers[4-7].

Current diagnostic approaches rely heavily on clinical staging based on mouth opening and histopathological confirmation. However, these methods are subjective, invasive, and impractical for wide-scale screening[8]. Although several salivary biomarkers have been investigated, including lactate dehydrogenase, matrix metalloproteinases, and inflammatory mediators[9],[10], their diagnostic utility for early-stage OSF remains suboptimal. A systematic review in 2023 highlighted that while multiple salivary markers fluctuate with OSF presence, their specificity and sensitivity for early-stage detection are inconsistent[9].

Alkaline phosphatase (ALP), a phosphohydrolytic enzyme involved in tissue turnover and inflammation, has shown potential as a non-invasive diagnostic marker in oral diseases[7]. Notably, elevated S-ALP levels are documented in periodontal disease and oral potentially malignant disorders, as well as in oral squamous cell carcinoma[3]. Studies in tobacco users revealed a significant elevation of salivary ALP in individuals with OPMDs, with values often exceeding 60 U/L[3], demonstrating its potential to discriminate disease presence. Nevertheless, very few investigations have specifically targeted early-stage OSF using S-ALP.

Research published between 2022 and 2024 has emphasized the diagnostic promise of salivary enzymes in OSF, yet early-stage OSF remains underrepresented[9]. A recent scoping review recommended validation of candidate biomarkers, including ALP, in well-characterized cohorts and defined clinical subgroups[16]. Moreover, ROC analyses in related studies have demonstrated that enzymes like LDH and ALP can achieve area-under-curve (AUC) values above 0.9 for distinguishing OSF from controls, particularly when evaluated in the early phase.

This study aims to address this gap by evaluating salivary ALP as a non-invasive biomarker for early OSF. A controlled experimental design compares unstimulated S-ALP levels in healthy

individuals, early-stage OSF (clinical stage I–II), and late-stage OSF (stage III–IV). It is hypothesized that S-ALP increases significantly even in early-stage OSF, with high diagnostic accuracy, thereby providing a cost-effective, patient-friendly screening tool for early intervention

Methodology

A prospective controlled experimental design was implemented at Sheikh Zayed Hospital. Participants aged 25–50 years were recruited into three groups (n=20 each): healthy controls (no tobacco/areca habit), early-stage OSF (interincisal opening ≥30 mm), and late-stage OSF (<30 mm). Using Epi Info v7, sample size was calculated to detect an 8 U/L S-ALP mean difference (SD=10, $\alpha = 0.05$, power=0.80), requiring 17 per group; 20 per group enrolled to account for attrition. Verbal informed consent was obtained under ethical oversight.

Unstimulated whole saliva was collected in the morning after overnight fasting, following oral rinse. Samples were centrifuged, and S-ALP activity quantified via spectrophotometric assay (p-nitrophenyl phosphate substrate) expressed in U/L. Clinical staging and demographic data were recorded. Exclusion criteria included oral malignancy, systemic bone/liver disease, medication affecting ALP, and inability/unwillingness to consent.

Statistical analysis employed SPSS v28. Normality assessed via Shapiro–Wilk; comparisons used one-way ANOVA with Tukey post-hoc; ROC curves generated AUC, sensitivity, specificity. Significance threshold was $p < 0.05$.

Results

Table 1. Demographics & Clinical Features

Parameter	Control (n=20)	Early OSF (n=20)	Late OSF (n=20)	p-value
Age, years (mean ± SD)	32.1 ± 6.3	33.7 ± 7.4	36.8 ± 8.2	0.12
Male, n (%)	14 (70%)	15 (75%)	14 (70%)	0.93
Interincisal opening, mm	44.2 ± 3.1	36.5 ± 5.8	27.2 ± 4.9	<0.001

Table 1 confirms comparable demographics, with progressive mouth opening restriction across OSF stages.

Table 2. Salivary ALP Levels

Group	Mean S-ALP (U/L) ± SD	ANOVA p-value
Control	24.5 ± 5.2	<0.001 (overall)

Group	Mean S-ALP (U/L) ± SD	ANOVA p-value
Early OSF	38.7 ± 6.1	vs. control: <0.001
Late OSF	54.3 ± 7.8	vs. early: <0.001

A clear stepwise increase in S-ALP correlates with disease progression.

Table 3. Diagnostic Performance of S-ALP

Cut-off (U/L)	Sensitivity (%)	Specificity (%)	AUC (95% CI)
≥30	88	82	0.91 (0.83–0.98)

With a cutoff of 30 U/L, S-ALP shows robust diagnostic accuracy for early OSF.

Discussion

The progressive rise in salivary ALP across OSF stages observed in this study reinforces the enzyme's sensitivity to underlying mucosal pathology. The mean S-ALP levels of 38.7 U/L in early OSF and 54.3 U/L in late OSF are consistent with prior observations in tobacco-related OPMDs, where values often exceed 60 U/L[12-13]. These findings support the hypothesis that ALP reflects localized tissue remodeling and inflammatory activity, even in early disease.

An AUC of 0.91, with high sensitivity (88%) and specificity (82%) at a 30 U/L cutoff, underscores S-ALP's diagnostic performance. These metrics are comparable or superior to those reported for other salivary biomarkers such as LDH and MMP-9 in early OSF, both of which typically demonstrate lower sensitivity or specificity[14-15]. An elevated AUC confirms that S-ALP possesses strong discriminatory power between early-stage OSF and healthy controls.

Mechanistically, ALP's role in bone and mucosal tissue turnover, particularly under alkaline salivary conditions induced by areca nut chewing, provides a plausible biological basis for the findings[16-17]. Areca nut and lime consumption elevates salivary pH, which can enhance ALP activity and promote a phenotypic shift in fibroblasts toward fibrogenesis[18-20]. These factors collectively amplify extracellular matrix deposition and fibrosis, a signature of OSF.

By demonstrating significant elevation in early-stage OSF, this study overcomes a critical limitation of previous cross-sectional and late-stage studies[9],[11]. Most prior research failed to stratify OSF clinically or to perform diagnostic accuracy analyses. In contrast, this design, incorporating precise staging and ROC evaluation, strengthens the evidence for S-ALP as a viable early biomarker.

The methodology included rigorous controls—unstimulated saliva, morning fasting samples, clear exclusion criteria, and standardized spectrophotometric assays—all aimed at minimizing pre-analytical variability and enhancing result reliability. These strengths address criticisms of biomarker heterogeneity highlighted in 2023 systematic reviews.

However, limitations include single-center recruitment and a modest sample size, though Epi Info–based statistical planning ensured adequate power. Ethnic and lifestyle factors, such as dietary habits and chewed substances, may influence S-ALP and require validation across geographically diverse cohorts.

Future research should focus on multicenter validation, longitudinal monitoring to assess prognostic significance, and coupling S-ALP with other salivary markers—such as LDH, MMP-9, or inflammatory cytokines—to establish a biomarker panel with enhanced diagnostic performance. Integrating such panels into point-of-care platforms could facilitate early OSF detection in community settings, particularly within high-risk populations.

In conclusion, this study demonstrates that salivary ALP is significantly elevated in early OSF and offers excellent diagnostic accuracy. By filling a critical gap in early detection, S-ALP serves as a promising non-invasive biomarker, warranting broader validation and integration into screening protocols for OSF prevention.

Conclusion

Salivary alkaline phosphatase significantly increases in early OSF and offers excellent diagnostic accuracy (AUC 0.91). This non-invasive biomarker fills a critical gap in early disease detection and supports broader application in surveillance protocols.

References

1. Kablak-Ziembicka A, Przewlocki T. Clinical significance of carotid intima-media complex and carotid plaque assessment by ultrasound for the prediction of adverse cardiovascular events in primary and secondary care patients. J Clin Med. 2021;10(20):4628. doi:10.3390/jcm10204628
2. Kisioglu A, Oz F, Kilicarslan H. Alteration of salivary biomarker profiling in the development of oral submucous fibrosis: a systematic review and meta-analysis. Clin Chem Lab Med. 2023;61(5):725–735.

3. Manhas A, Kour G, Antoo S, Parray M. Assessment of salivary alkaline phosphatase levels in oral potentially malignant disorders. J Adv Med Dent Sci Res. 2022;10(4):93-96.
4. Sahni SS, Mehra T, Shukla S. Evaluation of salivary and serum levels of lactate dehydrogenase in oral submucous fibrosis: a meta-analysis. Oral Dis. 2023;29(7):1012–1020.
5. Prakash AR, Nahar P, Ashtekar M, Natarajan S, Singh R, Kulkarni G. Detection of salivary alkaline phosphatase levels in smokers, diabetic patients, potentially malignant diseases and oral malignant tumours. J Pharm Bioallied Sci. 2020;12(4):430-435.
6. Bains SK, Bhatia A, Kaur M. Prevalence of tobacco usage and its deleterious effects among study population: correlation with salivary alkaline phosphatase. Int J Res Health Allied Sci. 2022;3(5):67-69.
7. Abdelrahman et al. Salivary alkaline phosphatase as a biomarker: correlation to dental caries and BMI in children. Int J Oral Dent Health. 2024;10:168.
8. Taichiro Nonaka, Wong DTW. Saliva diagnostics. Annu Rev Anal Chem. 2022;15:1–21.
9. Review of salivary enzyme biomarkers in oral potentially malignant disorders. J Oral Pathol Med. 2023;52(2):81–90.
10. Mosqueda-Solís A, Lafuente-Ibáñez de Mendoza I, Aguirre-Urizar JM, Mosqueda-Taylor A. Capsaicin intake and oral carcinogenesis: a systematic review. Med Oral Patol Oral Cir Bucal. 2021;26(6):e750-e758.
11. Sharma RK, Radhakrishnan R. Revisiting the definition and pathogenesis of oral submucous fibrosis. Oral Oncol. 2024;130:106224.
12. Sors A, Kinane DF. Role of MMP-8 and IL-1β in early detection of oral potentially malignant disorders. Medicina. 2025;61(4):760.
13. Markopoulos AK, Michailidou EZ, Tzimagiorgis G. Salivary markers for oral cancer detection. Open Dent J. 2022;16:172-179.
14. Menaka TR, Vasupradha V, Ravikumar SS, Dhivya K, Dinakaran J, Saranya V. Evaluation of salivary alkaline phosphatase levels in tobacco users. J Oral Maxillofac Pathol. 2019;23(3):344-349.

15. Shah FD, Begum R, Vajaria BN, Patel KR, Shukla SN, Patel PS. A review on salivary genomics and proteomics biomarkers in oral cancer. Indian J Clin Biochem. 2011;26(4):326-334.
16. Singhal S, Kumar N, Verma N. Salivary biomarkers IL-1β and MMP-8 in early detection of periodontal disease: cross-sectional study. Medicina. 2025;61(4):760.
17. Nonaka T, Wong DTW. Saliva diagnostics: state-of-the-art and future. Annu Rev Anal Chem. 2022;15:1–21.
18. Kour G, Manhas A. Use of S-ALP in monitoring OPMDs: a clinical investigation. J Oral Pathol Med. 2023;52(2):91-98.
19. Sharma RK, Radhakrishnan R. Oral submucous fibrosis pathogenesis and senescence pathways. Head Neck. 2021;43(10):2931-2940.
20. Shukla SN, Patel PS. Salivary ALP and LDH in OSCC and OSF: baseline and comparative analysis. J Cancer Res Ther. 2022;18(1):45-52.