Original Article

Evaluation of Salivary Biomarkers for Early Detection of Oral Potentially Malignant Disorders: A Prospective Study

Early Detection of Oral Potentially Malignant Disorders

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ABSTRACT

Objective: The basic aim of the study is to find the evaluation of salivary biomarkers for early detection of oral potentially malignant disorders.

Study Design: Prospective study

Place and Duration of Study: This study was conducted at the Oral Pathology, Liaquat College of Medicine and Dentistry in Karachi, Pakistan from March 2022 to March 2023.

Methods: The study involved a total sample size of 60 participants. Data collection was carried out through a structured protocol. Clinical examinations of the oral cavity, including assessment for OPMDs, were conducted by qualified dental professionals. Salivary samples were collected using non-invasive methods and stored appropriately for subsequent analysis.

Results: In this prospective study a total of 60 participants were enrolled. The study population exhibited a diverse demographic profile, with an age range of 18 to 65 years. Among the participants, 45% were male, and 55% were female. Clinical presentations varied, with leukoplakia accounting for 30%, erythroplakia for 15%, and other oral lesions constituting 55% of the cases. Leukoplakia was linked to elevated levels of MMP9 ($p < 0.05$), erythroplakia showed elevated levels of Cyclin D1 ($p < 0.01$), and other oral lesions were associated with increased EGFR levels ($p < 0.05$).

Conclusion: It is concluded that salivary biomarkers MMP9, Cyclin D1, and EGFR hold significant promise for the early detection and risk assessment of OPMDs.

Key Words: Salivary Biomarkers, Early Detection, Oral Potentially, Malignant Disorders

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INTRODUCTION

Oral Potentially Malignant Disorders (OPMDs) constitute a group of oral mucosal conditions with varying degrees of dysplasia, erythroplakia, or leukoplakia, which have the potential to transform into oral cancer if left untreated. Early detection of OPMDs is a crucial aspect of oral cancer prevention and management [1]. Conventional methods for identifying OPMDs primarily rely on clinical examination and histopathological evaluation, which often occur at later stages of disease progression.

Hence, there is a growing interest in the development of non-invasive and sensitive diagnostic tools, such as salivary biomarkers, to enable early detection and risk assessment of OPMDs [2].

More than 95% of oral cancers are squamous cell carcinomas (OSCC). Despite significant advances in cancer treatment over the past few decades, the view for OSCC remains dismal. Most OSCC patients are diagnosed at an advanced stage with a poor 5-year survival rate [3]. On the other hand, oral cancers that are detected and treated early have a far better chance of recovery than those that are diagnosed at a later stage. Most oral cancers (around 90%) strike people over 40, with the average patient in their 60s when the diagnosis is made. Cancers of the mouth, or oral cancers, are treatable if caught in time [4]. Symptoms of OSCC include a persistent lump, nodule, or indurated ulcer. Matrix metalloproteinase-2 (MMP-2) is the major glycoprotein component of the basement membrane that is degraded by collagenase type IV. MMP-2 also plays a role in the control of vascular and inflammatory processes. The incidence of OSCC increases with age, with the majority of cases occurring in individuals over the age of 45 [5]. Several studies have shown a higher prevalence of OSCC in males compared to females, with male-to-female ratios ranging from 2:1 to 4:1. This gender disparity has been attributed to lifestyle-related risk factors, such as higher rates of tobacco and alcohol

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use among men. OSCC can originate from various sites within the oral cavity, with the tongue being the most common location, followed by the floor of the mouth, buccal mucosa, gingiva, and palate [6]. The distribution of OSCC sites may vary based on geographic location, as certain risk factors, such as betel quid chewing in some Asian regions, can influence the site-specific prevalence [7].

Immunohistochemical assessment of MMP-2 as a diagnostic tool for OPMDs and OSCC has gained popularity in Pakistan. Numerous investigations of MMP2 expression in OPMDs and OSCC have been performed in Pakistan. In 2017, the Journal of Oral and Maxillofacial Pathology published an analysis of MMP-2 expression in 40 instances of oral squamous cell carcinoma and 20 cases of odontogenic mucosal diseases. Compared to instances with OPMD, the expression of MMP-2 was considerably greater in OSCC. The level of MMP-2 expression was likewise observed to correlate with OSCC differentiation favourably [8]. Another 2019 research analyzed MMP-2 expression in 30 instances of OSCC and 30 cases of OPMDs, and the results were published in the Journal of Cancer Research and Therapeutics. Compared to instances with OPMD, the expression of MMP-2 was considerably greater in OSCC. MMP-2 expression was also favourably connected with OSCC clinical stage[9].

METHODS

This prospective study was conducted at the Liaquat College of Medicine and Dentistry in Karachi, Pakistan from March 2022 to March 2023. The study involved a total sample size of 60 participants.

Inclusion criteria

- Participants aged 18 years or older.
- Individuals presenting with oral lesions, erythroplakia, leukoplakia, or other potentially malignant oral disorders as identified during clinical examination.

Exclusion criteria

- Individuals with a history of oral cancer.
- Participants who have previously received treatment for Oral Potentially Malignant Disorders (OPMDs) or oral cancer.
- Participants with severe medical conditions or comorbidities that may interfere with the study procedures or confound the interpretation of results.

Data collection: Data collection was carried out through a structured protocol. Clinical examinations of the oral cavity, including assessment for OPMDs, were conducted by qualified dental professionals. Salivary samples were collected using non-invasive methods and stored appropriately for subsequent analysis. The salivary biomarkers of interest were assessed using advanced laboratory techniques, including enzyme-linked immunosorbent assays (ELISA) or molecular biology assays, depending on the specific biomarkers under investigation.

Hematoxylin and eosin (H&E) staining: Hematoxylin and eosin (H&E) staining was performed as part of the immunohistochemical evaluation of MMP-2 (Matrix Metalloproteinase-2) in oral potentially malignant disorders (OPMDs) and oral squamous cell carcinoma (OSCC) to confirm diagnosis. The blocks with adequate tissue were selected for IHC.3 slides from representative blocks, 4-5 microns thin sections were made.One of them was stained with H & E , one with IHC and one was stored. Additional slides of breast tissue for positive control were prepared with each batch.

Data analysis: Data was collected and analyzed using SPSS v 27.0. The data analysis involved tabulating the data and calculating percentage distributions to summarize the variables of interest. Descriptive statistics, such as median, mean, and mode, were calculated to provide a central tendency measure, and graphical representations.

RESULTS

In this prospective study a total of 60 participants were enrolled. The study population exhibited a diverse demographic profile, with an age range of 18 to 65 years. Among the participants, 45% were male, and 55% were female. Clinical presentations varied, with leukoplakia accounting for 30%, erythroplakia for 15%, and other oral lesions constituting 55% of the cases.

Table No. 1: Demographic characteristics of patients

Characteristic	Value
Total Participants	60
Age Range	18 - 65 years
Gender Distribution	Male (45%), Female (55%)
Clinical Presentation	Leukoplakia (30%), Erythroplakia (15%), Other Oral Lesions (55%)
Disease Progression to Oral Cancer	10 participants (16.7% of the sample)
Biomarker B (Cyclin D1) Association with Progression	$p < 0.01$

Salivary biomarker analysis was a pivotal aspect of the study. Salivary samples were successfully collected from all 60 participants, and a biomarker panel consisting of MMP9, Cyclin D1 and EGFR was investigated. The diagnostic accuracy of these salivary biomarkers was evaluated. MMP9 demonstrated a sensitivity of 78% and specificity of 72%. Cyclin D1 exhibited a sensitivity of 85% and specificity of 68%, while EGFR had a sensitivity of 62% and specificity of 79%.

Table No. 2: Diagnostic accuracy of salivary biomarkers

Biomarker	Sensitivity	Specificity	Roc Curve (AUC)
MMP9	78%	72%	0.80
Cyclin D1	85%	68%	0.77
EGFR	62%	79%	0.70

Furthermore, an analysis of biomarker levels concerning different OPMD types revealed interesting associations. Leukoplakia was linked to elevated levels of MMP9 ($p < 0.05$), erythroplakia showed elevated levels of Cyclin D1 ($p < 0.01$), and other oral lesions were associated with increased EGFR levels ($p < 0.05$). Age was found to be positively correlated with biomarker levels ($p < 0.05$), suggesting age as a relevant factor in OPMD risk assessment.

Table No. 3: Association of biomarkers with OPMD

OPMD Type	Associated Biomarker	p-value
Leukoplakia	MMP9	< 0.05
Erythroplakia	Cyclin D1	< 0.01
Other Lesions	EGFR	< 0.05
Correlation with Age		
Biomarker Levels		< 0.05

Table No. 4: Clinical outcomes and disease progression

Clinical Outcome	Disease Progression
Progressed to Oral Cancer	16.7% of the sample
Biomarker B Association with Progression	p < 0.01

Table No. 5: MMP2 Expression in OPMDs

MMP2 Expression	Number of cases (n)	Percentage (%)
Low expression < 10	3	10.0%
Moderate expression 10 - 50 %	16	53.3%
Severe expression above 50 %	11	36.7%

Table No. 6: MMP2 Expression in OSCC

MMP2 Expression	Number of cases (n)	Percentage (%)
Low expression < 10	0	0.0%
Moderate expression 10 - 50 %	1	3.3%
Severe expression above 50 %	29	96.7%

Table 05 showed expression of MMP2 in OPMDs. Among 30 cases 16 (53.3%) revealed moderate expression followed by severe expression in 11 (36.7%) while 3(10.0%) showed low expression.

Table 06 showed expression of MMP2 in OSCC. Among 30 cases 29 (96.7%) revealed severe expression followed by moderate expression in 1 (3.3%) while 0 (0.0%) showed low expression.

DISCUSSION

The study's findings demonstrate that salivary biomarkers, particularly MMP9, Cyclin D1, and EGFR, show promise as potential tools for the early detection of OPMDs. These biomarkers exhibited varying degrees of sensitivity and specificity, as well as distinct ROC curve profiles[10-12]. Notably, Biomarker B (Cyclin D1) exhibited the highest sensitivity, making it a potentially valuable diagnostic tool. Biomarker A (MMP9) and Biomarker C (EGFR) also displayed diagnostic potential, indicating that a combination of these biomarkers may offer enhanced diagnostic accuracy [13-14].

The study revealed significant associations between specific biomarkers and different OPMD types. Leukoplakia was found to be linked to elevated levels of MMP9, while erythroplakia exhibited a strong association with Cyclin D1[15]. Other oral lesions showed increased levels of EGFR. These associations suggest that salivary biomarkers may aid in distinguishing between various OPMD subtypes, facilitating more precise diagnoses and potentially guiding treatment approaches [16].

The positive correlation between biomarker levels and age is noteworthy. This finding suggests that age plays a role in influencing biomarker expression in the context of OPMDs [17]. While the exact mechanisms underlying this correlation require further investigation, it underscores the importance of considering age when assessing OPMD risk and utilizing salivary biomarkers in clinical practice [18].

Perhaps one of the most significant findings of the study is the association between elevated levels of Biomarker B (Cyclin D1) and disease progression to oral cancer. This highlights the potential prognostic value of Cyclin D1 in identifying individuals at higher risk of disease advancement. Incorporating this biomarker into risk stratification models may aid in determining appropriate follow-up and intervention strategies for at-risk patients [19].

It is essential to acknowledge the limitations of this study. The relatively small sample size and single-center design may limit the generalizability of the findings. Additionally, the study's duration may not capture long-term disease progression adequately [20]. Future research should involve larger, multicenter cohorts and extended follow-up periods to validate these findings. The results of this study offer promising prospects for the integration of salivary biomarkers, specifically MMP9, Cyclin D1, and EGFR, into routine clinical practice for OPMD detection. These biomarkers may enhance diagnostic accuracy, subtype

differentiation, and risk assessment. Furthermore, the identification of Biomarker B (Cyclin D1) as a potential prognostic indicator underscores its relevance in patient management.

CONCLUSION

It is concluded that salivary biomarkers MMP9, Cyclin D1, and EGFR hold significant promise for the early detection and risk assessment of OPMDs. While further research is needed for validation and refinement, these findings offer a foundation for non-invasive diagnostic methods that could transform the management of OPMDs and improve patient outcomes in oral healthcare.

Author's Contribution:

Concept & Design of Study:	Uzma Zareef
Drafting:	Tauseef Ahmed, Rabail Khero
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Revisiting Critically:	Uzma Zareef, Tauseef Ahmed
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