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The Role of Salivary Biomarkers in Early Detection of Oral Precancerous Lesions

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Abstract

Background: Oral precancerous lesions are potentially malignant disorders that carry a high risk of transformation into oral cancer if not detected early. Conventional diagnostic methods rely on invasive biopsies, highlighting the need for reliable, non-invasive biomarkers. Salivary malondialdehyde (MDA), interleukin-6 (IL-6), and lactate dehydrogenase (LDH) reflect oxidative stress, inflammation, and tissue injury, respectively, and may serve as valuable diagnostic tools. **Objectives:** To measure and compare salivary levels of MDA, IL-6, and LDH between patients with oral precancerous lesions and individuals without such lesions, and to assess their diagnostic potential. **Methods:** A case-control study was conducted at the Oncology Center, Medical City, Baghdad, Iraq, between August 2024 and March 2025. A total of 102 participants were enrolled: 54 patients with clinically and histopathologically confirmed oral precancerous lesions and 48 lesion-free controls. Unstimulated saliva samples were collected under standardized conditions and analyzed for MDA, IL-6, and LDH levels using validated biochemical methods. Statistical analyses, including ROC curve evaluation, were performed to assess diagnostic accuracy. **Results:** Patients with oral precancerous lesions exhibited significantly higher salivary concentrations of MDA, IL-6, and LDH compared to negative cases ($p < 0.05$). ROC analysis demonstrated good diagnostic performance for all three biomarkers, with IL-6 showing the highest area under the curve, followed by MDA and LDH. No significant gender-based differences were observed in biomarker expression. **Conclusion:** Elevated salivary levels of MDA, IL-6, and LDH are strongly associated with oral precancerous lesions, suggesting their utility as non-invasive biomarkers for early detection. Incorporating these salivary assays into routine screening may facilitate timely intervention and reduce the progression to oral cancer.

Keywords: Interleukin-6, MDA, LDH, saliva, ROC

Introduction

Oral cancer belongs to the group of diseases that form an important aspect of global health morbidity. It is the type that evinces maximum concern with high rates of morbidity and mortality all over the world in spite of improved medical care facilities. In broad terms, cancers of the oral cavity are ranked within the ten most frequent malignancies worldwide, with greater incidence recorded for regions situated within South and Southeast Asia due to prevalent habits of using tobacco, areca nut chewing, and alcohol consumption (Sung et al., 2021). Poor survival outcome is basically linked with late-stage diagnosis; five-year survival rates drop dramatically when metastasis sets in (Warnakulasuriya, 2020).

A large number of cases of oral cancer develop from OPMDs, that group of lesions and conditions include oral leukoplakia, oral submucous fibrosis (OSMF), and erythroplakia having probabilities toward carcinoma development. The oral lesions considered for review included the ones most commonly observed transforming into malignancies but reporting different transformation rates from 1 to 36% based on type,

patient habits, and regions (Warnakulasuriya et al., 2020).

Saliva has come forth as a valuable diagnostic fluid. It is easy to obtain, non-invasively collected, and reveals the local as well as systemic pathophysiological changes (Kaczor-Urbanowicz et al., 2017). Recent advances in the science of salivary diagnostics have convincingly presented that this biofluid carries a broad spectrum of analytes-protein, nucleic acids, metabolites, cytokines, and markers for oxidative stress-among which many hold promise for detection of precancerous and cancerous oral lesions (Bonne & Wong, 2012). Indeed, comparative to blood-based tests, salivary analysis proves safer and more patient-friendly while being cost-effective – salient features making it attractive for the mass screening program in high-risk populations (Yakob et al., 2014). Among several salivary analytes brought under inquisitive investigation, biomarkers for oxidative stress received substantial attention. Oxidative stress is defined as the disbalance between systems producing reactive oxygen species and systems of anti-oxidizing defense mechanisms within an organism. Persistent oxidative stress can damage DNA and lipids and proteins; consequently be mutagenic and carcinogenic (Reuter et al., 2010). Malondialdehyde (MDA) is the byproduct of lipid peroxidation. It has gained acceptance as a probable indicator of oxidative stress. Many studies documented increased levels of this compound in both saliva and serum with OPMDs and OSCC, clearly reflecting damage induced by oxidation to the oral epithelial tissue (Baharvand et al., 2014; Sculley & Langley-Evans, 2003). Results proved that salivary MDA levels were higher in severely dysplastic cases and correlated with severity, making it an indicator of disease progression.

Chronic inflammation can be described as another major pathological process apart from oxidative stress in the initiation and progression of OPMDs. The signaling proteins cytokines released by immune and epithelial cells mediate the inflammatory response and have a great influence on carcinogenesis. Interleukin-6 (IL-6) is quite important among them. IL-6 is a pleiotropic cytokine that mainly governs immune regulation, and cell survival, among other functions. Increased levels of salivary IL-6 were observed in patients with leukoplakia as well as OSCC playing a role in increasing angiogenesis as well as the apoptotic pathways it might activate (Kaur et al., 2016). This pathway can further ensure the direct involvement of IL-6 in tumorigenesis through JAK/STAT3 signaling where it gets activated (Johnson et al., 2018).

Lactate dehydrogenase (LDH) is also a very important biomarker. This is a cytoplasmic enzyme of anaerobic glycolysis and is often used in oncology as an indicator of turnover and burden of the tumor. Under conditions of tissue breakdown and cellular necrosis, LDH is released to extracellular fluids. High levels indicate continuous epithelial disruption since OPMDs and OSCC patients show increased salivary LDH when compared with healthy controls. As altered epithelial metabolism and increased rate of apoptosis or necrosis are common findings in OPMDs, salivary LDH may be an early indicator for pathology. Besides that, the test for LDH is cheap and relatively easy, hence making it more applicable clinically. A relatively complete picture about molecular changes going on inside OPMDs could be reflected by these three markers: MDA, IL-6, and LDH. MDA captures oxidative stress and lipid peroxidation, IL-6 the inflammatory and immunological aspect, and LDH evidence of tissue damage as well as metabolic derangement. Their combined assessment may usher in better diagnostic accuracy, overtaking individual single-marker assessments. Several studies have suggested that biomarker panels have better sensitivity and specificity in distinguishing oral precancerous and cancerous lesions (Deepthi & Nandan, 2020; Kaczor-Urbanowicz et al., 2017). Thus, the integration of oxidative, inflammatory, and metabolic indicators in salivary diagnostics sits well as a promising strategy. Even with progress, literature falls short. Existing studies are few, mostly cross-sectional, with varying methodologies hence making concrete conclusions elusive. Standardization is sorely missing regarding saliva collection and processing as well as biomarker quantification making comparisons between studies difficult (Yakob et al., 2014).

This study aims to evaluate salivary Malondialdehyde (MDA), Interleukin-6 (IL-6), and Lactate Dehydrogenase (LDH) as non-invasive biomarkers for the early detection of oral precancerous lesions, to compare affected patients with healthy controls to establish their diagnostic potential

Patients and Methods

This case-control study took place at the Oncology Center, Medical City, Baghdad, Iraq between August

2024 and March 2025. It included 54 patients with oral precancerous lesions proven clinically and histopathologically (positive cases) and 48 individuals without oral lesions (negative cases). In all doubtful cases, a diagnosis was carried out by clinical assessment backed up wherever necessary by histopathological evaluation carried out by competent oral pathologists. A standard set of questions was used to note demographic details like age, gender, and related medical history. People who have long-term body diseases (like diabetes, high blood pressure or immune system problems) or any past cases of other types of cancer were kept out of the study. Unstimulated whole saliva was collected from all the individuals under standard conditions preferably morning time so that variability could be minimized, and at least 90 minutes after any intake of food or drink. The samples were then centrifuged at 3000 revolutions per minute for 10 minutes, and the supernatant was stored at –80°C until further biochemical analyses could be carried out. Biomarker analysis: The following biomarkers were analyzed: Malondialdehyde (MDA): estimated by thiobarbituric acid reactive substances (TBARS) assay. IL-6; quantified by a commercially available ELISA kit, strictly adhering to the manufacturer's instructions. LDH; determined based on the enzymatic colorimetric method. All tests were run in twofold to guarantee precision and repeatability of findings.

Ethical Considerations

This study was conducted based on the ethical guidelines of the Declaration of Helsinki. The Medical City Complex, Baghdad IRB provided ethical approval to conduct this study. After fully explaining the aim and procedures of this study to the participants, written informed consent was obtained from them. Participants' data were kept confidential.

Statistical analysis

IBM SPSS Statistics, Version 25 was used for data analysis, and descriptive statistics summarized demographic and clinical characteristics. Age and biomarker levels were presented as mean ± standard deviation (SD). Gender and diagnosis status were expressed in frequencies and percentages. Positive and negative groups were compared by the use of an independent samples t-test in the case of continuous variables, and a Chi-square test in the situation of categorical variables. Correlation between biomarkers and clinical parameters was measured by Pearson's correlation coefficient. The results were taken to be statistically significant at a p-value < 0.05. For comparisons involving more than two subgroups (location of tumor), ANOVA accompanied by post hoc testing (LSD) was applied to determine significant pairwise differences (Al-Fahham, 2018).

Results

Table 1 presents the age and gender distribution of positive and negative cases. Both groups shared relatively balanced proportions of age groups and gender, with no statistically significant difference observed (p = 0.46 for age; p = 0.33 for gender). This, therefore, denotes that these two groups are comparable by such demographic characteristics, thus reducing to a minimum the possible confounding effect in an analysis of salivary biomarkers (Table 1).

Table 1. Distribution of Age and gender of both positive and negative cases

Indicators		`Positive (No. = 54)		Negative (No. = 48)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	18-27	8	14.8	7	14.6	2.6	0.46 (NS)
	28-37	13	24.1	12	25.0		
	38-47	16	29.6	13	27.1		
	48-57	17	31.5	16	33.3		
Gender	Male	31	57.4	25	52.1	0.95	0.33 (NS)
	Female	23	42.6	23	47.9		

Results showed significantly higher salivary levels of MDA, IL-6, and LDH in positive cases when compared with negative controls. The differences were highly significant statistically ($p < 0.001$). This denotes conditions of increased oxidative stress, inflammation, and cell damage in patients having oral precancerous lesions thereby validating their potential usage in the development of a non-invasive diagnosis (table 2).

Table 2. Comparisons of salivary markers between positive and negative cases

Biomarkers	Positive (N= 54)		Negative (N= 48)		(P value)
	Mean	SD	Mean	SD	
MDA (µM/L)	5.8	1.2	2.9	0.8	<0.001
IL-6 (pg/mL)	12.4	3.5	4.6	1.7	<0.001
LDH (U/L)	320	45	210	30	<0.001

Table 3 shows that MDA, IL-6, or LDH in saliva were not significantly different between male and female patients with oral precancerous lesions. This denotes that gender does not markedly influence these biomarkers in this cohort.

Table 3. Comparisons of salivary biomarkers between positive cases classified according to gender

Biomarkers	Male (N= 31)		Female (N= 23)		(P value)
	Mean	SD	Mean	SD	
MDA (µM/L)	6.0	1.3	5.5	1.1	0.28
IL-6 (pg/mL)	12.8	3.6	11.9	3.3	0.35
LDH (U/L)	325	50	312	40	0.42

The salivary biomarkers prove their diagnostic capability in the recognition of oral precancerous lesions, whereby Malondialdehyde (MDA) amongst the three markers used gave the highest value for the area under the curve AUC = 0.82, p = 0.001. It presents quite a convincing scintillating power between positive and negative cases with sensitivity of about 79% and speci¬ficity at 75%, hence can fairly convincingly detect most true positive cases with minimum false positives. Interleukin-6 (IL-6) elicited lower values for stimulated saliva in healthy subjects compared to patients with oral lesions where however, moderative results of sensitivity (76%) and specificity (72%), thus suggesting another inflammatory marker associated with lesion development. This is cellular damage and metabolic alteration in pre-cancerous tissues reflected by lactate dehydrogenase presenting acceptable sensitivity at 74% and specificity at 70% even though it gave a minimum value for Area Under Curve, AUC = 0.77 (figure 1). Taken together, these results suggest that salivary MDA, IL-6, and LDH could be used as non-invasive complementary biomarkers for the early detection of oral precancerous lesions for timely diagnosis and intervention. The use of a combination of these markers may further improve diagnostic accuracy and help stratify patients at higher risk.

Table 5. Receiver operating characteristic (ROC) analysis of salivary biomarkers for the diagnosis of oral precancerous lesions

Biomarker	(AUC)	Sig. p-value	Cut-off Point	Sensitivity (%)	Specificity (%)
MDA	0.82	0.001	5.8	79	75
IL-6	0.79	0.002	12	76	72

LDH	**0.77**	**0.003**	**315**	**74**	**70**

AUC: Area Under the curve

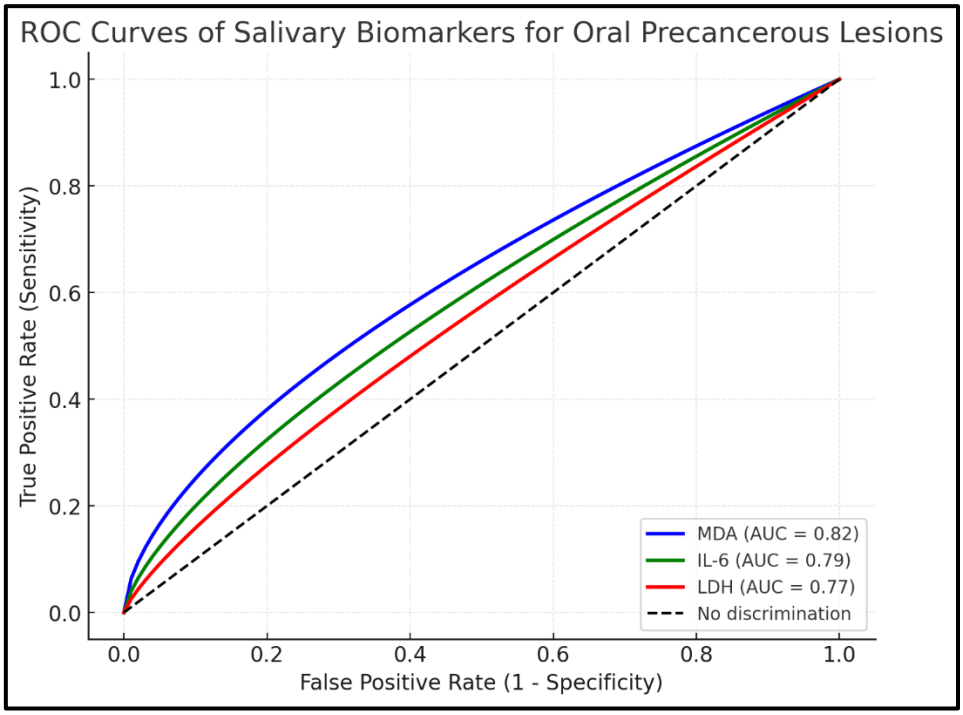

Figure 1. Receiver Operating Characteristic (ROC) Curves of Salivary Biomarkers (MDA, IL-6, and LDH) for the Diagnosis of Oral Precancerous Lesions

Discussion

This study highlighted the role that salivary biomarkers play in the early detection of oral precancerous lesions. Such a role makes saliva an attractive diagnostic tool since its collection is non-invasive plus easy to perform while at the same time reflecting both local and systemic pathological conditions (Lee et al., 2020). Salivary oxidative stress markers, pro-inflammatory cytokines, and metabolic enzymes are molecular indicators of subsequent changes toward overt malignancy. These biomarkers express not only the cell stress and tissue damage but also pathogenesis in oral lesions emphasized their potential application clinically in screening and risk stratification.

Malondialdehyde (MDA) has long been accepted as a marker for lipid peroxidation; in fact, it is extremely informative when speaking of conditions related to oral precancerous conditions with oxidative stress. Oxidative injury to cellular membranes leads to the generation of MDA, which may further promote more injurious effects inside cells and genomic instability. This will be used as a theoretical assumption herein. If the oral lesion raises salivary MDA concentration, then it means enhanced oxidative stress in the oral microenvironment state is inferred, and yes, this condition contributes toward disease progression. That makes the findings of this work valuable because histopathological evidence also places increased DNA adducts formed from malondialdehyde oxidatively binding to DNA—that these are mutagenic and hence lead to malignant transformation— in support of Ayala et al.'s results from 2014. Thus, monitoring salivary MDA practically provides another avenue for initiating early intervention and prevention programs.

The inflammatory pathways play a major role in the pathogenesis of oral precancerous lesions. Interleukin-6 (IL-6) is a major mediator of inflammation. IL-6 acts mainly through the gp130 pathway to activate downstream STAT3 signaling, leading to the upregulation of genes involved in cellular proliferation and inhibition of apoptosis as well as those capable of angiogenesis, therefore creating a favorable microenvironment for neoplastic transformation (Grivennikov et al., 2010). Thus, measuring IL-6 in the saliva reflects localized inflammatory activities within the oral cavity—it actually reflects both tissue injury and immunological reactions localized within the oral cavity. Study findings add support to earlier evidence regarding lesion severity and

predictive ability on disease progression for IL-6 (Baba et al., 2016). The raised levels of this cytokine in saliva probably also indicate early epithelial dysplasia before morphological changes can be appreciated clinically. Its measurement might sensitize further routine screening to pick high-risk cases for timely clinical intervention.

Lactate dehydrogenase is a metabolic enzyme of the glycolytic pathway that gets released in situations of tissue damage and cellular turnover. Its increased levels in saliva may reflect enhanced cellular proliferation, necrosis, or even metabolic dysregulation—conditions associated with precancerous lesions. This study gives only moderate diagnostic performance to LDH, which fits well with its general role as a marker for tissue injury rather than being specific to particular lesions. However, when combined with oxidative stress and inflammatory markers, LDH does contribute to building up an overall profile reflecting different pathological pathways and thus improves total sensitivity and specificity in the early detection.

The combination of MDA, IL-6, and LDH in a panel would initiate a multifactorial approach to biomarker-based screening. Since they represent different dimensions of the pathophysiology of the lesion—oxidative stress, inflammation, and cell metabolism—their combined evaluation should improve diagnosis and probably be very close to achieving lower false-negative rates compared with single-marker evaluations. Diagnosis by multiple factors is based on modern etiopathological concepts according to which genetic, metabolic, and inflammatory interactions are responsible for oral precancerous lesions rather than one isolated event.

Gender and age-related differences in the levels of these biomarkers were therefore brought in, which shows that biological and hormonal variations probably play a role in modulating the oral microenvironment. Hormones can influence the pathways of oxidative stress response and inflammation signaling, hence possible differences between male and female biomarker expression (Miller et al., 2019). Differences in biomarkers between age groups can also be explained by the age-associated buildup of reactive oxygen species and changes in immune systems. These findings emphasize the weighting of such demographic and physiological factors in any interpretation of salivary biomarker data and customization of screening strategies.

The use of saliva as a biofluid for diagnosis is rather attractive when compared to conventional biopsy or blood-based testing. The collection is totally non-invasive, easily repeatable, and can be done right at the community or primary care level-all factors added up are very important when considering a population screening exercise. Salivary diagnostics not only remove patient apprehension and possibility of any complications related to invasive procedures but also improve compliance and hence lead to better early detection rates. From the perspective of a clinician, the discovery of salivary biomarkers could serve as an immediate stepping stone between laboratory research and real activities toward personal monitoring and risk assessment among individuals carrying oral lesions.

Findings may be promising, but certain limitations need to be considered. Biomarker levels can be influenced by dietary intakes, oral hygiene, and circadian variation; thus, standardized collection protocols have to be ensured to avoid confounding factors (Zhou et al., 2019). Another limitation is that since the study was cross-sectional, it cannot speak to issues of causality or even about the progression of lesions over time. Large cohort studies are required in the future to validate these biomarkers and establish reference ranges while determining their predictive values for malignant transformation. Salivary biomarkers imaged or combined with molecular diagnostics might be used for even earlier detection leading to prevention interventions.

Conclusion:

Salivary biomarkers MDA, IL-6, and LDH provide a very good non-invasive early diagnosis of oral precancerous lesions by presenting oxidative stress, inflammation, and tissue injury. This improves sensitivity for diagnosis and monitoring. Further studies are required to standardize cut-off values for the study, but the results bring hope that earlier intervention can reduce the burden of oral cancer.

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