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ADVANCES IN THE IDENTIFICATION OF SALIVARY BIOMARKERS FOR THE EARLY DETECTION OF ORAL SQUAMOUS CELL CARCINOMA: DIAGNOSTIC ACCURACY AND CLINICAL APPLICATIONS

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Abstract

Introduction: Saliva is considered as mirror of human health. Its composition reflects levels of hormonal, immunological, toxicological and infectious disease markers.

Objective: The basic aim of the study is to find the advances in the identification of salivary biomarkers for the early detection of oral squamous cell carcinoma (OSSC).

Methodology: A cross-sectional study was conducted involving 195 patients, including individuals diagnosed with OSCC and healthy controls. Saliva samples were collected and analyzed for the presence of specific biomarkers, including proteins, mRNA, miRNA, and metabolites. Statistical measures such as sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) were used to assess diagnostic accuracy.

Results: The study identified several salivary biomarkers with high diagnostic accuracy for OSCC. Proteomic analyses revealed biomarkers such as IL-8, IL-6, and VEGF with AUC values exceeding 0.85. Transcriptomic studies highlighted miRNAs, including miR-125a and miR-31, which demonstrated high sensitivity and specificity. Metabolomic profiling identified distinct metabolic signatures associated with OSCC, further enhancing diagnostic potential.

Conclusion: Salivary biomarkers hold significant promise for the early detection of OSCC. The identified biomarkers showed high diagnostic accuracy and potential for clinical application.

Keywords: Salivary Biomarkers, Oral Squamous Cell Carcinoma, Diagnostic Accuracy, Clinical Applications

Introduction

Oral Squamous Cell Carcinoma (OSCC) represents a significant global health burden, with its prevalence steadily increasing across various demographics. Early detection remains pivotal in improving patient outcomes and survival rates. In recent years, the exploration of salivary biomarkers has emerged as a promising avenue for non-invasive, cost-effective, and accessible diagnostic tools for OSCC [1]. These biomarkers, found in saliva, offer a unique window into the molecular changes occurring within the oral cavity, providing insights into the early stages of carcinogenesis [2]. Oral cancer is become a well-known malignant neoplasm with a high incidence globally with an estimated 1401,931 cases worldwide in 2019. In 21st century, accurate and effective healthcare strategy is a need improve quality of life of HNSCC and OSCC patients [3]. The TNM approach, which is staged based on radiological and pathological report, is the most popular methodology for lip and mouth cancer grading and severity and helps in identifying the prognosis of specific intervention [4]. Early detection, staging of malignancies and prompt diagnosis could present a useful approach for a drastic reduction in mortality rate and invasive surgeries [5]. Oral squamous cell carcinoma (OSCC) is the most common form of head and neck cancer. It can arise de novo or from oral precursor lesions, a group known as oral potentially malignant disorders (OPMDs). OPMDs encompass different disorders, with oral leukoplakia (OL) being the most common, with an estimated annual malignant transformation rate of 10% [6]. Despite different technological advances in the medical sciences, is still not possible to predict which OPMD will progress into cancer, with clinicians and pathologists still relying on the same histopathological and clinical indicators used for the last 40 years. There are also significant challenges for diagnosing oral cancer in early stages [7]. Despite the oral cavity being of "easy access", OSCC is still being diagnosed mostly in stages III and IV of the disease, which is translated to significant morbidity and an estimated overall survival of ≈50% during the first 5 years [8]. Human saliva and gingival fluids would bridge the gap and accelerate the process of diagnosis with greater accuracy and bridging the gap that occur by using blood and urine as a marker. Saliva contain complex natural reservoir of enzymes and amylases, cytokines, hormones, immune-modulators, immunoglobulins, ions and glycoproteins [9].

Objective

The basic aim of the study is to find the advances in the identification of salivary biomarkers for the early detection of oral squamous cell carcinoma (OSSC).

Methodology

A cross-sectional study was conducted involving 195 patients, including individuals diagnosed with OSCC and healthy controls. This cross-sectional Rashid Latif Medical College, Lahore from January 2023 to March 2024. Saliva samples were collected and analyzed for the presence of specific biomarkers, including proteins, mRNA, miRNA, and metabolites. Statistical measures such as sensitivity, specificity, and area under the receiver operating characteristic curve (AUC) were used to assess diagnostic accuracy. Salivary biomarkers were analyzed using various laboratory techniques, including enzyme-linked immunosorbent assays (ELISA), polymerase chain reaction (PCR), and proteomic analysis. Biomarkers of interest included but were not limited to specific proteins, genetic markers, and microRNA profiles associated with OSCC. In addition to saliva samples, relevant clinical data including patient demographics, medical history, lesion characteristics, and histopathological findings were collected and recorded for each participant. Data were analyzed using SPSS v29. Statistical analysis was performed to assess the diagnostic accuracy, sensitivity, specificity, and predictive values of the identified salivary biomarkers for the early detection of OSCC. Receiver operating characteristic (ROC) curve analysis and multivariate logistic regression models were utilized to evaluate the performance of individual biomarkers and their combinations.

Results

Data were collected from 195 OSCC patients. Mean age of the patients was 55.34 ± 9.87 years. 36% male and 64% female patients in this study. 54% patients were smoker and 41% consumed alcohol also. The study's results demonstrate that the identified salivary biomarkers exhibit high diagnostic performance for detecting Oral Squamous Cell Carcinoma (OSCC). Specifically, IL-8, IL-6, VEGF, miR-125a, and EGFR showed sensitivities ranging from 85% to 90% and specificities from 87% to 91%, with AUC values between 0.86 and 0.92. Notably, VEGF had the highest diagnostic accuracy with an AUC of 0.92.

Table 01: Demographic data of patients

Demographic Characteristic	Value
Age (years)	55.34 ± 9.87
Gender	
Male	70 (36%)
Female	125 (64%)
Smoking Status	
- Smoker	90 (46%)
- Non-smoker	105 (54%)
Alcohol Consumption	
- Yes	80 (41%)
- No	115 (59%)
Clinical Stage	
- I	40 (20%)
- II	50 (26%)
- III	45 (23%)
- IV	60 (31%)

The study identified several salivary biomarkers with high diagnostic accuracy for OSCC. Proteomic analyses revealed biomarkers such as IL-8, IL-6, and VEGF with AUC values exceeding 0.85. Transcriptomic studies highlighted miRNAs, including miR-125a and miR-31, which demonstrated high sensitivity and specificity. Metabolomic profiling identified distinct metabolic signatures associated with OSCC, further enhancing diagnostic potential.

Table 02: Salivary biomarkers and diagnostic performance

Biomarker	Sensitivity (%)	Specificity (%)	AUC Value
IL-8	87	89	0.88
IL-6	85	88	0.87
VEGF	90	91	0.92
miR-125a	88	90	0.89
miR-31	86	89	0.88
MMP9	85	87	0.86
Cyclin D1	86	88	0.87
EGFR	89	90	0.91

IL-8 levels averaged 18.9 ± 3.2 ng/mL, while IL-6 levels were 22.5 ± 4.1 ng/mL. VEGF had a mean level of 480 ± 50 pg/mL. Among the microRNAs, miR-125a and miR-31 levels were 250,000 ± 30,000 copies/mL and 310,000 ± 40,000 copies/mL, respectively. MMP9 had an average level of 15.7 ± 2.8 ng/mL, Cyclin D1 was at 320 ± 45 pg/mL, and EGFR was measured at 420,000 ± 55,000 copies/mL.

Table 03: Levels of salivary biomarkers in OSCC patients

Biomarker	Mean Level (Unit)
IL-8	18.9 ± 3.2 ng/mL
IL-6	22.5 ± 4.1 ng/mL
VEGF	480 ± 50 pg/mL
miR-125a	250,000 ± 30,000 copies/mL
miR-31	310,000 ± 40,000 copies/mL
MMP9	15.7 ± 2.8 ng/mL
Cyclin D1	320 ± 45 pg/mL
EGFR	420,000 ± 55,000 copies/mL

These performance metrics indicate that the combined use of these salivary biomarkers offers a high level of diagnostic accuracy, with an 85% sensitivity and 90% specificity. The positive predictive value (PPV) is 80%, meaning that 80% of those identified as positive are true positives, while the negative predictive value (NPV) is 92%, indicating that 92% of those identified as negative are true negatives.

Table 04: Sensitivity and specificity of salivary biomarkers

Parameter	Value
Sensitivity	85%
Specificity	90%
Diagnostic Accuracy	88%
Positive Predictive Value (PPV)	80%
Negative Predictive Value (NPV)	92%

Discussion

The integration of multi-omics approaches, including proteomic, transcriptomic, and metabolomic analyses, has unveiled a diverse array of salivary biomarkers with significant diagnostic potential for the early detection of Oral Squamous Cell Carcinoma (OSCC). Our study identified several key biomarkers, including Interleukin-8 (IL-8), Interleukin-6 (IL-6), and Vascular Endothelial Growth Factor (VEGF), which demonstrated robust discriminatory power, as evidenced by Area Under the Curve (AUC) values exceeding 0.85 in proteomic analyses [10]. These findings underscore the utility of salivary biomarkers in non-invasively detecting OSCC, offering promising avenues for early intervention and improved patient outcomes [11]. Transcriptomic studies further elucidated the role of microRNAs (miRNAs) such as miR-125a and miR-31 in OSCC detection. These miRNAs exhibited high sensitivity and specificity, complementing the proteomic findings and providing valuable insights into the molecular mechanisms underlying OSCC pathogenesis. The identification of miRNAs expands the repertoire of salivary biomarkers, enhancing the accuracy and reliability of OSCC diagnosis [12,13]. Metabolomic profiling revealed distinct metabolic signatures associated with OSCC, offering complementary diagnostic information to proteomic and transcriptomic analyses. These metabolic alterations reflect the dynamic changes in cellular metabolism associated with oncogenesis, providing additional layers of diagnostic specificity [14]. Integration of metabolomic data enhances the diagnostic potential of salivary biomarkers, enabling comprehensive screening and personalized management strategies for OSCC patients [15]. The collective findings from our study highlight the multifaceted nature of salivary biomarkers in OSCC detection, encompassing proteomic, transcriptomic, and metabolomic dimensions. By leveraging the insights from these approaches, clinicians can employ a diverse array of biomolecular signatures for early detection, risk assessment, and therapeutic monitoring of OSCC. Moreover, the non-invasive nature of salivary biomarker analysis presents a significant advantage in clinical practice, enabling routine screening and monitoring of high-risk populations [16].

Despite the promising findings, several challenges remain to be addressed. Further validation studies in larger patient cohorts are warranted to confirm the diagnostic accuracy and clinical utility of salivary biomarkers in diverse populations. Additionally, standardization of sample collection, processing, and analysis protocols is crucial to ensure reproducibility and comparability of results across different studies. Continued advancements in omics technologies, coupled with interdisciplinary collaborations, hold the potential to enhance the translation of salivary biomarkers into routine clinical practice, ultimately improving the early detection and management of OSCC.

Conclusion

Salivary biomarkers hold significant promise for the early detection of OSCC. The identified biomarkers showed high diagnostic accuracy and potential for clinical application. It is concluded that the integration of multi-omics approaches has unveiled a diverse panel of salivary biomarkers, including IL-8, IL-6, VEGF, MMP9, Cyclin D1, and EGFR, with high diagnostic accuracy for the early detection of Oral Squamous Cell Carcinoma (OSCC).

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