Review

Review of Salivary Extracellular Vesicles (EVs) as Non-Invasive Biomarkers for Oral Cancer Detection

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Review of Salivary Extracellular Vesicles (EVs) as Non-Invasive Biomarkers for Oral Cancer Detection

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Abstract: Background and Objective-Unfortunately, there are few early detection techniques for oral squamous cell carcinoma (OSCC), which is still one of the most common and deadly cancers in the world. The capacity of salivary extracellular vesicles (EVs), especially exosomes, to transport tumor-specific molecular cargo has made them viable non-invasive biomarkers for early OSCC diagnosis. This systematic review summarizes the most recent data on the molecular makeup, isolation methods, and diagnostic accuracy of salivary EVs in order to assess their diagnostic potential in OSCC diagnosis and monitoring. Methods-This review compiles information from more than 60 peer-reviewed journals that were found using Web of Science, PubMed, and Scopus. The molecular content of EVs (such as proteins, tumor-specific DNA, and miRNAs), their diagnostic performance (specificity and sensitivity), and their use in clinical and point-of-care settings were also investigated. The final analysis only took into account research with clinical validation or human volunteers. Results-Salivary EVs from OSCC patients have been shown in numerous studies to be enriched with overexpressed proteins like EGFR and HSP70, as well as oncogenic miRNAs like miR-21, miR-31, and miR-125a. Sensitivities and specificities of 80–95% were found for these EV-based markers, indicating excellent diagnostic potential. The speed and precision of detection were improved by emerging diagnostic platforms, such as EFIRM, droplet digital PCR, and lab-on-a-chip technologies. There are still few but encouraging clinical trials. These studies demonstrated the diagnostic value of salivary EVs carrying tumor-specific biomarkers, such as miR-21, miR-31, miR-125a, EGFR, and HSP70. Reported sensitivity ranged from 72% to 95%, and specificity ranged from 70% to 90%, depending on the biomarker and detection platform used. Variability in EV isolation techniques and lack of standardization across studies were identified as key limitations. Conclusion-Salivary EVs provide an accurate, accessible, and non-invasive way to identify OSCC. Large-scale, multicentric clinical validations are required prior to widespread usage in routine diagnostics, even though the evidence from current investigations is strong. The most recent developments in salivary EV research are highlighted in this review, along with potential future directions for EV-based diagnostic integration in oncology. This review confirms that salivary EVs, due to their accessibility and molecular richness, represent a promising non-invasive diagnostic tool for OSCC. However, methodological inconsistencies and limited clinical validation remain barriers to their widespread adoption. Further large-scale, standardized studies are essential to establish the reliability, reproducibility, and diagnostic accuracy of salivary EVs in clinical settings and to facilitate their integration into routine cancer screening protocols.

Keywords: salivary biomarkers; extracellular vesicles (EVs); exosomes; non-invasive diagnostics; microRNAs (miRNAs); liquid biopsy; saliva-based cancer detection; oral squamous cell carcinoma (OSSC); and EV-associated proteins

1. Introduction

Oral squamous cell carcinoma (OSCC), the most common kind of oral cancer, continues to be one of the main causes of cancer-related morbidity and death globally. According to GLOBOCAN, IARC, and WHO, the number of new instances of oral and oropharyngeal cancers worldwide is

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377,713 and 98,412 yearly, respectively, and the number of deaths from these diseases is 177,757 and 48,143 (Sung *et al.*, 2021). Poor dental hygiene, alcohol intake, and tobacco use are prominent risk factors for oral cancer, which is especially widespread in underdeveloped nations (Irani, 2020). The 5-year survival rate for oral cancer is about 50%, and the death rate has not significantly dropped over the previous 40 years despite advancements in therapy and early detection technologies. The advanced stage at which oral cancers are frequently diagnosed—a considerable percentage of cases present with large tumors and regional lymph node involvement (N+), both of which complicate treatment options and adversely affect prognosis—is primarily to blame for this stagnation in survival rates (Chi, Day and Neville, 2015a), (Chow, 2020a). The most prevalent kind of oral cancer is oral squamous cell carcinoma (OSCC), which accounts for around 90% of all mouth cancers. Early detection is crucial since the stage at diagnosis has a significant impact on the prognosis of OSCC. Regretfully, around 50% of patients with OSCC receive a diagnosis at an advanced stage (T3/T4), when the tumor has gotten bigger and may have already migrated to neighboring lymph nodes, requiring more intensive treatments. Early detection of oral cancer, with a smaller tumor size and lymph nodes unaffected by metastases, should be linked to a higher survival rate because the oral cavity is an anatomical site that dentists and doctors can easily examine (Seoane *et al.*, 2012) (Gómez *et al.*, 2009).

Many reasons contribute to this late-stage diagnosis, such as the mild early signs of oral cancer, patient ignorance, and the difficulty in accessing screening programs in high-risk groups. In addition, almost 30% of people with oral cancer wait more than three months to seek medical help after seeing symptoms like ongoing mouth discomfort or ulcers, which causes the disease to worsen before a final diagnosis is established (Scott, Grunfeld and McGurk, 2005). These factors emphasize the urgent need for more efficient and accessible diagnostic methods that can facilitate the early detection of OSCC.

A significant obstacle in diagnosing OSCC is the dependence on invasive techniques, like tissue biopsy, which may not always be practical for routine screening, especially in populations with a high risk of oral cancer. Furthermore, biopsy operations may be uncomfortable for patients and can be expensive and time-consuming. CT and MRI scans are also used to measure the tumour's size, although they are not very good for detecting OSCC in its early stages or seeing micrometastases in nearby lymph nodes (Carreras-Torras and Gay-Escoda, 2015). These restrictions show how urgently non-invasive, reasonably priced diagnostic methods are needed to identify cancer in its early, curable stages and enable ongoing patient monitoring during the course of treatment (Carreras-Torras and Gay-Escoda, 2015) (Pierfelice *et al.*, 2024).

Saliva is an easily accessible and collectable biological fluid that has shown promise as a means of identifying biomarkers linked to oral cancer. A diverse range of substances, including proteins, lipids, and nucleic acids (DNA, RNA), are found in saliva and can represent both local and systemic changes in the body (Kumar, Gupta and Das, 2024). Extracellular vesicles (EVs), in particular exosomes, have garnered a lot of attention in cancer research because of their function in intercellular communication and capacity to transport molecular fingerprints unique to individual tumors (Mathew *et al.*, 2020). EVs are tiny, membrane-bound vesicles that are released into different body fluids, such as saliva, by all cell types. MicroRNAs (miRNAs), proteins, mRNAs, and lipids are among the many bioactive molecules they contain. These molecules are indicative of the condition of the parent cells, including their malignant transformation. EVs are excellent candidates for the early identification and surveillance of cancer, including OSCC, because of these molecular markers (Kalluri and McAndrews, 2023) (Wang, Li and Zhu, 2024).

A growing corpus of studies showing the presence of tumor-associated molecules in salivary EVs supports the possibility of these vesicles as biomarkers for OSCC identification. Salivary EVs from OSCC patients have been found to be enriched with particular microRNAs, including miR-21, miR-31, and miR-125a, which are known to have a role in metastasis, carcinogenesis, and the epithelial-to-mesenchymal transition (EMT) (Chi, Day and Neville, 2015b) (Chow, 2020b). Salivary EVs also contain proteins that are overexpressed in OSCC tissues, such as heat shock proteins (e.g., HSP70) and epidermal growth factor receptor (EGFR), in addition to miRNAs. According to these

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results, the molecular cargo that EVs carry in saliva can offer useful diagnostic data that represents the genetic and proteomic landscape of the tumor, allowing for the early diagnosis of OSCC while the tumor is still confined and the lymph nodes are unaffected (Kumar *et al.*, 2024) (Gai *et al.*, 2018) (Taha, Ono and Eguchi, 2019).

Recent developments in EV isolation methods, including size exclusion chromatography, immunoaffinity-based approaches, and ultracentrifugation, have significantly enhanced the capacity to extract high-quality EVs from saliva for further examination (Yáñez-Mó *et al.*, 2015). Furthermore, new molecular techniques such as reverse-transcription quantitative PCR (RT-qPCR), droplet digital PCR, and microarray technologies have made it possible to quantify and profile proteins, miRNAs, and other biomarkers in salivary EVs (Gardiner *et al.*, 2016). Because of these developments, EV-based diagnostic tests now have much higher sensitivity and specificity, which makes them a competitive alternative to more invasive procedures like biopsy. Furthermore, new technologies like lab-on-a-chip devices and electric field-induced release and measurement (EFIRM) may make point-of-care testing possible, which would speed up and increase the accessibility of EV-based diagnostics for clinical application (De Sousa *et al.*, 2023) (Van Deun *et al.*, 2014).

The purpose of this study is to provide an overview of recent developments in the use of salivary EVs for OSCC diagnosis, prognosis, and monitoring. We offer a critical evaluation of the clinical translational uses, the molecular markers found in EVs, and the isolation techniques.

2. Materials and Methods

2.1. Method for Searching for Literature Peer-reviewed literature was systematically searched for works published between January 2000 and February 2024 using a variety of sources, including PubMed, Web of Science, and Scopus. The search was conducted using the following keywords: "salivary biomarkers," "exosomes," "oral squamous cell carcinoma," "OSCC," "salivary extracellular vesicles," and "non-invasive diagnostics." When appropriate, MeSH keywords and Boolean operators (AND, OR) were used to improve the search approach. In order to find more relevant research, the reference lists of pertinent reviews were also manually examined.

2.2. Criteria for Inclusion and Exclusion (i) English-language studies; (ii) original research articles involving human saliva samples; (iii) studies that examined EVs or exosomes in relation to OSCC diagnosis or prognosis; and (iv) studies that reported diagnostic performance metrics (sensitivity, specificity, AUC) were among the inclusion criteria. Reviews, editorials, studies involving only animals, and papers lacking a clear diagnostic context were among the exclusion criteria.

2.3. Extracting Data and Evaluating Quality Author, study population, year, EV isolation method, biomarker type (miRNA, protein, DNA), detection method, and diagnostic performance (sensitivity, specificity, AUC) were among the data that were extracted.

3. Results

3.1. Selection of Studies 6,218 records in all were obtained. 84 full-text papers were evaluated following the elimination of duplicates and the screening of titles and abstracts. Fifty-one original research articles were chosen for this review based on inclusion criteria.

3.2. Types of Biomarkers in Salivary EVs- Several biomarkers linked to OSCC were shown to be present in salivary EVs. The miRNAs that were most commonly reported were miR-21, miR-31, miR-125a, and miR-200c. EVs from OSCC patients were found frequently abundant in proteins like EGFR, HSP70, and CD63.

3.3. Techniques for EV Isolation and Detection- The most popular isolation technique was still ultracentrifugation, which was followed by size exclusion chromatography and immunoaffinity-based

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methods. RT-qPCR for miRNA and protein profiling via ELISA, Western blotting, and mass spectrometry were the main methods used in molecular detection.

3.4. Performance of Diagnostics- Salivary EV-based biomarkers had a sensitivity of 72% to 95% and a specificity of 70% to 90%. High diagnostic accuracy was indicated by a number of studies that obtained area under the ROC curve (AUC) values above 0.85. Interestingly, as compared to individual biomarkers, the combination of several biomarkers frequently produced better results.

4. Discussion

The molecular markers of the tumour microenvironment are abundant in salivary EVs. They are excellent candidates for OSCC screening due of their non-invasive accessibility, especially in environments with limited resources. Salivary EVs' durability, high biomolecular composition, and simplicity of collecting make them a promising tool for early cancer detection. Their promise for non-invasive OSCC diagnosis is supported by the results of numerous investigations. Despite being the top standard, conventional biopsy is invasive and unsuitable for routine or mass screening. Variability in EV separation and analysis methods, however, makes clinical standardization difficult. Moreover, improved diagnosis accuracy may be possible through integrated omics (miRNA + proteomics + genomes), even if the majority of biomarker research concentrate on a small number of miRNAs or proteins. Utilizing Emerging Technology Integration, There are opportunities to create quick, point-of-care diagnostic instruments based on salivary EVs thanks to developments in microfluidics and biosensor technologies like lab-on-a-chip platforms and EFIRM. In primary care and community settings, such improvements could greatly improve screening. Large, varied populations should be included in future research, and uniform procedures should be used.

5. Conclusion

Salivary EVs have a high diagnostic value for OSCC because they carry tumor-derived molecular cargo. In at-risk populations in particular, they hold great promise for incorporation into standard screening and monitoring procedures. Their sensitivity, specificity, and viability for use in non-invasive oral cancer diagnostics are supported by consistent data from multiple research.

6. Future Prospectives

The development of standardized procedures for EV isolation and analysis, the validation of discovered biomarkers in extensive prospective trials, the integration of EV analysis with point-of-care technologies, and the investigation of EVs' function in therapeutic monitoring and recurrence prediction should be the main objectives of future research

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