REVIEW ARTICLE

Role of salivary biomarkers in the diagnosis of oral cancer: Mini review

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Abstract

Saliva is an oral fluid, comprising 99% of water and 1% of organic and inorganic constituents. Inorganic elements consist of minerals such as sodium, potassium, calcium, magnesium, fluoride, and phosphates. Organic elements comprise enzymes, hormones, immunoglobulins, proteins antioxidants, and coagulation factors are present in the saliva. These constituents are present in the saliva at a varying proportion and which tends to vary during oral cancer. Many of these constituents, their altered levels in oral cancer can serve as the potential biomarker and aid in its diagnosis. The aim of this review article is to highlight the importance of saliva in diagnosis, to overview different salivary biomarkers, its uses and acceptance for the early diagnosis of oral squamous cell carcinoma.

Keywords: Biomarkers, diagnosis, saliva

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common deadly diseases with a poor survival rate of 60%. Despite the improvement of therapeutic strategies, in the last couple of decades, the survival rate has not significantly improved. At present, no specific early detection techniques are available beyond conventional clinical oral examination. Diagnosis of oral cancer presently relies on thorough clinical examination combined with biopsy for histopathological interpretation. Majority of the cases of OSCC are diagnosed in their advanced stages. Early detection plays an essential role in successful therapy and to improve the quality of life of the patient.1

Only few biomarkers are available for detection of OSCC although open biopsy is the most accepted method to approve a diagnosis. However, biopsy has its definitive drawbacks such as invasiveness, false positivity, and poor patient acceptance level.1,2 Thus, a novel, non-invasive adjunctive screening aids (devices or tests) are the need of the hour.

Attributes of Saliva Suitable for Diagnosis

Saliva is a frothy, sparingly acidic (pH: 6–7) watery substance composed of secretions of all the three major and minor salivary gland of lips, buccal mucosa, and palate.1,3 About 1–1.5 L of saliva is produced daily, and it contains a cocktail of various salts, proteins, and minerals. Its composition, consistency changes continuously during health and disease, so it is widely known as the mirror of the mouth. Its collection is non-invasive and has been potentially used for discovery of oral cancer biomarkers. Considering its advantages as a diagnostic medium, the adoption of saliva in the surveillance of oral cancer has gained importance in the present time.4

Saliva is a composite fluid encompassing an array of hormones, enzymes, growth factors, immunoglobulins, and antibacterial components. Few of the mentioned factors gain entry to saliva through blood by paracellular or transcellular routes. Although saliva is a mixture of disparate elements with varied investigative attributes, their feeble concentration in comparison to serum often prevents the salivary diagnostics from being clinically practical; nonetheless, with the discovery of advanced and precisely sensitive procedures (e.g., nanodiagnostics diagnostics and molecular biology), the shallow concentration of salivary attributes, is no longer an inhibition for the routine salivary diagnostics.4

Various methods are advocated for the collection of saliva. Stimulated saliva can be collected by promoting mitigatory action through paraffin wax or chewing gum. However, this particular
method affects the original salivary consistency, pH, and its use is only restricted to patients who cannot produce sufficient amount of saliva without stimulation. Saliva can also be collected without exogenous stimulation and the flow rate varies among different individuals. The most accepted methods for the accumulation of saliva without stimulation are spitting, draining, and suctioning. Regardless of the method used, the patients should be advised to rinse the mouth before the collection of the sample to avoid contaminants. 

The compelling reasons for using saliva as a diagnostic aid are easy to sample collection, easy to use, inexpensive, non-invasive, and can be routinely adopted for mass screening in camps and epidemiological surveys. Furthermore, the health-care experts prefer a salivary test than using serum, because the latter is more likely to expose the technicians to various blood-borne diseases.

**Salivary Diagnostics in Oral Cancer**

**Background**

Since the last couple of decades, the role of saliva in the diagnosis of various diseases of the oral cavity such as tooth decay, periodontitis, and oral cancer is widely in research. An ideal diagnostic modality should possess the attributes of high sensitivity, specificity, and precision, and should satisfy the criteria of low cost, high output with a wide range of practical application. For salivary diagnostics, many of these objectives are fulfilled through assorted inventions in the terrains of biotechnology, physics, engineering, microbiology, and biochemistry.

**Challenges in the Routine Use of Salivary Biomarkers**

The biggest challenge for the researchers is to identify potential diagnostic markers in the saliva with high sensitivity and specificity. Next, it is equally important to identify effective equipment and devise standardized protocols for the estimation of these potential biomarkers. To empower salivary diagnostics in health surveillance, extensive research needs to be carried out at various scientific platforms for biomarker discovery, procedures for their estimation, and how to make them feasible for routine use.

**Comprehensive Salivary Analytics in OSCC**

Various researchers have conducted a thorough analysis of various salivary components such as calcium, inorganic phosphate, sodium, potassium, magnesium, albumin, total protein content of saliva, insulin growth Factor I, lactate dehydrogenase (LDH), amylase, total immunoglobulin content of saliva, salivary immunoglobulin A (IgA), and matrix metalloproteinases-2 and 9 (MMP-2 and MMP-9) in subjects having oral subjects and healthy controls. Results elucidated that oral cancer patients showed a higher salivary protein content than the healthy controls. The concentrations of salivary sodium, calcium, phosphate, and magnesium were significantly higher in the subjects with oral cancer. Amylase, potassium, and salivary IgA levels were significantly lower. Albumin, LDH, MMP-2, and MMP-9 levels showed 8–10-fold rise in oral cancer patients when compared with healthy controls. Thus, it can be contemplated that comprehensive salivary investigation can reveal an altered salivary constituent in oral cancer subjects, indicating that salivary analysis can serve as a valuable tool for the diagnosis of oral cancer.

**Tumor Markers in Saliva and OSCC**

A variety of tumor markers have been scrutinized in various studies showed a different level of sensitivity and specificity. Like, Kurokawa et al. evaluated circulatory carcinoembryonic antigen, inhibitors of apoptosis levels in subjects with oral cancer, and recorded the precision and sensitivity values of 76.4% and 80.4%, respectively. Hellner et al. expressed that the sensitivity of circulatory tumor cells in oral cancer patients is 24%. Zoller reported that the sensitivity of circulatory tumor cells and carcinoembryonic antigen in OSCC subjects was 42–33%, respectively. Different biomarkers upregulated and downregulated in oral cancer are mentioned in Table 1.

**Salivary DNA methylation in OSCC**

Disclosure of atypical DNA hypermethylation arrangement of oncogenes in the saliva is attainable; furthermore, it may serve as a potential tool for disease monitoring, as DNA hypermethylation is the primitive step in the development of oral cancer. The advantage of DNA hypermethylation over other diagnostic markers is it can yield a set of cancer-specific genes that can be employed for the early diagnosis of oral cancer. The methylation has a specific signature that highlights the stage of the tumor.

**Table 1**: Different biomarkers upregulated and downregulated in OSCC.

<table>
<thead>
<tr>
<th>Upregulated biomarkers</th>
<th>Downregulated biomarkers</th>
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<tbody>
<tr>
<td>Defensin 1</td>
<td>Salivary antioxidants</td>
</tr>
<tr>
<td>CEA</td>
<td>Amylase</td>
</tr>
<tr>
<td>CD 44</td>
<td>Potassium</td>
</tr>
<tr>
<td>IL-6, IL-8</td>
<td>IgA</td>
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<tr>
<td>IAP</td>
<td>ROS</td>
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<tr>
<td>RNS</td>
<td>LDH</td>
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<td>MMP-2, MMP-9</td>
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well as contemplates the histology of the tumor to delineate the
diagnosis. Finally, DNA methylation process can be reversed
using demethylating agents, which can be utilized for cancer
therapy together with traditional cancer chemotherapy.\cite{10,11}

**Salivary Loss of Heterozygosity (LOH) in OSCC**

Recently, LOH in presumptive tumor suppressor genes has
become a primitive indicator for oral potentially malignant
conditions and oral cancer. The occurrence of LOH is more
frequent than genetic mutations in oral and oropharyngeal
carcinomas. The frequency of transversion of TP53 protein in oral
cancer subjects increases with tobacco exposure. El-Naggar et al.
conducted a study and reported that LOH is frequently present
at least seven of the 25 markers studied.\cite{12} They concluded that
epithelial cells in saliva of oral cancer subjects can be a probable
material for gene analysis. The heterogeneity shown between
saliva and tumor occurs due to the genomic instability of the
mucosa of the patients. LOH at a particular chromosomal loci
has a positive correlation with tobacco chewing, smoking, and
consumption of alcohol.\cite{13}

**Salivary Proteomics in the Discovery of Oral Cancer
Biomarkers**

Analysis of DNA, RNA, and proteins in the saliva of OSCC
subjects raises the sensitivity index of these components to
serve as the potential biomarkers. The various methods used for
the analysis of salivary proteomics include capillary reversed-
phase liquid chromatography with quadrupole–time-of-flight
mass spectrometry, multianalyte profiling technology shotgun
proteomics on the basis of reverse-phase liquid chromatography,
and human genome-microarrays to prepare a profile of salivary
transcriptome.

Saliva of oral cancer subjects and healthy controls has shown
difference in proteomic signatures may be used as characteristic
biomarkers. Moreover, a large group of mRNAs can be identified
in the saliva which can be utilized by a unique diagnostic
modality, popularly known as salivary transcriptome diagnostics.
The mRNA markers in saliva are the transcripts of interleukin-1,
interleukin-8, and dual specificity protein phosphate 1. These
composite biomarkers possess high sensitivity and specificity
in distinguishing OSCC subjects from the healthy controls.
Furthermore, microarray analysis can be used to scrutinize
different levels of genetic expression between oral cancer patients
and healthy controls.\cite{1,14}

**Salivary Oxidative Profile in OSCC**

Stress alters the salivary composition in OSCC patients. Reactive
oxygen species (ROS) and reactive nitrogen species (RNS) levels increases, whereas salivary antioxidants level gradually goes down in oral cancer patients. Apart from their
relevance in the diagnosis, these salivary alterations throw
light on the disease pathogenesis as well. The presence of both
ROS and RNS at higher levels in the saliva may be the cause for
consumption of salivary antioxidant systems, thereby leading to
the extirpative destruction of the nucleic acids and proteins. The appearance of oxidized proteins in the oral fluid indicates
a correlation between salivary antioxidants and oral cancer.
Almadori et al. further investigated the glutathione and uric acid
levels in the saliva of OSCC patients. They found that urate
and glutathione levels in saliva were quite high in oral cancer
patients and suggested that it may serve as an epidemiological
marker for identifying the oral cancer subjects, for follow-up,
and chemoprevention also.\cite{1,2,15}

**Future Prospects of Saliva as Diagnostic Tool in Oral Cancer**

The diagnostic caliber of saliva was not regarded until its
advantage was noted in the last couple of decades, which
transformed saliva from a worthless fluid to a prime variable in
oral cancer diagnosis. Salivary diagnostics enable the clinicians
to detect, monitor diseases easily and have a crunch on the future
medical research and therapy. At present, promising evidence is
there on the diagnostic capability of saliva to detect oral cancer,
lung cancer, and breast cancer. Nonetheless, scientific validation
is required to standardize the diagnostic ability of saliva over
other body fluids. Based on the evidence of extensive research
reports, salivary diagnostics are presently a priority at National
Institute of Dental and Craniofacial Research. With the present
rate of evolution, we presume salivary diagnostics not only will
salvage life but also perpetuate the quality of lives that have been
salvaged.\cite{2,3}

**Conclusion**

Early detection of premalignant lesions can lead to prolonged
survival of the patients. Unfortunately, oral cancer is mostly
diagnosed in the advanced stages. Dependable primitive
biomarkers are not readily available. Potential biomarkers for
oral cancer will not only be useful in screening but also follow-up
patients following treatment. Moreover, salivary biomarkers can
be employed as an adjunct between biopsies to aid in supervising
the status of the disease from time to time. The use of salivary
biomarkers for screening of OSCC has shown promising results
and invokes extensive research in the future.

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