REVIEW ARTICLE

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

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Keywords:
Biomarkers, diagnosis, saliva

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Received: 04 April 2018;
Accepted: 03 May 2018

Doi: 15713/ins.jmrps.129

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.

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.[2,5]

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.[2,4]

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.[2]

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.[6]

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.[1] 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.[7] Hellner *et al.* expressed that the sensitivity of circulatory tumor cells in oral cancer patients is 24%.[8] Zoller reported that the sensitivity of circulatory tumor cells and carcinoembryonic antigen in OSCC subjects was 42–33%, respectively.[9] 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 as

Table 1: Different biomarkers upregulated and downregulated in OSCC

Upregulated biomarkers	Downregulated biomarkers
Defensin 1	Salivary antioxidants
CEA	Amylase
CD 44	Potassium
IL-6, IL-8	IgA
IAP	
ROS	
RNS	
LDH	
MMP-2, MMP-9	

OSCC: Oral squamous cell carcinoma, MMP: Matrix metalloproteinases, LDH: Lactate dehydrogenase, CEA: Carcinoembryonic antigen, RNS: Reactive nitrogen species, ROS: Reactive oxygen species, IAP: Inhibitors of apoptosis, IgA: Salivary immunoglobulin A, IL: Interleukin

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.[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.[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.[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.[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 ureate 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.[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.[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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How to cite this article: Ghosh S, Singh P, Nambiar KS, Haragannavar VC, Augustine D, Sowmya SV, Rao RS. Role of salivary biomarkers in the diagnosis of oral cancer: Mini review. J Med Radiol Pathol Surg 2018;5:7-10