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Saliva as a Biomarker in Oral Cancer

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Abstract

The prognosis for patients with oral cancer remains poor in spite of advances in therapy of many other malignancies. Early diagnosis and treatment remains the key to improved patient survival. The tumor markers are playing an increasingly important role in cancer detection and management. These laboratory-based tests are potentially useful in screening for early malignancy, aiding in cancer diagnosis, determining prognosis, surveillance following curative surgery for cancer, up-front predicting drug response or resistance, and monitoring therapy in advanced disease. Diagnostic tests for early detection include brush biopsy, toluidine blue staining, autofluorescence, salivary proteomics, DNA analysis, biomarkers and spectroscopy. This article focuses on biomarkers as valuable utility in risk assessment, clinical screening, diagnosis, and prognosis prediction of oral cancer in the era of precision medicine.

Introduction:

Cancer, a life-threatening global burden, is characterized by loss of control of cellular growth and development leading to excessive proliferation and spread of cells. It comprises a large group of diseases and affects all age groups. Although 100 different types of cancers were reported so far worldwide, oral cancer, skin cancer, mammary cancer, lung cancer, and cervical cancer are the most predominant cancers. The symptoms of cancer depend on its location, size, invasion, and metastasis. Early detection and diagnosis could definitely improve the survival rate as well as the life quality of the patients. Cancer patients, depending on the nature and location of tumors, are subjected to surgery, chemotherapy, radiotherapy, gene therapy, or immunotherapy[(1-5)].

Oral cancerrefers to a subgroup of head and neck tumors, accounting for about one-third of all such tumors. that develop on the surface of lips mucosa, mouth, tongue, buccal mucosa, upper and lower gingiva, retromolar trigone, soft and hard palate. Oral cavity has complex anatomy and its function such as speaking, swallowing, and face projection[(6,7)].The development of oral cancer is a multistep process, arising from pre-existing Potentially Malignant Disorders.

The actual cause for oral cancer is not known. However the two main factors which influence most diseases are genetic

and epigenetic factors. Development of oral or head and neck squamous cell carcinoma (HNSCC) and minor salivary gland carcinomas is influenced by both these factors namely tobacco, alcohol, diet and nutrition, viruses, radiation, ethnicity, familial and genetic predisposition, oral thrush, immunosuppression, syphilis, dental factors, occupational risks[8]. The incidence of OC is directly correlated with age of subjects. Rates rise dramatically after the age of 40-49 years and reach a plateau around the age of 70–79 years. OC is more frequent in men than women, and depending on its location within the oral cavity, males are two to six times more likely to be affected than females, largely owing to their higher intake of alcohol and tobacco[9].

Tumor markers are diagnostic aids that play a very important role in early diagnosis and interception of cancer. An increasing number of systemic diseases and conditions, amongst them oral cancer and pre cancer, have been shown to be reflected diagnostically in saliva which, unlike blood, is the most easily accessible, safe, noninvasive, and readily obtained bio fluid and is researched widely as one of the diagnostic media. Saliva is a unique fluid and interest in it as a diagnostic medium has advanced exponentially in the last 10 years[10-11].Salivary diagnostics has been implemented for more than 2000 years according to some traditional health-care systems[12].

Biomarkers are the main focus of current researches in biological characteristics that are easily measured in patients, and their discovery has laid the foundation for personalized medicine[13]. According to the National Institutes of Health (NIH) and the NIH Biomarkers Definitions Working Group (1988) [14], a biomarker is a "characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmaceutical response to therapeutic intervention. Since last few years, many researches are going on these and reported that biomarkers significantly increase during malignancy. Its implications are:

Biomarkers for Oral cancer applications:

- **a.** Biomarkers help in evaluating the preventive measures or therapies and the detection of the earliest stages of oral mucosal malignant transformation
- **b.** Reveal the genetic and molecular changes related to early, intermediate, and late end-points in the process of oral carcinogenesis
- **c.** Refine the ability to enhance the prognosis, diagnosis, and treatment of oral carcinomas - Monitor progression/ recurrence, treatment compliance
- **d.** Useful in early stages of cancer drug development
- **e.** Determine efficacy and safety of chemopreventive agents[15].

Saliva is an aqueous, hypotonic solution which protects all the tissues of the oral cavity[16].Saliva is a complex fluid containing various enzymes, electrolytes, proteins, nucleic acids, antimicrobial constituents, hormones, cytokines, and antibodies., In addition, some proteins are necessary for inhibiting the spontaneous precipitation of calcium and phosphate ions in the salivary glands and in their secretions[17,18]. It play vital role in maintaining oral homeostasis, it has different defense mechanisms, such as immunological and enzyme systems, defense mechanisms against bacteria, viruses, fungi, protection of the mucosa and it also promotes its healing properties[19].

Salivary biomarkers connote a very promising noninvasive approach to oral cancer detection and in monitoring the disease process and the therapeutic response increased attention has been placed on salivary biomarkers based on the convenient and noninvasive method of sample collections[20].

Rationale behind the use of salivary biomarkers include: Low chances of transmission compared to blood samples, non-invasive method of disease detection, saliva does not clot, saliva contains a wide range of compounds, easily accessible,

patient comfort , safe to handle ,and easy to store[21].

The salivary biomarkers for oral cancer can be broadly divided into protein and RNA based biomarkers. Protein based biomarkers include a group of biomarkers such as cytokines, fibroblast growth factor, cyfra 21-1, cancer antigen-125, tissue polypeptide antigen, endothelin, matrix metalloproteinases, glutathione transferase, and superoxide dismutase[22].

The proteome represents the complete set of proteins encoded by genome and proteomics which is the study of the proteome that investigates the cellular levels of all the isoforms and post-translational modifications of proteins that are encoded by the genome of the cell under a given set of circumstances[23].

Protein markers are differentiation antigens of corresponding normal tissue and characterize a certain stage of its maturation. They originate from live cells and show high tissue specificity. However, they may be detected in other pathologies as well. Salivary protein markers have shown moderate sensitivity and specificity as prognostic markers[24].

For example, defensins are peptides which possess antimicrobial and cytotoxic properties. They are found in the azurophil granules of polymorphonuclear leukocytes. Elevated levels of salivary defensin-1 were found to be indicative for the presence of OSCC, since higher concentrations of salivary defensin-1 were detected in patients with OSCC compared with healthy controls[25].

The cytoskeletal intermediate filaments present in almost all normal and malignant epithelial cells are termed as "cytokeratins." The activated protease increases degradation of cytokeratin free filaments into the blood in malignant epithelial cells. In OSCC, it has been found that levels of Cyfra 21-1 are increased in saliva [15].

Several salivary protein markers such as interleukins (8, 6, 1β), matrix metalloproteinase (MMP 2, 9), transforming growth factor (TGF-1), Ki67, cyclic D1, Cyfra 21.1, transferrin, α amylase, tumor necrosis factor (TNF-α) and catalase have been detected in oral squamous cell carcinoma by various studies[26].

Exosomes are cell-derived vesicles, 30–100 nm in diameter, generated by the endosomal pathway and released through exocytosis of multi vesicular bodies (MVBs) to the extracellular space and circulation[27].

More recently, salivary mRNA were localized inside salivary exosomes and these nucleic acids were protected against ribonucleases in saliva ;Moreover, saliva exosomes have been discovered to regulate the cell-cell environment by altering their gene expression allowing us to better understand the molecular basis of oral diseases[28]. Li *et al*. [29].found that exosomes derived from hypoxic OSCC cells increased the migration and invasion of OSCC cells in a HIF-1αand HIF-2α-dependent manner.

Salivary Genomics

In oral malignancy states, the genetic material like DNA can be obtained from the living cancer cells as Saliva is in constant contact with the oral cancer lesion. It was assessed that TP53 is a gene which shows loss of heterozygosity and mutations in oral cancer patients[30].

MiRNAs for Oral Cancer

MicroRNAs (miRNAs) are short RNA transcripts that range from 19 to 25 nucleotides. They were discovered during the early 90s in a transparent nematode. Until now more than 1000 miRNAs have been profiled and their dysregulation was found to affect cell growth, apoptosis, differentiation, motility, and also immunity [31]. Plasmic and salivary miRNAs are protected from ribonucleases present in the plasma/saliva by macromolecules called exosomes, which are known to package and transport miRNAs. The presence of miRNAs in human body fluids, especially in saliva, is an emerging field for monitoring oral diseases. Liu *et al*. [32].reported that salivary levels of miR-31 were significantly up regulated in pre-surgery OSCC patients compared with the levels

in healthy individuals. In this study, not significant differences were observed between oral verrucous leukoplakia patients and control group, but a significant lower value was observed respect to OSCC patients.

mRNA

mRNA is the direct precursor of protein and as the corresponding levels are correlated in cells and tissue samples, hence salivary mRNA for dual specificity phosphatase 1 (DUSP1), H3 histone, family 3A (H3F3A), IL-1B, IL-8, ornithine decarboxylase antizyme 1 (OAZ1), spermidine/ spermine N1-acetyltransferase (SAT) and S100 calcium binding protein P (S100P) are documented in literature as the biomarkers of oral cancer. Studies have reported that certain mRNA molecules up-regulated in the saliva samples of patients suffering from Oral cancers[33].

Salivary Oxidative Profile in oral cancer

Oxidative stress,a pathophysiologic imbalance in the production of oxidants or free radicals and antioxidant molecules in the body would result in OS state.Oxidative stress is associated with the development of severaldisorders including cancer. It is the most important causative factor for the induction of cellular apoptosis.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. Diagnosis of oral cancer can be assessed by oxidative stress. Salivary reactive nitrogen species are remarkably raised with the patient of oral cancer in comparison to the control group. Meanwhile, other salivary anti-oxidants are noticeably decreased (because of utilization), that lead to the oxidative destruction of DNA and proteins, thus helps in oral cancer detection[34,35].

Conclusion

The research should focus on finding a distinction between biomarkers for cancer diagnosis, and therapeutic targets are recommended. Development of clinically valid candidate biomarkers, with greater clinical utility values, for oral cancer screening is highly recommended because early identification of oral cancer will help to reduce patient morbidity and mortality.

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