Review Article

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ORAL CANCER – THE EPIDEMIOLOGY AND PREVENTION, CLASSIFICATION OF SALIVARY BIOMARKERS AND THE ROLE OF SALIVARY MIR-31

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ABSTRACT

This article focuses on saliva as a diagnostic medium of choice and the various biomarkers that are elevated in oral cancer. The outcome of Research proves that 90 % of the oral cancers are oral squamous cell carcinoma. The causative factors, risk factors, and screening for oral cancers are elaborated. Salivary biomarkers can be classified into genomic and protein biomarkers. Among Genomic biomarkers the role of micro RNAs are specified and the sensitivity of various protein biomarkers such as matrixmetalloproteinases, cytokines are studied.

KEYWORDS: Oral squamous cell carcinoma; risk factors; biomarkers; genomic; protein

INTRODUCTION

An ulcer can be defined as a loss of continuity in An ulcer can be defined as a loss of continuity in the skin, mucous membrane or oral mucosa. Head and neck cancers are cancers that start in the tissues and organs (Fig. 1) the head and neck.[1] They include cancers of the larynx (voice box), throat, lips, mouth, nose, and salivary glands. Most types of head and neck cancer begin in squamous cells that line the moist surfaces inside the head and neck[1] (for example, the mouth, nose, and throat). Tobacco use, (Fig. 2) heavy alcohol use, and infection with the human papillomavirus (HPV) increase the risk of many types of head and neck cancer.[1] The risk factors for oral cancer depends on the gender, age, prolonged Sun exposure, tobacco use and alcohol, diet, HPV infection[2] areca nut chewing ,and lodging the betel quid of the arecanut (Fig. 2) in the buccal vestibule leading to precancerous and cancerous lesions due to chronic irritation and significant changes in the oral mucosa adjacent or underneath it. This results in Extrinsic stains (Fig. 3a), loss of esthetics requiring scaling and oral prophylactic measures. The most common symptom is a sore or an ulcer (Fig. 3b) in the mouth that does not heal easily and which discharges or bleeds either on examination or spontaneously. Screening for oral cancer should include a thorough history and physical examination. The clinician should visually inspect and palpate the head, neck, oral, and pharyngeal regions. This procedure involves digital palpation of neck node regions,[2] bimanual palpation of the floor of mouth and tongue, and inspection with palpation and observation of the oral and pharyngeal mucosa with an adequate light source; mouth mirrors are essential to the examination. Forceful protraction of the tongue with gauze is necessary to visualize fully the posterior lateral tongue and tongue base. The clinician should review the social, familial, and medical history and should document risk behaviours (tobacco and alcohol usage), a history of head and neck radiotherapy,[3] familial history of head and neck cancer, and a personal history of cancer. Patients over 40 years of age should be considered at a higher risk for oral cancer.[3]

DIAGNOSIS

Most oral cancer screening programs include the simple visual inspection, whereas others attempt the use of toluidine blue, brush biopsy (exfoliative cytology) chemiluminiscence and fluorescence imaging. The last three screening methods in fact deal with the diagnosis of lesions that have already been detected by the patient, dentist or other clinician but a definitive diagnosis can only be made by a tissue biopsy. Diagnosis can be delayed by several months or more if the clinician treats the patient's complaints

Fig. 1: Multiple abscesses observed in the hatsheputxray, ancient Egypt

Fig. 2: Few of the Factors inducing malignant lesions of the oral cavity

Fig. 3a: Malignant Lesions as a result of lodging the quid in the buccal vestibule

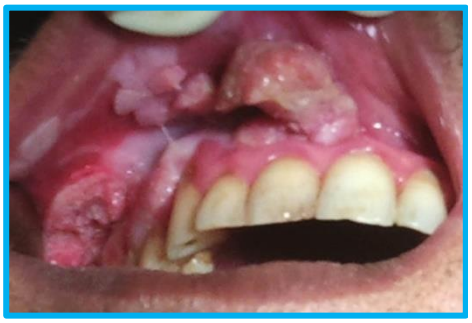

Fig. 3b: Malignant Lesions as a result of lodging the quid in the buccal vestibule

Fig. 4: Effects of Oral Cancer - DNA

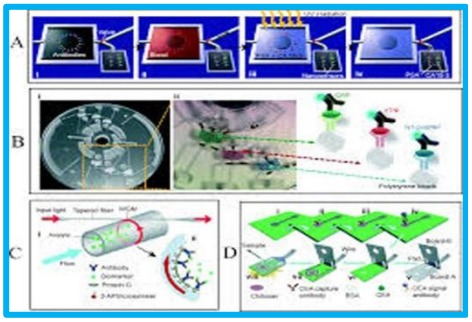

Fig. 5: Cellular Derangements

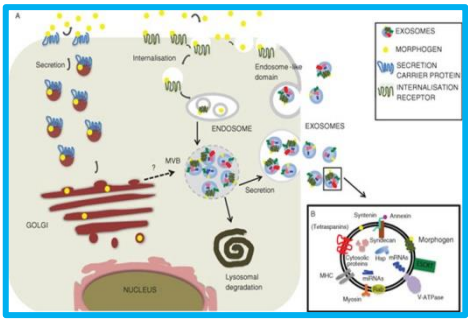

Fig. 6: Salivary Biomarkers

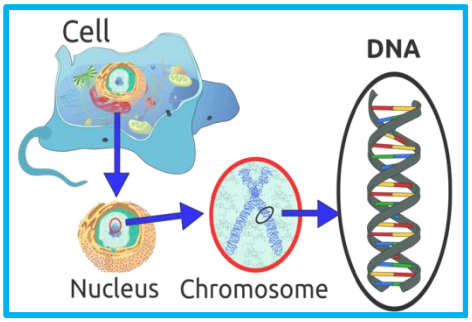

Fig. 7: Alterations at the cellular level

empirically with drugs instead of providing a thorough physical examination and workup.[4] Patients with complaints lasting longer than 2-4 weeks should be referred promptly to an appropriate specialist to obtain a definitive diagnosis. If the specialist detects a persistent oral lesion, a biopsy should be performed without delay. There are invasive and noninvasive methods of detecting oral cancer. Biopsy is defined as removal of tissue from a living individual for diagnostic examination. The health history, history of the lesion, clinical examination, radiographic examination, laboratory investigation, and Biopsy. Biopsies are of various types oral brush Biopsy, incisional wedge biopsy, excision biopsy, aspiration biopsy, tissue

Table 1

EARLY	LATE
Persistent red and/or white patch Nonhealing ulcer	Indurated area
Progressive swelling or enlargement Unusual surface changes	Paresthesia, dysesthesia of the tongue or lips, altered vision
Sudden tooth mobility without apparent cause	Airway obstruction
Unusual oral bleeding or epistaxis Prolonged hoarseness	Chronic earache (chronic serous otitis media) /otalgia, Persistent pain or referred pain

stabilization, specimen care, surgical closure. The diagnostic medium of choice is saliva because of the fact that the levels denote the systemic health and disease status. The first report of saliva as a diagnostic medium for oral cancer was published by Liao et al. who identified mutations in exon4, condon63 in 5 out of 8 patients with oral squamous cell carcinoma. Saliva can be utilized for early detection of oral cancer as this body fluid maintains continuous contact with these lesions. Diagnosis of OSCC is currently based on biopsy test, which is an invasive method. There is a need for developing a noninvasive screening tool[5] (biomarker test) for early detection of squamous cell carcinoma. The many signs and symptoms of oral cancer are usually divided into early and late presentation. They can be so diverse that the differential diagnosis may not lead to oral malignancy.

FREQUENT SIGNS AND SYMPTOMS

Table 1 summarizes the signs and symptoms

Oral Brush Biopsy

Brush cytology can be a noninvasive means of diagnosing dysplasia and early carcinoma in those patients who are either asymptomatic or in those with minor symptoms who do not warrant immediate biopsy. The mechanism of cytology, regardless of its application to cervical, bladder or oral mucosal lining, is based upon the fact that dysplastic and cancerous cells tend to have fewer and weaker connections to each other and to their neighboring normal cells in the surrounding tissue. Dysplastic and cancerous cells therefore, tend to "slough off" or exfoliate preferentially and can easily be collected from the surface of the lesion. A sample of these cells applied to a microscope slide will often contain abnormalities if harvested from a dysplastic or cancerous lesion.

SALIVARY BIOMARKERS

Saliva as a diagnostic fluid meets the demands for inexpensive, noninvasive, and accessible diagnostic methodology. The presence of HPV (human papilloma virus) and Epstein Barr virus genomic sequences have been identified as possible DNA (Fig. 4) molecular markers in detecting OSCC and tumor. The salivary biomarkers (Fig. 5) 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. RNA based biomarkers include a group of recently discovered biomarkers, which include messenger RNAs and micro RNAs. In this review, we present the importance of ribose nucleic acids in saliva and their role as biomarkers in the diagnosis of oral squamous cell carcinoma. The 90% of oral cancers are oral squamous cell carcinoma. This cancer, when found early, has an 80 to 90% survival rate. Despite this fact and as the great treatment advances, the World Health Organization has reported oral cancer as having one of the highest mortality ratios amongst other malignancies.[7] Molecular markers for the diagnosis of OSCC can be quested in 3 levels; (I) changes in the cellular DNA, which result in (II) altered mRNA transcripts, leading to (III) altered protein levels (intracellularly, on the cell surface or extracellularly).[7,8] Typical changes in the host DNA of dysplastic or cancer cells include point mutations, deletions, translocations, amplifications and methylations, cyclin D_1, epidermal growth factor receptor (EGFR), microsatellite instability and HPV {human pappiloma virus} presence.[8-15] Allelic loss on chromosomes 9p has been observed in OSCC

{oral squamous cell carcinoma}. Mitochondrial DNA mutations have also been useful targets to detect exfoliated OSCC cells in saliva. They have been identified in 46% of head and neck cancers. The same mitochondrial DNA mutations were detected in 67% of saliva samples from OSCC patients by direct sequencing alone. p53 gene mutations are also present in approximately one-half of head and neck cancers. Using plaque hybridization, Boyle *et al*., identified tumor specific p53 mutations in 71% saliva samples from patients with head and neck cancer.[8-15] RNA for years was thought to quickly degrade in saliva due to the various RNAses that saliva contains. Despite the opposite reports, cell-free RNA molecules however, seem to exist in saliva both intact but also fragmented. An intriguing question that remains to be answered is the mechanism by which mRNA in saliva is protected by degradation. A speculation is that salivary mRNA is contained in apoptotic bodies, or actively released in exosomes or microvesicles (Fig. 6). Lately microRNAs, small RNA molecules, 18-24 molecules in length, that seem to regulate transcription were also discovered existing in saliva.[8-15] Moreover mRNA markers (Fig. 5) in the saliva have been proposed for the diagnosis of primary Sjögren's syndrome and for the identification of sleep drive both in flies but also in humans. Several salivary protein markers for OSCC have been investigated in various studies and have shown relatively moderate sensitivity and specificity values relative to prognosis prediction. For example, defensins are peptides which possess antimicrobial and cytotoxic properties. They are found in the azurophil granules of polymorphonuclear leukocytes.[8-15] 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. A group of leading researchers - using new and sophisticated approaches, such as, Luminex Multianalyte Profiling (xMAP) technology, shotgun proteomics, capillary reversed-phase liquid chromatography with quadruple time-of-flight mass spectrometry and matrix-assisted laser desorption/ionization–mass spectrometry (MALDI–MS), has contributed significantly in recent years to the research in saliva for cancer diagnosis. Their studies have shown that saliva contains proteins that may serve as biomarkers for OSCC, since 46 peptides/proteins were found at significantly different levels between the OSCC and control groups. For example Arellano-Garcia *et al.*, using Luminex xMAP technology showed that both IL-8 and IL-1β were expressed at significantly higher levels in OSCC subjects.[16] Other salivary biomarkers which have been shown to be significantly altered in OSCC patients as compared with healthy controls are inhibitors of apoptosis (IAP), squamous cell carcinoma associated antigen (SCC-Ag), carcino-embryonic antigen (CEA), carcino-antigen (CA19-9) CA128 serum tumor marker (CA125), intermediate filament protein (Cyfra 21-1) tissue polypeptide specific antigen (TPS) reactive nitrogen species (RNS) and 8-OHdG DNA damage marker lactate dehydrogenase (LDH) and immunoglobulin (IgG) s-IgA insulin growth factor (IGF) metalloproteinases MMP-2 and MMP-11.[16-18]

Role of Salivary miRNA 31 in oral cancer

Micro RNAs[19] miRNAs are small noncoding RNAs that are produced naturally by the cell. miRNAs are short noncoding RNA molecules that play important roles in regulating a variety of cellular processes. Dysregulation of miRNAs are known to be associated with many diseases. miRNAs were found present in the saliva of OSCC patients and could serve as potential biomarkers for oral cancer detection. They function by sequence-specific binding of a seed sequence to the 3′ end of the untranslated region (UTR) of a target mRNA, causing the mRNA to then be degraded or to be translationally inhibited. The microRNAs have been thought to regulate two-thirds of the entire protein coding genome. The expression of miRNAs themselves can also be regulated similarly to that of protein coding genes. Whether through genetic or epigenetic shifts, the expression levels of miRNAs[20] are often altered in many cancers, resulting in abnormal increases or decreases. These alterations have been shown to play a part in almost all facets of cancer development and progression. Recent research has shown that tumor suppressors, such as phosphatase and tensin homolog (PTEN) and p53, can be potential targets of miRNAs. Typically downregulated in many cancers, the loss of these critical tumor

suppressors can greatly increase cell proliferation and tumor progression. Furthermore, the link between some tumor suppressors and survival genes, such as the link between PTEN and the survival effector, AKT (also known as protein kinase B or PKB), may indicate therapeutic means of targeting metastasis, tumor growth, and cancer survival. And indeed, current research is examining these miRNAs[21] that target tumor suppressors both on their own and in combination with traditional therapies, such as cisplatin, etoposide, and ionizing radiation. Additionally, certain families of miRNAs have been implicated in epithelial-to-mesenchymal transition (EMT), a critical component of cancer metastasis. EMT is typically marked by changes in morphology and cytoskeletal rearrangement. The microRNAs are short noncoding RNA molecules[21] that play important roles in regulating a variety of cellular processes. Dysregulation of miRNAs are known to be associated with many diseases. Those miRNAs that were found present in the saliva of OSCC patients and could serve as potential biomarkers for oral cancer detection.[21]

Prevalidation of Salivary Biomarkers

In order to substantiate the development of salivary biomarkers, we assessed the original putative OSCC markers in 395 subjects, in five independent validation cohorts. In this article, we describe two important validation steps: firstly we independently validated the behavior of these biomarker candidates in multiple cohorts. Second, we demonstrated the reproducibility and robustness of the assays in an outside reference laboratory.[22] Oral cancer is the sixth most common cancer with a five-year survival rate of approximately 60%. Presently there are no scientifically credible early detection techniques beyond conventional clinical oral examination. The goal to validate is if the 7 mRNAs and 3 proteins previously reported biomarkers are capable[22] of discriminating patients with oral squamous cell carcinomas (OSCC) from healthy subjects in independent cohorts and by a National Cancer Institute (NCI)- Early Detection Research Network (EDRN) Biomarker Reference Laboratory (BRL).[22]

Salivary Metabolomics

Saliva is an informative biofluid that can be used to monitor a wide range of oral and systemic diseases. This medium has gained much attention as a diagnostic fluid because of its simple collection, non-invasiveness, and low cost. Many studies have been conducted to identify diagnostic markers in saliva for several diseases,[23] including HIV, diabetes, viral hepatitis and, importantly, cancers. The recently developed comprehensive molecular profiling approaches, such as transcriptomics and proteomics, can accelerate these studies. Metabolomics, profiling of all intracellular metabolites, has become a powerful new omics tool that can provide insight into cellular functions and facilitate biomarker discovery. Although there are several approaches to profiling metabolites, capillary electrophoresis time-of-flight-mass spectrometry (CE-TOFMS) has a prominent advantage because it enables comprehensive, simultaneous and quantitative analysis of key metabolites in various pathways, such as glycolysis,[23] pentose phosphate pathway, tricarboxylic acid cycle and urea cycle, as well as amino acid and nucleotide metabolism. Thus, this method is well-suited to biomarker discovery in cancer cells because there are diverse metabolic aberrations related to energy metabolism and cellular proliferation in cancer cells. We recently applied CE-TOFMS-based metabolomic analysis to evaluate the potential of using the salivary metabolomic signature to detect oral cancer (oral squamous cell carcinoma cancers). The metabolic fingerprints embedded in the salivary specimens showed a prominent difference between oral cancer and healthy controls.[23]

TREATMENT AND DISCUSSION

Recent Innovations in Oral Cancer early Detection and Cure

The treatment modalities for cancer especially head and neck cancer depends on the TNM staging assessment, metastasis, Biopsy results, radiotherapy, chemotherapy. It is well known fact that Prompt detection of head and neck squamous cell carcinoma is vital to successful management. Microsatellite analysis of the DNA (Fig. 7) of exfoliated mucosal cells and pretreatment oral rinse helped to detect cancer.[24] The outlook for oral chemotherapy[25] is positive. Having been for many years the poor relation, oral chemotherapy is poised to become a major force. Encouraging clinical trial results indicate that for the first time many of the oral anti-cancer drugs in development are actually better drugs rather than

pale imitations of i.v. treatments. Many of these new agents, especially signal transduction inhibitors or angiogenesis inhibitors, will be available only as oral treatments with an i.v. dosage form either impractical or inappropriate. Oral treatment will reduce the number of in-patient and out-patient hospital visits with their associated medical and nursing administrative costs, avoid the expense of disposables[25] (e.g. infusion equipment, pumps) and decrease the pharmacy workload. Chemotherapy costs account for only a small proportion of the direct cost of cancer care so it should be possible to set increased drug costs against the substantial savings that will be made elsewhere. Drug budgets are, however, easily identified and this process will be easier in some countries than in others. This article reviews the diagnostic aids, medium of choice for investigation both invasive and noninvasive, role, validation and prevalidation of salivary biomarkers,[25] drug delivery systems, metabolomics recent developments in oral chemotherapy, both of traditional cytotoxics and novel, targeted agents, from the viewpoint of patients, physicians, and health-care providers.[25]

CONCLUSION

Oral cancer refers to the malignancies that occur in the head and neck, the tongue, the lip, the oral mucosa and the other oro pharyngeal structures. Salivary biomarkers play a vital and noninvasive method of early detection of cancer. The changes in cellular level of DNA, the micro RNA profile, messenger RNA and the salivary biomarkers are discussed in detail. Salivary Metabolomics focuses on cellular functions. The recent innovations like micro satellite analysis of exfoliated cells during pretreatment rinse and electrochemical sensor have been beneficial in the treatment modalities in addition with the traditional modalities. Oral cancer has a low five-year survival rate. Early detection of oral cancer could reduce the mortality and morbidity associated with this disease. Saliva, which can be sampled non-invasively and is less complex than blood, is a good potential source of oral cancer biomarkers.

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The author declares that there is no source of funding and there is no conflict of interest among all authors.

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