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SALIVARY BIOMARKERS FOR EARLY DIAGNOSIS OF ORAL CANCER

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ABSTARCT

Oral cancer is one of the major global public health problems and is the sixth most common human malignancy. Oral squamous cell carcinoma (OSCC) accounts for more than 90% of all oral cancer. The high morbidity rate in OSCC can be attributed to the delay in the diagnosis of the disease. Saliva, a watery and frothy substance produced in the mouth of human is in direct contact with the oral cancer lesion. Hence, the abnormal DNA, RNA, protein molecules discharged by the cancer cells can be easily acquired from saliva. Detection of oral cancer by The use of salivary biomarker can serve as a noninvasive, cost effective and valid method which could easily employed for early detection, monitoring post therapy status, prognosis of oral cancer patients. This article aims to discuss various salivary biomarkers and their implications in oral cancer.

KEYWORDS: Oral Cancer, Biomarkers, Saliva, DNA, Mrna.

INTRODUCTION

Worldwide, cancers of the oral cavity and pharynx are the 6th most common type.[1] Its five-year survival rate has remained around 62% in contrast to the five year survival rates for

breast cancer (89%) and prostate cancer (99%).[2] Oral squamous cell carcinoma (OSCC) accounts for more than 90% of all oral cancers.[3] Each year about 5,75,000 new cases are diagnosed and as a consequence 3,35,000 deaths occur worldwide. Its high frequency in Central and South East Asian countries (India, Bangladesh, Sri Lanka, Thailand, Indonesia and Pakistan) has been well documented. Oral cancers are seen predominantly in both sexes accounting for one third of all the cancers in South East Asian countries. India has one of the highest incidences of oral cancer in the world, with estimated incidence of 12.48 cases per 1, 00,000 population in males and 5.52 per 1,00,000 populations in females.[4] Oral cancer is usually first diagnosed when it becomes symptomatic. Unfortunately, by this stage approximately 2/3rd of the patients would have already developed advanced disease with regional metastasis.[5] Delayed detection is likely to be a primary reason for the high morbidity and mortality rates of oral cancer patients, and this strongly supports the need to perk up early detection of oral cancers. The gold standard for the diagnosis of OSCC is still a biopsy of the suspicious lesion. This procedure is not suited for screening purposes for early oral cancer detection due to its invasive nature, high cost, and need for specially trained medical personal and equipment.[6] There is a constant search for biomarkers in saliva, a body fluid that can be easily collected, for noninvasive detection of oral cancer and precancer.[7] For this purpose recently developed technology such as proteomics, transcriptomics and metabonomics are being explored. The development from normal to OSCC cells can lead to altered expression of proteins.[8,9,10] These cytokines have also been linked with increased tumor growth and metastasis, and could thus contribute to the pathogenesis of this disease.[11] Detection of oral cancer by the use of salivary biomarkers can serve as a noninvasive and cost effective and valid method which could easily be employed to screen large populations.

MATERIALS AND METHODS

Literature search was carried out in Medline, PubMed (NCBI) and Scholar Google using the key words salivary biomarkers, tumor markers in saliva, oral cancer, salivary diagnostics, proteomics, transcriptomics and metabonomics. Some basic information was also obtained from textbook and medical university websites.

DISCUSSION

Whole human saliva, a multi-constituent oral fluid, is secreted primarily by three major glands that is parotid gland, sub mandibular gland and sublingual gland. [12,13] Generally, 1–1.5 L of saliva is produces by salivary glands. It contains approximately 99% water with

minerals, nucleic acids, electrolytes, mucus and proteins.[14] It is one of the most intricate, versatile, and significant body fluids, supplying a large range of physiological requirements. Therefore, saliva is also called the "mirror of the body" or "a window on health status". One of the great advantages of saliva is its non-invasive collection as a diagnostic medium, especially when repeated samples must be taken for specific assessments. Moreover, it has the advantages of easy to store and inexpensive compared to blood sample collection.[15] The non-invasive saliva collection techniques dramatically reduce anxiety and discomfort of the patients. Saliva is also easier to handle for diagnostic procedures because no special equipment is needed for saliva sample collection and it does not clot, thus reducing the manipulations which may be required for biochemical analysis.[16]

Biomarkers

The United Nations' World Health Organization defines a biomarker as any substance, structure or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease.[17]

Criterion for Biomarker[18]

1. A stable product, not vulnerable to artefactual induction, not easy to lose, or not changeable during storage.
2. Determined by an analytical assay that is specific, sensitive, reproducible and robust
3. A major product of oxidative modification that may be implicated directly in the development of the disease.
4. Representative of the balance between oxidative damage generation and clearance
5. Accessible in a target tissue or a valid surrogate tissue such as a leukocyte
6. Free of confounding and interference factors from dietary intake
7. Detectable and measurable within the limits of detection of a reliable analytical procedure.

Biomarkers for Oral Cancer-Applications[19]

1. Biomarkers help in evaluating the preventive measures or therapies and the detection of the earliest stages of oral mucosal malignant transformation

2. Reveal the genetic and molecular changes related to early, intermediate, and late end-points in the process of oral carcinogenesis

3. Refine the ability to enhance the prognosis, diagnosis, and treatment of oral carcinomas - Monitor progression/ recurrence, treatment compliance

4. Useful in early stages of cancer drug development

5.Determine efficacy and safety of chemopreventive agents

Potential salivary biomarkers for oral cancer detection

Hitherto, more than 100 potential OSCC salivary biomarkers have been described in the literature, based mainly on comparing the levels found in oral cancer patients to the levels found in normal controls. The research methodology involved so far in investigating these potential OSCC salivary biomarkers have been grouped according to the types of biomarker, as follows[20]

1. Non-organic compound biomarkers
2. Peptide or protein biomarkers
3 DNA, mRNA or microRNA biomarkers
4. Metabolomic biomarkers
5. Miscellaneous biomarkers (chemical and enzyme activity)

Table 1 Potential salivary biomarkers for oral cancer detection [20]

Category	Potential OSCC salivary biomarkers
Non-organic compound	Na, Ca, F, and Mg
Peptide	Defensin-1
Proteins	P53 autoantibody α-amylase IL-8 TNF-α IL-1 IL-6 Basic fibroblast growth factor Statherin Cyfra 21.1 TPA CA125 Endothelin-1 IL-1β CD44 IGF-1 MMP-2 MMP-9 CD59 Catalase Profilin S100A9/MRP14 M2BP CEA Carcinoma associated antigen CA-50

	Salivary carbonyls Cyclin D1 Maspin 8-oxoguanine DNA glycosylase OGG1 Phosphorylated-Src Ki-67 Lactate dehydrogenase Transferrin Zinc finger protein 501 peptide Hemopexin Haptoglobin Complement C3 Transthyretin α1-antitrypsin
DNAs	P53 gene codon 63 Loss of heterozygosity in the combination of markers D3S1234, D9S156, and D17S799 Mitochondrial DNAs (cytochrome c oxidase I and cytochrome c oxidase II) Hypermethylation of promoters in tumor suppressor genes: DAPK, DCC, MINT-31, TIMP-31, TIMP-3, p16, MGMT, CCNA1 Presence of HPV, EBV
mRNAs	IL-8 IL-1β DUSP1 H3F3A OAZ1 S100P SAT (spermidine/SAT EST)
MicroRNAs	miR-125a miR-200a miR-31
Long non-coding RNAs	HOTAIR
Oxidative stress-related molecules	RNS such as NO, NO2 and NO3 Peroxidase GST SOD 8-OHdG Glutathione MDA
Glucocorticoid	Cortisol
Metabolomics	Cadaverine, alpha-aminobutyric acid, alanine, C5H14N5, piperidine, taurine piperideine, pipercolic acid, C4H9N, C8H9N, pyrroline hydroxycarboxylic acid, betaine, C6H6N2O2, leucine+isoleucine, tyrosine, histidine,

	tryptophan, beta-alanine, glutamic acid, threonine, serine, glutamine, choline, carnitine, C4H5N2O11P
	Phenylalanine
	Valine Lactic acid
Glycosylation related molecules	Sialic acid α-L-fucosidase
Other	Telomerase activity
OSCC: Oral squamous cell carcinoma, IL: Interleukin, TNF-α: Tumor necrosis factor-α, TPA: Tissue polypeptide antigen, CA125: Cancer antigen 125, IGF-1: Insulin growth factor 1, MMP: Matrix metalloproteinase, CEA: Carcinoembryonic antigen, HPV: Human papillomavirus, EBV: Epstein-Barr virus, DUSP1: Dual specificity phosphatase 1, H3F3A: H3 histone family 3A, OAZ1: Ornithin decarboxylase antizyme1, S100P: S100 calcium binding protein P, SAT: Spermine N1-acetyltransferase, RNS: Reactive nitrogen species, NO: Nitric oxide, NO2: Nitrites, NO3: Nitrates, GST: Glutathione S-transferase, SOD: Superoxide dismutase, 8-OHdG: 8-hydroxy-2-deoxyguanosine, MDA: Malondialdehyde	

Clinical implications of salivary biomarkers of oral cancer

Numerous salivary tumor markers are found to be significantly increased in the saliva of oral cancer patients. Several reports on these salivary biomarkers in oral cancer have shown significant clinical usefulness for oral cancer. Shiptzer Tet al in 2009 found that various biomarkers namely CycD1, Ki67, LDH, MMP-9, OGG1, Maspin were significantly altered in oral cancer and found to be useful as a supportive tool for diagnosis, prognosis and post-operative monitoring.[21] Authors[22,23] in other studies found that Methylation array analysis of saliva can produce a set of cancer related genes that are specific and can be used as combined biomarkers for early detection of oral cancer. An assay was developed that could rapidly quantify the promoter hypermethylation of the gene of interest and could potentially be applied into a clinical setting. Sudbo, *et al*.,[24] and Femiano, *et al*.,[25] have found that premalignant lesions with aneuploidy convert into cancer more frequently than lesions with normal DNA content irrespective of the histopathological grade of dysplasia. DNA aneuploidy appears to be associated with advanced stage carcinomas and lymph node metastasis.[26] Hence, DNA content of a tumor may help in predicting the aggressiveness of the cancer. Zhao M et al in their study concluded that detection of HPV in salivary rinses has Potential for development of molecular screening of HPV related oral cancer.[27] Mitochondrial DNA mutations have also been useful to detect exfoliated OSCC cells in saliva.[28] Such mutations have been identified in 46% of head and neck cancer and in 67% of saliva samples from OSCC patients by direct sequencing.[29] Zhong et al. [30] found 75% positive expression of telomerase in saliva of oral cancer patients suggesting its utility as a

supportive marker to diagnose oral cancer and also suggested that human telomerase reverse transcriptase (hTERT) analysis may be a potential biomarker for the diagnosis of oral cancer. The Jou YJ et al concluded that actin and myosin are promising salivary biomarkers for distinguishing premalignant and malignant oral lesions. Salivary transferrin is also validated as a biomarker for detection of early stage oral cancer.[31] Many research have also been carried out on Carbonylation (indicative of oxidative damage to proteins) because of its irreversible and irreparable nature, which becomes cytotoxic and is associated with cancer.[32] It is presently reported that a substantial increase in salivary carbonyls (246%) is seen in OSCC patients and there is a significant free radical attack to which the epithelial cells are exposed.[33]

Many studies have investigated the use of salivary proteins as potential diagnostic biomarkers for OSCC [34,35,36,37] The levels of salivary soluble CD44 were shown increased in the majority of OSCC and could distinguish cancer from benign disease with high specificity.[34] Three tumor markers, cytokeratin 19 fragment Cyfra21-1, tissue polypeptide antigen, and cancer antigen 125, were found significantly raised in the saliva of OSCC patients, and combined use of these markers resulted in similar diagnostic value as sera of OSCC patients.[35] The level of p53 autoantibody in saliva was also found correlated with its serum levels in OSCC and examination of p53 autoantibody in saliva may present a specific method for recognition of a subset of OSCC with p53 aberrations.[36]

Researchers have found seven mRNA molecules; IL-8, and IL1β which play a part in signal transduction, proliferation, inflammation and apoptosis, S100P (S100 calcium binding protein P) with a role in protein binding and calcium ion binding, DUSP1 (dual specificity phosphatase 1) which takes part in signal transduction, protein modification and oxidative stress, OAZ1 (ornithine decarboxylase antizyme 1) helps in polyamine biosynthesis, H3F3A (H3 histone, family 3A) possessing a DNA binding activity and SAT (spermidine/spermine N1-acetyltransferase) which is included in enzyme and transferase activity, to be significantly increased in OSCC patients in comparison to healthy controls. [37,38]

Challenges in OSCC salivary biomarker research[20]

1. A lack of standardization of settings and methods of saliva sample collection, processing, and storage

2. Variability in the levels of potential OSCC salivary biomarkers in both non-cancerous individuals and OSCC patients, suggest unknown confounding factors

3. The need for further validation of OSCC salivary biomarkers
4. Validation in the presence of other types of human cancers.

CONCLUSION

A number of tumor markers in serum for OSCC have been investigated in the recent past with the results demonstrating moderate sensitivity and specificity with respect to the diagnosis, prognosis prediction and post therapy status. Saliva as a sample has many advantages over serum and tissue. It is relatively easy to obtain fluid that can be collected in sufficient quantities. The collection process is noninvasive, simple, not technique sensitive and is easily adaptable for use even in outpatient clinics and laboratories. Measurement of molecular markers in saliva can thus be used as a tool for screening large populations. Though at present no single tumor marker can validate the presence or prognosis of disease, a panel of biomarkers would be more helpful and standardization of methods and validation of these biomarkers should be carried out to increase the standards of diagnostic and therapeutic research.

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