Importance of Salivary Biomarkers in the Detection of Oral Cancer (Review Article)

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Abstract: *Aim*: This article aims to provide a brief overview of various salivary biomarkers and their importance in early detection of oral cancer.

Background: The tumor markers are playing an important role in cancer detection and management. The cancer biomarker is produced either by the tumor itself or by other tissues, and consider as molecular signature indicating the physiologic and pathologic changes in a particular tissue or cell during the development of cancer. The direct contact between the saliva and the oral cancer lesions makes the salivary biomarkers the best sensitive and specific test for primairy screening method in diagnosis, staging and follow-up of oral cancer.

Materials and Methods: Studies were conducted by searching for reviews in salivary biomarkers of oral malignancy in the past 5 years in Google Scholar, Medline, and PubMed. The references were also crosschecked for the relation of salivary biomarkers and oral cancer. Articles were identified and subjected to qualitative and quantitative analyses.

Review Results: More than 100 potential Oral squamous cell carcinoma (OSCC) salivary biomarkers have been reported in the literature. The proteomics analysis of saliva proteins is significance for early diagnosis of Oral squamous cell carcinoma (OSCC). Saliva contains reliable amounts of cells, mainly exfoliated from the oral cancer, which can provide early diagnosis and detection of oral malignancy.

Conclusion: The goal of cancer screening is to detect tumor at an early stage, when treatment is most likely to be successful. Salivary biomarkers will help to differentiate patients who clinically have no detectable disease but are potential candidates for oral cancer.

Keywords: Oral squamous cell carcinoma (OSCC), proteomics, tumor biomarkers, salivary biomarkers.

1.INTRODUCTION

Most oral cancers are detected at a later stage leading to lower survival rates. Early detection of oral cancer is important to reduce the incidence of mortality of fatal disease, and will allow 90% of oral cancer to be curable with low effective cost for treatment [1].

Early detection of Oral squamous cell carcinoma (OSCC) is a key factor in improving the prognosis and survival rate of the patients; it represents one of the most promising approaches to reducing the growing cancer, and improves the treatment outcome.

The tumor markers are playing an increasingly important role in cancer detection and management. The cancer biomarker, is produced either by the tumor itself or by other tissues, and consider as molecular signature indicating the physiologic and pathologic changes in a particular tissue or cell during the development of cancer. There are several types of biomarkers, including diagnostic biomarker, prognostic biomarker and stratification biomarker. Biomarkers can be a molecular or a series of molecular, which can be found in a variety of fluids, tissues and cell lines. Biomarkers can be DNA, mRNA, proteins, metabolites, or processes such as apoptosis, angiogenesis or proliferation.

Proteins produced by cancer cells or their microenvironment may eventually enter the circulation system and the expression patterns of these proteins could be assessed by Quantitative proteomics tests which is allowed to quantify and identify all the proteins expressed by a whole genome. saliva examination shows the greatest benefit for detection of oral cancer because of its direct contact with oral cancer

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lesions. The most important point for selecting saliva as a diagnostic tool is that it also contains the fallen cells in oral cavity which allow saliva to be the first choice of screening and identification of potential biomarkers in the oral cancer. The proteomics analysis of saliva proteins is significance for early diagnosis of OSCC. Saliva contains reliable amounts of cells, mainly exfoliated from the oral epithelium, which can provide adequate amounts of protein for proteomics studies. These biomarkers will help to differentiate patients who clinically have no detectable disease but are susceptible to be potential candidates for oral cancer.

2. SALIVARY BIOMARKERS

Saliva is the main source of oral fluid, It consists not only of secretions from the three major salivary glands (parotid, submandibular and sublingual) and the minor glands, but also gingival crevicular fluid, oral mucosa transudate, secretions from nasal and pharyngeal mucosa, non- adherent bacteria, desquamated oral epithelial cells, keratin debris, blood cells [2]. Saliva, consider as an aqueous biological fluid which is in direct contact with the oral cancer lesion. Saliva has been found to contain constituents that reflect the disease or physiological state of the human body, and hence could be utilized for diagnostic purposes [3].

Hence, the abnormal DNA, RNA, protein molecules released by the malignant cells can be easily obtained from saliva [4]. This fluid has multiple functions, including: anti-bacterial, anti-viral and anti-fungal properties; buffering capacity for plaque acids; digestive activity (amylase, protease, nuclease enzymes) needed for food mastication; mineralizing agents for protection and repair of hard tissues; lubricant and viscoelastic properties essential for the maintenance of oral health; and protective and repairing fluid for mucosal surfaces [5].

So far, more than 100 potential Oral squamous cell carcinoma (OSCC) salivary biomarkers have been reported in the literature, based mainly on comparing the levels found in OSCC patients to the levels found in non-OSCC normal controls [2]. Most categories of Potential salivary biomarkers for oral cancer detection are: Non-organic compound (Na, Ca, F, and Mg) [6], peptid (Defensin-1) [7], proteins (IL-1, IL-6, IL-8, TNF-α, Endothelin-1,...) [8-22], DNAs (P53 gene codon 63,…) [23-24], mRNAs (IL-1β, H3F3A,…) [25], Oxidative stress-related molecules (Glutathione, Per-oxidase,…) [26], Glucocorticoid (Cortisol) [27], Meta-bolomics (Lactic acid, Valine,….) [28], Glycosylation related molecules (Sialic acid, α-L-fucosidase) [29-30]. A comprehensive description of most potential oral cancer biomarkers are described in the Table **1** summary of which have been reported in the literature [31].

3. MATERIALS AND METHODS

Studies were conducted by searching for reviews in salivary biomarkers of oral malignancy in the past 5 years in Google Scholar, Medline, and PubMed. The references were also crosschecked for the relation of salivary biomarkers and oral cancer. Articles were identified and subjected to qualitative and quantitative analyses.

4. EXCLUSION CRITERIA

The following studies were excluded: (1) those that used different biological media such as blood or body fluids instead of saliva as potential media diagnostics (2) those that were not related to salivary biomarkers and oral cancer (3) those that reported associations between saliva and cancer in experimental studies (*et al* or *in vivo* animal studies).

5. REVIEW RESULTS

The tumor markers are playing an increasingly important role in cancer detection and management. These biomarkers are detected by laboratory tests (blood, saliva). The biomarkers for early cancer detection must meet the following criteria: (a) the altered can be objectively measured; (b) must be measurable in small specimens; (c) must be altered in the high-risk tissues, but not in the normal tissues; and (d) must be altered in the early stages of cancer development [2]. saliva examination for detection oral cancer shows the greatest benefit because of its direct contact with oral cancer lesions, makes it a most sensitive and specific, screening method in diagnosis [32]. The most important point for selecting saliva as a diagnostic tool is that it also contains the fallen cells in oral cavity which allow saliva to be the first choice of screening and identification of potential biomarkers in the oral cancer [3]. The salivary tumor markers in oral cancer include genomic markers, transcriptome markers, protein markers and microbiota. Salivary biomarkers have many advantages over serum because they are inexpensive, non-invasive, and easily accessible media and plays a vital role in diagnosis, prediction of prognosis and monitoring of patient's health [33].

Table 1: Potential Salivary Biomarkers for Oral Cancer Detection

Potential Salivary Biomarkers in OSCC	Category
Na, Ca, F, and Mg	Non-organic compound
Defensin-1	Peptide
α-amylase	Proteins
IL-1 , IL-8 , IL-6 , IL-1β	
TNF-α	
Basic fibroblast growth factor	
Endothelin-1	
Cancer antigen 125 (CA125)	
Carcinoembryonic antigen (CEA)	
Carcinoma associated antigen CA-50	
8-oxoguanine DNA glycosylase (OGG1)	
Phosphorylated-Src	
Salivary carbonyls	
Transferrin	
α1-antitrypsin	
P53 gene codon 63	DNAs
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	
Loss of heterozygosity in the combination of markers D3S1234, D9S156, and D17S799	
IL-8, IL-1β	mRNAs
DUSP1 (dual specificity phosphatase 1)	
OAZ1 (ornithin decarboxylase antizyme 1)	
H3F3A (H3 histone family 3A)	
MicroRNAs	MicroRNAs
miR-200a	
miR-31	
Peroxidase	Oxidative stress-related molecules
Glutathione S-transferase (GST)	
Malondialdehyde (MDA)	
Glucocorticoid	Glucocorticoid
Phenylalanine	Metabolomics
Valine	
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	

More than 2300 proteins and peptides have been found in human saliva and among them than 100 salivary biomarkers have been reported as potential OSCC salivary biomarkers [2]. The screening tools should be sufficiently noninvasive and inexpensive to allow widespread applicability. Salivary screening can be the best choice as the primary screening test for the high-risk cases of OSCC, since the collection procedure is noninvasive and low cost [31].

6. DISCUSSION

Despite the fact that more than 100 potential OSCC salivary biomarkers have been reported [6], but there has been no standardization regarding the condition of the subjects from whom the saliva samples are collected (e.g., the timing in regard to prior food and drink intake, or the use of oral hygiene products). Similarly, a uniform method has not been established for how the saliva samples are collected, processed and stored prior to measurement and comparison of the biomarker levels in the groups studied.

The differences in these factors among the different studies raises the question as to whether the levels of the potential OSCC salivary biomarkers reported in any one lab could be compared to the levels of the same biomarker reported in any other lab. In fact, for the reported potential biomarkers which have been investigated by more than one study, a wide variability was found in the levels of both the diseased and the control groups among different labs. Variability in the levels of potential OSCC salivary biomarkers in both non-cancerous individuals and OSCC patients, suggest unknown confounding factors.

Without standardization and validation of biomarkers, valuable research resources are being squandered, and the experts in this research field is needed to discuss this issue and find a way to establish the standards protocol [33].

Salivary biomarkers represent a promising non-invasive approach for oral cancer detection, and an area of strong research interest. However, some issues (These issues include a lack of standardization for saliva sample collection, processing, and storage) need to be resolved in order to establish this approach as a reliable, highly sensitive and specific method for clinical use [33]. Until present there is no consensus regarding which salivary biomarkers have the best diagnostic value [34].

The interest is based on several advantages of saliva versus other body fluids, for example, serum or urine such as straightforward sample collection, sufficient quantities for analysis, and the lower costs of storage and shipping than those for serum or urine [35].

Previous studies have shown that IL-6, IL-8 are post-inflammatory cytokines, play a prominent role in immune host defense responses to infection. These chemokines are found to stimulate angiogenesis, influence tissue remodeling and take part in the regulation of cell proliferation and differentiation. They are essential mediators of cancer development and powerful activators of apoptotic and anti-apoptotic signaling cascade. Hence, IL-6 and IL-8 have been implicated in early detection of oral pre malignancies and OSCC [36-37].

It is impossible to determine what ranges of salivary IL-6 or IL-8 levels are likely to indicate OSCC development. Inherent biological variations are known to create difficulties in determining the reference values for biological samples in clinical laboratories [38]. Such variations in salivary constituents could be attributed to differences in ethnic background, geographic locations, age, gender, non-neoplastic systemic diseases, dietary habits, medications being taken or other factors.

Screening test for oral cancer using whole saliva samples, which can be easily and noninvasively collected. A proteomic studies performed to assess changes in protein expressions of whole saliva, revealing that some proteins are either expressed or unexpressed specifically in oral squamous cell carcinoma (OSCC). Two-dimensional electrophoresis (2-DE) and peptide-mass fingerprinting (PMF) employed to identify biomarkers that specifically change expression in OSCC, the Results showed that ten protein spots were specific to the preoperative whole saliva of OSCC patients, but were absent in the whole saliva of healthy individuals and OSCC patients after surgery. Detection of enolase 1 in the saliva was shown to be significantly higher for OSCC patients than for healthy individuals. Immuno histo chemical staining confirmed that appearance of enolase 1 was significantly higher in OSCC tissue than in healthy tissue [38].

CONCLUSION

Salivary biomarkers help in evaluating the preventive measures or therapies and the detection of the earliest stages of oral mucosal malignant transformation. Early detection of suspicious lesions will result in earlier diagnosis, less aggressive treatment, and decreased need for complicated post-treatment management. These biomarkers will help to differentiate patients who clinically have no detectable disease but are potential candidates for oral cancer. Therefore identification of OSCC protein biomarker during cancer initiation and progression could aid the diagnosis and treatment of OSCC.

SOURCE OF SUPPORT

Nil

CONFLICT OF INTEREST

None

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