SALIVARY TUMOUR MARKERS IN ORAL CANCER: BRIEF REVIEW

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Abstract

Oral cancer accounts for over 30% of all cancers in India. Early detection followed by appropriate treatment, can increase cure rates to 80 or 90%, and greatly improve the quality of life by minimizing extensive, debilitating treatments . Circulatory epithelial tumour markers were previously investigated in the serum of OSCC patients for early detection of cancers. Scientists are now searching for biomarkers in saliva, an easy to obtain body fluid, for noninvasive detection of oral cancer which makes the measurement of tumour markers in saliva an attractive alternative to serum testing for early detection, post treatment monitoring and monitoring high risk lesion.

Keywords: saliva, biomarkers, oral cancer.

Introduction

Oral cancer represents only about 3 % in US population but in India it accounts for over 30% of all cancers.[1]. Also India accounts for 86% of worlds oral cancer cases(National Institute of Public Health February 2011). Thus oral cancer is a common human malignancy with an increasing incidence and with high mortality rate of ~ 50% which has not changed significantly in more than 50 years. Its survival rates increase significantly when it is detected and treated early.

An estimated 40, 250 new cases of oral cancer will be diagnosed in the United States in 2012, and an estimated 7,850 people will die of the disease. This form of cancer accounts for about 3% of cancers in men.[2] and 1.5% of cancers in women[3]. Oral cancer occurs more frequently in blacks than in whites.[4,5]. In India oral cancer ranks first among all cancer cases in males and is the third most common among females in many regions.[6].

The estimated annual worldwide number of incidence of oral cancers is about 275,000, with an approximately 20-fold variation geographically. South and Southeast Asia (India, Sri Lanka, Pakistan, and Bangladesh), France, and Brazil have particularly high rates. In most countries, men have higher rates of oral cancer than women (due to tobacco use) and higher rates of lip cancer (due to sunlight exposure from outdoor occupations)[7]

Significant proportions of cancers in the initial stages are asymptomatic and are not diagnosed or treated until they reach an advanced stage. Therefore early detection is the most effective means to reduce death from this disease. Currently diagnosis depends on a thorough oral examination and histopathological examination by taking a biopsy. Even though a definite diagnosis is based on biopsy, it would be beneficial if it could be done through non invasive techniques like salivary tumour marker analysis.

Saliva, a multiconstituent oral fluid, has high potential for the surveillance of

general health and disease. Particularly, it represents a promising diagnostic fluid for the screening of various oral diseases. Moreover, saliva is readily available and easily collected without specialized equipment or personnel. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting caries risk, periodontitis, oral cancer,breast cancer, salivary gland diseases and systemic disorders such as hepatitis and the presence of human immunodeficiency virus (HIV) or hepatitis C virus. The simple and non-invasive nature of saliva collection and its high-sensitivity assay development has led to an emphasis on the promise of salivary biomarkers. It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases.

Discussion

There has been ever growing effort dedicated to the basic research of oral cancer, focusing on the identification of biological indicators for diagnosis of its biological nature and aggressiveness. Salivary tumour biomarkers can be classified into proteome, transcriptome,micro RNA,metabolome and microbe[8]. Metabolome is the complete set of small-molecule metabolites such as metabolic intermediates, hormones and other signalling molecules, and secondary metabolites which is found in a biological sample. Recent studies in salivary protein research have shown that , in addition to major salivary protein families , saliva contains hundreds of minor proteins or peptides. Even though these proteins are present in low concentration salivary proteomic technology play an important role in the discrimination of disease. Proteome is the protein complement of the genome and proteomics is analysis of the portion of the genome that is expressed. Using proteomics profiling, Hu et al identified 309 saliva proteins from a healthy participant including 220 proteins with known biological functions.[9,10]

In addition to salivary proteome, salivary transcriptomes are also used in salivary diagnostics. They are unusually stable in saliva. They include mRNA molecules that cells use to convey the instructions carried by DNA for subsequent protein production. 3000 mRNAs are discovered in normal salivary transcriptome. Of these 180 are common between different normal subjects contributing the normal salivary transcriptome core.[11]

P53 is a tumour suppressor protein which is produced in cells exposed to various types of DNA damaging stress. Inactivation of this suppressor is considered a frequent occurrence in the development of human cancer. As a result, accumulation of inactive p53 protein is observed, which in turn may lead to the production of antibodies directed against this protein. Besides its presence in sera, p53 can also be detected in the saliva of patients diagnosed with oral squamous cell carcinoma(OSCC) and can thus assist in the early detection of and screening for this tumour.[12]

Salivary defensin 1 were found to be indicative of the presence of OSCC. Defensins are proteins which possess antimicrobial and cytotoxic properties. They are found in the azurophil granules of polymorphonuclear leukocytes. Higher concentration of salivary defensin 1 were detected in patients with OSCC in comparison with the defensin 1 concentration in the saliva of patients with adenocarcinoma and in healthy controls. A high positive correlation was observed between salivary defensin 1 levels and serum levels of OSCC related antigen .[13]

In addition to IL 8(Interleukin 8),six cancer associated genes have been upregulated in saliva from oral cancer patients such as IL1B(Interleukin 1 ß),DUSP1(Dual Specificity Phosphate 1),H3F3A(H3 histone, family 3A), OAZ1(Ornithine decarboxylase antizyme 1), S100P(S 100 calcium binding protein P),SAT(Spermidine/spermine N1-acetyltransferase) in a study by Yank li et al. DUSP1 gene encodes a dual specificity

phosphatase and has been implicated as a mediator of tumour suppressor PTEN signalling pathway. OAZ1 is predicted as a tumour suppressor based on its known inhibitory function to ornithine decarboxylase. SAT and S100P are associated with prostate cancer progression. The expression of IL1B is also associated with cancers. Chen *et al* previously demonstrated the elevation of IL 8 protein expression in head and neck cancer tissues. The serum level of IL1B has been shown to be higher in patients with OSCC. Identification of cancer associated genes that are consistently changed in cancer patients will provide us not only with diagnostic markers but also with insights about molecular profiles involved in head and neck cancer development.[14]

Certain bacterias were found to be in higher levels in saliva of oral cancer patients. They are C gingivalis, P. Melaninogenica, S mitis. Reason for this finding is not clear. One explanation may relate to the altered cell surface receptors observed in cancer cells. Alterations in tumour cell receptors could change the adhesion of certain species of bacteria.[15]

Cyfra 21-1, TPS(Tissue Polypeptide Antigen) ,CEA(Circulatory Carcinoembryonic Antigen), SCC, CA125(Carbohydrate Antigen 125) and CA19-9(Carbohydrate Antigen19-9) are the most often studied serum circulatory epithelial tumour markers. Rafael Nagler *et al* studied about the presence of these markers in saliva. A significant increase was noticed for Cyfra 21-1, TPS and CA19-9.Other markers also showed an increase in concentration but were not significant.With these results they concluded that eventhough a definite diagnosis of oscc is based on biopsy , it would be highly desirable and beneficial if salivary tumour marker analysis could be done on a routine basis between biopsies.[16]

Using Proteomic profiling Hu *et al* have identified 309 saliva proteins from a healthy participant Their study also revealed the co existence phenomenon between saliva proteins and mRNAs[17]

Free radicals such as reactive oxygen species(ROS) and reactive nitrogen species(RNS) are main contributers to oral carcinogenesis. In OSCC patients salivary RNS levels showed an increased level while all salivary antioxidants were substantially reduced. 8- hydroxydeoxyguanosine (8-OHdG) marker, a widely used indicator of DNA oxidation is also seen increased in OSCC patients(Bahar G *et al*, 2006).RNS in the form of nitrosamines and ROS such as superoxide radicals, hydroxyl radicals and hydrogen peroxide can cause DNA base alterations , strand breaks, damaged tumour suppressor genes and an enhanced expression of proto oncogenes. Thus they play a key role in human cancer development.[18]

In a study to evaluate biochemical and immunological parameters in the saliva of OSCC patients salivary median total protein concentration was found to be in a significantly higher level along with the increased level of Na(sodium), Ca(calcium), P(inorganic phosphate) and Mg(magnesium). Alb(Albumin), salivary LDH (lactate dehydrogenase), total IgG(Immunoglobulin G) also showed increased level while Sec Ig A(Secretory Immunoglobulin A) showed a decreased level. Concentrations of IGF(Insulin Growth Factor), MMP-2,MMP-9(metalloproteinases) were significantly higher in OSCC patients.[19]

Jamee MS *et al*(2007) studied about the salivary levels of proinflammatory cytokines like TNFα, IL1α, IL6 and IL8 in OSCC patients. They noticed a significant increase in the concentration of salivary IL 6.Eventhough others showed an increase, they were not significantly increased. In an earlier study investigators found that serum concentration of IL1α,IL6,TNFα(Tumour Necrosis Factor alpha),soluble TNF receptor I(sTNF-RI) and C reactive protein(CRP) were higher in patients with OSCC and the increased serum levels appeared to be related to the clinical stage of disease.Since various studies showed a controversy between results of investigations, they concluded that further

studies with large samples are needed to accept or reject the utility of these cytokines in predicting or diagnosis of OSCC or evaluation of treatment. [20]

Substractive proteomics approach to profile salivary proteins from oral cancer and matched healthy subjects followed by immunoassay validation revealed a panel of candidate protein biomarkers for potential detection of OSCC. They are M2BP(Mac 2 Binding Protein), MRP14(Myeloid Related Protein 14), CD59, Catalase, Profilin. Substractive proteomics refers to direct profiling of proteins expressed in samples from two cellular or pathologic states using multidimensional LC separation and data dependent MS/MS analysis. In a previous study M2BP , tumour antigen was found significantly upregulated in nasopharyngeal carcinoma. An increased level of MRP14 has been previously reported in tissue cells of oral tongue cancer.CD59(protectin) is one of the compliment restriction factors that are overexpressed on tumour cells , and they enable tumour cells to escape from compliment-dependent and antibody mediated killing. Profilin 1 is a regulator of the microfilament system and is involved in various signalling pathways via interactions with cytoplasmic and nuclear ligands..Catalase protects the cell against oxidative stress and altered levels of catalase are evident in many human tumours and are fundamentally involved in carcinogenesis and tumour progression. [21]

Sanjay PR etal (2008) conducted a study to evaluate the role of salivary biochemical parameters as markers of OSCC.They have found a significantly higher amount of free sialic acid in well differentiated OSCC patients compared with those of moderately differentiated OSCC suggesting a correlation of elevated salivary sialic acid levels to the progression of OSCC. Sialic acid is a constituent of many salivary glycoproteins. Studies have reported elevated salivary levels of sialic acid in pregnancy, Down's syndrome and Diabetes mellitus. Dablesteen *et al* have reported elevated salivary sialic acid in oral cancer. Baxi *et al* studied the sialic acid levels in various cancers and reported elevated levels of sialic acid compared to healthy subjects.PR Sanjay et al also observed an increased salivary levels of total protein and sugar in OSCC patients. [22]

Being a filtrate of the serum, saliva has been found to have an abundance of biomarkers with major clinical significance.The biomarker concentration in whole saliva can be lower than that of serum.To indicate their presence, a more sensitive protein sensor is needed. So confocal optical protein sensors can be used to detect biological markers with lower concentration [23]

Five markers were found to be increased in saliva of cancer patients in a study by Shptizer *et al*. They are carbonyls, lactate dehydrogenase (LDH), MMP 9(Metalloproteinase 9), Ki67(Antigen Ki 67), and CycD1(Cyclin D).An increase in salivary carbonyls is pointing at the significant free radicals attack to which the epithelial cells have been exposed.CycD1 and Ki67 are cell cycle regulators, which have been shown to be correlated with cellular proliferation and tumour progression, metastasis and poor prognosis. LDH was found to increase in the serum of various malignancies and has been identified as the main recurrent adverse prognostic factors. MMP was earlier shown to be elevated in saliva. MMP 9 are metalloproteases that have been shown to participate in cancer pathogenesis as they degrade type IV collagen, a major component of basement membrane as well as other types of collagens, elastin and fibronectin.They are highly expressed in stromal cells surrounding the invading front of metastasing tumors and their levels are elevated in tumour endothelium and in urine of cancer patients. [24]

There were previous reports that salivary mRNAs can be used as biomarkers for oral cancer. To enhance the diagnostic power of saliva for oral cancer, Park NJ etal profiles

salivary mi RNAs(micro RNAs) to determine if any miRNAs could be used as potential diagnostic markers. Study showed that endogenous plasma miRNAs are more stable than exogenous miRNAs. They detected about 50 miRNAs in both whole and supernatant saliva. Of these they noticed that two salivary miRNAs , miR -200a and miR-125a were present at lower levels in oral squamous cell carcinoma patient saliva than in healthy controls. Through transient transfection studies , miR-125a along with it homologue miR-125b , have been shown to reduce ERBB2 and ERBB3 oncogenic protein levels in SKBR3 cell, a human breast cancer cell line.miR200a has been reported to be differentially expressed in head and neck cancer cell lines and other cancer cells. [25]

Via bioinformatic analysis and biochemical validations, de Jong EP etal (2010) have identified myosin and actin as promising salivary biomarkers capable of distinguishing between subjects with premalignant and malignant lesions.Actin and myosin are key cytoskeletal proteins enabling cell motility and invasion,behaviour central to epithelial tumorigenesis. Both proteins displayed increases in their abundance levels in soluble saliva from subjects with malignant lesions. Additionally both proteins showed abundance increases in the exfoliated cells from the saliva of same subjects. These findings provided critical evidence that the differences observed in the soluble fraction of saliva are most likely a direct result of protein differences occurring within the epithelial cells during the transition from a pre-malignancy to malignancy .[26]

A panel of five salivary metabolites including Y- aminobutyric acid, phenylalanine, valine, n-eicosanoic acid and lactic acid were selected for study by Wie J *et al* (2011).The predictive power of each of the five salivary metabolites was evaluated by receiver operating characteristic curves for OSCC. Valine, lactic acid and phenylalanine in combination yielded satisfactory accuracy, sensitivity, specificity and positive predictive value in distinguishing OSCC from controls. The utility of salivary metabolome diagnostics for oral cancer is successfully demonstrated in this study and these results suggest that metabolomics approach compliments the clinical detection of OSCC , leading to an improved disease diagnosis and prognosis. [27]

Cd44 proteins, one of the adhesion molecules are released in soluble form (sCD44) via proteases and are detectable in normal circulation.The interaction of CD44 with many receptor-mediated signalling pathways has been shown to promote various tumor progression behaviors, including tumor cell growth, migration, invasion, and metastasis.In the study by Ghalwash DM et al ,levels of soluble CD44 (sCD44) were measured in whole unstimulated saliva (WUS) using an enzyme linked immune- assay (ELISA)and a significant increase in salivary sCD44 level was found in oral cancer patients making it a potential molecular marker for oral cancer detection[28].

Conclusion

The significant increase in salivary tumour markers is encouraging in light of the many advantages of saliva analysis in comparison with serum analysis. Biopsy remains the gold standard for definitive diagnosis of oral squamous cell carcinoma but it would be highly desirable and beneficial if salivary tumour markers may be made as a diagnostic tool, especially when a concurrant analysis for significantly increased markers is done. That is because salivary harvesting is noninvasive which may make it an attractive, effective alternative to serum testing. However , caution should be taken and note made of the fact that this suggested salivary analysis may be regarded as an aid and not as a replacement for other well established diagnostic tools available for oral squamous cell carcinoma.

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