SHORT COMMUNICATION

Oral carcinogenesis and non-invasive biomarkers for the diagnosis of oral squamous cell carcinoma

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Abstract

Oral squamous cell carcinoma (OSCC) is the most prevalent cancer in Pakistani population because of consumption of different tobacco-containing products whether smoked or chewed. These patients commonly report at a late stage of the disease. The patient's survival only depends upon early-stage diagnosis. Literature has reported that there is an increased tendency of transformation of oral potentially malignant disorder (OPMD) into OSCC. Biopsy is the gold standard measure for diagnosis but for OPMD cases biopsy was not recommended and most of the times the patients were also not willing to have a biopsy done. So, along with the biopsy there is a need for non-invasive protein biomarker that might aid in the early detection of oral cancer as well as highlight the high-risk individuals. This short communication focuses on the role of early diagnostic biomarkers present in literature, such as synuclein-γ (SNCG), Squamous cell carcinoma antigen (SCCAg), p53, MMPs-12, and IL-6. Furthermore, application of these biomarkers in multi-centre longitudinal studies is needed to establish their role as a non-invasive diagnostic biomarker for OSCC.

Keywords: Mouth neoplasms; Squamous cell carcinoma of head and neck; Oral potentially malignant disorders; Early diagnosis; Biomarkers.

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Introduction

Cancer of the oral cavity is the second most common reported cancer in Pakistan. To be exact, it is the most common cancer in males and second in females. The diagnosis of oral cancer is usually made by clinical examination and histopathological evaluation. Majority of the cases reported are histopathological squamous cell carcinoma.[1]

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Oral cancer is highly aggressive with increased risk of metastasis so early diagnosis, in pre-cancerous stage is necessary because the prognosis of this cancer depends upon the stage of the tumour. Stage I and II have better prognosis as compared to stage III and IV. Oral potentially malignant disorder (OPMD) is a term used for asymptomatic white or red-white oral patches, sometimes ulcers, that have an increased risk of malignant transformation. On histopathological examination these lesions show hyperkeratosis, epithelial proliferation dysplasia, and carcinoma in situ. Clinically, these lesions are classified as leukoplakia, erythroplakia, and oral submucous fibrosis.[2]

Factors associated with the oral carcinogenesis are tobacco intake and infections such as human papilloma virus. Besides these factors, some other conditions including genetic susceptibility of patients and presence of OPMD lesions in the oral cavity may also cause this malignancy. The molecular pathogenesis of oral carcinogenesis occurs due to increased expression of certain oncogenes and proto-oncogenes; some of these are epidermal growth factor receptors (EGFR/c-erb), members of the Ras gene family, C-myc, int-2, Hst-1, Prad-1, and BCL-1. The primary mechanism of oral carcinogenesis is mutations in the cell cycle regulatory proteins, such as p53 and K-Ras that inhibit cell apoptosis and uncontrolled proliferation. The secondary mechanism is the increased expression of myc and other proto-oncogenes that causes down-regulation of E-cadherin and leads to metastasis.[3]

Figure illustrates the histopathological transformation of the oral epithelium into hyperplasia, dysplasia, carcinoma in situ and then metastasis because of different oncogenes and proto-oncogenes.[3]

Recent advancements in the molecular diagnostic field make it much easier now to detect different molecules involved in oral carcinogenesis. These molecules are mainly protein-based and inflammatory products that are released in patients' biological samples, such as serum and saliva. Detection of these molecules in patients' biological samples might predict the malignant transformation of cancer at an early stage.[4] This short

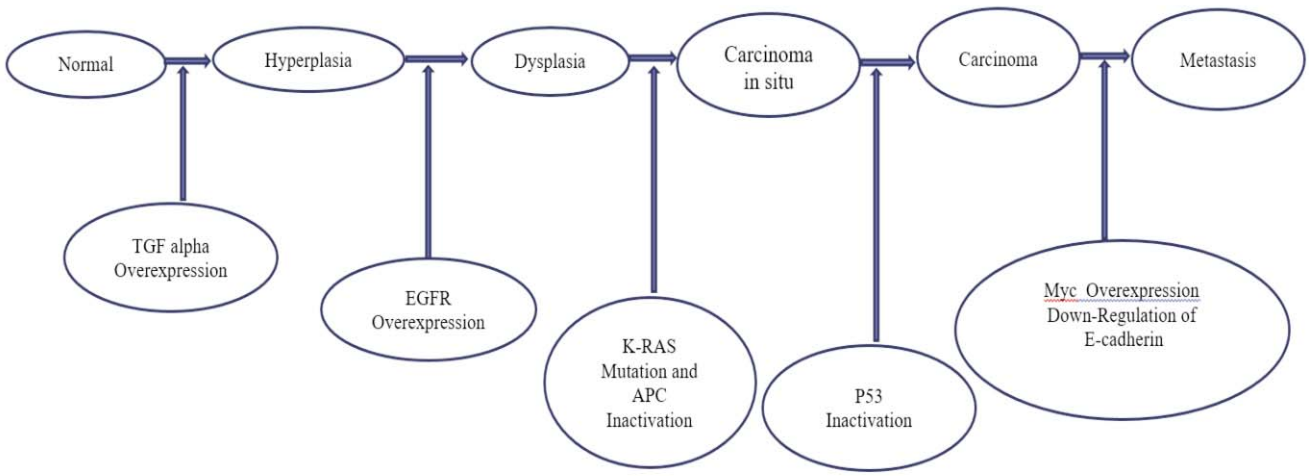

Figure: The histopathological changes of the oral epithelium into hyperplasia, dysplasia, carcinoma in situ carcinoma and then metastasis because of different oncogenes and proto-oncogenes.[3]

communication emphasises the utilisation of these non-invasive biomarkers in clinical settings along with routine diagnostic procedures.

Methods and Results

For this short communication, an electronic literature search was done from August 2022 to November 2022 on PubMed and Google scholar by using keywords such as OSCC, biomarker, serum, and saliva; manuscripts published within 10 years and in English were included.

Furthermore, it emphasises on clinical implantation of non-invasive protein biomarkers with routine diagnostic measures for screening of high-risk patients so that the cancer can be detected early.

Endothelium 1 (ET-1) is an angiogenic molecule. Literature reported that it has a role in carcinogenesis in various solid tumours, including OSCC. ET-1 is released from cancer cells and promotes its proliferation, migration and breakdown of the extracellular matrix, leading to invasions and metastasis.[5]

A case-control study from India highlighted the role of ET-1 in OSCC; they assessed serum levels of ET-1 through sandwich enzyme-linked immunosorbent assay (ELISA) between OSCC cases and healthy controls and the results show that a higher value of ET-1 among cases is statistically significant as compared to healthy control groups.[5] Another comparative study from India accessed the diagnostic role of ET-1 in OSCC and OPMD cases, and the author suggested that ET-1 might be used as a screening test.[6]

Despite the significant results both the studies did not analyse the sensitivity and specificity of this marker.

C-reactive protein (CRP) is an acute phase plasma protein that can be utilised as an indicator of immune system activation. Having a comparatively short plasma half-life, the CRP is an outstanding potential marker for inflammation because of its robust and consistent response to inflammation. Typically, static CRP sampling has been employed in clinical investigations, and they can predict disease presence or recurrence, particularly for several malignancies given the well-established association between inflammation and cancer.[7] Different studies estimated serum CRP levels in OSCC and OPMD conditions.[7,8,9,10]

Contradictory results of serum CRP were noted between the OSCC and OPMD patients.

Some studies suggested that it can be used as a non-invasive marker for diagnosis and measured the development of OSCC among OPMD cases.[7,8] While another plot study from India conceded that CRP cannot be used as a predictive biomarker for progression of OPMD into OSCC. It can be only utilised as a non-specific inflammatory marker for systemic disease.[9] A clinical study from Germany assessed the role of CRP as a diagnostic biomarker with some other markers; the study suggested that it can be used as a non-invasive biomarker for oral cancer detection, but the diagnostic accuracy of CRP is inadequate with area under the curve value 0.39.[10]

Synuclein is a protein that consists of three small proteins: α-synuclein, β-synuclein, and γ-synuclein. It was first discovered in brain tissue, peripheral nerve tissue, and retina. The synuclein protein family is associated with neurodegenerative illnesses and malignancies. Extensive studies have linked synuclein-γ (SNCG) overexpression to the progression of several cancers, including OSCC.[11,12]

Squamous cell carcinoma antigen (SCCAg) is an ovalbumin-family serine proteinase first found in metastatic cervical squamous cell carcinoma. Its expression was present in the normal epithelium as well as cancerous epithelium.[13]

A retrospective study from China demonstrated serum levels of SNCG and SCCAg in patients with OSCC and OPMD; they discovered that SNCG and SCCAg could be served as a biomarker to predict OPMD transformation into OSCC. The cut-off value of serum SNCG for diagnosing OSCC was 3.434 µg/L with 92 % sensitivity and 57% specificity. Serum SCCAg cut-off value for diagnosing OSCC cases amongst OPMD cases were 1.354 ng/L having 85.06% sensitivity and 86.67% specificity.[12]

The significance of p53 is notable, as it has been classified in the scientific literature as the 'molecule of the year, as an 'apoptotic superhero' as 'the protector of the genome', policeman of oncogenes and as the 'caretaker and gatekeeper gene'. p53 tumour suppressor functions are maintained by cell cycle arrest, DNA repair, senescence, and apoptosis. A case control study from Pakistan investigated p53 gene among OSCC, OPMD patients, and healthy individuals. They concluded that p53 levels can be used to predict the malignant transformation in OPMD patients with 98.0% of sensitivity and 66% specificity.[14]

Cytokines are pro-inflammatory cells that are not just secreted by immune cells but are also secreted by tumour and their microenvironment cells.[15]

Several cytokines have been studied widely including Interleukin 1b, 6, and 8.[15,16] Clinical application of these salivary and serum cytokines levels as a biomarker for early diagnosis of OSCC need to be done.

A recent meta-analysis used descriptive data and concluded that these three cytokines' markers can be utilised as a diagnostic marker for the prediction of malignant transformation of OPMD into OSCC.[16]

Extensive research is present that proposed that IL levels can be used as a screening test for diagnosis of early stage of oral cancer. The sensitivity of salivary IL-6 for identifying OSCC cases amongst OPMD cases were reported as 99%, while the specificity of this marker was noted as 96% with cut-off value 75 pg/ml. The author further suggested that longitudinal and multi-centre studies are required to validate these markers as a screening test. Studies that compared IL-6 levels with histopathological grading observed a strong correlation. Serum and saliva IL-6 levels increased from well to moderately and poorly differentiated tumours.[15]

Matrix metalloproteinases (MMPs) are a subclass of proteases classified into five families according to their substrate specificity: collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and 11), matrilysins (MMP-7), and membrane-type proteases (MMP-4) (MMP-14 to -17, -24 & -25). So far, around 25 unique MMP genes have been observed in humans.[17]

An observational cross-sectional study from Pakistan estimated the serum levels of MMPs 1-13 in OSCC and healthy groups through ELISA. Patients with OSCC had significantly higher serum levels of MMP-1, -8, -10, -12, and -13 than healthy group. Area under the curve (AUC) for MMP-12 in predicting OSCC presence was 0.836 (95% CI [0.733 to 0.911]), with 80% sensitivity and 78.9% specificity at a cut-off value of 16.13 pg/ml. Therefore, amongst all the subclasses of MMPS 1-13, MMPs subclass 12 value can be utilised as a non-invasive diagnostic marker for OSCC. The author concluded that further multi-centre studies with larger sample size and expression of different subclasses of MMPs will give proper understanding for its role as a diagnostic and therapeutic agent.[17]

Conclusion

Non-invasive biomarkers such as serum and saliva can be utilised along-with clinical and histopathological diagnostic methods; alternatively, these can be used for screening among high-risk population. Because of the importance of early results and minimum invasiveness, there is a need for serum and saliva collection from patients.

Furthermore, implantation of these biomarkers in out-patient department can be done. However, multi-centre and longitudinal studies are needed to confirm precise association of serum and salivary biomarker role with oral carcinogenesis before being employed as screening test so that the burden of OSCC can be reduced in a high prevalence population like Pakistan.

The limitation of the current study is that it is just a short communication that was relevant to the electronic database present in literature on protein biomarkers.

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Author's Contributions
SK: Concept, drafting of review article.
MMA: Proofreading and final approval.