Recent Advances in the Diagnosis of Oral Pre-malignant and Malignant Lesions - A Review

*Iqbal A. Muhammad Khyani, **Masood A. Qureishi, ***Talat Mirza, ****M. Rafiq Khanani, *M. Umar Farooq,

ABSTRACT: Oral cancer is a fatal disease and is the eighth most frequently occurring cancer worldwide, with approximately 400,000 new cases diagnosed each year. It is the most common cancer and constitutes a major health problem in developing countries, representing the leading cause of death. Oral cancer most commonly occurs in middle aged and older individuals, although a disturbing and alarming number of this malignancy is also being documented in younger adults in recent years. More than 95% of oral tumours are squamous cell carcinoma which arises from oral mucosal lining. A significant number of it developed from pre-malignant or potentially malignant lesions such as leukoplakia, erythroplakia, lichen planus and oral submucosal fibrosis. Early detection and prompt treatment offer best hope for the patient. This improves survival and diminishes the morbidity. Clinical examination and histopathology of the suspected lesion remains the gold standard for diagnosis and identification of malignant oral lesion. Biopsy being an invasive technique has its surgical and technical limitation for professionals and psychological implications for most patients. These issues can be addressed by detecting the biomarker with non-invasive techniques. Accurately identified biomarker may provide new avenues and constitute major target for early cancer detection and risk assessment. Different methods have been used to obtain DNA & RNA for biomarker identification. Oral rinse, swab, brush biopsies and recently saliva have been employed for this purpose. In this review article, we describe the various conventional and novel methods of diagnosis of oral pre-malignant lesions and oral cancer. We discussed the advances in diagnosis on molecular levels by identifying the biomarker through non-invasive methods which are expected to affect choice of treatment and patient’s outcomes.

Key Words: Pre-malignant lesions, Oral cancer, Molecular diagnosis, Salivary diagnosis, Biomarkers.

INTRODUCTION AND BACKGROUND: Oral cancer is the eight most frequently occurring cancer worldwide, with approximately 400,000 new cases diagnosed each year. It is the most common cancer and constitutes a major health problem in developing countries, representing the leading cause of death. Although constituting 2-4% of the malignancy in the west, this carcinoma accounts for almost 40% of all cancers in the Indian subcontinent. Carcinoma of oral cavity is the second most frequent malignant tumor for both the gender in Pakistan. The incidence is reported highest worldwide. It constitutes 20-35% of all cancers seen in various public hospitals in Karachi and slightly less in other regions of Pakistan and increasing cases are being consistently reported in younger age groups. Never the less it is a major killer in our population. It most commonly occurs in middle aged and older individuals, although a disturbing and alarming number of this malignancy is also being documented in younger adults in recent years. This paper reviews the conventional and recent advances in techniques for early detection of pre-malignant lesions and oral cancer which may predict morbidity and mortality.

PATHOLOGY & PATHOGENESIS: Over 95% of oral tumours are squamous cell carcinoma (SCC) which arises from the oral mucosal lining. A significant proportion of oral squamous cell carcinoma (OSCC) developed from pre-malignant lesions such as leukoplakia, erythroplakia, oral submucosal fibrosis and lichen planus. Multiplicative effects of nutritional deficiency, dietary habits, bad oral hygiene, mal-directed sharp, gagged teeth and infection with human papilloma virus (HPV) has been implicated for contributing in oral carcinogenesis but their exact role has not been fully established. In Pakistan, the major risk factors for oral cancer are areca nut (betel nut, chalia, Supari), betel quid (Paan), tobacco chewing, naswar, paan masala (Ghutka, Mawa) and poor nutrition. Alcohol consumption is not a prevalent habit in Karachi, therefore, not a major risk factor.

The natural history of oral cancer seems to gradually evolve from normal epithelium through precursor lesion to full blown metastatic phenotype. There exists a complex web of inter-relationship among habits, diet, nutrition, chemical carcinogens, viruses, immune status and genetics in carcinogenesis. Link between human papilloma virus and squamous cell carcinoma of the head and neck was suggested more than 20 years ago. HPV-16 was the most detectable virus in salivary samples, serum and biopsy...
cell blocks of the patients with pre-neoplastic lesions & OSCC. The oncological potential of high risk HPV is attributed to its ability to insert specific DNA fragments (early gene E6 and E7) into the host cellular genome. Investigators showed a strong association between high risk HPV infection and OSCC in Mexican, Swedish, Japanese, and Chinese populations. The role of high-risk oncogenic HPV in pre-malignant and malignant oral lesions has been an issue of extreme controversy with conflicting data reported by numerous studies. Its prevalence reported in OSCC vary from <5% to 100%. This wide range may be due to a variety of reasons such as the choice of primers used in the PCR, inherent differences in the populations being studied, and the methods used for HPV detection. PCR techniques are very sensitive and in the absence of a quantitative method such as Real-Time PCR, insignificant virus (with no role in carcinogenesis) can be identified. In terms of sensitivity and specificity, in situ detection techniques of HPV in paraffin-embedded tissue sections of OSCC (using in situ PCR and in situ hybridization PCR) are more powerful in detecting HPV than the liquid-phase PCR methods. In contrast, Enzyme Linked Immune Assay (ELISA) lacks acceptable sensitivity in detecting clinically meaningful HPV infections. Certain proinflammatory, proangiogenic cytokines such as tumour necrosis factor alpha (TNFα) interleukin (IL6 & IL8) are raised in OSCC and has important role in carcinogenesis. There are evidences that these cytokines are produced in a disregulated fashion in oropharyngeal SCC and that they have roles in cell growth, invasion, interruption of tumour suppression, immune status and even survival.

PRESENTATION: Pre-cancerous lesions and early oral cancers are often subtle and asymptomatic and many patients do not present for diagnosis and treatment until they have stage III or IV disease. Therefore it is important for a clinician to maintain a high index of suspicion, especially when risk factors are present. Invasive OSCC is often preceded by the presence of clinically identifiable pre-malignant changes of oral mucosa as white or red patches. As the neoplastic process progress, patient may notice a non-healing ulcer or mass. Later stage symptoms includes: Pain, bleeding, loosening of teeth, burning mouth, restricted opening of mouth, intolerance to spicy food, difficulty in wearing dentures, referred earache, dysphagia dysarthria, odynophagia and development of neck mass.

Detailed history is of paramount importance. Duration of symptoms and complete history of possible risk factors like smoking (type, frequency and duration), alcohol consumption (type, amount, frequency and duration), smokeless tobacco (paan, betel quid, betal nut, areca nut, gutka, mawa, naswar etc) use along with frequency, duration and habit of placement in oral cavity, presence of dental pain and caries all should be recorded. Family history, socio economical status and dietary habits should be recorded which may be an aid to reach diagnosis and further management.

**IMPORTANCE OF EARLY DIAGNOSIS:** Despite ready accessibility of the oral cavity to direct examination, these malignancies still are often not detected until a late stage and the survival rate for oral cancers has remained essentially unchanged over the past three decades. In spite of advances in accessibility, methods of diagnosis and treatment (Surgery, radiation and chemotherapy) the five years survival rate for OSCC has not improved significantly over the past several decades and it remained at about 50-55 percent. Early detection can improve patient survival and diminishes the morbidity of treatment required for advance disease. Early diagnosis depends upon an astute clinician or patient who may identify a suspicious lesion or symptom while it is still at an early stage. Early diagnosis is perhaps the greatest factor contributing to outcome for treatment of this disease. Prompt detection is also vital for effective surveillance after treatment.

**DIAGNOSTIC METHODOLOGY:**

1. **Vital Staining:** When on physical examination, a suspicion area of neoplastic activity is seen especially in high risk patient, one can use the vital staining of the suspected area with Toluidine blue to strengthen the doubt. Toluidine blue is a water soluble dye that stains the malignant cell giving them dark blue colour while normal mucosal cells remains unchanged. Two mechanism of Toluidine blue staining are proposed;

   1. It is believed that the nuclei of malignant cells take up the dye manifesting increased DNA synthesis.
   2. Second hypothesis is that dye penetrates the crevices as a result of randomly arranged tumour cells.

Toluidine blue rinses stain malignant cells that appear dark blue. It can increase the sensitivity of detecting malignant transformation from 26.6% by visual inspection alone to 96.7% with staining.

2. **Photo Spectrometry:** Different photo spectral techniques have shows promise for early detection of OSCC. Its use is still in the early stages of clinical testing. Unlike visual inspection method, this spectral analysis is claiming to detect pre-malignant conditions well before any visually detectable morphological change forming frank clinical lesion. The basic idea of spectral technique is that electromagnetic radiations are used to illuminate the area of oral tissue and a photo detection system records the wave length spectrum of the radiation reflecting the area of illumination during and immediately after illumination period.

3. **Biopsy for Histopathology:** When, on inspection, a suspicious lesion is identified, a conventional biopsy remains the best and most accurate means of assessing it. Biopsies of normal and malignant tissue, scrapings by brush biopsies containing exfoliative buccal cells have been explored for many years. Histological examination of the tissue remains the gold standard for diagnosis and identification of malignant oral lesions. Biopsy is an invasive technique with surgical implications, technique limitations for professionals and
psychological implications for most patients. It also presents limitation when there is trismus due to submucosal fibrosis and when the lesion is large, whereby, it is important to select the most appropriate site of biopsy. Furthermore, even though the biopsy study is fundamental, it is a diagnostic method with limited sensitivity where one of the most important factors is the subjective interpretation of the examining pathologist. These issues underlie the importance of discovering and developing new diagnostic methods, improving the existing ones and discovering new therapeutic targets for oral neoplastic diseases\textsuperscript{23}.  

\textbf{d) Molecular Diagnosis (Biomarkers):} In recent decade have seen a dramatic switch from histopathology to molecular methods of disease diagnosis and exfoliative cytology has gained importance as a rapid and simple method for obtaining DNA samples. Accurately identified biomarker may provide new avenue and constitute major target for early cancer detection and risk assessment. Identification of high risk oral pre-malignant lesion and intervention at pre-malignant stages could constitute one of the keys for reducing the mortality, morbidity and cost of treatment associated with OSCC. Absence of definite early warning signs in most head and neck cancer suggests that sensitive and specific biomarkers are likely to be important in screening high-risk patients. The rationale for molecular targeted prevention of oral cancer is strong. Biomarkers of genomic instability can accurately measure the cancer risk of oral pre-malignant lesion or intra-epithelial neoplasm\textsuperscript{20}. Screening and early detection of pre-neoplasm in population at risk have been proposed to reduce morbidity and mortality associated with oral cancer\textsuperscript{41}. Screening and early detection of pre-neoplasm in population at risk have been proposed to reduce morbidity and mortality associated with oral cancer\textsuperscript{41}. Despite the improvement in the scraping methods used for collecting oral cytological material, this methodology still presents problem in diagnosing oral cancer. There are controversies related to the real value of this technique in the early detection of OSCC. The problems of exfoliative cytology by this method are mainly due to existence of false negative obtained as a result of a non-representative samples, subjectivity of the cytological evaluation, patients discomfort and reluctance and in-accessibility and / or limited approach due to trismus especially in case of submucosal fibrosis. Liquid based cytology was developed in 1990. It offers significant advantages over conventional exfoliative cytology, as it reduces the problems related to sampling error, poor transfer and fixation of cellular samples.

\textbf{LIMITATIONS OF SCREENING BY BIOMARKERS:} There exits some limitations to our ability to detect cancer in its earliest stages with biomarker, as none of the markers has been shown to universally identify OSCC in its early stage. Furthermore, most of these markers have been identified either in cancer cell lines or in biopsy specimens from late invasive and metastatic cancers. Moreover, the invasive nature of a punch or brush biopsy and blood collection, that is discomforting and distressing for the patient, makes it unsuitable for cancer screening in high risk population. This suggests an imperative need for developing new diagnostic tool that would improve early detection. The goal of a cancer-screening program is to detect tumour at a stage early enough that treatment is likely to be successful. Screening tools are needed that exhibit the combined features of high sensitivity and specificity. Moreover, the screening tool must be sufficiently easy, non-invasive and cost-effective to allow widespread applicability. Identification of molecular markers in bodily fluid that would predict the development of cancer in its earliest stage or in pre-cancerous stage would constitute such a tool.  

\textbf{SALIVA AS A TOOL FOR MOLECULAR DIAGNOSIS:} It has been shown that identical mutation present in the primary tumour can be identified in the body fluids of affected patients. Significant development of biotechnology and improvement in our basic understanding of the cancer initiation and progression now enable us to identify tumour signatures, such as oncogenes and tumour suppressor gene alteration in bodily fluid that drain from organs affected by the tumour\textsuperscript{32}. Recently saliva has been used as prospective source in molecular diagnostics by analyzing genomics, proteomics and salivary transcriptomes. Oral fluid (Saliva) meets the demand for non-invasive, easily accessible biofluid of human body that nurtures a wide spectrum of biological analytes, informative for clinical diagnostic application. It provides highly efficient diagnostic medium. Oral fluid is a perfect medium to be explored for health and disease surveillance\textsuperscript{33}. Compelling reasons exist to use saliva as a diagnostic fluid. It meets the demands for inexpensive, non-invasive and easy-to-use diagnostic method. As a clinical tool, saliva has many advantages over serum including ease of collection, storing and shipping and it can be obtained at low cost, in sufficient quantities for analysis. For patients, the non-invasive collection techniques dramatically reduce the anxiety and discomfort thus simplifies procurement of repeated samples for monitoring overtime (Longitudinal studies). For professionals, saliva collection is safer than blood, it is easy to handle and as it does not clot and thus lessening the manipulation with less chance of exposure of health care workers\textsuperscript{34}. Saliva has been demonstrated to be a promising body fluid for early detection of diseases and salivary diagnostics has been exhibited tremendence potential in clinical application as a credible diagnostic tool. Saliva is often regarded as mirror of the body, is a perfect surrogate medium to be applied for clinical diagnostics\textsuperscript{35,36}. A great need exists for this convenient
and accurate point-of-care diagnostic tool that can be used in a non-invasive manner. This is of particular relevance in the developing countries like Pakistan, where many health risks and illnesses remains poorly defined and patient receive inappropriate treatment. In addition reliable indigenous epidemiological data is grossly deficient in developing countries about the burden of the disease to guide population wide health decisions. Human DNA biomarkers has been identified in saliva and were used for the detection of oral cancer. In this one of the most important applications of the salivary diagnostic approach is to detect the cancer conversion risk of oral pre-malignant lesion. The overall malignant transformation rates range from 11-70\%.

**IMAGING OF ORAL CANCER:** Once the diagnosis is confirmed by histopathology, different imaging techniques are used to evaluate the primary tumour and regional metastasis in cervical lymph nodes and to search for distant metastasis. Plain radiology, Orthopentogram (OPG), Computerized tomographic Scan (C.T Scan), Magnetic resonance imaging (MRI), Positron emission tomography (PET Scan), Radionuclear Scan and ultrasound scan are utilized at the merit of the situation. Different diagnostic strategies in oral pre-malignant and malignant lesion are summarized in table-1.

**PRESENT STATUS:** In Pakistan, Saliva has never been explored as a diagnostic medium to detect biomarkers for OSCC. As genetic alteration can be grossly deficient in developing countries about the risk factor for squamous cell carcinoma of the head and neck cancer. Curr Opin Oncol 2001;13:183-8.

- Santonlis PK, Kastramkis NG, Touruas AD. Advance in biology and genetic heterogeneity can be detected in the saliva oral cancer cells are immersed in the salivary milieu when many health risks and illnesses remains poorly defined and patient receive inappropriate treatment. In addiction reliable indigenous epidemiological data is grossly deficient in developing countries about the burden of the disease to guide population wide health decisions. Human DNA biomarkers has been identified in saliva and were used for the detection of oral cancer. In this one of the most important applications of the salivary diagnostic approach is to detect the cancer conversion risk of oral pre-malignant lesion. The overall malignant transformation rates range from 11-70\%.

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<th>Table 1: Diagnostic strategies in oral Pre-malignant lesions</th>
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<td>1. Physical Examination</td>
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<td>- Mirror Examination</td>
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<td>- Palpation</td>
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<td>2. Vital Staining Toulidine Blue</td>
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<td>3. Photomicroscopy</td>
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<td>4. Cytology &amp; Histopathology</td>
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<td>5. Imaging</td>
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<tr>
<td>- Plain radiology</td>
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<td>- Orthopentogram (OPG)</td>
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<td>- Radionuclear Scan</td>
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<td>- Ultrasound Scan (U.S)</td>
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