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# ADVANCES IN TUMOR MARKERS IN ORAL ONCOLOGY – A REVIEW

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## INTRODUCTION

Oncology is defined as a branch of medicine that deals with the prevention, diagnosis and treatment of cancer.[1] It involves the study of tumors, their development, effects on body, as well as the various treatment modalities such as chemotherapy, radiation therapy, surgery, and immunotherapy. Cancer is characterized by uncontrolled growth and spread of abnormal cells in body.[2] Tumor an abnormal mass of cells that occur in any part of the body, which can be benign or malignant. Metastasis is the process where abnormal cells invade and destroy surrounding tissue, and potentially spread to other parts of the body.[3]

Oral cancer refers to a tumor cells that develops in tissues of mouth or throat.[4] This can include cancer of lip, floor of the mouth, tongue, cheek, soft and hard palate, sinuses, and pharynx. Types of oral cancer are squamous cell carcinoma, verrucous carcinoma, adenocarcinoma, lymphoma, melanoma, and sarcoma. The most common type of oral cancer is squamous cell carcinoma, accounting for nearly nine out of ten cases. The disease burden of oral cancer globally is 389,846 new cases and 188,438 deaths.[5] In India oral cancer is high, 143,759 new cases and 79,979 death.[6]

## Literature review

### Prevalence of oral cancer

Worldwide, the prevalence of oral cancer reported to be 1,094448 cases with proportion of 2.0 per 100,000 population.[5]

In India, the prevalence of oral cancer is reported to be 370,106 cases, with a proportion of 11.4 per 100,000 population.[6]

### Disease burden

In world – Oral cancer is the sixteen most common cancer in world wide accounting the incidence rate with 389,846 (2%) and mortality rate with 188,438 (1.9%).[5]

In India – Oral cancer is the second most common cancer accounting incidence rate with 143,759 (10.2%) and mortality rate with 79,979 (8.7%).[6]

### Chair side investigation for oral cancer

Inspite of advancement in radiotherapy, chemotherapy, and surgical management the disease burden of oral cancer is very high. One reliable reason is delay in oral cancer diagnosis. Early detection at potentially malignant disorder or initial stage of malignancies is the key for good prognosis. Hence chair side diagnostics should be given utmost importance.

### Chair side diagnostic aids

**Vital staining:** Vital staining is procedure where living cells takes up the staining.

Commonly used vital stains are Toluidine blue, Methylene blue, Lugol's iodine and Rose bengal stains.[7]

**Biopsy:** It is surgical procedure for obtaining living tissue sample for performing diagnosis. There are different types of biopsy like incisional biopsy, excisional biopsy, oral brush biopsy, punch biopsy, fine needle aspiration biopsy.[8]

**Light based detection system:** It is non invasive procedure in detecting premalignant and cancerous lesion. Most commonly used is vizilite which is non toxic chemiluminescent light.[9]

**VELscope:** The device is hand held, non magnifying device which uses 400 – 460nm wavelength light. The light emitted from the device reaches the mucous

membrane and excites the endogenously present auto fluorescent substance called fluorophore.[10]

**Auto fluorescence imaging –** In auto fluorescence imaging the tissues are illuminated with a light source, in the near UV to the green spectral range. The fluorescence images which are produced by the tissue are recorded in the camera.[11]

**Tumor marker**

Tumor marker is a substance found in tissue, blood, bone marrow, or other body fluids that may be a sign of cancer or certain benign (non cancer) conditions. Many tumor markers are proteins made by both normal cells and cancer cells, but they are made in higher amounts by cancer cells.[12]

**History of tumor marker**

- The first known attempt to find markers for malignancy was made 2000 years ago and it is described in an Egyptian Papyrus where breast cancer was distinguished from mastitis.[13]
- The first tumor marker in modern medicine was identified by Bence – Jones, who in 1846 detected a heat precipitate in sample of acidified urine from patients suffering from 'Mollities ossium'.
- 1928 – WH Brown – ectopic hormone syndrome
- 1930 – B Zondek – HCG (human chorionic gonadotropin)
- 1932 – H Cushing – ACTH
- 1949 – K.oh – Uti – deletions of blood group antigens
- 1959 – C Markert – isoenzymes
- 1963 – GI Abelev – AFP (alpha fetoprotein)
- 1965 – P Gold and S Freeman – CEA (carcino embryogenic antigen)
- 1969 – R Heubner and G Todaro – oncogenes
- 1975 – H Kohler and G Milstein – monoclonal antibodies
- 1980 – G Cooper, R Weinber, M Bishop – oncogene probes and transfection
- 1985 – H Harris, R Sagar, and A Knudson – suppressor gene.

**Ideal Concept of tumor marker**

1. They should have highly sensitive and should have low false negative.
2. They should be highly specific and should have low false positive.
3. They should have high positive and negative predictive value.
4. It should be accurate in differentiating between healthy individual and tumor patients.
5. They should be able to differentiate between neoplastic and nonneoplastic disease and show positive correlation tumor volume and extent.
6. They should predict early recurrence and have prognostic value.
7. Their level should preceding the neoplastic process, so that it should be useful for screening early cancer.
8. They should be either a universal marker for all types of malignancies or specific to one type malignancy.
9. Be easily assayable and able to indicate all changes in cancer patient receiving treatment.[14]

**TUMOR MARKER IN ORAL CANCER**

Biomarkers for oral premalignant and malignant lesions can be used as a screen to detect early asymptomatic changes in the mucosa.

**ADVANTAGES OF TUMOR MARKER**

1. Body fluids such as saliva and urine (non-invasive) can be used to diagnose cancer through biomarkers.
2. At a lesser time, a large number of samples can be automated assay using many markers.
3. Markers will provide qualitative and quantitative results with measurable values.
4. Marker assays are less expensive compared to other investigation procedures such as radiography, computed tomography scan, and endoscopic procedure.
5. Biomarkers' results are precise, reproducible, and reliable.[15]

**CLASSIFICATION OF TUMOR MARKER IN ORAL CANCER**

**According to Schliephake H.[16]**

**Tumor growth markers**

1. Epithelial growth
2. Cyclin
3. Nuclear cell proliferation antigens
4. AgNORs (Agryophilic nucleolar organizer region)
5. Skp2 (S-phase kinase-interacting protein 2)
6. HSP 27 and 70 (Heat shock protein).

**Markers of tumor suppression and antitumor response**

1. Retinoblastoma protein
2. Cyclin-dependent kinase inhibitors
3. p53
4. bax
5. Fas/FasL.

**Angiogenesis markers**

1. Vascular endothelial growth factor/receptor
2. Platelet-derived endothelial cell growth factor
3. Fibroblast growth factor.

**Markers of tumor invasion and metastatic potential**

1. matrix metalloproteases
2. Cathepsins
3. Cadherins and catenins
4. Desmoplakin.

**Cell surface markers**

1. Carbohydrates
2. Histocompatibility antigen

3. CD57 antigen.

**Intracellular markers**
1. Cytokeratins.

**Markers of anomalous keratinization**
1. Filagrins
2. Invoulcrin
3. Desmosomal proteins
4. Intercellular substances antigen
5. Nuclear analysis.

**Arachidonic acid products**
1. Prostaglandin E2
2. Hydroxyeicosatetraenoic acid
3. Leukotriene B4.

**Enzymes**
1. Glutathione S-transferase.

**Tumor marker types**

➢ **Epithelial tumor marker**

- Cytokeratin - Currently 54 out of 60 cytokeratin genes identified in human genome are functional genes. They help in the diagnosis of spindle cell tumours, malignant melanoma, HNSCC, leiomyosarcoma, ameloblastoma (solid multicystic, desmoplastic with squamous differentiation, adenomatoid-ductal, tubular, whorled patterns), mixed salivary gland tumour.[18]

➢ **Growth factors**

- EGFR- Epidermal growth factor receptor (EGFR) is overexpressed in more than 90% of HNSCC cases.[19] Although many molecular agents are available in the treatment of HNSCC, Cetuximab was the first to be used in standard practice.[20]

- EGFRvIII- Not yet validated in clinical use but may have an effect on sensitivity to cetuximab.[21]

➢ **p53**

Mutation in p53 tumour suppressor gene and its expression provides an invaluable marker for detection of recurrence and second primary in HNSCC. It can also predict the tumour resistance to radiotherapy.[22]

➢ **Cyclin D1/EMS1 (11q13), EGFR and Neu**

Lymph node metastasis in HNSCC.[23]

➢ **E-cadherin**

Nodal metastasis can be detected by the failure of expression of E-Cadherin in primary tumor.

➢ **CD 44**

The tendency of tumor to metastasize has been strongly correlated with down regulation of CD44v6 expression in head and neck squamous cell carcinoma[24] and CD44 h expression in laryngeal[25] and tongue cancer.[26]

➢ **Salivary IL 8 and Serum IL 6**

It is 99% sensitive and 90% specific in detecting oropharyngeal cancer. It is 91% sensitive in early detection of oral cancer.[27,28]

**Recent advances in tumor marker**

Over past few years, technological advance provided new avenue for early diagnosis. These advances have also improved the early diagnosis of oral cancer. Advanced tumor marker are quantitative polymerase chain reaction (qPCR), mass spectrometry, microarray, enzyme linked immunosorbent assay (ELISA), biosensor, lab on a chip.

**1. Quantitative polymerase chain reaction (qPCR)**

qPCR is the most widely used technique for the amplification and real-time quantification of nucleic acid. It is used to quantify expression of specific mRNAs and microRNAs (miRNAs) through their reverse transcription into complementary DNA (cDNA).[29] Currently, qPCR represents the conventional method for the analysis of liquid biopsy samples and the discovery of novel oral cancer biomarkers.

**2. Microarray**

Microarray represents a valuable biomedical platform with several applications, ranging from the evaluation of gene expression to DNA methylation and non-coding RNA expression profiles.[30] DNA microarrays are based on the principle of complementarity and in situ hybridization, which allows for the evaluation of thousands of genes in a single assay. Microarrays have also been applied to liquid biopsy samples for the detection of oral cancer-related biomarkers. Salazar C et al. (2014) analyzed salivary samples using microarray technology and identified three differentially expressed miRNAs (miR-9, miR-134, and miR-191) in HNSCC patients and healthy subjects.[31]

**3. ELISA**

ELISA is an immunoenzymatic assay widely used in both research and clinical settings. ELISA assays represent the gold standard for the detection of various circulating tumor markers such as prostate-specific antigens (PSAs) and carcinoembryonic antigen (CEAs).[32] ELISA assay is an effective tool for the detection of new oral cancer-associated biomarkers, which could improve the early diagnosis and management of this malignancy. Sivadasan P and colleagues (2020) focused their attention on the salivary proteomic profile of patients with dysplastic leukoplakia and OSCC.

**4. Biosensor**

Biosensors are analytical tools used to detect several biological molecules including nucleic acids, enzymes as well as antibodies and antigens. Depending on the detection method, biosensors can be classified into six main types: electrochemical biosensors, surface plasmon resonance (SPR) biosensors, colorimetric biosensors,

surface-enhanced Raman scattering (SERS) biosensors, immunofluorescence biosensors, and nuclear magnetic resonance (NMR) biosensors.[33]

**5. Lab on chip (LOC)**

LOC technology integrates several analytical laboratory procedures on a single chip, providing a miniaturized and automated system for the detection of cellular and molecular elements. LOC system showed high sensitivity and specificity for several circulating biomarkers (miRNAs and proteins), paving the way for the development of an effective technology for the early diagnosis of oral malignancies.[34]

➢ **Molecular biomarker**

Although histological investigations on tissue biopsies still represent the gold standard for the diagnosis of oral cancer, the clinical relevance of circulating tumor biomarkers for the early detection of cancer as well as for the monitoring of treatments and patient prognosis was widely demonstrated in the last few years.

**6. Circulating DNA (ctDNA)**

Circulating free DNA (cfDNA) refers to the extracellular DNA released into the bloodstream by apoptotic and necrotic cells under both physiological and pathological conditions. In tumors, the rapid turnover of cancer cells causes a constant release and accumulation of cfDNA and ctDNA in the tumor microenvironment and in body fluids.[35,36] In this field, many studies have also investigated ctDNA as a novel biomarker for oral cancer. Although several methods have been proposed in the last few years, the major challenge will be to develop cost-effective and highly sensitive technologies to detect ctDNA levels at early cancer stages and simultaneously analyze different mutations.

**Uses of tumor marker**

1. Screening in general population
2. Diagnosis of primary tumour
3. Differential diagnosis of suspicious lesions
4. Clinical staging of cancer
5. To identify the undetected tumour metastasis
6. Estimating tumour volume
7. To indicate the prognosis of disease progression
8. Evaluating the success of treatment
9. Detecting the recurrence of cancer
10. Monitoring responses to therapy
11. Radioimmunolocalization of tumour masses.[37]

**Limitations of tumor marker**

a. False elevation may occur in non- neoplastic conditions as many tumour markers are proteins, over expressed not only by cancer cells but also by normal tissues.
b. Early detection difficult, since low levels are seen in normal individuals.
c. Large volume of cancer needed for significant elevation above normal.
d. Many tumour markers are not specific to a particular type of cancer.
e. Tumour marker levels are not elevated in every person.
f. No simple tests are yet available with sufficient specificity to detect the presence of a cancer.[38]

**CONCLUSION**

Tumor markers that are the biochemical substances thus not only help in detecting the malignancy but also differentiate the nature of malignancy involved. Identifying specific molecular changes in different premalignant lesions and malignant tumours that could potentially guide management has been made possible by advances in understanding the genetics and molecular basis of human malignancies. It is therefore important for us to continuously update our knowledge regarding the effective usage of tumour markers in day-to-day practice in order to do justice to our patients.

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