Review Article

Tumor Markers in Oral Squamous Cell Carcinoma as an Adjunct to Diagnosis: An Insight

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Abstract

The topic of tumor markers is an immeasurable one, and there is an opulence of data collected till now. It has been deduced that tumor markers can suffice as an acceptable screening test for initiating definitive diagnostic procedures with a goal of making an “early diagnosis.” Through modern techniques of sensitive immunoassays such as radioimmunoassay and enzyme-linked immunosorbent assay, quantitative as well as qualitative evaluation of these markers is possible. Most tumor markers are substances produced by some types of nonneoplastic cells, although perhaps in much lower quantities than they are produced by tumor cells. The review article on tumor markers in oral squamous cell carcinoma (OSCC) as an adjunct to diagnosis is grounded totally on our analysis, consultation, experience, exploration, reviews, and original articles on the subject. Guidelines have been developed by various national skillful and international Oracle groups for the use of tumor markers for cancers, but none of these are currently formalized to maneuver in OSCC. It has been concluded that tumor markers cannot be maneuvered as fundamental modalities for the diagnosis of oral cancer. Tumor markers’ main profitability in clinical medicine has been a laboratory test to support the diagnosis; further detailed studies are required to determine their practical usefulness in clinical workflow. It cannot be used as a sole diagnostic tool but can be used as an adjunct to routine histopathology using hematoxylin and eosin stain. Instead by combining various tumor markers, we can achieve a great specificity and sensitivity in the follow-up of one type of malignancy, for example, OSCC.

Keywords: Cancer, head and neck, malignant, oral cancer, tumor markers

INTRODUCTION

Tumor markers are entity that are produced either by the tumor itself or by the body in retaliation to the presence of cancer or certain benign noncancerous conditions that can help in the diagnosis of cancer and in the evaluation of tumor burden. In certain types of cancer, tumor markers are detected in higher than normal amounts in the blood, urine, or body tissues. Quantification of Tumor markers can be helpful in the detection and diagnosis of some varieties of cancer.

Abundance of data related to the topic of tumor markers is available in the literature as its a vast topic. Tumor markers can be reviewed in an arbitrary way for the role of tumor markers in the prevention and detection of tumors of head and neck. There are variety of tumor markers present that have been developed primarily focusing on the tumors of head and neck. Tumor markers are a paramount part of the secondary prevention (i.e., detection) efforts. If a simple laboratory test could be devised that would (based on a sample of blood or urine) indicate the presence of cancer, with a high degree of specificity and sensitivity, before the occurrence of metastasis in the body.[1] Brief review of the article as provided by different authors is tabulated in Table 1.

According the given data present in the literature, head-and-neck cancer is the sixth most common human cancer representing “3% of all types of cancer.”[2]

Head-and-neck cancer is located in the oral cavity in 48% of cases and 90% of these are oral squamous cell carcinoma (OSCC).[3]

OSCC develops from tobacco; it is a multistep and multifocal process involving field cancerization and carcinogenesis.[3]

It could serve as an adequate screening test for initiating definitive diagnostic procedures with a goal of making
an “early diagnosis” and when simple removal of the tumor would result in normal survival characteristics for a population of patients. There is no such test available, although number of efforts have been made and number of tumor markers have been reported, among which most are proteins.\[4\\]

Tumor markers may be present as intracellular substances in tissues or may be released into the circulation and appear in the serum. Through modern techniques of sensitive immunoassays but with a limitation that most tumor markers are substances produced by some types of nonneoplastic cells, although perhaps in much lower quantities than they are produced by tumor cells.\[5\\]

**DEFINITION**

- According to the National Cancer Institute, a biomarker is “a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease,” such as cancer\[6\\]
- Tumor marker is defined as a substance present in or produced by a tumor or by the tumor’s host in response to the tumor’s presence that can be used to differentiate a tumor from the normal tissue or to determine the presence of a tumor based on measurement in the blood or secretions\[7\\]
- Tumor markers can also be defined as “specific novel or structurally altered cellular macromolecules or temporarily spatially or quantitatively altered normal molecules that are associated with malignant (and in some cases benign) neoplastic cells”\[8\\]
- Cellular products that are abnormally elaborated by malignancies that can be detected in various body fluids and on the surface of the cancer cells\[9\\]
- Tumor markers can also be broadly defined as “biological or molecular attributes of tumor cells that distinguish them from normal cells”\[10\\]

**CLASSIFICATION**

Malati’s classification is as follows:\[4\\]

1. Oncofetal antigens (e.g., alpha-fetoprotein [AFP], carcinoembryonic antigen [CEA], pancreatic oncofetal antigen, and fetal sulfoglycoprotein)
2. Tumor-associated antigens/cancer antigens, for example, CA125, CA19-9, CA15-3, CA72-4, and CA50
3. Hormones, for example, beta human chorionic gonadotropin, calcitonin, and placental lactogen
4. Hormone receptors (e.g., estrogen and progesterone receptors)
5. Enzymes and isoenzymes (e.g., prostate-specific antigen [PSA], prostatic acid phosphatase [PAP], neuron-specific enolase [NSE], glycosyl transferases, placental alkaline phosphatase [PALP], terminal deoxy nucleotidyl transferase, lysozyme, alpha amylase)
6. Serum and tissue proteins (beta-2 microglobulin, monoclonal immunoglobulin/para proteins, glial fibrillary acidic protein [GFAP], protein S-100, ferritin, and fibrinogen degradation products)

7. Other biomolecules, for example, polyamines.

Reddy *et al.*’s classification is as follows:\[11\\]

- Proliferative markers: Ki-67, proliferating cell nuclear antigen (PCNA), p27 Kip/gene, DNA polymerase alpha, p105, p120, and stain
- Oncogenes: c-erb-gene, ras gene, myc gene, and bd-2 gene
- Growth factors and receptors: Epidermal growth factor receptor, transforming growth factor-β, hepatocellular carcinoma, fibroblast growth factor (FGF) receptor, insulin and insulin-like growth factor receptor
- Tumor suppressor genes: p53, retinoblastoma-susceptibility suppressor gene
- Serological tumor markers: Markers associated with cell proliferation, markers related to cell differentiation (carcinoembryonic proteins such as carcinoembryonic Ag, α-feto protein), markers related to metastasis, markers related to other tumor-associated events, markers related to malignant transformation, inherited mutation, monoclonal Ab-defined tumor markers.

Schliephake’s classification is as follows:\[11\\]

a. Tumor growth markers
   a. Epithelial growth factor (EGF)
   b. Cyclin
   c. Nuclear cell proliferation antigens
   d. Argyrophilic nucleolar organizer region (AgNORs)
   e. S-phase kinase-interacting protein 2
   f. HSP 27 and 70 (heat shock protein)
   g. Telomerase.

b. Markers of tumor suppression and antitumor response
   a. Retinoblastoma protein (pRb)
   b. Cyclin-dependent kinase inhibitors
   c. p53
   d. bax
   e. Fas/FasL.

c. Angiogenesis markers
   a. Vascular endothelial growth factor/receptor
   b. Platelet-derived endothelial cell growth factor
   c. FGFs.

d. Markers of tumor invasion and metastatic potential
   a. Matrix metalloproteases
   b. Cathepsins
   c. Cadherins and catenins
   d. Desmoplakin.

e. Cell surface markers
   a. Carbohydrates
   b. Histocompatibility antigen
   c. CD57 antigen.

f. Intracellular markers
   a. Cytokeratins.

g. Markers of anomalous keratinization
   a. Filagrins.
Negi, et al.: Tumor markers in OSCC

b. Invouclrin
c. Desmosomal proteins
d. Intercellular substance antigen
e. Nuclear analysis.
h. Arachidonic acid products
   a. Prostaglandin E2
   b. Hydroxyeicosatetraenoic acid
c. Leucotriene B4
d. Enzymes
e. Glutathione S-transferase.

Classification of Spieght and Morgan (1993) is as follows:[12]
• Proliferative markers: PCNA, Ki67, BrU, histones, and AgNORs
• Genetic markers: Ploidy
• Oncogene: C-myc
• Tumor suppressor markers: P53 mutations
• Cytokines
• Blood group antigens
• Integrin extracellular matrix ligands.

Waxman’s classification is as follows:[13]
1. Oncofetal antigens
   a. AFP
   b. CEA.
2. Hormone catecholamines
   a. Calcitonin
   b. b-hCG.
3. Glycoproteins
   a. CA125
   b. CA15-3
   c. CA19-9
   d. CA72-4
   e. PSA.
4. Metabolites
   a. Vanillylmandelic acid
   b. Hydroxy indole acetic acid.
5. Tumor-associated antigen
   a. Viral antigens – Polyoma, SV40
   b. MHC-related antigens – H-2k antigen
   c. Enzymes – PAP, NSE, PLAP
d. Oncogene products – c-myc, c-erbB2
e. Cytogenetic products – Philadelphia.
6. Tumor-associated markers
   a. Proteins – Immunoglobulins, b-2M
   b. Enzymes – Lactate dehydrogenase, alkaline phosphatase, pteridines, pterins
c. Acute-phase proteins – C-reactive protein, ferritin
d. Inflammatory markers – Erythrocyte sedimentation rate (ESR), viscosity.
7. Ultrastructural components
   a. Intermediate filament components – Desmin, vimentin

Manikantan et al.’s classification is as follows:[10]
1. Epithelial markers
   a. Cytokeratins
   b. Epithelial membrane antigen
c. Oncofetal antigens
   i. AFP
   ii. CEA.
d. Desmoplakin.

2. Mesenchymal markers
   a. Muscle antigens
      i. Desmin
      ii. Actin
      iii. Myoglobin
      iv. Myosin.
b. Vasculator antigens
   i. CD34
   ii. CD31.
c. Neural antigens
   iii. S100
   iv. NSE
   v. GFAP
   vi. Synaptophysin
   vii. Nerve growth factor receptors.

3. Prognostic markers
   a. Cell adhesion molecules
      i. Cadherins
      ii. Integrins
      iii. Selectins.
b. Proliferative markers
      i. PCNA
      ii. Ki67
      iii. AgNORs.

4. Biochemical markers
   a. Enzymes and isoenzymes
      i. PAP
      ii. PSA
      iii. PALP.
      iv. Lysozyme.
b. Protein
      i. Ferritin
      ii. Glycoprotein
      iii. Beta-protein
      iv. Immunoglobulins.
c. Hormone receptors
      i. Estrogen receptor
      ii. Progesterone receptor
d. Epithelial markers.

**Ideal Characteristics of Tumor Markers**

During the course of time, only a few tumor markers have stood the test of time and entered into the diagnostic or management algorithms for clinicians.[14-16] Table 2 Ideal Characteristics Of tumor Markers.[15] Most important Ideal Characteristics and use of Potential markers are as follows.[15]

1. It should be highly specific to a given tumor type
2. It should provide a lead time over clinical diagnosis and
3. It should be highly sensitive to avoid false-positive results.[45]
USES

• Estimating the risk of developing cancer
• Screening
• Differential diagnosis
• Determine the prognosis of disease
• Predict response to therapy
• Monitor for disease recurrence
• Monitor for response or progression in metastatic disease.

The methods of detection are classified as follows:\[14,16-18\]

• Serology: Enzyme assays
• Immunological: Immunohistochemistry, radioimmunoassay, enzyme-linked immunosorbent assay
• Flow cytometry cytogenetic analysis: Fluorescent in situ hybridization, spectral karyotyping, comparative genomic hybridization
• Genetic analysis sequencing (automated): Reverse transcription gel electrophoresis and DNA microarray analysis
• Proteomics: Surface-enhanced laser desorption/ionization.

TO KNOW HOW WELL TREATMENT IS WORKING

On the other hand, if the marker level goes up, then the cancer is not responding and the treatment may need to be changed. (And exception to such cases is if the cancer is very sensitive to certain chemotherapy treatment, in that case, the chemo can cause many cancer cells to die and release large amounts of marker into the blood, which in turn causes the level of the tumor marker to rise for a short time.)\[19\]

When there is:

• No change – Tumor marker does not fall to <50% of pretreatment concentration
• Improvement – Tumor marker falls to <50% of pretreatment concentration
• Response – Tumor marker falls to <10% of pretreatment concentration
• Complete response – Tumor marker falls to nonmalignancy reference values,\[20\]
• Difficulty in identifying minute quantities of particular substances in serum
• Existence of proliferation-related rather than tumor-associated antigen
• Cross-reactive antigens, for instance, a common domain in different proteins
• Cross-reaction with degradation products of normal proteins taken up by tumor cells
• Malignant tumors with extensive necrosis have increased hydrolytic enzymes. Antigenic degradation products may then form which would normally be absent from nonnecrotic control tissue
• The financial and psychological cost to the society of routine screening for early cancers using currently available tumor marker would be prohibited.\[21\]

No tumor marker is ideal and the fact that the substances that are being applied as tumor markers are not synthesized exclusively as a consequence of malignancy. Some of the most common factors (in addition to malignant disease) that affect serum concentrations of tumor markers are as follows:\[22\]

False-positive results

• Presence of inflammatory processes
• Benign liver diseases and consequential disturbances in metabolism and excretion (AFP, TPA, CEA, CA19-9, CA15-3)
• Disturbances of renal function (beta-2-microglobulin, calcitonin, PSA, CEA, CA19-9, CA15-3)
• Extensive tumor necrosis
• As a consequence of diagnostic and therapeutic procedures (digitorectal examination, mammography, surgery, and radio and chemotherapy)
• As a consequence of different physiological conditions (pregnancy – HCG, CA125, CA15-3, MCA, AFP, and menstrual cycle – CA125).

False-negative results

• Complete absence of production (e.g., CA19-9 in Le (a-b-) persons)
• Insufficient expression of a certain antigenic determinant (or production in only some of the tumor cells)
• Insufficient blood circulation in the tumor
• Production of immune complexes with autoantibodies
• Rapid degradation and clearance of antigens
• Recommendations for ordering a tumor marker test
• A single value or test is unreliable in itself.\[23\] It is noteworthy that in most situations, elevations of markers in nonmalignant diseases are often transient, whereas elevations associated with cancer either remain constant or continuously rise. Ordering serial testing can help detect falsely elevated levels due to transient elevation\[24-26\]
• It is imperative to be certain that the marker in question was, in fact, elevated before relying on it for monitoring disease activity, the reason being that none of the tumor markers are 100% sensitive (may not be elevated in some disease activity, the reason being that none of the tumor markers are 100% sensitive (may not be elevated in some patients).\[16,18,27\]
• In tumors with multiple raised markers measured before definitive therapy, the marker showing the highest elevation should be used for follow-up\[18,27\]
• If in a given case, tumor markers were not evaluated in the pretreatment setting, it is advisable to use multiple markers for monitoring in the posttherapy setup\[16,18,27\]
• As a general guideline, the time interval between serial determinations should be 3 months; but in case of an abnormal value, a repeat estimate can be ordered within 2–4 weeks irrespective of the initial reading\[25\]
• Use of multiple markers based on the combination pattern for the selected malignancy will improve sensitivity and specificity of the detection\[28,29\]
• An important interfering factor to be considered is the presence of a Hook effect.\[30\]
It is true in cases, where the value of tumor markers is not found in co relation to the clinical situation. Hook effect is an inherent flaw of certain methods of detection (specifically immunoassay) due to which the serum tumor marker levels may be reported to be falsely low if the concentration rises above a particular level.[30,31]

**General Points about Tumor Marker Tests According to Association of Biochemist in Ireland Scientific Committee Guidelines**

- No serum marker in current use is specific for malignancy
- In general, serum marker levels are rarely elevated in patients with early malignancy. With a few exceptions, high levels are usually found only when patients have advanced disease
- No cancer marker has absolute organ specificity. PSA, however, appears to be relatively specific for prostate tissue but not for prostate cancer
- Apart from possibly hCG in choriocarcinoma, no marker is elevated in 100% of patients with a particular malignancy
- Requesting of multiple markers (such as CEA and the CA series of antigens) in an attempt to identify metastases of unknown primary origin is rarely of use
- Tumor marker assays should not be carried out on biological fluids such as peritoneal fluids, pancreatic juice, and ovarian cystic fluids as reliable reference ranges are currently unavailable for these types of specimen
- Reference ranges for cancer markers are not well defined and are used only for guidance. Please note that a level below the reference range does not exclude malignancy, while concentrations above the reference range onto necessarily mean the presence of cancer. Changes in levels over time are likely to be more clinically useful than absolute levels at one point in time
- As many tumor markers lack agreed international reference preparations (e.g., CA125, CA15-3, and CA19-9), different assay kits may give different results for the same sera
- Laboratories carrying out tumor marker tests should state the assay used on their report form.

**Conclusion**

It has been concluded that a large number of molecular markers are associated with occurrence, progression, and prognosis of squamous cell carcinoma. Tumor markers of increased proliferation in oral carcinoma have been identified and explored for more than a decade now. Although a large body of literature exists on the association of these markers with tumor grading and different degrees of dysplasia in premalignant lesions, it is surprising to note that there are only few markers that have an impact on prognosis. Nevertheless, markers of cellular proliferation are difficult to interpret as an independent scale for judgment of tumor prognosis.

Another more recent approach to oral carcinogenesis is focused on the escape of malignant cells from apoptotic signals. p53 alone is not particularly valid in predicting prognosis. Additional markers of apoptosis such as FAS and FAS ligand and Bax as well as anti-apoptotic molecules such as BCL2/BAG-1 are reported to have significant prognostic.

There is an evergrowing number of molecular markers for OSCC. Nevertheless, a number of studies have shown that it is not only the presence of tumor markers as such that make up the prognosis of disease, but also the location of these markers within the tumor. Particularly, the invasive tumor front of tumor appears to be of great importance for prognosis. Tumor markers cannot be construed as primary modalities for the diagnosis of oral cancer. Their main utility in clinical medicine has been a laboratory test to support the diagnosis. A host of tumor markers have been described, and new ones appear every year. New investigative techniques at the cellular and molecular levels show great promise at defining potentially malignant lesions, but further prospective, in-depth studies are required to determine their practical usefulness in clinical workflow. Tumor markers are reliable in monitoring

| Table 1: Brief history of tumor markers[^1] |
|-------|------------------|
| Year  | Author           | Marker                              |
| 1846  | H. Bence Jones   | Bence Jones protein                 |
| 1928  | W.H. Brown       | Ectopic hormone syndrome            |
| 1930  | B. Zondek        | HCG                                 |
| 1932  | H. Cushing       | ACTH                                |
| 1949  | K. Oh-Uti        | Deletions of blood group antigens   |
| 1959  | C. Markert       | Isoenzymes                          |
| 1963  | G.I. Abelev      | AFP (fetoprotein)                   |
| 1965  | P. Gold and S. Freeman | CEA                      |
| 1969  | R. Heubner and G. Todaro | Oncogenes               |
| 1975  | H. Kohler and G. Milstein | Monoclonal antibodies              |
| 1980  | G. Cooper, R. Weinberg, M. Bishop | Oncogene probes and transfection |
| 1985  | H. Harris, R. Sager, and A. Knudson | Suppressor gene                   |

| CEA: Carcinoembryogenic antigen, AFP: Alpha-fetoprotein, HCG: Human chronic gonadotropin, ACTH: Adrenocorticotropic Hormone |

| Table 2: Ideal characteristics of tumor marker[^15] |
|------------------|------------------|
| Characteristics  | Remarks          |
| Highly specific  | Detectable only in one tumor type |
| Highly sensitive | Nondetectable in physiological or benign disease states |
| Long lead time   | Sufficient time for alteration of natural course of disease |
| Levels correlate with tumor burden | Prognostic and predictive utility of the tumor marker |
| Short half-life  | Frequent serial monitoring of the marker levels after 5-6 half-lives |
| Simple and cheap test | Applicability as screening test |
| Easily obtainable specimens | Acceptability by target population |
the treatment response, as well as in the early detection of disease recurrence (before the development of clinically notable signs) even it can to some extent help in determining the pathogenesis of the malignancy. Tumor markers cannot be used as a sole diagnostic tool, but they can be used as an adjunct to routine histopathology using hematoxylin and eosin stain. They can also be used in combination with the diagnostic methods to confirm the malignancy and help in grading it. Besides, by combining various tumor markers, we can achieve a greater specificity and sensitivity in the follow-up of one type of malignancy, the underlying mechanism that leads to an aggressive phenotype, which is not yet fully understood. Hence, possible biomarkers are much in need to predict prognosis.

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Conflicts of interest
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