REVIEW ARTICLE

TUMOR MARKERS FOR HEAD AND NECK CANCER: A REVIEW

Monica Mehta

ABSTRACT:
Oncologists all over the globe, relentlessly research on the methodologies for early detection of cancer and precise localization of cancer therapeutics with minimal adverse effects to healthy tissues. The chances of patient survival increase with early diagnosis of cancer rather than late diagnosis. Hence, it becomes imperative to develop means for early and accurate detection. Tumor markers since their discovery have made a great impact on the field of oncology and they are hailed as important diagnostic and prognostic indicators of the future. Markers may take a number of chemical forms and have cellular (tissue-based) or extracellular (circulating) expression, and henceforth require different approaches for recognition and quantitation. Thus, nucleic acids, proteins (antigen and hormone), enzymes, altered metabolites, and cellular components are all examples of tumor markers.
Key words: cancer, malignancy, tumor marker.

Corresponding author: Dr. Monica Mehta, E mail: drmonicamehta@gmail.com

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INTRODUCTION:

According to the WHO Report in 2004, cancer accounted for 7.1 million deaths in 2003 and it is estimated that the overall number of new cases will rise by 50% in the next 20 years[1]. India has the highest number of the oral and throat cancer cases in the world. Every third oral cancer patient in the world is from India.[2] Despite efforts and improvements in many fields of tumour research, the surviving time of cancer patients is strongly related to the stage of disease more than to therapeutic strategies. Therefore, screening procedures for early diagnosis of asymptomatic patients have been proposed, ranging from biochemistry (onco-developmental proteins) to molecular biology (oncogenes). From these studies the term "marker" has been introduced for indicating a sensitive, specific, easy to perform, recognizable substance related to the presence of the tumor.[2] Some of the most well-known tumor markers are Bence-Jones proteins, Prostate Specific Antigen (PSA), Alpha-fetoprotein (AFP) and Carcinoembryonic Antigen (CEA).

Varying definitions of 'Tumor Markers' have been given by different authors. According to Schwartz et al (1989) "Tumor markers are defined as substances which can be measured quantitatively by biochemical or immunochemical means in tissue or body fluids to identify the presence of cancer, possibly the organ where it resides, to establish the extent of tumor burden before treatment as well as to monitor the response to therapy".[3]

Huber et al (1994) described "tumor marker as any measurable parameter that differentiates a transformed cell from its biological progenitor".[4]

According to T. Malati (2007) "Tumor Markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process. These markers can be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A tumor marker produced by the tumor and, when present in significant amounts, indicates the presence of a cancer".[5]

According to National Cancer Institute (2008), "Tumor markers are substances produced by tumor cells or by other cells of the body in response to cancer or certain benign (non-cancerous) conditions. These substances can be found in the blood, in the urine, in the tumor tissue, or in other tissues".[6] Different tumor markers are found in different types of cancer, and levels of the same tumor marker can be altered in more than one type of cancer.

IDEAL TUMOR MARKER

The search for tumor marker begins with outlining the qualities that are desired in them. An ideal tumour marker theoretically should have the following criteria[4;5] .

1. It should be highly sensitive and should have low false negatives.
2. It should be highly specific and should have low false positives.
3. It should have high positive and negative predictive value.
4. 100% accuracy in differentiating between healthy individuals and tumor patients.
5. It should be able to differentiate between neoplastic and non-neoplastic disease and show positive correlation with tumor volume and extent.
6. It should predict early recurrence and have prognostic value.
7. It should be clinically sensitive i.e. detectable at early stage of tumor.
8. Its levels should be preceding the neoplastic process, so that it should be useful for screening early cancer.
9. It should either be a universal marker for all types of malignancies or specific to one type of malignancy.
10. It should be easily assayable and be able to indicate all changes in cancer patients receiving treatment.

APPLICATIONS AND LIMITATIONS OF TUMOR MARKERS:

The hope in the search for tumor markers was that all cancers could someday be detected by a single blood test. Presently, they are being used in screening, diagnosis, evaluation of prognosis of cancer and also predicting the therapeutic response. However, their use, although important, have been limited. In the next paragraphs are some of current applications and the limitations of various tumor markers[3;7].

In screening, there is a need for a definitive diagnostic method that will separate positives from false positives. If this procedure is invasive (e.g. surgery) and/or expensive, patients will not accept it. Thus, a marker is needed that is elevated at early disease stages, when the disease is localized and potentially curable. Most circulating cancer markers (with the exception of PSA) are elevated significantly in the late stage disease. Thus, diagnostic sensitivity is usually low for early-stage disease. Also, most cancer markers are not specific for a particular tissue and elevations may be due to diseases of other tissues, including benign and inflammatory diseases. Thus, diagnostic specificity may be low, leading to many false positives.

Tumor markers have lower diagnostic sensitivity and specificity, same as above. However, for selected subgroups of high risk patients, tumor markers analysis may aid the clinician in ordering more elaborate testing. E.g. imaging techniques or laparoscopic investigations.

Most cancer markers have prognostic value but their accuracy is not good enough to warrant specific therapeutic interventions. E.g. higher pre-operative levels of PSA are associated with capsular penetration, higher Gleason score, positive surgical margins, and positive lymph node status, but the decision to treat with two different modalities (e.g. radical prostatectomy vs. non-surgical approaches) cannot be taken based on tumor marker data alone. Same applies to many other cancers.

Despite the importance of using biomarkers in predicting response to anti-estrogens and Her-2/neu application for predicting response to Herceptin in breast cancer patients, we need more predictive markers to individualize therapy and maximize clinical response.

Biomarkers can be very useful for detecting cancer relapse. Current markers are limited by the following:

a. Lead time is short (weeks- few months) and does not significantly affect outcome, even if therapy is instituted earlier.

b. Therapies for treating recurrent disease are not effective at present.

c. In certain groups of patients, biomarkers are not produced and do not detect relapse.

d. Sometimes biomarkers provide misleading information, e.g. Clinical relapse occur without biomarker elevation, or biomarker is elevated non-specifically, without progressive disease, leading to either over-treatment or discontinuation of a current treatment protocol.

For patients with advanced disease, who are treated with various modalities, it is important to know if therapy works. In this regard, biomarkers usually provide information that is readily interpretable, and more economical, more sensitive and safer than radiological or invasive procedures. For certain cancers, this may facilitate increased enrollment of patients into therapeutic clinical trials.

TYPES OF TUMOR MARKERS

The appearance of tumour marker and their concentration are related to the genesis and growth

of malignant tumours in patients. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum. Continuing search for suitable tumour markers in serum, tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies. Broadly describing, tumor markers can be of following two types:

1. Biochemical /serological markers: Markers which can be detected in the blood or body fluids of patients harbouring an underlying malignancy are thus called as Serological markers. There are a large number of tumour markers present in the blood circulation.

2. Histochemical /tissue markers: Markers which can be detected in the tissue by immunological tests are called Histochemical markers.

Attempts have been made by authors at classification of tumor markers.

Gerhard Siefert (1987) in his book 'MORPHOLOGICAL TUMOR MARKERS' described following categories of tumor markers[8]:

1. Epithelial Tumor Markers e.g. cytokeratins, tissue polypeptide antigen, markers of glandular differentiation.
2. Mesenchymal Tumor Markers e.g. intermediate filaments, myosin, S-100.
3. Biochemical Tumor Markers e.g. tumor associated antigens, enzymes and isoenzymes, oncogene products.
4. Proteoglycans and Intercellular matrix
5. Basal Membrane Antigen as Tumor Marker
6. Lectins and Blood Group Substances e.g. Peanut agglutinin, Europeaus agglutinin.

Huber (1994) categorized tumor markers[4] as:

I. Markers in extracellular compartment
   a. Serum markers
   b. Urinary markers
   c. Salivary markers
   d. Other extracellular markers
      i. Enteric fluid(gastric, pancreatic)
      ii. Third space(pleural fluid, ascites)
      iii. Cerebrospinal fluid

II. Cellular markers demonstrable immunohistochemically
   a. Peptides
   b. Intermediate filaments
   c. Antigens
   d. Receptors

III. Cytogenetic markers

IV. Molecular markers (detection techniques)
   a. Blot analysis
   b. In situ hybridization
   c. Immunological analysis of genes
   d. Polymerase chain reaction

T. Malti (2007) gave the following classification of tumor markers[5]:

1. Oncofetal antigens (e.g. AFP, CEA, Pancreatic oncofetal antigen
2. Tumor associated antigens /Cancer antigens e.g. CA125, CA19-9, CA15-3 etc.
3. Hormones e.g. Beta human chorionic gonadotropin, calcitonin etc.
4. Hormone receptors (e.g. estrogen and progesterone receptors)
5. Enzymes and isoenzymes (prostate specific antigen, prostatic acid phosphatase, neuron specific enolase, glycosyl transferases, lysozyme, alpha amylase
6. Serum and tissue proteins (beta-2 microglobulin, protein S100, ferritin, fibrinogen degradation products
7. Other biomolecules e.g. polyamines

KERATINS: Recently, in diagnostic histopathology molecular components that are specifically expressed in epithelial cells have acquired great importance as epithelial tumor markers or, more correctly, epithelial differentiation markers. Such markers have two

main applications, i.e., in distinguishing epithelial from non-epithelial tumours, and in distinguishing different types of epithelial tumours. Franke et al. (1978, 1979) proposed the use of intermediate-filament antibodies for demonstrating whether a given tumour is of epithelial origin.[9]

Keratin, intermediate filament forming proteins that includes five classes – cytokeratins, vimentin, desmin, glial filaments and neurofilaments, which are used as tumour markers. Using available broad-spectrum keratin antibodies it has been found that malignant epithelial tumors, including their metastases, consistently maintain the expression of keratin-type intermediate filaments. Numerous studies have further confirmed and demonstrated that the expression of keratin filaments is a constant feature of all carcinomas, irrespective of their degree of differentiation. According to BE Sundström and TI Stigbrand (1994), Cytokeratins 8, 18 and 19 are the most abundant cytokeratins in carcinomas[10]. They are released into necrotic areas and can be found intratumorally and in blood, circulating as partially degraded complexes, and can as such be used as tumour markers.[10] In their publication Crevillini et al(2003) said that K13 is positive in epithelial islands with squamous metaplasia in ameloblastoma, AOT and ameloblastic fibroma.[11] They also found coexpression of vimentin and K14 in Calcifying epithelial Odontogenic tumour(CEOT) AND adenomatoid odontogenic tumour (AOT) and concluded that the significance of this coexpression remains to be elucidated.[11] According to Crevillini et al(2003) and Crevillini et al(2009), K14 is the typical intermediate filament of Odontogenic epithelium and is also found to be positive in ameloblastoma, adenomatoid odontogenic tumour (AOT), ameloblastic fibroma and calcifying epithelial odontogenic tumour.[11,12]

TISSUE POLYPEPTIDE ANTIGEN (TPA): which was originally prepared from the insoluble tissue residue of pooled carcinomas, was one of the first serum tumour markers. According to BE Sundström and TI Stigbrand (1994), TPA is a molecular complex containing CK 8, 18 and 19 and determinations of TPA in serum samples can be used in the follow-up of patients with many types of cancer.[10] It is often elevated in patients with carcinomas and appears to be correlated with the proliferative activity of carcinomas. TPA has therefore been regarded as a marker of proliferation. Nagler et al (1999) reported the potential use of tissue polypeptide specific antigen (TPS) as diagnostic marker of early head and neck cancer.[13] According to Rosatti et al (2000), in patients with head and neck cancer TPA has proven useful for monitoring the response to chemotherapy in patients with head and neck cancer, in particular for undifferentiated tumours.[14]

EPITHELIAL MEMBRANE ANTIGEN: EMA has proved to be a global marker of epithelial cells of normal and neoplastic origin and can be detected by the use of both polyclonal and monoclonal antibodies. According to Pinkus and Kurtin (1985), EMA is an excellent marker of epithelial differentiation, appears to be highly reliable for discriminating between poorly differentiated carcinomas and malignant lymphomas, and is especially helpful in characterizing small cell anaplastic carcinomas. EMA immunoreactivity is well preserved in paraffin sections of routinely processed tissues, facilitating application of this technique in diagnostic surgical pathology.[15] All tumours with glandular differentiation are EMA positive. It is found in benign and malignant salivary gland tumours. Gusterson et al (1982) demonstrated the positivity for EMA in pleomorphic adenoma, adenoid cystic carcinoma and mucoepidermooid tumours. They also found mesenchymally derived tumours to be negative for EMA.[16]

S-100: S-100 is normally present in cells derived from the neural crest (Schwann cells, melanocytes, and glial cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, dendritic cells, and keratinocytes. Gaynor et al (1980) has demonstrated the presence of S100 in human malignant melanoma.[17] Hashimoto et al (1984) have suggested that the immunohistochemical demonstration of S-100 protein is a useful diagnostic tool, particularly for the assessment of vacuolated tumour cells and for the diagnosis of myxoid tumours.[18]

DESMIN AND VIMENTIN: Desmin and Vimentin are members of intermediate filament family of proteins. According to Altmannsberger et al (1982) the use of antibodies to desmin could be very helpful in the future for the diagnosis of undifferentiated rhabdomyosarcomas[19]. Parham et al (1992) reported a case where desmin staining was positive in neuroctodermal tumor of childhood.[20] Caselitz et al (1981) demonstrated that the tumor cells of pleomorphic adenoma of parotid are vimentin positive and that of the mucoepidermoid tumors and squamous cell carcinomas are prekeratin-positive but vimentin-negative.[21]

MATRIX METALLOPROTEINASES: Kumamto et al (2006) have suggested that matrix-

degrading enzymes (MMPs) may contribute to the local invasiveness of odontogenic tumors.[22] Vincent et al (2007) suggest that matrix metalloproteinase MMP-7 and MT1-MMP are useful markers for progression in OSCC, and that they may be useful in identifying patients who would benefit from treatments based on MMP inhibitors. They also proposed that MMP-7 and MMP-14 combined with other markers may be used to predict the metastatic potential of OSCC.[23]

BASAL MEMBRANE ANTIGEN: From the viewpoint of diagnostic pathology, basal membranes are regarded as structural barriers to the invasion and metastatic procedure of malignant cells. Separating the epithelial and other cells from the surrounding stroma, they are obstacles to invasion from tumor cells. For many substances which are directly or indirectly associated with the basal membrane, there is some evidence that the invasive progression of a tumor is accompanied by a loss of these substances. Thus, demonstration of these substances can be used as a tool for diagnosis of cancer. Martinez – Hernandez and Amenta (1983) have divided the substances composing the basal membrane into those which are intrinsic (collagen type IV, laminin, heparin sulfate proteoglycan, entactin) and those which are extrinsic (fibronectin and type V collagen).

Type IV collagen, along with laminin, plays an important role in cell adhesion, migration, differentiation, and growth. According to Erlandson et al (1984), in pleomorphic adenomas, a considerable amount of basal membrane associated material, collagen type IV and laminin, is localized in the stroma and around the tumor cell groups. The labeling for fibronectin is especially intense at the border of cell groups and duct-like structures. At the morphological level it cannot be decided whether fibronectin is 'epithelial' or 'mesenchymal' by origin. A possible source of part of the basal membrane associated substances is the myoepithelial cell. The staining of the basal membrane substances seems to be parallel to, but not always identical with, the staining for elastic fibers.[24]

Stenman and Vaheri (1981) found single cells in sarcomas which were surrounded by fibronectin. It was abundant in the connective tissue but Labat-Robert et al (1981), found a loss of the pericellular labeling and a decreased staining of fibronectin in the extracellular matrix. Biphasic synovial cell sarcoma is reported to exhibit extracellular linear collagen type IV and laminin. In the basal membrane of neurofibromas, collagen type IV and laminin were localized.[25]

According to Seifert (1987)[25] and Birembaute et al (1984)[26] In Preinvasive carcinomas: Intrinsic components (collagen type IV, laminin, heparin sulfate) present as basal membrane labeling with interruptions. Extrinsic component (fibronectin) is present in the stroma.

According to Seifert (1987)[25] in invasive and metastatic carcinomas of the head and neck, collagen type IV displayed focal thickening, reduplication, aggregation, attenuation and segmental defects. Focal interruptions of laminin and collagen type IV in the basal membranes are found in dysplasia of the laryngeal mucosa. A production of collagen type IV is found in tumors with a high degree of differentiation. In solid basal cell carcinomas, the cells still appear to preserve their characteristic production of basal membrane material, but seem to have lost the ability of polar distribution of this material.

According Skalova et al (1992), some tumors (like mixed salivary gland tumors) with myoepithelial like cells display an augmentation of basal membrane material, although arranged in quite a different manner (e.g. pseudocysts in adenoid cystic carcinomas). This special arrangement may be a possible marker for insidious behavior of the adenoid cystic carcinoma in the head and neck region.[27] In other types of malignant carcinomas of the salivary glands, the staining for laminin, collagen type IV, and for fibronectin is generally interrupted in the region of the basal membrane.

Shinohara et al (2004) conducted a study in which six distinct alpha (IV) chains of collagen in the basement membrane (BM) of adenoid cystic carcinoma (ACC) of the salivary gland were immunohistochemically examined. In the BM of normal salivary ducts, alpha chains were continuously stained, but the staining was irregular in ACC. These results suggest that BM irregularity with the differential expression of alpha (IV) chains in ACC closely relates to cell proliferation, cell differentiation and histological structure.[28]

A.J. d'Ardenne (1989) said that in benign proliferations, laminin, collagen type IV and fibronectin are found in all vascular basal membranes and around all muscular and Schwann cells and adipocytes in a linear pericellular layer.[29] Staining for laminin and collagen type IV is found around the vessels in malignant mesenchymal tumors. However, there is a decrease in the pericellular staining of the muscle cells, Schwann cells and adipocytes, except fibronectin which persists as intracellular and

pericellular material in most sarcomas, especially in cases with fibroblastic differentiation.

CELL ADHESION MOLECULES: In recent years much attention has been directed towards cell adhesion molecules (CAM's), including the role of the cadherins, a family of Ca-dependent transmembrane proteins that mediate intercellular adhesion, like CD-44, E-cadherin, P-cadherin and Catenins. Mareel et al (1995) and Smith et al (1997), have proposed that, in carcinomas, E-cad functions as an invasion-suppressor molecule such that its loss permits or enhances the invasion of adjacent normal tissues.[30;31] Williams et al (1998), suggested that the reduced expression of P- and E-cadherin and the catenins in severe dysplasia and carcinomas in situ adjacent to infiltrating carcinomas is a late event associated with tumour invasion. In the carcinomas, reduction or loss of E-cadherin and the catenins is also associated with loss of differentiation and with metastases. Loss of E-cadherin at the advancing front of the carcinomas appears, to be an indicator for metastatic spread and possibly a prognostic indicator. The of loss of cadherin and catenin expression shows potential as biological markers for metastatic spread or as prognostic indicators.[32] According to Lim et al (2004) it seems reasonable to stratify the patients into a high- and low-risk group, and place patients with tumour thickness 4 mm, mode of invasion grade 3 or 4, and low expression of E-cadherin in the high-risk group. Combination of these factors may be a useful marker for deciding on salvage treatment during wait-and-see follow-up.[33] Munnoz-Guerra et al (2005), suggest that in the early stages of oral carcinogenesis, the reduction of P-cad expression is a prognostic marker for a disease recurrence-free interval. They also suggest that in OSCC, changes in E-cad and P-cad expression at the invasive tumour front may promote invasion and metastasis.[34] Masarelli et al (2005), in their study demonstrated that E-cadherin loss in conjunction with p27 loss was associated with a significantly shorter time to disease progression, possibly due to the strong predictive influence exerted by p27 loss.[35] Kawano et al (2005) observed that the determination of sCD44 serum concentrations may be a useful predictive or prognostic parameter of head and neck cancer.[36] Ksunen et al (2007) said that irregular staining of CD44 predicted more advanced disease and shortened survival of the patients.[37]

CONCLUSION

A patient diagnosed with cancer at an early stage has a better chance of survival than when diagnosed at later stages. Thus, it becomes essential to devise a mode for early and accurate diagnosis of cancer. Over past few decades, a large number of tumor markers have been identified for diagnosing cancer, predicting the nodal metastases and the survival. These markers have to be used in conjunction with each other to serve various purposes. However, the ideal tumor marker still remains elusive.

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