Introduction

The first recognized biomarker was Bence–Jones protein, discovered in 1847 by precipitation of a protein in acidified boiled urine.[1] This protein is produced by tumor plasma cells and is still used for the diagnosis of multiple myeloma. Biomarkers are biochemical substances expressed by tumor cells or normal body cells either due to the cause or due to effect of the malignant process. They are substances that can be detected in higher than normal amounts in the blood, urine, or body tissues of patients with certain types of cancers. These markers can be normal endogenous products that are formed at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A biomarker may be produced by tumour itself or by the body in response to the tumour. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum. Continuing search for suitable tumor markers in serum, tissue, and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies.

Biomarkers Tests

The biomarker tests are mainly to help diagnose the condition to monitor the disease process, stage the disease, assess the prognosis, guide and monitor the treatment, and determine the recurrence. Evaluation of biomarkers can be of valuable aid in diagnosis, prognosis, and staging and in monitoring the growth of the tumor. Once the patient is positive for a particular marker before instituting therapy, the effective clinical use becomes evident only after its continued measurement throughout the patient’s clinical course. Certain biological markers are isolated by means of Immunohistochemistry (IHC), polymerized chain reaction (PCR), and enzyme-linked immune-sorbent assay (ELISA) techniques, which are helpful for laboratory diagnosis of certain diseases like (Temporomandibular Joint) TMJ disorders and premalignant lesions. The diagnostic efficiency of biomarkers depends on variety of factors relating to its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

The sensitivity of tumor marker is the probability of its detection in test results of patients possessing tumors. Specificity of tumor marker as a screening parameter indicates whether it may be used for describing in what percentage of healthy individuals the test result is negative. The PPV
describes the probability that the disease in question is actually present if the test result is positive. NPV describes the probability that the disease is not actually present if the test result is negative.

At present, there is dearth for an ideal biomarker that is suited for general screening, though some are used to monitor those with a strong family history of a particular cancer. Genetic markers help predict risk in family members. In patients with symptoms, biomarkers are used to identify the source of the cancer. For instance, CA-125 is tested for ovarian cancer and help differentiate it from other conditions. If a patient is suffering from cancer, biomarker elevations help determine how far the cancer has spread into other tissues and organs. Some biomarkers are used to determine the aggressiveness of the cancer and others provide information regarding the type of treatment the patient is likely to respond to. For instance breast cancer patients who are Her2/neu positive are more likely to respond to Herceptin therapeutic drug treatment. Biomarkers are also used to monitor the effectiveness of treatment, especially in advanced cancer cases.

However, the measurement of most tumor marker levels alone is often insufficient to diagnose cancer. This is because the levels can be elevated in people with benign conditions. Also, tumor marker levels are not elevated in every person with cancer, especially in the early stages of the disease. Many tumor markers are not specific to a particular type of cancer and the level of a tumor marker can be elevated by more than one type of cancer.

Monitoring through biomarkers

Molecular markers linked to the occurrence and progression of malignancies have been used in molecular tumor staging or grading, or for molecular targeting of therapy, but little is known regarding the prognostic and therapeutic value of biomarkers in the diagnosis and treatment of oral cavity cancer. Monitoring is achieved by determining the level of biomarker, if the marker level drops the treatment is working and if it stays elevated, adjustments in the treatment may be considered. Whilst monitoring, the information of the marker must be interpreted with care. CEA is a biomarker that is used to monitor bowel cancer, but not every bowel cancer patient will have elevated levels of CEA. If the marker level is not initially elevated with the cancer, it is not used later as a monitoring tool. Currently one of the commonest uses of biomarkers is to monitor cancer recurrence. If a tumour marker is elevated before treatment, low after treatment, and then begins to rise over time then it is likely that the cancer is returning. Likewise, if it remains elevated after surgery then chances are that the cancerous lesion was not completely removed.

Ideal biomarker

Unfortunately, none of the tumor markers reported to date can be considered as an ideal biomarker. In general, tumor markers are not specific to any single malignancy. Most of them are specific to a group of malignancies or a single organ.

An ideal biomarker theoretically should have the following criteria.

1. Highly sensitive and low false negative values
2. Highly specific and low false positive results
3. Possess high positive and negative predictive values
4. Hundred percent accurate in differentiating between healthy individuals and tumor patients
5. Able to differentiate between neoplastic and non-neoplastic conditions
6. Able to show positive correlation with tumor volume and extent
7. Predict early recurrence and have prognostic value
8. Clinically sensitive and detectable at early stages of the tumor development
9. Its levels should be preceding the neoplastic process, so that it is be useful for screening early cancer
10. Either a universal marker for all types of malignancies or specific to one type of malignancy
11. Easily assayable and be able to indicate all changes in cancer patients receiving treatment.

Types of biomarkers

The spectrum of biochemical tumor markers reported is very wide. Biomarkers can be epithelial markers, mesenchymal markers, muscle markers, neural markers, endothelial markers, melanocytic markers, lymphoid markers, neuro-endocrine markers, metastatic tumor markers, minor salivary gland tumor markers, Ewings tumor and primitive neuroectodermal tumor markers. They can also be classified as Oncofetal antigens, tumor associated antigens/cancer antigens, hormones, hormone receptors, enzymes, isoenzymes, serum, and tissue proteins. They are also classified based on their use in dentistry as markers for odontogenic cysts and tumors, markers for oral cancer, markers for TMJ diseases, markers for bone activity, markers for TB, auto antibodies as markers, micronutrients as markers, and miscellaneous Markers.

Markers of oral cancer

14-3-3r

14-3-3r is an intracellular phosphoserine binding protein, which regulates different cellular signaling processes. It is a marker that is involved in cancer development. 14-3-3r belongs to a family of highly conserved 28-33 kDa acidic polypeptides that spontaneously self-assemble as homo- or heterodimers and are expressed in all eukaryotic cells. The family consists of seven closely related genes, encoding b, e, g, c, s, f, and r isoforms. They can bind to more than 200 functionally diverse cellular proteins and thereby play an important role in various cellular processes such as signal transduction, maintenance of cell cycle checkpoints, apoptosis, the onset of cell differentiation and senescence, cytoskeleton organization and malignant transformation. 14-3-3r high expression has been demonstrated as an independent prognostic marker for overall survival in colorectal cancer patients suggesting 14-3-3r as a potential oncogene for this cancer.
**Hypoxia inducible factor**

Hypoxia is characteristic of solid tumors, arising from a less-ordered vasculature and necrosis. Clinically relevant hypoxia has been detected in approximately 50% of all solid tumors. Tumor hypoxia has been associated with poorer outcomes in patients with head and neck carcinomas. It is an important factor determining the response of head and neck SCC to surgery, chemotherapy, and radiotherapy. Among the endogenous hypoxia markers used to evaluate microenvironmental conditions of head and neck tumors are Hypoxia-inducible factor (HIF)-1α, HIF-2α, Carbonic anhydrase (CA)-9, Glucose transporter (GLUT)-1, and Erythropoietin receptor (EPOR). The over expression of these markers has been associated with solid tumor aggressiveness. They have also been used to noninvasively identify tumors showing poor response to radiotherapy and/or chemotherapy.

**P53**

P53 is a tumor suppressor gene located on the short arm of chromosome 17p, which encodes a 53-kd protein involved in the control of multiple cellular events. The cellular events include cell-cycle regulation, apoptosis, cell senescence, DNA repair, cell differentiation, gene amplification, DNA recombination, chromosome segregation, and angiogenesis, through mechanisms including the transcriptional activation or inhibition of the expression of numerous target genes. This gene, known as the ‘guardian of the genome’[3,4] essentially monitors and regulates the cell cycle so that the cell responds in an appropriate manner to the stress and genetic damage to which it is exposed. Mutation or inactivation of the tumor suppressor gene p53 is frequently observed in malignant neoplasms and is known to be involved in the early stages of carcinogenesis. Cyclooxygenase-2 (COX-2) is an induced proinflammatory enzyme involved in various steps of carcinogenesis such as cell proliferation, reduction in apoptosis rates, and promotion of tumor angiogenesis. Correlation between COX-2 and p53 expression in several malignant neoplasms exists. Over expression of COX-2 has been demonstrated during the transition process the oral mucosa undergoes during carcinogenesis. Correlation between COX-2 over expression and the presence of mutations in p53 gene of neoplastic cells has been established. This over expression of COX-2 may contribute to the development and progression of tumors as a result of its inhibitory effect on apoptosis induced by p53 in response to DNA damage. The antitumor effect of some COX-2 inhibitors, such as NS-398, potentiate apoptosis induced by p53 in the presence of genotoxic stress and even act on the transcriptional activity of p53.

**E-Cadherin**

E-Cadherin[5,6] is a calcium-dependent adhesion molecule, a transmembrane glycoprotein that plays a role in normal epithelial polarity, anchoring the cytoskeleton of adjacent cells via cytoplasmic protein in cadherin. It functions in proliferation, migration, polarization, and differentiation through cell adhesion and signaling regulation of epithelial cells. Expression of E-cad has been reported in various malignancies and is associated with the advance stage, lymphatic, and hematogeneous spreading. Differential expression with cytoplasmic shifting is seen with oral squamous cell carcinomas and some premalignant lesions compared with normal mucosa. It can be regarded as a differential marker, which can be used in grading carcinomas. During metastatic process in invasion stage, there is loss of E-cad, Integrin and other adhesion molecules that seem to be crucial for the detachment of tumor cells from primary lesion. Re-colonization of metastatic cells in lymph nodes might require an adaptation to environmental changes such as hypoxia, acidosis, and demands for angiogenesis and reduction in anchorage. Vast majority of metastatic deposits had higher E-cad expression than their counterparts in primary lesion. Cadherin can help the growth of an OSCC cell in an anchorage-independent system.[4]

**Markers of odontogenic tumors**

High levels of proliferating cell nuclear antigen (PCNA) activity is found in ameloblastic carcinomas.[5] PCNA is suggestive of invading islands in the walls of cystic ameloblastoma and is the biological basis for more vigorous treatment of cystic with tumor proliferation within the fibrous wall. Lectins UEAl and BSA-1[6] help to distinguish cystic ameloblastoma from ameloblastomas originating in non-neoplastic cystic epithelium with more certainty. The cystic and solid ameloblastomas bind the lectins differentiating them from non-neoplastic odontogenic cysts which do not bind these lectins.[6] Aggressive clear cell odontogenic carcinomas show raised levels of tumor markers such as SCC associated antigens TA-4 and CEA. Platelet derived growth factors such as endothelial cell growth factor (ECGF), thymidine phosphorylase (TP) and angiprotien 1 and 2[7] are important factors in the development and progression of odontogenic tumors. In tooth germs and ameloblastic tumors, these factors participate in tooth development and tumor progression by regulating angiogenesis. Altered expression of PD-ECGF/TP and angiprotien in ameloblastic tumors may be involved in Oncogenesis. Malignant potential and tumor cell differentiation.[7] IIHC showed positive reactivity of some cells for Keratin and EMA epithelial membrane antigen, but Negative for S-100, actin, vimentin and myosin. Undulin a glycoprotein associated with mature collagen fibrils and differentiated tissues is positive for ameloblastic fibroma and fibro sarcoma.[7]

**Markers of odontogenic cysts**

Radicular cysts contain foci of hyaline refractile material described Dewey[9] as Ruston Bodies (RB). Microscopically, RB assume linear, circinate, hairpin and irregular forms. Their presence in the epithelium suggests epithelial origin of the cysts, however, hematological origin can also be considered. Cytokeratin 18[5,7,10] is present in some dentigerous cysts and focally in ameloblastomas. It is also expressed in Botryoid odontogenic cyst epithelium but absent in gingival epithelium. Bone resorption factors (BRF) such as PGE2, E3 and Collagenase in the walls of keratocysts contribute for the growth of the cyst growth. They are markers for expanding cysts.
Tenascin\cite{11} is a multifunctional glycoprotein involved in cell to cell and cell to extracellular matrix interaction. It is temporally expressed in a site restricted manner at the epithelial–mesenchymal interface during embryonic and fetal development, wound healing and in various solid tumors. In ameloblastomas, immunoreactivity of tenascin is observed in the tumor epithelial island basement membrane-connective tissue interface, tumor stroma and in stellate reticulum like cells. Tenascin is also expressed in the epithelium–connective tissue interface of oral leukoplakia and in squamous cell carcinoma \(\text{(SCC)}\)\cite{11}. In addition, composite odontomes, periodontal ligament fibers and mesenchyme adjacent to areas of calcifications show intense immunoreactivity for tenascin.

**Markers of temporomandibular joint diseases**

Cytokines, enzymes, and tissue break down products that are identified in synovial fluid could potentially serve as diagnostic markers of pathologic changes occurring in TMJ\cite{12}. In early stages of osteoarthritis microscopic breakdown of articular surface occurs and Chondrocytes clustering is seen. This is followed by matrix depletion affecting proteoglycans and then altering collagen fiber architecture. The articular surface undergoes vertical and horizontal splitting, fibrillation, and thinning with the result of denudation of subchondral bone. Synovial alteration includes both proliferation and inflammation. Osteoarthritis (OA) process in TMJ is triggered by certain traumatic events, which lead to oxidative stresses and generation of free radicals within the joint. These radicals possess the capacity to modify cellular milieu, both directly by engagement of signaling pathway such as P21, and activation of pleiotropic transcription nuclear factor KB (NFKB), thus, indirectly causing oxidation of sugars and lipids resulting in chemical modification of proteins and lipids. Such events lead to the generation of advanced glycation end products (AGE’s) that cause distinct cellular effects. These AGEs have the capacity to directly modify basement membranes and components of the extracellular matrix as well as ability to engage specific cellular receptors such as receptors for AGE (RAGE).\cite{13} RAGE is a multiligand signaling receptor of the immunoglobulin super family of cell surface molecules. Most prevalent AGE product found in \textit{vivo} is \(\text{N}^\epsilon\)– Carboxymethyllysine\cite{12} (CML) adduct, which specifically engages RAGE.

RAGE also serves as signaling receptor for S 100/calgranulin polypeptides by activated leukocytes and mononuclear phagocytes. A member of this family, which is an extracellular newly identified RAGE-binding protein (EN-RAGE) also known as S 100 A12, can bind with activated RAGE. Synovial fluid levels of S100/calgranulins are markers of disorders such as Juvenile RA\cite{12}.

**Cytokines**

TNF \(\alpha\) and IL-1\(\beta\) are inflammatory cytokines critically important to the pathogenesis of joint injury. These cause fever, and directly stimulate resorption of bone. They also regulate and activate several extra cellular matrix-degrading enzymes such as Matrix Metalloproteinase (MMP’s) in chondrocytes, synovial cells and fibroblasts. There exists a relationship between the extent of elevation of TNF in TMJ synovial fluid and degree of pain and surgical outcome. Soluble receptors like TNF\(\alpha\), IL 6, IL 1\(\beta\), STNF 1, STNF2, IL 6sR, and receptor antagonist like IL 1sR type II and IL1RA have been aspirated from the synovial fluid of patients exhibiting internal derangement and OA changes. Apart from these cytokines, inflammatory mediators such as PGE2 and COX-2 can also be present in inflammatory TMJ disorders βglucuronidase, Ig A, Ig Gwas aspirated several symptomatic temporomandibular dysfunction (TMD) patients. It is believed that these inflammatory mediators are the result of serum exudates as well as the infiltration of chronic inflammatory cells into the joint space.

**Enzymes**

Proteolytic enzymes like MMP’s\cite{12} are linked to tissue injury in the inflamed joint. These are capable of degrading extracellular matrix components particularly collagen, gelatin, and proteoglycans. Transcription of several MMP genes is unregulated by various cytokines and is repressed by steroid hormones. These MMP’s consists of 20 zinc dependent enzymes out of which three are collagenases such as, MMP 1 (Fibroblast type) MMP 8 (Neutrophil type) MMP 13 (Collagenase 3), and Stromelysin 1 (MMP 3), 2 (MMP10), 3 (MMP 11). Recently identified MMP 8 and MMP 13 are produced by articular chondrocytes from osteoarthritic cartilage.

**Proteoglycan break down products**

Proteoglycan breakdown products are Aggrecan, Keratan sulfate, and Chondroitin sulfate. Aggrecanmetabolic fragments generated by MMP’s and Aggrecanase enzyme were identified in articular cartilage of patients suffering with OA and rheumatoid arthritis (RA). Rheumatoid factor (RF) is an antibody that recognizes Ig G as an antigen and is the most characteristic serum lab abnormality seen in RA. Anti-citrilline containing peptide (Anti-CCP) is a post transcriptionally modified arginine residue. Anti-CCP antibodies when used in conjunction with RF may be useful in diagnosis of polyarthritis.

C-reactive proteins (CRP) has been used for many years to monitor disease activity in RA. CRP production also correlates with the extent of destruction observed on radiographs in RA. Serum Hyaluronan, a product of synovium is elevated in joint disease and correlates with disease activity. Apart from these, Anti perinuclear Factor (APF), Anti Keratin antibody \(\text{(AKA)}\), Anti Neutrophil Cytoplasmic Antibodies \(\text{(ANCA)}\) and Anti-RA3, Anti-Sa, Anti-P68 \(\text{(BiP)}\) antibodies are useful biomarkers for RA.

**Markers of Sjogrens**

The presence of nonspecific markers of auto immunity such as ANA, RF, Elevated IgG, and ESR are suggestive of Sjogrens.\cite{13,14} In addition, the presence of antibodies against the more specific extractable nuclear antigens SS-A/rO and SS-B/rA are definitive for Sjogrens.\cite{19} It is estimated that 80% of Sjogrens are likely to display ANA and 60% SS A/rO. Serum amylase is also a feature in several salivary gland disorders and
some time in inflammatory state of glands. CD 1a antibody mAb 010 found in histiocytes in paraffin-embedded sections, is used as a marker in cosinophilic granuloma and Letterer- Siwe disease. Diffuse mucosal aggregates of monotypic Ig G- producing λ and κ light chain restricted plasma cells are associated with ulcerative stomatitis.[13,14] Serological findings like raised ESR, Rouleaux formation of RBC’s in blood sample, raised plasma protein level resulting from Ig over production is a common finding in multiple myeloma.

**Squamous cell carcinoma antigen**

The squamous cell carcinoma (SCC) antigen concentration is elevated in squamous cell carcinomas of head and neck, lung, esophagus, and anal canal. The highest concentration of SCC antigen is found in patients with metastases. The marker elevation is also observed in 70% patients with advanced cervical cancers. Serial serum SCC antigen determination helps in determining progression and regression of cervical cancer following chemotherapy. The antigen levels are also raised in some patients of extensive liver disease. The combined use of CEA and SCC antigen has helped in increasing sensitivity for detection and monitoring of lung tumors.[16]

**Oral biomarkers in the diagnosis and progression of periodontal diseases**

Gingival crevicular fluid (GCF) is an inflammatory exudate from the gingival microcirculation that crosses inflamed periodontal tissues and en route collects molecules of potential interest from the local inflammatory reaction. The collection and analysis of GCF samples provides a noninvasive means to assess the pathophysiological status of the periodontium in a site-specific manner. GCF is easily collected by means of paper strips, absorbent points and micropipettes from gingival crevices of teeth. More than sixty five GCF constituents have been identified in GCF. Differences in the GCF concentration of markers such as PGE2, IL‑1, IL‑6, IL‑8, and TNF. In addition, pro-inflammatory and anti-inflammatory cytokines are also identified in GCF. Differences in the GCF concentration of PGE2 in patients with gingivitis compared with periodontitis have been reported. Subsequently, it was found that there was a correlation between increased PGE2 concentration and clinical attachment loss in patients who were diagnosed with moderate to severe periodontitis. Proinflammatory cytokines in particular IL‑1β, may play an integral role in the etiology of periodontal disease. With an increase in gingival index and probing, there was a corresponding increase in IL‑1β in both the gingival tissue and GCF. GCF IL‑1β expression is genetically influenced and not solely a result of local clinical parameters. Also, a GCF level of ILS was found to be higher in periodontal diseases and was influenced by local IL‑1β activities. Loos and Tjoa reviewed potential diagnostic markers of periodontitis present in GCF. Their review identified eight potentially valuable markers, including alkaline phosphatase, β-glucuronidase, cathepsin B, collagenase-2 (matrix metalloproteinase, MMP-8), gelatinase (MMP-9), dipeptidyl peptidase (DPP II and III), and elastase.[17]

**Conclusion**

Biomarkers as yet may not be suitable as isolated cancer or disease screening tools. When appropriately used alongside clinical findings and other investigations, they can be useful adjuncts in diagnosis, prognostic evaluation, and monitoring treatment response and as markers of recurrence.

**References**