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BIOMARKER: VALIDATION, CHARACTERISTICS, PITFALLS AND IMPORTANCE IN ORAL SQUAMOUS CELL CARCINOMA

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ABSTRACT: Since the emergence of the so-called omics technology, thousands of putative biomarkers have been identified and published, which have dramatically increased the opportunities for developing more effective therapeutics. These opportunities can have profound benefits for patients and for the economics of healthcare. Clinical biomarkers can serve a variety of functions that correspond to different stages in disease evolution. Biomarkers can assist in the care of patients without apparent disease (screening biomarkers), with suspected disease (diagnostic biomarkers), or with overt disease (prognostic biomarkers). Present review focuses on the validation, characteristics, major pitfalls and limitations of biomarkers and their importance in oral squamous cell carcinoma.

KEYWORDS: Biomarker, validation, screening, diagnostic, prognostic, oral squamous cell carcinoma.

INTRODUCTION: Diseases with high complexity such as cancer are associated with increased incidence rates worldwide. Recent data reveal that approximately 7.6 million deaths caused by cancer occurred in 2008, with this number corresponding to 13% of all deaths.¹ The prevalence of cancer in India is estimated to be around 2.5 million, with about 8,00,000 new cases and 5,50,000 deaths per annum; this high morbidity rate can definitely be attributed to late diagnosis of the disease.² Based on these numbers, there is substantial room for improvement in the current strategies for development of biomarkers capable of being introduced into clinical practice. According to the National Cancer Institute, a biomarker is defined as "a molecule detected in body fluids or tissues that are associated with a special process (normal or abnormal), a condition or disease".¹

Early detection of various forms of cancer before they spread and become incurable is an important incentive for physicians and research scientists. One of the best ways to diagnose cancer early, aid in its prognosis, or predict therapeutic response, is to use serum or tissue biomarkers.³

A biomarker is a pharmacological or physiological measurement that is used to predict a toxic event; a specific molecule in the body, which has a particular feature that makes it instrumental for measuring disease progression or the effects of treatment.⁴

Cancer biomarkers can be DNA, mRNA, proteins, metabolites, or processes such as apoptosis, angiogenesis or proliferation. The markers are produced either by the tumor itself or by other tissues, in response to the presence of cancer or other associated conditions, such as inflammation. Such biomarkers can be found in a variety of fluids, tissues and cell lines.³ Biomarkers have been categorized following the recommendation by the Committee on Biological Markers of the National Research Council/National Academy of Sciences. They fall into broad groups that detect exposure, progression, susceptibility to carcinogens, and/or the responses by the target cellular populations.⁵

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DIFFERENT KINDS OF BIOMARKERS: The utility of a biomarker lies in its ability to provide an early indication of the disease, to monitor disease progression, to provide ease of detection, and to provide a factor measurable across populations.⁶ Biomarkers include gene expression products, metabolites, polysaccharides and other molecules such as circulating nucleic acid, in plasma and serum, single nucleotide polymorphism and gene variants.⁷

Based on a combination of all biomarkers, biomarkers can be divided into three Categories:

1. Biomarker specific to a disease.
2. Biomarkers that are nonspecific to a disease but seems to demonstrate an abnormal condition.
3. Biomarkers that are randomly seen that may be a result of diversities or variations between control-diseased samples, sample treatment protocols, and mass spectrometry platforms.⁴

Biomarkers can also be categorized as pharmacodynamic, prognostic and Predictive:

- Pharmacodynamic biomarkers indicate the outcome of the interaction between a drug and a target, including both therapeutic and adverse effects.
- Prognostic biomarkers were originally defined as markers that indicate the likely course of a disease in a person who is not treated; they can also be defined as markers that suggest the likely outcome of a disease irrespective of treatment.
- Predictive biomarkers suggest the population of patients who are likely to respond to a particular treatment.⁷

Characteristics of an ideal biomarker and basic statistical methods for Evaluation;

- An ideal biomarker should be safe and easy to measure.
- The cost of follow-up tests should be relatively low, there should be proven treatment to modify the biomarker.
- It should be consistent across genders and ethnic groups.⁸

Specifications of Biomarkers⁹;

- **Specificity:** The proportion of control (normal) individuals who test negative for the biomarker.
- **Sensitivity:** The proportion of individuals with confirmed disease who test positive for the biomarker.
- **Receiver operating characteristic (ROC) curve:** A graphical representation of the relationship between sensitivity and specificity. This curve is used to evaluate the efficacy of a tumor marker at various cut-off points. An ideal graph is the one giving the maximum area under the curve (AUC). In the given example, the red curve represents a useless test (AUC = 0.5). The green curve represents a useful (AUC <1.00) but not perfect (AUC = 1.00) test. (Figure 1)

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Figure 1: Receiver operating characteristic (ROC) curve⁹

BIOMARKERS FOR ORAL SQUAMOUS CELL CARCINOMA: Biomarkers help in the detection of the earliest stages of oral mucosal malignant transformation. They reveal the genetic and molecular changes related to early, intermediate, and late end points in the process of oral carcinogenesis. These biomarkers enhance the prognosis, diagnosis, and treatment of oral carcinomas. Genetic and molecular biomarkers also determine the effectiveness and safety of chemo preventive agents. Unlike other drugs, chemo preventive agents reduce the incidence of diseases before clinical symptoms occur. This development is critical for the understanding of early oral mucosal transformation.¹⁰ Figure 2 shows the opportunities of identifying biomarkers in the process of carcinogenesis and Table 1 shows potential biomarkers for oral carcinogenesis. Biomarkers will also reduce the number of patients and the time for long-term follow up required to define a significant clinical response to a chemo preventive agent. The markers may, therefore, clarify the types, doses, frequencies, and regimens to achieve the maximum level of benefit from chemo preventive agents.¹⁰

Many studies have been reported in the past few years to explore the potential use of saliva to discover biomarkers for OSCC. Salivary tumour biomarkers can be classified into proteome, transcriptome, micro RNA, metabolome and microbe. Metabolome is the complete set small-molecule metabolites such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites which are found in a biological sample. Proteome is the protein complement of the genome and proteomics is analysis of the portion of the genome that is expressed. Using proteomics profiling, Hu et al identified 309 saliva proteins from healthy participant including 220 proteins with known biological functions.¹¹ Table 2 enumerates few salivary biomarkers with their possible uses.¹²

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Figure 2: The process of carcinogenesis, showing opportunities of identifying biomarkers.¹²

CATEGORY	MEASURES
Genomic biomarker	Micronuclei, DNA adduct, DNA content, and chromosomal aberration (Polymorphism, alleic loss, gain, and amplification)
Oncogenic biomarker	Oncogenic expression, modified tumor suppressor genes, and Src genes
Proliferation biomarker	Nuclear and cyclin-related antigens, mitotic frequency, ornithine decarboxylase (ODC), and polyamines
Differentiation biomarker	Cytokeratins, transglutaminase Type I, and transcription factor (AP)-1
Oxidative stress biomarker	Glutathione S-transferase, stress proteins (HSPs), and Superoxide dismutase
Apoptosis biomarker	Bcl-2 family, chromatin condensation factors, caspases, and nucleosome formation
Immunologic Biomarker	Cytokines

Table 1: Potential biomarkers for oral carcinogenesis¹⁰

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Saliva/Oral Fluid Biomarkers	Possibilities for Use
DNA	<ul style="list-style-type: none"> • Standard genotyping • Bacterial infection • Diagnosing carcinomas of the head and neck • Forensics
RNA	<ul style="list-style-type: none"> • Viral/bacterial identification • Carcinomas of the head and neck
Proteins	<ul style="list-style-type: none"> • Diagnosing periodontitis • Diagnosing carcinomas of the head and neck • Detecting dental cavities
Mucins/glycoproteins	<ul style="list-style-type: none"> • Diagnosing carcinomas of the head and neck • Detecting dental cavities
Immunoglobulins	<ul style="list-style-type: none"> • Diagnosing viruses (HIV, hepatitis B and C)
Metabolites	<ul style="list-style-type: none"> • Diagnosing periodontitis
Drugs and their metabolites	<ul style="list-style-type: none"> • Monitoring drug abuse • Detecting of drugs in the body
Viruses, bacteria	<ul style="list-style-type: none"> • Epstein-Barr virus reactivation (mononucleosis)
Cellular material	<ul style="list-style-type: none"> • Diagnosing carcinomas of the head and neck

Table 2: Salivary Biomarkers with their Possibilities for Use¹²

BIOMARKER DATABASES AND VALIDATION: Building of reliable biomarker databases and integration of information from the genome programs expand the scientific frontiers on etiology, health risk prediction, and prevention of environmental disease. Biomarker validation may be performed in a number of ways: bench-side in traditional labs; web-based electronic resources such as gene ontology; literature databases; and clinical trials (Phan et al. 2006). Biomarker databases have potential value for pharmaceutical research and development.¹³

The biomarker database of GVK Bio (Hyderabad, India) holds information from published literature relating to clinical and preclinical biomarkers and may be used in biomarker design and validation research. Under an agreement, the FDA (Food and Drug Administration) uses this database as part of its Voluntary Exploratory Data Submission Program and in internal research projects.¹³

PITFALLS AND LIMITATIONS: Following are the major pitfalls in the translation from biomarker discovery to clinical utility:

1. Lack of making different selections before initiating the discovery phase.
2. Lack in biomarker characterization/validation strategies.
3. Robustness of analysis techniques used in clinical trials.⁷

SUMMARY: To summarize, biomarkers are defined as biological molecules that (1) correlate with the presence or absence of a disease state, (2) are prognostic correlating with a disease course, or (3) predictive of a tumor's response to a specific therapy. Biomarkers should be objective, independent and require validation by clinical testing and patient outcome. Ideally biomarkers should be easy to analyze, quantitative, affordable, and must be subject to quality control and assurance.¹⁴

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CONCLUSION: However, the transfer of biomarkers from discovery to clinical practice is still a process filled with lots of pitfalls and limitations, mostly limited by structural and scientific factors. To become a clinically approved test, a potential biomarker should be confirmed and validated using hundreds of specimens and should be reproducible, specific and sensitive. The technology to discover the ideal biomarkers does not exist yet. Humans make hundreds of thousands of proteins and peptides some of which are a trillion-fold more common than others, and the most informative biomarkers are probably among the least abundant. The techniques necessary to enrich and fractionate low- abundance proteins are immature. Current attempts to validate biomarkers are inefficient and require improvement. To improve on this current situation, a well-defined study design has to be established driven by a clear clinical need, while several checkpoints between the different phases of discovery, verification and validation have to be passed in order to increase the probability of establishing valid biomarkers.

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