REVIEW

RECENT ADVANCES IN DIAGNOSIS OF ORAL CANCER

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ABSTRACT:
Oral carcinogenesis is a stepwise accumulation of genetic damage over time. Technological and therapeutic advances are needed to improve the poor outcomes associated with oral cancer due to our inability to diagnose and treat this disease at an early stage. Advances in molecular biology over the past decade have helped us to enhance our understanding of the complex interplay between genetic, transcriptional, and translational alterations in human cancers. This review provides a summary of comprehensive diagnostic modalities that can be used for early detection, which is crucial for its ultimate control and prevention.

KEYWORDS: genomics, microarrays, next generation sequencing, PCR, proteomics

INTRODUCTION:
“Oral cancer” encompasses all malignancies originating in the oral cavity. Oral cancer ranks sixth in the overall incidence for the ten most common cancer sites worldwide and third in the developing countries. Most of the oral cancers are squamous cell carcinomas and the majority is unequivocally associated with tobacco chewing and an early diagnosis greatly increases the probability of cure, with minimum impairment and deformity. They are of utmost concern as the mortality rate of the oral cancer for the past three and a half decades has remained high (over 50%) in spite of new treatment modalities. Despite numerous advances in treatment, the 5-year survival has remained approximately 50% for the last 50 years. Annually 75000 to 80000 new oral cancer cases develop in India.

Based on the increasing incidents of head and neck cancers, problems associated with late diagnosis and the public health dilemma they present, it seems prudent to enact screening protocols to check people at risk. Early diagnosis would allow for conservative therapeutic approaches with a brief recovery and a more favorable prognosis. Suspected patients should be more intensively examined and treated. The current trends in diagnosis focuses in the areas of molecular biology and advanced diagnostic aids will transform our traditional approaches to oral and dental disease management.

The approaches in the early detection of oral cancer includes screening programs and employing specific diagnostic tools that identify asymptomatic patients with suspicious lesions.

Traditional diagnostic aids:
Oral brush biopsy uses the concept of exfoliative cytology to provide a cytological evaluation of cellular dysplastic changes. To help localize the optimal site for brushing an abnormality, conventional oral brush biopsy combined with the application of toluidine blue is used to localize suspected mucosal areas. Chemiluminescent illumination technique, photodiagnosis, Velscope system, flow cytometry helps in detection of oral pre cancer and cancer by luminoscopy, spectroscopy or tissue autofluorescence. But these traditional aids are technique sensitive and may yield false negative results, hence definite treatment cannot be planned.

Modern diagnostic techniques are introduced to meet the following objectives:
- To develop & maintain an oral cancer database and tissue bank
- To strengthen multidisciplinary research culture by establishing a vigorous vital research platform
- To prevent and detect oral cancer with advanced validated diagnostic tools at a molecular level
• To comprehend the genetic susceptibility of individuals for oral cancer
• To predict the disease course by identifying genetic links that aid in developing personalized medicine and cancer vaccine
• To generate a consortium of comprehensive panel w.r.t genomic abnormalities in oral cancer.

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**Shifting the paradigm:**

**Genomics and proteomics:**

Regular histopathological evaluation gives very limited information in terms of rate of proliferation, capacity for invasion and metastases, and development of resistance mechanisms to certain treatment agents. Biomarkers help in early detection of cancer by providing valuable information about the status of a cell at any given point in time. As the cell transforms from non-diseased to neoplastic, distinct changes occur that could be potentially detected through the identification of the appropriate biomarkers. Proteomics is the study of expressed proteins, including identification and elucidation of the structure–function relationship under normal or disease conditions, such as in cancer. It also provides an avenue to understand the interaction between the functional pathways of a cell and its micro environment.

Cancer proteomics encompasses the identification and quantitative analysis of differentially expressed proteins relative to healthy tissue counterparts at different stages of disease, from preneoplasia to neoplasia. Proteomic technologies can also be used to identify markers for cancer diagnosis, to monitor disease progression, and to identify therapeutic targets. It is valuable in the discovery of biomarkers because the proteome reflects both the intrinsic genetic program of the cell and the impact on its immediate environment of protein. At the protein level, distinct changes occur during the transformation of a healthy cell into a neoplastic cell, ranging from altered expression, differential protein modification, and changes in specific activity, to aberrant localization, all of which may affect cellular function. Identifying and understanding these changes are the underlying themes in cancer proteomics.7, 8

**PCR:**

The polymerase chain reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and DNA paternity testing) and the detection and diagnosis of infectious diseases.

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase, which the method is named after, are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations.9, 10

**Microarrays:**

New technologies are developed, such as, DNA microarray and DNA chips, that give hundreds to thousands more genetic information in a shorter period of time than the original PCR techniques. Microarrays are needed to appropriately classify tumor subtypes, molecular information can be extracted and integrated to find common patterns within a group of samples. They can be used in combination with other diagnostic methods to add more information about the tumor specimen by looking at thousands of genes concurrently. This new method is revolutionizing cancer diagnostics because it not only classifies tumor samples into known and new taxonomic categories, and discovers new diagnostic and therapeutic markers, but it also identifies new subtypes that correlate with treatment outcome.9, 10

**Nanodiagnostics:**

Use of nanodiagnostics is cost effective and has increased sensitivity. The tools include quantum dots (QDs), gold nano particles and cantilevers. QDs are semiconductor nano crystals characterized by high photo stability, single wavelength excitation, and size tunable emission. Bar coding of specific analytics can be done by QDs and magnetic nano particles. Hence they are bio bar code assay which has been proposed as a future alternative to PCR. The applications of QDs being used for tumor detection, tissue imaging, intracellular imaging,
immunohistochemistry, infectious agent detection, multiplexed diagnostics and fluoroimmuno assays\textsuperscript{11}.

**Next-generation sequencing (NGS):**

It is arguably one of the most significant technological advances in the biological sciences. They have advanced rapidly to the point that several genomes can now be sequenced simultaneously in a single instrument run in under two weeks. Targeted DNA enrichment methods allow even higher genome throughput. The sensitivity, speed and reduced cost per sample make it a highly attractive platform compared to other sequencing modalities\textsuperscript{12}.

**Conclusion:**

The detection, diagnosis, and management of oral diseases is complex. There are several obstacles to be addressed before genomics and proteomics reach an optimal yield and be beneficial for the patients. The requirement of fresh or frozen tissue samples to protect and obtain high quality genetic material to use in high-throughput techniques limits their wide spread use. The facilities for immediate freezing of tissue samples with high quality sample banks are not readily available in all hospitals Developing better techniques in order to utilize paraffin-embedded tumor tissue can be another way to circumvent this problem. In the future, methods used in genomics and proteomics will be useful for the discovery of tumor specific marker genes and refinements and continued research will undoubtedly improve our ability to detect any disease at the earliest possible stage. New technologies may emerge which will prove much more valuable in early diagnosis with probability of cure, with minimum impairment and deformity.

**References:**