



Clinical Diagnostic Tools in Detection of Oral Cancers and Potentially Malignant Disorders

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ABSTRACT

Oral cancers are one of the most common cancers worldwide today. Oral malignancies are usually well advanced at the time of diagnosis. Prognosis depends on early diagnosis and the disease is life threatening, with high morbidity resulting from late treatment. Early detection and treatment gives the best chance for its cure. The clinical examination is one of the best modalities in suspecting the pathology but the biggest disadvantage in the diagnosis lies in deciding whether or not a biopsy is required in these lesions. Techniques that are promoted or assessed to improve earlier detection and diagnosis of oral malignancy include Toluidine Blue, Vizilite Plus With Tolidine Blue, Velscope, Brush Biopsy, etc. The gold standard remains tissue biopsy and histopathological examination. Advanced techniques include Lab On A Chip, Optical Coherence Tomography, DNA Ploidy and Salivary Biomarkers. The purpose of this review article is to discuss various clinical diagnostic aids in diagnosis of oral cancer and potentially malignant disorders.

Keywords: Oral Cancer, Diagnostic Aids, Potentially Malignant Disorders.

INTRODUCTION

Oral cancers are one of the most common cancers worldwide today. They are usually neglected by the common population when compared to systemic cancers such as the lung cancer, colon cancer etc. However, they also may be extremely fatal if left untreated even at a very initial stage of the lesion. ^[1] Oral cancer is life threatening and most commonly involves the tongue, floor of the mouth, buccal mucosa, gingiva and lips. Approximately 94% of all oral malignancies are Squamous cell carcinoma. Dental Surgeons have a duty to detect benign and potentially malignant oral lesions such as oral cancer and are generally the best trained health care professionals in this

field. Prompt referral to an appropriate specialist allows for the best management but, if this is not feasible, the dental practitioner should take the biopsy which should be sent to an oral pathologist for histological evaluation.^[2]

This article is based on information gathered from various literatures on Early Dignostic aids in detecting oral cancers and potentially malignant disorders

Clinical Methods

- Vital staining – Toluidine Blue/Lugol's Iodine
- Vizilite
- Brush Cytology

Visualization Adjuncts Tissue Auto fluorescence

- VEL scope
- In Vivo Confocal Microscopy

Saliva-based oral cancer diagnostics

- Molecular Methods
- DNA Ploidy & Quantification of nuclear DNA content
- Tumor Markers & Bio Markers
- PCR-Based diagnostic aids

Photo Diagnosis

- Auto fluorescence Spectroscopy
- Fluorescence Photography

Newer Methods

- Lab on a chip
- Liquid Biopsy
- PET Scan
- Micro Array^[1]

VITAL TISSUE STAINING

TOLUIDINE BLUE STAINING & LUGOL'S IODINE

Oral carcinoma in situ and early invasive oral carcinoma shows affinity for toluidine blue dye. Lugol's iodine and toluidine blue have been used together in the detection of early carcinomas and other oral lesions. Toluidine blue is an acidophilic meta chromatic dye which selectively stains acidic tissue components, thus staining DNA and RNA. As it binds to nucleic acids (DNA or RNA) , it helps in better visualization of high risk areas especially with rapid cell proliferation of OSCC and potentially malignant lesions . It stains mitochondrial DNA, cells with greater than normal DNA content or altered DNA seen in dysplastic and malignant cells. Lugol's solution is used for delineation of the malignant change which produces a brown black stain when the iodine reacts with the glycogen content. The use of toluidine blue and Lugol's iodine serves as a useful adjunct in the diagnosis of patients who are

at risk and for selecting the site for biopsy with wide field cancers prior to treatment .^[3,4]



Fig.1: Toluidine blue staining in a potentially malignant lesion.

VELSCOPE

VEL scope is a hand-held device which was approved by Federation Dentaire Association for direct visualization of autofluorescence in the oral cavity. Only recently it was introduced in the market as a diagnostic adjunct for oral cancer detection. The VEL scope Vx is one of the most powerful tools available today for assisting in oral abnormalities especially oral cancer. The distinctive blue-spectrum light causes the soft tissues of the mouth to naturally fluoresce. The use of VEL scope Vx is a safe and simple technique and the entire examination can be done in about two minutes. However, it is a relatively new device and so far only a limited number of studies have been done on its effectiveness as a diagnostic adjunct for oral cancer .^[5]

VIZILITE

Vizilite is a non toxic chemiluminescent light. Today, vizilite Plus examination, in combination with the regular visual examinations, provides a comprehensive oral screening procedure for those patients who are at increased risk for oral cancer. Vizilite Plus technology helps in identifying soft tissue abnormalities which is shined inside the mouth. This shows glowing of abnormal tissue different from that of normal tissue thus making it more visible. The technique is painless and fast

and can help in saving life. However, it cannot necessarily tell if they are potentially cancerous or not. To improve early detection of oral cancer, the use of a dilute acetic acid rinse and observation under a chemiluminescent light such as ViziLite is usually recommended.^[6,7]



Fig.2: Lesion under normal incandescent light and vizilite illumination.

BRUSH CYTOLOGY

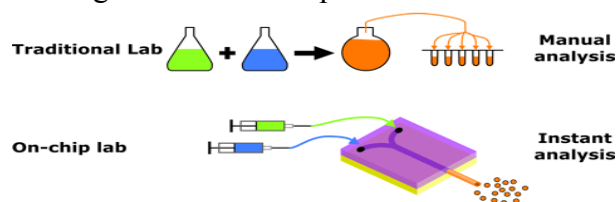
Brush cytology, developed in 1999, is useful in the assessment of dysplastic changes in various suspected lesions especially in oral cancer. As majority of oral cancers are squamous cell carcinomas, Cytological study of oral cells is a relatively inexpensive, simple, noninvasive and also risk-free technique which is well accepted by the patient and medical practitioner today. The oral cells can be obtained by the use of a cytobrush. With brush cytology, sensitivity for detecting oral epithelial dysplasia or Oral squamous cell carcinoma is high. But, the technique has attracted lots of controversies and more incidences of false negative results with this technique has been encountered.^[8,9,10]

DNA PLOIDY AND QUANTIFICATION OF NUCLEAR DNA CONTENT

DNA ploidy is the measurement of nuclear DNA content that provide a measurement of gross genetic damage. If the chromosomes are not uniformly distributed to the daughter cells during mitosis or if some parts of chromosomes become detached, the chromosomal segregation becomes unbalanced and aneuploidy is seen which is commonly observed in many cancers. DNA image cytometry shows high sensitivity and serves as a non-invasive method for cancer. Potentially malignant lesions such as oral leukoplakias, the nuclear DNA distribution patterns can be analyzed by flow-cytometry, showing different rates of dysplasia, however the quantity of specimens should be more for the examination. Even cytology with DNA-cytometry has emerged as a highly sensitive and non-invasive method for the early diagnosis of oral epithelial neoplasia and hence in oral cancer (Mar.^[11]

LAB ON A CHIP

Microfluidics technology, also referred to as lab-on-a-chip or micro-total-analysis systems (TAS) is the adaptation, miniaturization, integration and automation of analytical laboratory procedures into a single device or “chip”.



Redrawn from: Brivio, M., Verboom, W., & Reinhoudt, D. N. (2006). Miniaturized continuous flow reaction vessels: influence on chemical reactions. *Lab on a Chip*, 6, p. 329.

Fig.3: Advantage of Lab on a chip and traditional method.

Microfluidics technology, also referred to as lab-on-a-chip or micro-total-analysis systems (TAS) is the adaptation, miniaturization, integration and automation of analytical laboratory procedures into a single device or “chip”. Microfluidics is often regarded as the chemistry or biotechnology equivalent of the silicon integrated silicon chip that has revolutionized electronics, computers and communications. Microfluidics are by definition

suitable for handling living cells (whose typical diameter is a few micrometers) in a three-dimensional, biologically relevant environment. This microfluidic chip accepts saliva sample, can be operated by minimally trained personnel, and can provide a diagnostic answer in an automated and timely fashion. The detection of oral pre-cancer (dysplastic) and cancer cells within the chip will take advantage of membrane-associated cell proteins that are singularly expressed on cell cancer cells. The measured profile is compared with archived gene transcription profiles to determine the cancer type and stage.^[2]

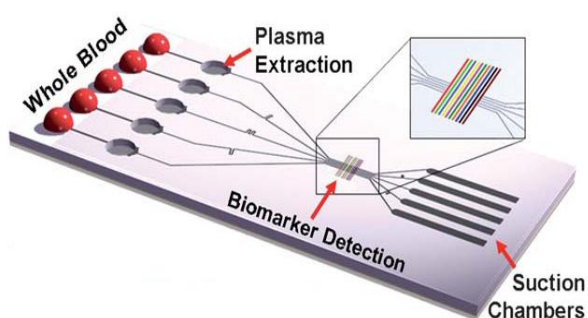


Fig.4: Mechanism of Lab on A Chip.

LIQUID BIOPSY

A liquid biopsy, also known as fluid biopsy or fluid phase biopsy, is the sampling and analysis of non-solid biological tissue, primarily blood. Like traditional biopsy this type of technique is mainly used as a diagnostic and monitoring tool for diseases such as cancer, with the added benefit of being largely non-invasive making use of the circulating tumour cells. In cancer studies, circulating tumor cells (CTCs) and/or cell-free tumour DNA (ctDNA) are collected^[2]

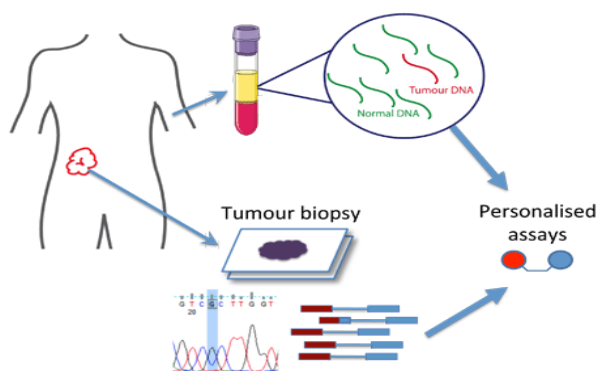


Fig.5: Mechanism of Liquid Biopsy.

SALIVARY ANALYSIS

There is an attractive possibility that saliva testing may be an effective, non-invasive screening test for oral cancer. The principle is based on the fact that salivary composition is altered in patients with oral cancer. The approach relies on measuring specific salivary macromolecules, enzymes, cytokines, growth factors, metalloproteases, endothelin, telomerase, cytokeratins, messenger ribonucleic acid (mRNA) and DNA transcripts. It has been shown that the salivary levels of total sugar, protein-bound sialic acid, free sialic acid, sodium, calcium, immunoglobulin G, albumin and lactate dehydrogenase are significantly higher in patients with oral cancer than those with healthy mucosa. In addition, patients with oral cancer have been found to have significant alterations of various oxidative stress-related salivary parameters, epithelial tumor markers CYFRA 21-1, tissue polypeptide-specific (TPS) antigen, and various RNA transcripts (e.g. insulin-like growth factor, matrix metalloproteinases (MMP-2 and MMP-9), interleukin-8 and 1B) [34-36]. These biomarkers may be used as a tool for the diagnosis, prognosis, and post-operative monitoring of oral cancer. However, well-designed studies are still required to investigate whether salivary analysis could prove to be a feasible and cost-effective tool for these purposes.^[12,13]

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.^[14,15]

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.^[16]

CONCLUSION

Unfortunately, no technique or technology to date has provided definitive evidence to suggest that it improves the sensitivity or specificity of oral cancer screening beyond clinical oral examination. Further detailed investigations to estimate the sensitivity, specificity, positive and negative predictive values for each of these adjunctive tests against standard pathology reporting (cancer or dysplasia) will allow us to judge the accuracy of these chair side or

laboratory tests in detecting cancer or oral potentially malignant lesions.

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