

# Portable Non Invasive Methods for Diagnosis of Potentially Malignant Disorders

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## ABSTRACT

Most common malignancies worldwide is Oral cancer and its early detection and treatment is imperative. Commonly neglected cancer is oral cancer among population when compared to systemic cancers such as the lung cancer, colon cancer etc. Survival rate of Oral cancer has remained to be 5-year. Poor survival rate is because of reason fact that in most of the oral cancers, are not diagnosed until the advanced stage despite the general accessibility of the oral cavity, Most effective means to improve clinical outcome and cure most patients is early detection of the oral tumors and its precursor lesions. Early diagnosis and detection are currently based on clinical examination, histopathological evaluation of the biopsy material and molecular methods. Early detection of oral cancer have been developed over the years by several diagnostic aids. The purpose of this article is to development of the promising tools for the early detection of oral cancer and to review the current knowledge about the commercially available diagnostic adjuncts in diagnosis of potentially malignant disorders.

**KEYWORDS:** Biopsy Material, Diagnostic Adjuncts, Molecular Methods, Oral Cancer

## INTRODUCTION

Oral cancer is a life threatening disease. Primary lesion in any part of the oral cavity or oropharynx which may arise by metastasis from a distant site of origin. It is a part of group of head and neck cancer. Most commonly involved parts in oral cavity are the tongue, floor of the mouth, buccal mucosa, gingiva and lips. Risk factors for developing oral cancer in many Asian countries, especially India, are chewing betel, paan and Areca nuts.<sup>1</sup> Various signs, symptoms and risk factors for oral cancer lack of awareness in the public of the are all believed to be responsible for the diagnostic delay in a long venture. Routine clinical examination are often difficult to diagnose potential malignant diseases. Microscopic study of cells and tissues definitive diagnosis of these diseases.

Historically, conventional pattern been relied upon oral examination and screening of patients with signs and symptoms of oral cancer and various potentially malignant lesions. Immunohistochemistry has proved to be a useful tool, Laboratory diagnosis in oral cancer is by the use of antigens and antibodies. Advanced diagnostic adjuncts are helpful tool for the early diagnosis of oral cancer to the medical practitioners in treating patients suffering from it. Early detection of these cancers helps in better and faster treatment for improving the prognosis to patients suffering from potentially malignant disorders. First line of screening of oral cavity abnormalities is performed by visual inspection, which is subjective.<sup>2,3</sup>

Secondly clinical endoscopic examination and invasive needle biopsies followed by histopathological analysis

remains the gold standard for diagnosis and surveillance of oral cavity cancer.<sup>4</sup>

But these conventional techniques has their own limitations such as:

- (i) Difficulty to distinguish benign from malignant lesions;
- (ii) Difficulty in determining margin of lesions;
- (iii) Can be subjective, especially during histopathological analyses; and also
- (iv) Tissue biopsies can be invasive and painful for the patients.<sup>5</sup>

Considering these limitations, and much needed to reduce the resulting mortality rate it is imperative that a new rapid and accurate diagnostic method for early oral cavity cancer detection. Therefore any technique that improves the diagnosis should be used to improve the screening ability among large population, Hence optical techniques that are robust, accurate, of low cost, portable and easy to handle can be used effectively for clinical applications. This article will give a mini review of currently available diagnostic adjuncts which will help in development for early detection of oral cancer.

## VARIOUS DIAGNOSTIC AIDS

### Clinical Methods

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

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**Future Diagnostic Aids for Oral Cancer****Visualization Adjuncts Tissue Auto fluorescence**

- VELscope
- 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

**Clinical Methods**

**Vital Tissue Staining – Toluidine Blue Staining:** One of dye that binds to DNA in vital tissues is Toluidine blue, it is a metachromatic dye. It has been reported to stain cells with relatively increased amounts of DNA and possibly altered DNA in premalignant and malignant epithelial lesions<sup>5</sup> but its not cancer specific. Toluidine blue has been used widely in many countries except in US as its has not been approved FDA.<sup>6</sup> It is used for decades in screening abnormal epithelium in the cervix. It mostly used for screening oral cancer. Sensitivity and specificity of toluidine blue for detecting pre-malignant and malignant lesions have been investigated by many studies. However, the results varied and were inconsistent.<sup>7,8,9</sup> In most the studies toluidine blue staining appears to be useful as an adjunct to clinical examination, with a sensitivity ranging from 77-100% and a specificity ranging from 62- 100%.<sup>5,7,8,9</sup> However, a low positive predictive value of 43.5% for potentially malignant lesions and a false negative rate as high as 20.5% for pre-malignant lesions also have been reported. (Figure 1)

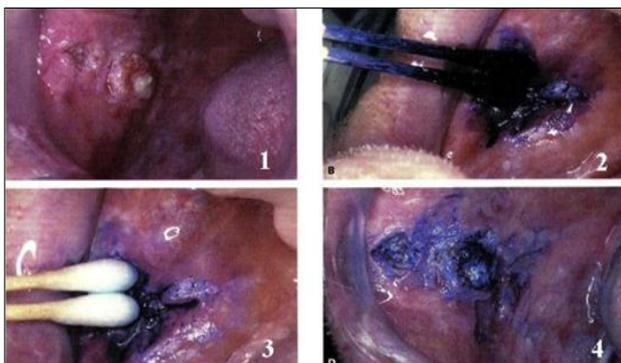


Fig 1: Toluidine Blue Staining

**Vizilite:** The second method is use ViziLite system and MicroLux these products have been approved by the FDA to enhance the identification of oral mucosal lesions. They are derived from an adjunct for diagnosing cervical lesions (speculoscopy).<sup>10</sup> Procedure for both systems, where the patient is asked to rinse with a 1% acetic acid solution and then the oral cavity is examined by the clinician under the illumination of an activated chemiluminescent light (ViziLite) or a battery-operated light source (MicroLux DL). There is emission of two colours in the procedure to differentiate between normal

and abnormal oral epithelium. Blue-white light generated by two light sources. The normal epithelium appears light bluish whereas the abnormal epithelium appears distinctly white (aceto-white) as shown in figure 2. Marking the abnormal epithelium which is aceto-white is done by using lesion a toloum chloride solution which is present in ViziLite where in biopsy can be done. (Figure 2)

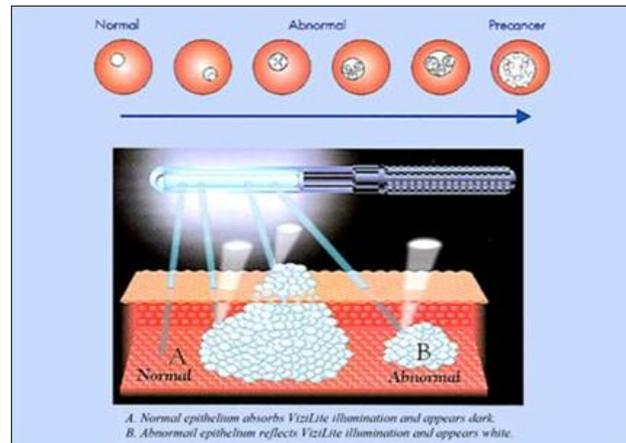


Fig 2: ViziLite Application

**Brush Cytology:** Brush cytology (Oral CDX), developed in 1999, has become popular in dental offices in recent years.<sup>11-14</sup> In this method there is collection epithelial cells by rotating a provided circular brush against the lesion until pinpoint bleeding is seen clinically, indicating penetration of the basement membrane and a collection of cells from all layers of the epithelium.<sup>11</sup>

Fixation of cells are done and sent to the company for a computer-assisted specimen analysis. Interpretation of results as follows which falls into one of four categories: inadequate, negative, atypical, or positive; and the a follow-up is done scalpel biopsy for patients with an atypical or a positive result if company recommends.

**Future Diagnostic Aids for Oral Cancer**

**Visualization Adjuncts Tissue Auto Fluorescence:** One of the hand-held device is VEL scope which was approved by Federation Dentaire Association for direct visualization of autofluorescence in the oral cavity (Figure 3). Recently introduced diagnostic adjunct for oral cancer detection is VEL scope Vx. It is one of the

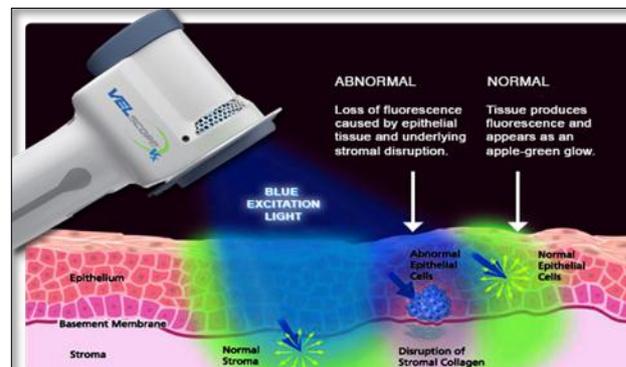


Fig 3: Velscope

most powerful tools available today for assisting oral cancer and some oral abnormalities in oral cavity. Entire examination can be done in about two minutes the use of VEL scope Vx is a safe and simple technique. It has distinctive blue spectrum light causes the soft tissues of the mouth to naturally fluoresce.<sup>15</sup>

**Confocal Reflectance Microscopy:** Another non-invasive image tissue optical tool in vivo is confocal reflectance microscopy (CRM) with near histological resolution.<sup>16</sup> Natural difference in refractive indices of organelles and other subcellular structures within the tissues by natural difference determines the image contrast.<sup>17</sup> The difference between CRM and conventional microscope as it uses a laser to illuminate a small spot within tissue. The procedure is such that Light that is backscattered from the tissue is captured through an aperture, which matches the size of the illuminated spot placed in front of the detector. The present in CRM aperture spatially filters light returning from out of focus planes within the tissue, such that only the plane in focus is imaged. Hence image viable tissue with high-resolution and contrast without performing a biopsy and processing the tissue, as in standard histology is done by CRM. (Figure 4)

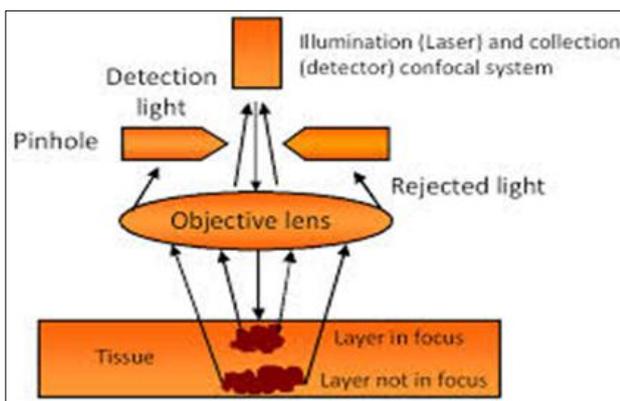


Fig 4: Confocal Reflectance Microscopy

### Saliva-Based Oral Cancer Diagnostics

**Molecular Imaging:** Most promising non-invasive modality that can image and quantify molecular changes associated with diseases is molecular imaging. Molecular imaging is used for the early detection of malignancies, staging tumors, and also for monitoring the efficacy of treatment. There are three important elements are needed for molecular imaging that includes a labelled probe that can be detected with high sensitivity and a ligand that has high affinity and specificity to the target, a method to amplify the signal from the label and a high-resolution imaging modality to detect the label. Diagnosis of disease has gained importance by using molecular methods as disease changes occur at the molecular level before they are seen under the microscope and before clinical changes occur. Identification specific genetic anomalies is done by analysis of molecular alterations.<sup>18</sup>

**DNA Ploidy:** Nuclear DNA content can be measured by using DNA ploidy. This diagnostic aid may provide a surrogate measure of gross genetic damage and this could

act as a surrogate for individual molecular markers.<sup>19</sup> In many cancers commonly observed chromosomes are not uniformly distributed to the daughter cells or if parts of chromosomes become detached, the chromosomal segregation during mitosis is termed unbalanced, a situation termed aneuploidy.<sup>18</sup> The method in DNA ploidy can be measured fairly simply with automated image cytometry of nuclei obtained from routinely processed tissue samples and the expertise is available in many pathology laboratories. Unfortunately there has been controversy over the ploidy results published from one Norwegian laboratory, which must now be repeated by other workers.<sup>16</sup>

**Tumor Markers & Bio Markers:** Host response to cancerous substances tumor markers are released by cancer cells. They may be present in blood circulation, body cavity fluids, cell membranes and cell cytoplasm. Tumors markers are used in identification of a cancerous growth. Some of the potential tools which help in prediction for the prognosis of patients with OSCC are tumour Suppressor Genes, oncogenes, cell proliferation markers, angiogenic markers and cell adhesion molecules. Use of cytokeratin markers are also used in detecting OSCC by the help of analyzing the altered keratin expression in the oral site especially the buccal mucosa according to a study.<sup>1</sup>

**PCR-Based Diagnostic Aids:** The scientific technique in molecular biology is polymerase chain reaction (PCR) which can be used in the diagnosis and study of infectious diseases and malignancies associated with micro organisms. Clear understanding of the pathogenesis of neoplasia in the study of cancer is provided by PCR and is also used in to detection mutations in cancer-associated oncogenes (e.g., K-ras, Nras), tumor suppressor genes (e.g., p53, p16) etc. It aids as an important detection tool. This technique has increased range and sensitivity of diagnostic procedures the but still with a major drawback, as contamination and amplification artefacts may give rise to difficulties in the interpretation of the desired results. In chronic myelogenous leukemia the diagnosis and prognosis of other lesions such as has also been useful. With the introduction of polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR) and other molecular techniques.

### Photo Diagnosis

**Auto Fluorescence Spectroscopy:** Another promising tool for oral cancer detection is Auto fluorescence spectroscopy. This system consists of a small optical fiber which produces various excitation wavelengths and a spectrograph which receives and records on a computer and analyzes it with the help of software, the spectra of reflected fluorescence from the tissue. Auto fluorescence spectroscopy seems to be very accurate for distinguishing lesions especially malignant tumors from healthy oral mucosa, with a high sensitivity and specificity and also an non-invasive aid in the detection of various alterations in the structural and chemical compositions of cells

indicating the presence of a diseased tissue. This technique is useful in guiding the clinician in identifying the optimal location for biopsy. However, the technique is controversial and often found with unclear results.<sup>1,19</sup> (Figure 5)

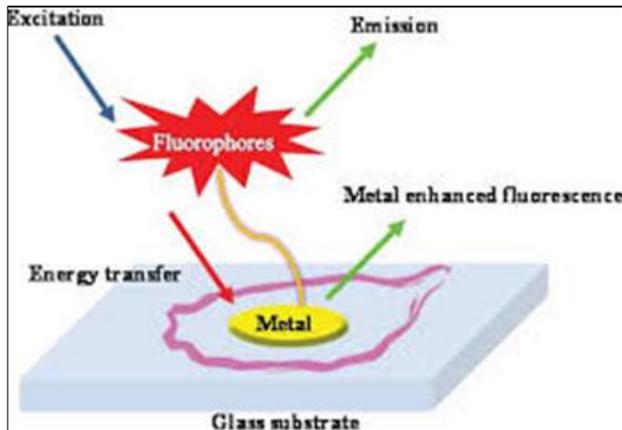


Fig 5: Auto Fluorescence Spectroscopy

**Fluorescence Photography:** Detection of oral cancer can be achieved by Fluorescence photography. Which is non-invasive, rapid, simple and reproducible method in Fluorescence positivity can show enlargement of carcinomas and progression of the disease as in squamous cell carcinoma which helps in diagnosis. But biopsies are still necessary.<sup>1,19</sup>

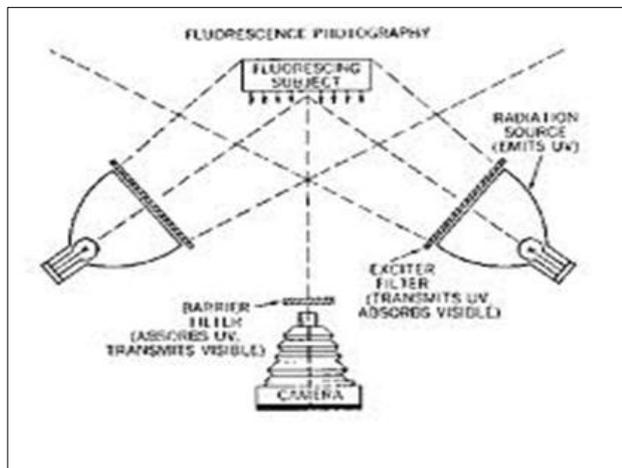


Fig 6: Fluorescence Photography

## CONCLUSION

To conclude this review, it is still unknown what really causes its development regardless of all the type of oral cancer. Tobacco usage may be the leading cause of oral cavity and oropharyngeal cancer as mentioned in recent studies, and it is also believed that smokers are more likely to develop oral cancer than nonsmokers. Leading cause of cancer in lungs, esophagus and several other organs Carcinogens, which are present in high concentration in tobacco and its products. Various diagnostic aids as mentioned here will help in Screening and early detection of oral cancer and will also help decrease the risk of morbidity and mortality associated

with cancer. Hence this article gives mini review in various diagnostics aids which helps in early detection of life threatening cancers.

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