**REVIEW ARTICLE**

**Detection of Oral Potentially Malignant Disorders and Oral Cancer: A Review On Non Invasive Diagnostic Aids**

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**Abstract**

The long term prognosis for patients with oral cancer is discouraging with overall survival following definitive treatment approaching only 30% at 5 years. Early detection of cancer should lead to less damage from cancer treatments and to a better prognosis. Improving oral cancer detection and diagnosis have long been major challenges facing both dental and medical providers around the globe. A variety of commercial diagnostic aids and adjunctive techniques are available to improve the clinical and cytological diagnosis of oral precancerous lesions. The present paper reviews the main techniques such as toluidine blue, brush cytology, tissue chemiluminescence and autofluorescence etc in detection of potentially malignant and malignant disorders.


**Introduction**

The long term prognosis for patients with oral cancer is discouraging with overall survival following definitive treatment approaching only 30% at 5 years. Death usually results from failure to control the primary lesion, cervical lymph node metastases, development of multiple primary tumors and the advanced extent of the disease at the time of diagnosis. Detection of oral cancer at its earliest clinical manifestation is essential because there is a direct correlation between survival and the stage of the disease at the time of diagnosis. Also early detection provides the patient with the best opportunity for successful management and positive cure and is crucial to improve the patient’s survival rate. Recent studies have demonstrated that early lesions have a characteristic appearance and can be predictably recognized by the development and use of diagnostic aids & tests that could help the general dentist identify or assess...
persistent potentially malignant disorders and oral cancer at its initial stages\textsuperscript{4,5,6}.

Thus this paper aims to review the various non invasive technological advances in the detection of potentially oral malignant disorders and oral cancers.

**Vital Staining**

The most popular stains used for detection of premalignant and malignant lesions of oral cavity includes Lugol’s iodine, toluidine blue (tolonium chloride), methylene blue.

**Vital Iodine Stain (3\% Lugol’s Iodine Solution)**

Lugol’s iodine, named after the French physician J.G.A. Lugol was first developed in 1829. In 1928, Schiller reported the use of Lugol’s Iodine solution in carcinoma of the uterine cervix to identify dysplastic epithelium and this test is called as Schiller’s test\textsuperscript{3,4}. The basic principle with iodine staining is its affinity for carbohydrates and starch in the tissues as iodine infiltrates and reacts with the glycogen mainly in the upper superficial layer of thenon-keratinized epithelium\textsuperscript{5}. As malignancy is associated with reduction in the glycogen content of the tissues, the malignant tissue remains unstained and on the contrary the normal epithelium gets stained brown or black\textsuperscript{3,6,7,8}. This selective staining delineates the inflammatory and carcinomatous epithelium from the normal epithelium\textsuperscript{7}. This technique however cannot be used for the detection of keratinized mucosa and subepithelial infiltrating tumors\textsuperscript{6}. It can also be applied topically on the suspected areas with the help of cotton swab or applicator. The more intensely stained areas should be the ones elected to be biopsied\textsuperscript{10}.

The area which is dark blue in color suggests malignancy whereas pale blue indicates benign lesion\textsuperscript{10,11,14}. Mashberg suggests some areas not to be considered positive if it retains stain. These areas include the nucleated scales covering the papillae on the dorsum of the tongue, pores of seromucinous glands in the hard palate, dental plaques and gingival margins around each tooth\textsuperscript{11}. False positive results are rarely observed in squamous cell carcinoma, inflammatory and ulcerative lesions\textsuperscript{10,15,16}. Its use has been contraindicated in hypersensitivity, pregnant and lactating females and patients suffering from liver and renal insufficiency and mental and physical abnormalities\textsuperscript{7,12,13,14}. It is an easy, inexpensive and harmless technique and still remains one of the most commonly used routine staining techniques for the detection of malignant tissues\textsuperscript{12}.

**Toluidine Blue**

Toluidine Blue (also known as tolonium chloride) is a vital metachromatic dye of the thiazine group. It consists of toluidine blue- 1 gm, acetic acid- 10 cc, absolute alcohol- 4.2 cc, distilled water- 86 cc. It was first used by Richart in 1963 to stain uterine cervical carcinoma in situ and dysplasia. It is an acidophilic dye that selectively stains acidic tissue components (carboxylates, sulfates, and phosphate radicals) such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)\textsuperscript{3,9}. TB is a cationic dye and attaches to phosphate bonds and the extent of binding depends on the amount of DNA, which is related to number and size of nuclei present in the superficial layers\textsuperscript{5}.

It has been used for decades as an aid in epithelium dysplasia identification\textsuperscript{2,10} and appears to improve precancerous lesion visualization by showing high-risk areas (areas of high cell proliferation), therefore guiding biopsy and for the identification of mucosal abnormalities of the cervix as well as in the oral cavity\textsuperscript{2,3,11}.

The TB solution can be prepared in the laboratory or its also available commercially as ready to use kit, which consists of three component systems. One component is 1\% TB solution and the other two are the pre and post-rinse solutions consisting of 1\% acetic acid\textsuperscript{12,13}. The patient is asked to rinse his mouth with 1\% of acetic acid for 20 to 30 second followed by water for another 20 to 30 seconds. Then he is asked to rinse his mouth with 1\% of toluidine blue for 20 to 30 seconds followed by 1\% of acetic acid and water. It can also be applied topically on the suspected areas with the help of cotton swab or applicator. The more intensely stained areas should be the ones elected to be biopsied\textsuperscript{10}.

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Methylene Blue

Methylene Bluestaining was originally described by Japanese investigators for improving the diagnosis of early gastric cancer. Its application has been reported recently in detecting some gastrointestinal abnormalities (Barrett’s oesophagus, gastric cancer, prostate cancer), and bladder cancer. However, its application in detecting oral lesions by far is very limited\textsuperscript{17,18}. The physicochemical properties and chemical structure of Methylene Blue are similar to Toluidene Blue except that, it is less toxic to the human body. The uptake of Methylene Blue dye in epithelial cells is still not very clear. It is acidophilic in nature and may penetrate into cells with an abnormal increase in nucleic acids, thus resulting in different uptake between normal and highly dysplastic and malignant cells\textsuperscript{17}.

Methylene Blue dye system includes two bottles of solution. Bottle A- the dye rinse solution containing Methylene Blue and bottle B- containing pre and post rinse solution. The application of Methylene Blue dye in epithelial cells is still not very clear. It is acidophilic in nature and may penetrate into cells with an abnormal increase in nucleic acids, thus resulting in different uptake between normal and highly dysplastic and malignant cells\textsuperscript{17}.

Methylene Blue dye system includes two bottles of solution. Bottle A- the dye rinse solution containing Methylene Blue and bottle B- containing pre and post rinse solution. The application of Methylene Blue involves\textsuperscript{17} rinsing with bottle B for 20 seconds to remove food debris and excess saliva and gently draining the target area with gauze and power air spray to ensure that the lesion is not contaminated with saliva. Thereafter, rinsing with 1\% MB dye; bottle A for 20 seconds and then rinsing again with bottle B for 20 seconds to wash out the excess dye. Local, stippled, patchy, and deep blue stains are marked as positive reaction. Wide, shallow, or faint blue stains are marked as negative reaction. If the blue stain is washed out, negative reaction is recorded\textsuperscript{18}.

It is indicated for early detection of oral cancer and precancerous lesions. It has recently been used for intraoperative detection of canal isthmuses in molars during endoscopic periradicular surgery and to identify the areas of incomplete excision during peripheral osteotomy of aggressive lesions like odontogenic keratoctyst (OKC) and ameloblastoma\textsuperscript{17,18}. The authors suggested that being economical, Methylene Blue can be used as a useful diagnostic adjunct in a large, community based oral cancer screening program for high risk individuals\textsuperscript{17}.

Acetowhite Staining

Acetic acid staining has been used as a part of colposcopic examination since 1938. It is also used as a component in other staining techniques such as Toluidene Blue and chemiluminescence for cancer screening, where it is used in the concentration of 1\% acetic acid both pre and post applications of Toluidene Blue stain or the light stick. With these techniques, it functions to remove the ropey saliva and to reduce the extent of mechanically retained stain. Since it is relatively inexpensive and easy to use, interest has emerged in using acetic acid alone in the assessment of premalignant and malignant lesions\textsuperscript{17}.

It acts by causing dehydration of the cells, thereby producing a white appearance. The acetic acid removes the mucus by areas. It also cause swelling of the epithelium and reduces its transparency by producing a transient coagulation of nuclear proteins. Thus, the higher nuclear content in premalignant and malignant lesions reacts with the acetic acid producing an Acetowhite appearance\textsuperscript{17}.

Acetic acid is used in the concentration of 3-5\%. A piece of gauze soaked with 5\% of acetic acid is applied on to a cleaned and dried lesion for 60 seconds. A positive finding is designated as a lesion that changes color to opaque white, while a negative finding is a lesion that shows no change or changes to transparent white\textsuperscript{17}.

It has been used in detecting oropharyngeal squamous cell carcinomas, oral HPV infection\textsuperscript{17}.

Cytopathology

Microscopic study of cell samples collected from mucosal surfaces can be obtained by exfoliative cytology (via smears, scrapings, or lavage) and fine-needle aspiration, cytomorphometry.

Oral Exfoliative Cytology

Study and interpretation of the characteristics of cells that flake off, whether naturally or artificially, from the oral mucosa is known as exfoliative cytology. It was 1st used in cervical cancer by George N Papnicolau (1941)\textsuperscript{1}.

The rationale for this is epithelial physiology. Normal epithelium undergoes exfoliation of its
superficial cell layer due to physiological turnover. Cells of deeper layer are adherent to each other normally. However in case of pathology cells lose their cohesiveness and cells of deeper layer may also shed along with superficial layer16.

Procedure involves collection and fixation of smear. Fixation of smears is done by Alcohol (90%), spray cyte or equal parts of ether and alcohol. Drying time is 30 minutes (air drying). Staining of smear is done by Mayer’s hematoxylin, orange G and Eosin. Reporting is done as follows as Class 1, class II, Class III, class IV and class V19.

It is a quick, simple, painless and a bloodless procedure. It checks on false negative biopsies and can be used for follow up and detection of recurrent carcinomas. However the extent of invasion cannot be assessed1.

**Cytomorphometry: Computer-Assisted Analysis Brush Biopsy**

Cytomorphometry, computer-assisted analysis brush biopsy, was introduced in 1999. It is a method used for the analysis of cellular samples collected by brush biopsy. A disposable specialized circular plastic brush that collects transepithelial cellular samples composed of free cells and clusters is used for this technique10,20.

The clinician rubs or rotates the brush against the lesion until pinpoint bleeding is absorbed21. The samples are fixed onto a glass slide and sent to a laboratory where they are stained (via a modified Papanicolaou test), scanned, and analyzed microscopically by means of a computer-based imaging system that can rank cells on the basis of their degree of abnormal morphology10.

The analytical results and representative examples are then referred to a pathologist. Results are reported as “negative” or “benign,” “positive” or “atypical.” Abnormal diagnoses have included “positive” (defined as definitive cellular evidence of epithelial dysplasia or carcinoma) and “atypical” (defined as abnormal epithelial changes of uncertain diagnostic significance) results20.

The brush biopsy is intended for innocuous (Class II) lesions that would not otherwise be biopsied after the automated analysis. The occurrence of positive findings, or lesion progression despite negative findings, signals that the patient needs to be referred to a specialized clinic where a surgical biopsy should be performed, followed by histopathological analysis. Histopathology remains the gold standard for the definitive diagnosis of oral malignant lesions21.

**Light Based Detection System**

Light based detection system are based on the metabolic and structural changes occurring in the mucosa during carcinogenesis thereby giving rise to different profiles of refraction and absorption when exposed to different waves of light and energy2,12. It detects minimal changes such as nuclear/cytoplasmic ratio, redox status, expression of specific biomarkers, tissue architecture and composition, chemical changes (e.g. mineralization), vascularity/angiogenesis and perfusion. These properties are ideal for the detection of minimal (early) changes, for assessing the margins of lesions and potentially the presence of subclinical abnormalities beyond the clinical margins, for repeated non-invasive monitoring of existing lesions, and for rapidly examining at-risk populations. This system incorporates techniques such as Chemiluminescence, Light emission techniques such as Microlux D and Led and Visually enhanced lesion scope (VELscope)2,17.

**Chemiluminescence**

Chemiluminescent light source was approved by the US Food and Administrative (FDA) - 2002 for head and neck regions. In June 2005 it got approval for dental application. It improves visualization, identification and monitoring of precancerous lesions. It consists of emission of light which is based on chemical reaction between hydrogen peroxide and acetylsalicylic acid in a capsule light stick action of which lasts for about 10 minutes. This reaction emits blue/white light (430-580 nm) based on reflective properties of tissue (abnormal cellular alterations) and high nuclear cytoplasmic ratio. Normal tissue appears dark or blue in color as it absorbs light whereas malignant lesions show acetowhite light1,2.

The patient is asked to wash oral mucosa with 1% acetic acid for about 1 min which will remove the glycoprotein barriers and desiccates the oral mucosa) followed by exposure to the chemiluminescent stick3.
These systems are also available as vizilite test kit and Toluidene Blue oral lesion marking kit in which it is combined with TB (ViziLite Plus) to improve visualization. However, it is contraindicated in hypersensitivity, pregnant and lactating females, patients with renal and liver insufficiency and physical and mental abnormalities.\textsuperscript{12,16,22}

It is an easy, safe and non-invasive system capable of helping the dentist to better visualize lesions along with its edges.\textsuperscript{3,14} The main advantage of this technique is that it significantly improves the sharpness of the lesions’ margins.\textsuperscript{13,14} One disadvantage is that this system is expensive and a stick is used for each patient. Furthermore, chemiluminescence light seems to be nonspecific as it does not identify the lesion etiology whether inflammatory, neoplastic benign, or neoplastic malignant and this could lead to unnecessary biopsies\textsuperscript{3,14}.

**Light Emission Technique (Microlux-D, LED)**

The Light Emission Technique (Microlux DL) seems to operate on a principle of light emission similar to that of chemiluminescence light and helps to sharpen the lesion edges as well as to improve visualization\textsuperscript{5}. It emits blue/white light (430-580 nm) and improves visualization and sharpens edge. Patient is asked to rinse the mouth with 1% acetic acid and then a battery powered light source is used which is reusable and enhances visibility. However it is a poor discriminator or between malignant and inflammatory/traumatic/ulcerative lesions.\textsuperscript{16,17,23}

LED (Orascoptic) uses a rechargeable battery to screen the oral mucosa and claims to have a better visualization when compared to Microlux D\textsuperscript{17,24}.

**Velscope (Visually Enhanced Lesion Scope)**

The Narrow-emission tissue fluorescence (VELscope) technique involves tissue exposure to different wave lengths (400 to 460 nm) in order to observe differences between normal and abnormal mucosa.\textsuperscript{2} It works on the principal of autofluorescence. Autofluorescence is the property of cells due to which mitochondria, lysosomes, porphyrins in erythrocytes) of cells absorb specific wavelength/ light and move in to excited state. When they return back to resting state energy is released in form of fluorescence emission. Cancer cells have different fluorescence pattern which leads to a technology to detect oral lesions. Approximately 30 years ago, it was observed that the autofluorescence of tissues (tissue fluorescence) could potentially be used for cancer detection.\textsuperscript{2,3,9}

While the normal mucosa glows and emits color (pale green), the abnormal mucosa shows decreased levels of fluorescence and acquires a dark magenta, brown, or black color, as it absorbs fluorescence\textsuperscript{2,9,10}.

This technique seems to be helpful in lesion detection, but it cannot be used for the differentiation of malignant from benign lesions.\textsuperscript{2,3} Despite its applicability, the system is expensive, and color interpretation is difficult, which could lead to an erroneous diagnosis.\textsuperscript{5}.

**Conclusion**

Screening and early detection in populations at risk have been proposed to decrease both the morbidity and mortality associated with oral cancer. There is a growing realization that some premalignant and early cancerous lesions are not readily detectable to the naked eye. As such, additional screening aids for oral cancer are desperately needed. Improving oral cancer detection and diagnosis have long been major challenges facing both dental and medical providers around the globe. In this paper we approached different non invasive techniques which may be useful in the diagnosis of precancerous lesions. However all have shown one or two limitations therefore further research should be done to prove their efficacy in the early detection of potentially malignant disorder and cancer.

**References**


