An Insight into the Recent Innovations in Early Detection of Oral Cancer

1Pallak Arora, 2Lalita Yadav, 3Vandana Sharma, 4Varun Rastogi, 5Geet P Kaur, 6Vimal Kumar

ABSTRACT

Early diagnosis of oral squamous cell carcinoma (OSCC) and potentially malignant lesions and conditions is an attractive strategy to decrease patient morbidity and mortality, and thus scientists engage to find efficient diagnosis and preclinical screening approaches. The screening of patients for potentially malignant and malignant lesions by traditional methods is a historic concept. Appreciation of subtle surface changes of clinically abnormal mucosal lesions using conventional methods in conjunction with new evolving diagnostic tools has gained a lot of enthusiasm in the recent past. Various search engines for the preparation of this manuscript included PubMed, PubMed central, Science Direct, Springerlink, and google.com. This paper highlights the importance of recent adjunctive techniques used in the field of oncology for early diagnosis of oral mucosal lesions.

Keywords: Autofluorescence, Narrow band imaging, Oral cancer, Spectroscopy, Tumor marker, VELscope, ViziLite.


INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide and the majority of cases occur in India and Southeast Asia. The high mortality rate in cancers, such as OSCC is commonly attributed to the difficulties in detecting the disease at an early treatable stage. In asymptomatic patients seeking dental care, there is insufficient evidence to determine whether screening by means of visual and tactile examination to detect potentially malignant and malignant lesions alters disease-specific mortality. The goal of cancer screening is to detect the tumors at a stage early enough that treatment is likely to be successful. Moreover, the screening tool must be sufficiently noninvasive and inexpensive to allow widespread applicability. A number of promising recent technologies have been proposed to improve the effectiveness of early oral cancer detection. This paper overviews such techniques and methods (Table 1) for the detection of oral cancer, with special emphasis on recent advances.1

TOLUIDINE BLUE

Toluidine blue is an acidophilic metachromatic dye of thiazine group that selectively stains acidic tissue component, thus staining deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It shows increased dye uptake in areas of tissue with high nucleic acid content, such as those undergoing dysplastic or malignant changes. In addition, malignant epithelium may contain widened intracellular canal than normal epithelium, which may facilitate penetration of the dye.2

It is available in ready-to-use kits OraScan, OraScreen, and OraTest in three component systems. One component is a flavored 1% toluidine blue “o” 10 mL solution, the other two are pre- and postrinse solution containing 1% acetic acid.

Toluidine blue stains two types of lesions: Squamous cell carcinoma (SCC) and inflamed traumatic areas. The purpose of prerinse is to remove excess saliva and provide a consistent oral environment. The postrinse reduces the overall background levels of staining and facilitates identification of suspect lesions.3 False-positive staining (when lesions stain blue, but no carcinoma is identified after a biopsy is taken) occurs in 8 to 10% of cases associated with keratotic lesions and the regenerating edges of ulcers and erosions. Use of toluidine blue has improved the sensitivity and specificity of visual examinations when used in selected cases, in which suspicious mucosal characteristics are present. Sensitivity in the published data ranged from 93.5 to 97.8%, and the specificity ranged from 73.3 to 92.9%.4-6
Several studies have shown that toluidine blue vital staining of suspicious oral epithelial lesions can help in the detection of OSCC in high-risk populations, including patients with a history of a previous oral cancer.\textsuperscript{7,8}

**LUGOL’S IODINE**

Richart used Lugol’s solution for delineation of malignant change. Normal tissue stains brown, but proliferative epithelium is poorly stained. These solutions produce a brown-black stain by reaction of the iodine with glycogen. Glycogens content is inversely proportional to the degree of keratosis.

The combined use of toluidine blue and Lugol’s iodine is as an adjunct to visual examination of oral cancer patients and assess high-risk patients with suspicious oral lesion.\textsuperscript{3}

**METHYLENE BLUE**

Methylene blue\textsuperscript{9} is a heterocyclic aromatic chemical compound with molecular formula C\textsubscript{16}H\textsubscript{18}ClN\textsubscript{3}S. It is used as a dye for a number of different staining procedures, such as Wright’s stain and Jenner’s stain. Since it is a temporary staining technique, it can also be used to examine RNA or DNA.

As toluidine blue is hazardous if swallowed, it has shown toxicity to fibroblasts. Methylene blue is less toxic and does not intercalate in the nucleic acid chain.

It is available in three component systems of solution (three bottles). The first bottle is a prerinse solution containing 1% lactic acid, raspberry flavor, and purified water. The second bottle is a rinse solution containing active ingredient methylene blue 1%. The third bottle is postrinse solution containing 1% lactic acid, raspberry flavor, and purified water.\textsuperscript{9}

**Indications**

- For screening high-risk patients for oral cancer and suspicious lesion
- Site selection for biopsy
- Assist in identifying outer margin of cancer prior to planning the appropriate treatment.

**Disadvantages**

- Not US Food and Drug Administration (FDA) approved
- High rates of false positives if patient is not followed up adequately
- Works well on erythroplakia but not on leukoplakia, which is where clinicians need more help
- Better than visual acuity but does not identify the true margins of the field
- Toxic if swallowed.

**Advantages**

- Simple and rapid to perform
- Low cost
- Noninvasive
- Helps to better define gross extent of areas of lesions.

**CHEMILUMINESCENCE**

Chemiluminescence means “emission of light as a result of a chemical reaction at environmental temperatures.” This technique is now getting used in the development of new diagnostic aid for oral carcinoma. Chemiluminescent diagnostic aids have been adopted as an adjunct to conventional examination in the diagnosis of early-stage carcinoma.

This includes the following test:

- ViziLite and MicroLux
- ViziLite Plus.

**ViziLite**

**Background History**

ViziLite equipment was developed by Trylon Corporation for use in detecting abnormal growths on the uterine cervix. In 2001, the FDA cleared the ViziLite\textsuperscript{TM} Comprehensive Examination Tray for marketing for identification, evaluation, and monitoring of oral mucosal abnormalities in a patient population i.e., at increased risk for oral cancer.

<table>
<thead>
<tr>
<th>Clinical methods</th>
<th>Photodiagnosis</th>
<th>Histopathological methods</th>
<th>Molecular methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vital staining</td>
<td>5-ALA-mediated fluorescence endoscopic imaging</td>
<td>Exfoliative cytology</td>
<td>Quantification of nuclear DNA content</td>
</tr>
<tr>
<td>Chemiluminescent light</td>
<td>5-ALA-mediated digitalized fluorescence endoscopic imaging</td>
<td>Oral CDx system</td>
<td>Tumor marker</td>
</tr>
<tr>
<td>Narrow band imaging (VELscope)</td>
<td>Autofluorescence spectroscopy</td>
<td>Fine needle aspiration cytology</td>
<td>Microsatellite markers</td>
</tr>
<tr>
<td></td>
<td>Autofluorescence imaging</td>
<td>Biopsy</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Classification of diagnostic aids
ViziLite Test Kit Components

ViziLite kit is a single-use product that consists of an acetic acid rinse, retractor (sheath and handle), and light stick.

Procedure

- The patient rinses with the ViziLite acetic acid solution and expectorates.
- The ViziLite light stick is activated by bending until the inner capsule breaks.
- The examiner shakes the stick until it glows, then inserts the light stick into the hollow end of the retractor.
- After dimming the lights, the oral cavity is examined using the ViziLite device.
- The light is reported to impart a blue hue to normal tissue, while lesions become clinically discernible and take on an “acetowhite” appearance.

Physics of ViziLite

Normal epithelium absorbs ViziLite and appears dark; abnormal epithelium reflects ViziLite and appears acetowhite; as a cell becomes more dysplastic, the nucleus becomes larger compared with the rest of the cell. The enlarged nucleus reflects light, and thus appears aceto white.

ViziLite screening was done to determine the characteristics of clinically obvious OSCC when visualized under chemiluminescent light and to screen for the possibility of change in other apparently normal mucosa. The technology used in this device is based on similar technology utilizing chemiluminescent light to evaluate dysplastic and malignant squamous cell lesions in the cervix.3

Ram and Sian10 examined the use of ViziLite as a diagnostic aid in the detection of oral cancer and potentially malignant epithelial lesions (PMELs) by comparing it against 1% tolonium chloride mouth rinse and concluded that ViziLite is a more reliable diagnostic tool than tolonium chloride in the detection of oral cancer and PMELs, and for follow-up of patients treated for the same.11

ViziLite Plus

In November 2004, the FDA approved the ViziLite Blue Oral Lesion Identification and Marking System, a three-component swab system used as an adjunct to the ViziLite Test. This system consists of three swab components: Two swabs of 1% acetic acid rinse, including a postdye decolorizer and one swab with a metachromatic vital tissue dye and tolonium chloride (also called toluidine blue). The dye is applied to ViziLite identified white lesions to allow the health care provider to visualize the lesions with incandescent light. The ViziLite Blue Plus is indicated as an adjunct to the ViziLite Test for oral mucosa lesions for further evaluation and monitoring of lesions by providing physical marking of lesions already differentially identified with ViziLite in a population at increased risk for oral cancer.12

NARROW BAND IMAGING

Narrow band imaging (NBI) is a novel method of imaging that has the potential to improve the diagnostic capability of standard white light endoscopy. It was initially developed for use in the gastrointestinal tract, but recently NBI has gained a lot of enthusiasm for screening and examining patients for mucosal SCC in the head and neck. It is an innovative optical image-enhanced technology system that uses narrow bandwidth NBI filters.13

Advantages

- Narrow band imaging increases tissue contrast by specifically identifying superficial capillaries and neoangiogenesis in abnormal mucosa14 (Fig. 1).
- Requires no special dyes.
- Serves as an accurate endoscopic tool during biopsy examination to target areas with suspicious superficial vascular morphology, or enable excision biopsies to be more accurate.13

Various studies have shown that NBI has value in the detection of malignant disease and in the determination of surgical margins.15 Several outcomes from these studies include the development, FDA approval, and marketing of an NBI instrument and VELscope (LED Dental Inc., Atlanta, USA) that is designed for use in general practice settings.16,17

VELscope

The mortality rate associated with oral cancer has remained unchanged for over 30 years. This has created a dire need for an improved oral mucosal screening procedure that would make it possible for clinicians to accurately identify tissue changes at and below the surface before they become apparent under white light examination, which led to the development of visually enhanced lesion scope by LED DENTAL – also called as VELscope. It is based on the direct visualization of tissue fluorescence and the changes that occur when abnormal cells are present. The VELscope system is a revolutionary handheld device that provides an easy-to-use adjunctive mucosal examination system to the dentists, hygienists, and other oral health care professionals for the early detection of abnormal tissue.
Physics of VELscope

The VELscope Handpiece emits a safe blue light (400–460 nm) into the oral cavity, causing tissue fluorescence from the surface of the epithelium through to the basal membrane – where premalignant changes typically start. By utilizing selective (narrow-band) filters in the VELscope handpiece, the clinician is able to immediately view the different fluorescence signatures in the oral tissue to help differentiate between normal and abnormal cellular activity. Typically, healthy tissue appears as a bright apple green, while the suspicious regions are identified by a loss of fluorescence, which thus appear dark\textsuperscript{18} (Fig. 2).

Indications

- VELscope is to be used by a dentist or health care provider as an adjunct to traditional oral examination by incandescent light to enhance the visualization of oral mucosal abnormalities that may not be apparent or visible to the naked eye, such as oral cancer or premalignant dysplasia.
- VELscope is further intended to be used by a surgeon to help identify diseased tissue around a clinically apparent lesion and thus aid in determining the appropriate margin for surgical excision.\textsuperscript{19,20}

Advantages

- The ability to detect abnormal activity or changes, not yet visible in white light or to the naked eye, makes this another excellent early screening tool.
- It allows for photodocumentation of suspicious areas to facilitate follow-up and further action.
- It is FDA approved.

Disadvantage

- Low specificity.

OPTICAL TECHNIQUES

A variety of optical techniques have recently been utilized for the diagnostic study of cancerous tissue. These include fluorescence spectroscopy, Raman spectroscopy, light scattering spectroscopy, and Fourier transform infrared spectroscopy.
FLUORESCENCE SPECTROSCOPY

This is a new diagnostic modality with the potential to bridge the gap between clinical examination and invasive biopsy. Tissue architecture and biochemical composition can be evaluated in near real time using optical spectroscopy. By scanning the tissue with a small, flexible, fiberoptic probe, subtle alterations induced by dysplasia or inflammation can be detected noninvasively. This is accomplished by analyzing the spectrum of the fluorescence emitted by the tissue. The development of software algorithms allows automated data analysis of various types of spectra to provide instantaneous tissue diagnosis.21

Photodynamic diagnostics is a modern diagnostic method based on detection of different autofluorescence of tissues (pathological – especially premalignant lesions and malignant tumors vs normal one) or fluorescence after previous local or systemic administration of photosensitizers, selectively accumulating in pathological foci. In this method, the fluorescence of endogenous fluorophores and exogenous photosensitizers is induced by monochromatic light and then analyzed digitally with the use of special equipment.22

Porphyrin-enriched tumor tissue irradiation with a fluorescence excitation system leads to the emission of pink-red fluorescence. This principle is used as a diagnostic procedure and is called photodynamic diagnosis (PDD), also known as fluorescence diagnosis, which is a more precise denomination.23

Among all photosensitizers, two have a high specificity and sensitivity for tumor diagnosis: m-Tetra (hydroxyphenyl)chlorin (Foscan) and Photofrin and delta-aminolevulinic acid (ALA) and Levulan.3 Doses and route of administration of photosensitizers are described in Table 2.

5-ALA-INDUCED FLUORESCENCE ENDOSCOPIC IMAGING

5-Aminolevulinic acid-induced protoporphyrin IX (PpIX) fluorescence improves the differentiation of tumor and normal tissue in the oral cavity. Aminolevulinic acid is present virtually in all human cells. 5-Aminolevulinic acid itself is not a photosensitizer but serves as the biological precursor of the photosensitizer, PpIX, in the heme biosynthesis pathway. The synthesis of ALA is normally tightly controlled by feedback inhibition of ALA synthetase by intracellular heme levels.24,25

Image Acquisition

Fluorescence images are recorded with an intensified and integrated charge-coupled device camera. Diagnostic illumination lasted 5 to 30 seconds. The highly suspicious lesion will show bright reddish fluorescence, while a normal mucosa exhibits blue color background in the fluorescence images.

Studies of 5-ALA-induced PpIX fluorescence have shown a sensitivity of 95 to 100% for oral cancer diagnosis, but the specificity is only about 50 to 60%.26

5-ALA-MEDIATED DIGITIZED FLUORESCENCE ENDOSCOPIC IMAGING

To improve the diagnostic specificity, a 5-ALA-mediated digitized fluorescence endoscopic imaging system was built to enable the online image acquisition, analysis, and fluorescence quantification for the early detection of neoplasms in the oral cavity.27,28

Advantages

- It allows estimating the existence and size of epithelial pathologies, which might be partly or completely invisible in white light.21
- To guide biopsy from representative samples.
- Usefulness of 5-ALA-induced porphyrin fluorescence in preoperative demarcation of ill-defined clinical tumor margins and as a control after photodynamic therapy.
- Spectral diagnosis can provide imaging and point spectroscopic information in both morphological and biochemical data modes.
- The advantage of using exogenous fluorophores is that the photophysical and pharmacokinetic properties can be selected and are known.

Disadvantages

- A certain waiting time after application is necessary for the fluorophore to reach its optimal fluorescence intensity.
- The application of photosensitizers leaves the patient temporarily sensitive to light, which negatively affects his/her daily life. This makes the technique impractical, especially for use in regular screenings of high-risk patient groups.
- Finally, the specificity of the photosensitizers appeared to be less than expected.29 Photodynamic diagnostics

<table>
<thead>
<tr>
<th>Photosensitizer</th>
<th>Dose</th>
<th>Application</th>
<th>Time till PDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ALA</td>
<td>20% ALA cream 0.4% ALA solution</td>
<td>Topical application Rinse the mouth (20 minutes)</td>
<td>2–8 hours 1–2 hours</td>
</tr>
<tr>
<td>Hematoporphyrin derivative</td>
<td>2.5–5 mg/kg Intravenous</td>
<td>3–72 hours</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Doses and types of application of photosensitizers23
can be used for a complete visualization of malignant lesions after the topical or systemic application of a tumor-selective photosensitizer. It has been shown to be highly effective in malignant superficial skin and mucosal lesion diagnostics.30

AUTOFLUORESCENCE SPECTROSCOPY AND IMAGING

Optical spectroscopy allows noninvasive physical and chemical characterization of biological tissues. The structural and chemical composition of cells and tissues strongly influences their optical features, and therefore, alterations in the optical characteristics may indicate the presence of diseased tissue. Optical spectroscopy may provide possibilities in the early detection of cancerous tissues in humans.

Autofluorescence

Autofluorescence is the fluorescence of tissues to which no chemical substances have been applied: It is the natural fluorescence of the tissue itself (“auto”).30,31

Biological Origins of Tissue Autofluorescence

Autofluorescence of tissues is produced by fluorophores that naturally occur in living cells after excitation with a suitable wavelength. The fluorophores can be located in the tissue matrix or in cells. The invoked intrinsic autofluorescence profile is altered by absorption and scattering events in the tissue before measurement. The absorption in tissue is mainly attributed to oxyhemoglobin and deoxyhemoglobin, which have different absorption profiles. The scattering is due to inhomogeneities of refraction index caused by cell nuclei and cell organelles. The presence of disease changes the concentration of the fluorophores, as well as the light scattering and absorption properties of the tissue due to changes in blood concentration, nuclear size distribution, collagen content, and epithelial thickness.29

Instrument

The spectroscopic system incorporates a fiber-optic probe, two nitrogen-pumped dye lasers, and an optical multichannel analyzer. The probe consists of a central fiber surrounded by six fibers. Three fibers deliver excitation light at wavelengths of 337, 365, and 410 nm. The probe illuminates a 1-mm-diameter spot on the tissue surface, and a quartz shield at the tip of the probe maintains a fixed distance between the fibers and the tissue. The laser has 5 ns pulse duration and a repetition rate of 30 Hz. The average transmitted pulse energies at 337, 365, and 410 nm were 15.2, 3.3, and 17.4 μJ respectively. The light from the four emission-collection fibers is sent to a polychromator, which disperses the light onto an array of diodes. The diodes collect and digitize the fluorescence to produce an emission spectrum.21

This technique may be able to differentiate between normal mucosa, hyperkeratosis, and premalignant lesions of the mucosa at an early stage. Autofluorescence spectroscopy can be a useful tool for guiding the clinician to the most dysplastic location for biopsy. It seems to be very accurate for distinguishing lesions from healthy oral mucosa (sensitivity 82 to 100%, specificity 63 to 100%). This is especially true for distinguishing malignant tumors from healthy mucosa, for which sensitivities and specificities >95% are no exception.29

Disadvantages of Autofluorescence Spectroscopy

- Autofluorescence spectroscopy is for practical reasons not suitable to detect new lesions or to demarcate lesions.
- It is not feasible to scan the complete oral cavity using the small sampling area that results from the use of an optical fiber. Emerging lesions are very small and therefore, each and every part of the mucosa would have to be measured separately.
- Procedure is time consuming.
- It would be impossible to mark which locations have already been measured.

Oral CDx Brush Test System

Standard exfoliative cytology of oral mucosal lesions, including oral precancer, has for years been criticized for not producing adequate and reliable results. In recent years, new techniques, particularly the brush biopsy technique, have been developed. Computer-assisted transepithelial oral brush biopsy (Oral CDx) is a transepithelial oral biopsy designed to test lesions in their early stages. This method utilizes a small circular brush, i.e., designed to penetrate the superficial, intermediate, and basal cell layers with minimal discomfort. The resulting sample is then placed onto a slide for computer analysis. These samples are fixed onto a glass slide and sent to a laboratory where they are stained, scanned, and analyzed microscopically by means of a computer-based imaging system that can rank cells on the basis of the degree of abnormal morphology.

Advantages

- The OralCDx oral brush biopsy is a rapidly conducted chair-side procedure that results in minimal or no bleeding and requires no topical or local anesthetic.
An Insight into the Recent Innovations in Early Detection of Oral Cancer

- A transepithelial brush biopsy is not a difficult or demanding procedure to master.
- OralCDx testing can be reliably used on oral lesions with epithelial abnormalities as a method of confirming their benign nature, and more importantly, revealing those that are precancerous and cancerous when they are not clinically suspected of being so.
- As dysplastic and cancerous oral lesions frequently have an overlying keratin layer, cellular abnormalities in the deep basal layer of the epithelium are best sampled with this instrument.

**TUMOR MARKERS**

Tumor markers are defined as biochemical substances (e.g., hormone, enzyme, or proteins) synthesized and released by the cancer cell or produced by the host in response to cancerous growth. It may be present in blood circulation, saliva, body cavity fluids, cell membrane, and cell cytoplasm. The tumor markers can be DNA, messenger RNA, protein, or processes (apoptosis, angiogenesis, proliferation, etc.) measured quantitatively or qualitatively by an appropriate assay.

Tumor markers can be used for one of four purposes:

1. Screening a healthy population or a high-risk population for the presence of cancer
2. Making a diagnosis of cancer or of a specific type of cancer
3. Determining the prognosis in a patient
4. Monitoring the course in a patient in remission or while receiving surgery, radiation, or chemotherapy

Common methods for identifying tumor proteins

- Immunohistochemistry
- Fluorescent in situ hybridization (FISH)
- Reversed transcriptase and polymerase chain reaction (RT-PCR)
- Ploidy
- Genetic
- Proliferating cell nuclear antigen (PCNA)
- MIB1

**TUMOR MARKERS**

Markers of increased proliferation in oral carcinoma have been identified and explored for more than a decade. Although a large body of literature exists on the association of these markers with tumor grading and different degrees of dysplasia in premalignant lesions, it is surprising that there are only a few studies that have shown an impact on prognosis. In this respect, argyrophilic nucleolar organizer regions had been confirmed as relevant for prognosis in more reports than proliferating cell nuclear antigen (PCNA) or MIB1.

Another more recent approach to oral carcinogenesis is focused on the escape of malignant cells from apoptotic signals. p53 has been extensively researched in this respect and there is broad evidence for its role in the manifestation of oral carcinoma. Additional markers of apoptosis, such as Fas and Fas ligand and Bax, as well as antiapoptotic molecules, such as bcl2/BAG-1 are reported in a smaller number of publications showing significant correlation with prognosis. Although it may not be justified to disregard molecular markers in the assessment of individual prognosis of oral cancer, their current role is more likely to be beneficial if they are used in association with traditional means of histopathological evaluation.

**MICROSATELLITE ANALYSIS**

Microsatellite allele losses are characteristic features of head and neck SCC. Microsatellite alterations have been used as markers of clonality and to detect cancer cell DNA in a background of normal cells. Microsatellite analyses can reveal either loss of heterozygosity or microsatellite instability in the amplified microsatellite repeat locus. Microsatellite instabilities, hot spot mutations, and epigenetic changes (including methylation) of different oncogenes and tumor suppressor genes have been linked with oral precancer and cancer and are often reliably used to screen precancer.

One of the more sensitive techniques available for studying clonal changes in tumors and premalignant lesions is the use of PCR-based microsatellite analysis for allelic loss. The advantage of the PCR-based microsatellite analysis is that, it requires only small quantities of DNA yet yields valuable data.
on the loss of chromosomal regions that contain putative suppressor genes. Hence, we can obtain information on critical genetic events even before the identification of the actual suppressor gene. This approach has been used frequently in head and neck cancers.50

CONCLUSION

With increasing incidence of oral cancer and the era of conservation, there is an increasing demand for early diagnosis of carcinoma for a better prognosis and good treatment. Increased use of these adjunctive tools by dentists would likely improve the early cancer detection statistics, thereby lowering the death rates. The future is promising for further development and evolution of oral lesion diagnostic aids to enhance the quality of patient care provided by all clinicians.

REFERENCES


