Review Article

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Non Invasive and Reliable Diagnostic Aids in Detecting Potentially Malignant Disorders: A Review

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ABSTRACT

Background: Oral cancers are most common cancers worldwide today and its morbidity and mortality rates are still high in most countries. Typically, oral cancer is preceded by a premalignant state for a long time. The survival rates for oral cancer patients will significantly be improved provided lesions are detected and treated at the infancy stage. The World Health Organization has clearly identified prevention and early detection as major objectives in the control of the oral cancer burden. Early diagnosis is therefore of paramount importance. It is important to screen patients for these conditions so as to detect malignant changes early. Previously, the screening of patients for oral cancer and potentially malignant disorders (PMD) has relied mainly on conventional oral examination. Nowadays, many newer techniques are available to potentially assist in the screening of healthy patients for evidence of oral cancer. In recent years, there has been a growing and persistent demand for new non-invasive, practical diagnostic techniques that might facilitate the early detection of oral PMDs. The present paper reviews the main techniques used to improve premalignant lesion diagnosis.

Keywords: Potentially malignant disorders, non invasive, chair side investigations, oral cancer, toludine blue, vizilite.

INTRODUCTION

Cancer of the head and neck, including all oral, laryngeal and pharyngeal sites, is the sixth most common Cancer.[1] The incidence of oral cancer differs widely in various parts of the world with 2-10 per 100,000 populations per year. High incidence of oral cancer is seen in South Asian countries like Srilanka, India, Pakistan, and Bangladesh. In India the incidence is 7-17/100,000 persons/year with 75,000-80,000 new cases registered annually.[2] Detecting oral cancer in its early stages dramatically affects survival rates compared with detecting it in later stages.[2] Five-year survival of oral cancer varies from 81% for patients with localized disease to 42% for those with regional disease and to 17% if distant metastases are present. Because of the delay in diagnosis fewer than 50% of patients with oral and pharyngeal cancers survive more than 5 years. This rate has remained disappointingly low and relatively constant during the last few decades.[1] The early detection and diagnosis of oral PMDs allow clinicians to monitor and treat oral carcinogenesis at intraepithelial stages, including mild, moderate, and severe dysplasia, as well as carcinomain situ. This is crucial for improving the survival rate and reducing morbidity, disfigurement, function loss, treatment duration, and hospital costs of oral squamous cell carcinoma (OSCC) patients.[3] Early detection therefore necessitates raising awareness in the general public and improving access to oral health services for all segments of the population.[4]

Screening is 'the process of identifying apparently healthy people who may be at increased risk of a disease or a condition. It involves checking for the presence of disease in a person who is symptom

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free, with the intention of diagnosing treatable conditions at the earliest stage. Oral cancer is a potential candidate for screening as the majority of OSCC are thought to be preceded by an PMD.[5] In a epidemiological study by Rao for Oral Cancer states that it is important to undertake programs to prevent and control Oral Cancer by screening for early diagnosis.[6]

In 2005 Sankaranarayanan and co-workers reported the first solid evidence that periodic examination of the oral cavity can reduce mortality from oral cancer in high-risk individuals.[7] Their 9-year screening study reported a significant 32% reduction in mortality in high-risk individuals in the intervention group (42% when only male tobacco/alcohol users are considered), suggesting that oral visual screening in high-risk patients could prevent about 40,000 deaths from oral cancer worldwide.[1]

Approaches to Early Detection of Dysplasia and Oral Cancer[4]

There are two approaches in the early detection of oral dysplasia and cancer: 1) oral cancer screening programs that identify asymptomatic patients with suspicious lesions and 2) employing specific diagnostic tools toidentify dysplasia and early oral cancers in asymptomatic patients with an oral abnormality.

Screening aids of oral cancer and PMD are classified as follows.

Classification of screening aids to oral cancer and precancer[2,8]

1. Standard screening test
a. conventional oral examination (COE)
2. Established screening adjuncts
a. Oral cytology
b. Oral brush biopsy
3. Vital staining
a. Toluidine blue (TB)
b. Lugol's Iodine
c. Methylene Blue
d. Rose Bengal
e. Acetic acid
4. Light-based detection systems
a. Chemiluminescence- vizilite, vizilite plus, microlux/DL, orascoptic DK
b. VELscope
5. Photodiagnosis
a. 5-Aminolevulinic acid mediated fluorescence endoscopic imaging
b. 5-Aminolevulinic acid mediated digitized fluorescence endoscopic imaging
c. Autofluorescence spectroscopy
d. Fluorescence photography

1. Standard screening test

a. conventional oral examination

Conventional oral examinations, including inspection and palpation, are the routine methods for the screening of oral lesions. However, subtle lesions may pass undetected, and it is difficult to make a distinction among benign, PMD. It has been reported that dysplasia or micro-invasive carcinoma can occur in clinically normal-appearing mucosa.[3] Conventional oral examination using normal (incandescent) light is the most commonly applied and accepted screening method for oral squamous cell carcinoma.[9] This is easily available and a cheap method has found to be equally effective in detecting PMDs across all levels of training, including junior and senior dentists as well as trained healthcare workers or auxiliaries.[9] However, COE tends to pick up lesions that in a majority of cases that are clinically and histopathologically benign, that is, the conventional leukoplakias, with a small number that are progressive or definitely malignant. Conversely, COE may also miss areas of histological changes that appear clinically normal (false negative).[9]

A meta-analysis of the data showed a weighted pooled sensitivity of 0.848 (95% CI 0.73, 0.92) and specificity of 0.965 (95% CI 0.93, 0.98) indicating a satisfactory test performance for an oral examination.[10] A meta-regression analysis of heterogeneity indicated no differences between studies suggesting that trained auxiliaries are able to screen with a degree of accuracy similar to dental practitioners.[10]

A Cochrane review of visual oral examination identified several serious potential biases in the study.[11] These included a lack of standardized diagnostic criteria, the absence of outcome data blinding, only a 63% rate of compliance with the referral and no active follow-up, and only a minority

of subjects being biopsied to confirm the diagnosis of a potentially malignant oral disorder. They concluded that there is insufficient evidence to recommend inclusion or exclusion of screening for oral cancer using a visual examination in the general population.[11]

Advantages:

1. Simple and easy
2. Cost effective

2. Established screening adjuncts

a. Oral cytology:

Cytopathology is the microscopic study of cell samples collected from mucosal surfaces obtained by exfoliative cytology (via smears, scrapings, or lavage) or from internal sites via fine-needle aspiration. Exfoliative cytology was first designed for cervical cancer early detection and it has been primarily applied in oral medicine practice to detect early changes in oral mucosa related to malignancy.[12] Cytology is cheaper and easy procedure that can be carried out at out-patient department to diagnose malignancy at early stage.[2] cotton swab, wooden spatula, metal spatula were used to collect smear.[2,8]

According to Mehrotra et al., sensitivity and specificity of conventional exfoliative cytology in carcinoma's suspected lesions, ranged between 76.8%–100%, and 88.9%–100%, respectively.[12] This technique collects only cells of the superficial layers, So methods of collection of cells were modified by the use of oral brush biopsy(oral CDx brush).

b. Oral brush biopsy :

The oral brush biopsy, also known as OralCDx Brush test system, consists of a method of collecting a trans-epithelial sample of cells from a mucosal lesion with representation of the superficial, intermediate and parabasal/basal layers of the epithelium.[1] The oral CDx cytobrush claims to collect the basal layer cells non-invasively and assess the dysplasia by computer-assisted network, in order to eliminate subjective misinterpretations. A specially designed brush is the non-lacerational device used for epithelial cell collectionand samples are eventually fixed onto a glass slide, stained with a modified Papanicolaou test and analyzed microscopically via a computer-based imaging system. Results are reported as "positive" or "atypical" when cellular morphology is highly suspicious for epithelial dysplasia or carcinoma or when abnormal epithelial changes are of uncertain diagnostic significance respectively. Results are defined as negative when no abnormalities can be found.The test is considered an intermediate diagnostic step as ascalpel biopsy must follow when an abnormal result is reported.[1]

Indeed current data show that OralCDx's cytologic test is highly sensitive and specific in detecting dysplastic changes in high-risk mucosal lesions (due to clinical findings suggestive of malignancy), but when used in a low risk population with benign-appearing oral epithelial lesions, the accuracy is reduced and the rate of false-positive findings increases.[1]

3. Vital staining:

Vital staining is a procedure where living cells take up certain dyes, which selectively stain some elements in the cells like mitochondria, lipid vesicles, lysosome, etc.[13] In vivo staining was first applied in 1963 by Reichart for uterine cervical carcinoma in situ. Application of vital stains to detect oral premalignant and malignant lesions was first reported by Neibel HH and Chomet B in 1964.[13]

Applications of vital stains[13]

- To highlight the PMD.
- To detect multicentric or second primary tumors
- To outline the full extent of dysplastic epithelium or carcinoma prior to excision
- Selecting the biopsy sample site in PMD
- Help in follow up of the patients with oral cancer
- Useful in obtaining the marginal control of carcinoma
- To identify early lesions which could be missed out on clinical examination.
- Can be used as effective screening modality to assess the intra operative margins alterative to the frozen sections
- Recognition of post-treatment recurrence
- Also in educating the patient

a. Toludine blue:

Toluidine blue is also known as tolonium chloride, methylanaline, aminotoluene. Its principal use was in dye industry after its discovery by William Henry Perkin in 1856.[13]Toluidine blue, an acidophilic metachromatic dye of thiazine group selectively stains acidic tissue components (sulfates, carboxylates and phosphate radicals), thus staining DNA and RNA. Toluidine blue has been established as a diagnostic adjunct in detecting oral lesions related to invasive carcinomas, carcinoma in situ or early asymptomatic oral carcinomas.[14]

Mechanism:

Binding with DNA:

It is an acidophilic, metachromatic nuclear dye of Thiazine group thatselectively stains acidic tissue components particularly nucleic acid such asDNA and RNA. Dysplastic areas contain more DNA.[15]

Intracellular canal:

Malignant epithelium contains wider intracellular canal which facilitates the penetration of dye.[15]

Procedure: [8,15,13]

1. Rinse the mouth twice with water for 20s to remove debris.
2. Apply 1% of acetic acid for 20seconds to remove ropey saliva.
3. Apply 1% toluidine blue either with cotton swab or can be given as rinse.
4. Rinses with 1% acetic acid are done to reduce the mechanically retained stain.
5. Finally rinse the mouth with water. Then the color change is assessed.

Interpretation: [8,15,13]

It is based on the color change of mucosa.

Positive - Dark royal blue
Doubtful - light blue is doubtful
Negative - no color

False positive results are seen with following lesions:[13]

Epithelial hyperplasia, hyperkeratotic lesions, inflammatory and traumatic lesions, hyperplastic candidiasis can retain 60% of stain. The decision making can also be attributedto the experience of the clinician. Repeat the test after 10-14 days to allow the inflammatory lesions to resolve. This reduces the false positive by 8.5%.

False negative results are recognized in:Low grade dysplasia, lichenoid dysplasia. [13]

Advantages:[2,13]

- Indicates area for biopsy
- Low cost
- Non-invasive
- Disposable device (no cross-contamination)
- 100% sensitivity for pathology

Disadvantages:[2,13]

- TB stain can remain in mouth for 4-6 h
- Unpleasant taste
- Specificity: Not meant to be a diagnostic tool; it is a screening device only.
- Filiform papillae retain the dye due to high protein synthesis rate.

b. Lugol's iodine:

Lugol's iodine was first made in 1829, which is named after the French physician Lugol (1786–1851). Upto the end of 19th century, it was used as an antiseptic and disinfectant.[13] The vital dye with Lugol's solution is also called Schiller's test.[2.12]

Composition: 5 g iodin (I2), 10 g potassium iodide, Distilled water 100 ml

Mechanism:

The principle is based on glycogen content of the cytoplasm and the reaction is known as the iodine–starch reaction, visualized by a colour change. As there is enhanced glycolysis in cancer cells, do not promote the iodine–starch reaction. Hence there is no color change in dysplastic epithelium, whereas due to high glycogen content of normal epithelial cells, brown color can be noticed. The vital dye with Lugol's solution is also called Schiller's test.[13]

Procedure:

Rinse with water/ carbocisteine syrup 250 mg/5 ml and dry with a gauze to clear the mucin. Then apply

Lugol's iodine until parakeratinized epithelium is stained a brown or black. After one to two minutes, interpret the stain reaction. Most effective method is to stain the lesion with 3% Lugol's solution followed by 5% Lugol's solution. Lugol iodine is cheap, widely available, easy to use and only about 5 min are necessary to perform the staining procedure.[13]

Advantages:[2]

- It can be used for non-keratinized stratified squamous epithelium
- Simple, easy-to-learn approach that is minimally reliant upon infrastructure
- Low start-up and sustaining costs
- Many types of health care providers can perform the procedure
- High sensitivity results in a low proportion of false negatives
- Test results are available immediately
- Decreased loss to follow-up.

Disadvantages:[2]

- It is an irritant that damages normal epithelium.
- Patient complaints of abdominal pain, heart burn and nausea.
- Allergic reaction to iodine
- Induces shock some times.

c. Methylene blue

It is a heterocyclic aromatic chemical compound. At room temperature it appears as a solid, odorless, dark-green powder, which yields a blue solution when dissolved in water. Methylene blue was first used in detecting oral mucosal lesions.[15] Similarly to TB, methylene blue also stains tissue with large quantities of nucleic acids.[16] Methylene blue staining is useful for screening oral cancer in high-risk individuals, and shows high sensitivity in detecting oral PMDs Combined with plant auxin (indole-3-acetic acid), methylene blue is being investigated for the photodynamic treatment of cancer. Considering its low toxicity and the fact that it is cheaper than TB, it may be convenient to substitute it for TB in large-scale oral screening in high-risk patients.[1]

d. Rose Bengal test(RB):

It is 4, 5, 6, 7 tetrachloro-2, 4, 5, 7 tetraiododerivate of fluorescein, can stain the desquamated ocular epithelial cells. RB staining is used to delineate the extent of the corneal and conjunctival neoplasms and even oral epithelial dysplasia and OSCC.[13] It stains desquamated ocular epithelial cells, dead or degenerated cells, but not healthy epithelial cells.[3] Study done by Mittal et al demonstrated that RB staining may be a valuable diagnostic technique in the detection of oral PMDs and oral cancer.[17] In a study done by Du et al, authors had investigated the efficacy of RB staining in the detection of oral PMDs and oral cancer in 132 patients, and concluded that RB staining seemed promising for the detection of epithelial dysplasia in oral leukoplakia, lichen planus, and leukokeratosis.[18] Recently, a rose-bengal- conjugated gold nanorod (RB-GNR) platform was developed for the optical detection of cancer cells.[19]

e. Acetic acid staining:

Composition

100 ml of 1% acetic acid rinse contains 1 ml of glacial acetic acid with 99 ml distilled water.[13]
Principle

Application of acetic acid causes reversible coagulation / precipitation of cellular proteins and causes swelling of the epithelial tissue, particularly abnormal squamous epithelial areas, dehydration of the cells and it helps in coagulating and clearing the mucous secretions. The normal squamous epithelium appears pink and the columnar epithelium red, due to the reflection of light from the underlying stroma, which is rich in blood vessels. If the epithelium contains a lot of cellular proteins, acetic acid coagulates these proteins, which may obliterate the colour of the stroma. The resulting acetowhite is seen distinctly as compared with the normal pinkish colour of the surrounding normal squamous epithelium.[13]

Procedure

A piece of gauze soaked with 5% acetic acid is applied to a cleaned and dried lesion for 60 seconds. After that gauze is removed and characteristic color change can be noted. Color change to opaque white is considered positive and transparent white as negative. The Sensitivity, specificity and accuracy is

about 83.33%, 84.21% and 83.64% respectively.Advantage is associated by false positive reaction with small aphthous-like ulcerated lesion that might not routinely be biopsied turned opaque white after the application of acetic acid and the histopathologic result was moderate epithelial dysplasia.[13]

4. Light based detective system

A number of light-based detection systems have been developed for the identification of oral PMDs and oral cancer at their earliest stage.[4] Clinical inspection of oral mucosa with the aid of chemiluminescent blue/white light was recently suggested to improve the identification of mucosal abnormalities with respect to the use of normal incandescent light.[1] Mucosal tissues undergoing abnormal metabolic or structural changes have different absorbance and reflectance profiles when exposed to various forms of light sources, enabling the identification of oral mucosal abnormalities.[3] Vizilite Plus withTBlue system (Zila Pharmaceuticals, Phoenix, Arizona, U.S.), VELscope (LED Dental, White Rock, BritishColumbia, Canada) Microlux/DL (AdDentInc, Danbury, Connecticut) and Orascoptic DK (Orascoptic, Middleton,WI) are commercially available light-based systems that are based upon the assumption that abnormal metabolic or structural changes have different absorbance and reflectance properties.[4]

a.Chemiluminiscence

Chemiluminiscence refers to the emission of light from a chemical reaction which is of varying degrees of intensity with colors that span the visual spectrum.[2] Commercially available chemiluminescence techniques include ViziLite, ViziLite Plus, Microlux/DL and Orascoptic DK. The main difference between these techniques is that ViziLite and ViziLite Plus involve a single use chemiluminescent stick, while Microlux/DL and Orascoptic DX provide a blue-white light-emitting diode (LED) fiber-optic light.[3]

ViziLite system (Zila Pharmaceuticals, Phoenix, AZ):

The ViziLite kit contains a vial of 1% acetic acid solution, a capsule, a retractor. The capsule has an outer shell of flexible plastic and an inner vial of fragile glass. To activate it, the capsule is bent to break the glass vial, so that the chemical products react with each other and produce bluish-white light with a wave length of 430-580 nm that lasts for around 10 min.[3]

Procedure:

Oral cavity is rinsed with 1% acetic acid solution for 1 minute followed by the examination of the oral mucosa under diffuse chemiluminescent blue/white light (wavelength of 490 to 510 nm). The theory behind this technique is that the acetic acid removes the glycoprotein barrier and slightly desiccates the oral mucosa, the abnormal cells of the mucosa then absorbing and reflecting the blue/white light in a different way with respect to normal cells.[1]

Interpretation: [1]

- Normal mucosa appears blue
- Abnormal mucosal areas reflect the light (due to higher nuclear/cytoplasmic ratio of epithelial cells) and appear more acetowhite with brighter, sharper and more distinct margins

Vizilite plus:

The only difference between the Plus and the earlier version is that the latter contains a TB staining solution. TB can further delineate ViziLite-positive lesions, thus improving the specificity.[3]

Microlux/DL:

Shares the basic principles of ViziLite. The oral cavity is examined with a battery-powered LED fiber-optic source that emits a blue-white light.[3] In a study of 599 tobacco users, using biopsy as the gold standard, the sensitivity and specificity of Microlux/DL in detecting oral PMDs were 100% and32.4%, which indicated that it seemed useful for enhancing lesion visibility, but couldn't discriminate between benign and malignant lesions.[20] Microlux/DL is also a poor discriminator for inflammatory, traumatic, and malignant lesions.[3]

Orascoptic DK:

The Orascoptic DK system also requires an acetic acid rinse and a three-in-one, battery- operated, hand-held LED instrument to improve the visualization of oral lesions. There is no published evidence regarding its utility in oral PMD detection.

Advantages:[2]

- Vizilite has the advantage in that it is capable of delineating the sharp borders between normal and abnormal oral mucosa and often extended beyond the clinically identified outline.
- Malignant lesions could be recognized without the aid of adjunctive diagnostic tools.
- To screen for the possibility of field cancer change in other parts of the apparently normal mucosa.

Applications:[2]

- It is used to diagnose leukoplakias and radiation mucositis
- Identification of asymptomatic and clinically non-evident lesions
- Diagnostic aid for the detection of oral cancer and PMD.

VELscope:

VELscope is a handheld device that uses visible light in the 430 nm wavelength in order to cause fluorescent excitation of certain compounds in the tissues.[4] Autofluorescence is due to the presence of endogenous fluorophores in cells, which produce a fluorescent emission when exposed to light of a specific wavelength. Within the oral mucosa, the most relevant fluorophores are nicotinamide adenine dinucleotide and flavin adenine dinucleotide in the epithelium, and collagen cross-links in the stroma. Mucosal abnormalities can alter the absorption and scattering properties of light as a result of changes in tissue architecture and concentrations of fluorophores.[3]

Interpretation: when viewed through filters normal cells show a pale green fluorescence, while abnormal cells show a loss of autofluorescence and appear dark.[3]

Advantages:[2]

- The VELscope examination takes only 1-2 min and is painless and non-invasive, with no stains or rinses required
- Improves the distinction between normal and abnormal tissues (both benign andmalignant)
- Useful adjunct to a thorough visual and digital soft tissue clinical examination.
- Possesses useful benefit in the determination of surgical borders and post-surgical evaluations.
- It covers large surface area
- Non invasion method
- Small lesions can be identified

Disadvantages:[2]

- Heat from prolonged and close tissue examination may cause patient discomfort.

5. Photodiagnosis

a. 5-Aminolevulinic acid mediated fluorescence endoscopic imaging

Aminolevulinicacid (ALA), anantecedentin the biosynthesis of heme, causes the production of the endogenous photosensitizer protoporphyrin (PPIX). Once the topical application is done within the dysplastic cells the synthesis of PPIX takes place. These changes will promulgate the existence of these cells and tumours. So, ALA can be used as a reliable detective aid of neoplasms and tissues with dysplastic changes.[8]

b. 5-Aminolevulinic acid mediated digitized fluorescence endoscopic imaging

It has the ability to capture high quality online images and can quantify the fluorescence intensity of the diseased oral tissues. This modality would be an added asset to diagnose pathological cases, decision making in biopsies and for evaluating the surgically resected margins of oral cavity lesions. This has been proved by a study conducted by weizheng et al on the diagnosis of early oral neoplasms.[8]

c. Autofluorescence spectroscopy:

It is a promising tool for oral cancer detection. It is non-invasive and easily applicable for detection of alterations in structural and chemical composition of cells which might indicate the presence of diseased tissue.[8] The autofluorescence spectroscopy system consists of a small optical fiber that produces various excitation wavelengths and a spectrograph that receives and records on a

computer and analyzes, via a dedicated software, the spectra of reflected fluorescence from the tissue.[1] This technique has the clear advantage of eliminating the subjective interpretation of tissue fluorescence changes.[1] Overall, autofluorescence spectroscopy seems to be very accurate for distinguishing lesions from healthy oral mucosa, with high sensitivity and specificity, especially when malignant tumors are compared to healthy mucosa.[1]

CONCLUSION

Oral cancer is the sixth most common cancer worldwide. Early diagnosis is one of the important objectives to improve the survival rate and its one of major challenging faced both dental and medical providers around the globe. Screening and early detection in population at risk have been proposed to decrease both morbidity and mortality associated with the oral cancer. Many diagnostic methods are tried to eliminate surgical biopsy which is the golden standard, but these tests are not the alternative for surgical biopsy rather, they can be adjuvant. All the newer methods should be analyzed with methodologically sound clinical-trails, which should be compared, qualified and quantified with that of surgical biopsy.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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