Early Detection Of Precancerous And Cancerous Lesions – A Review

Yachana Vipul Patel*, Vivek Sunil Nair*, Madhura Jathar**

*Dental Surgeon, **Lecturer, Department of Oral Medicine and Radiology, Sinhgad Dental College and Hospital, Pune 411041, Maharashtra, India.

Abstract: Oral cancer screening should be a routine part of every annual dental examination. These regular check-ups, including examination of the entire oral cavity, are essential in the early detection of precancerous and cancerous lesions. Over 90% of these tumours are squamous cell carcinomas, which arise from the oral mucosal lining. In spite of easy accessibility of the oral cavity to direct examination, these lesions are often missed and not detected until late stage. The survival rate for oral cancer has remained essentially unchanged over the past three decades. New technologies have provided an exciting new array of diagnostic tools for localizing or emphasizing abnormal mucosa in the dental office. Some of these technologies claim to identify atypical cells prior to biopsy, even before there are clinically visible mucosal changes, hence, can allow a more confident assessment of risk and localization of the most "suspicious" area to biopsy. In essence, molecular-level detection of dysplastic oral mucosal change appears to be moving into the practitioner's office. Recently, there has been an increasing trend of optical spectroscopy methods which depends on the optical spectrum derived from any tissue that contains information about their histological and biochemical make-up of that tissue. Toluidine blue staining and cytological analysis are the main investigations for screening. Oral cancer has a tendency to be detected at a later stage which is detrimental to the patients because of its high mortality and morbidity rates. Most technologies are beneficial but must be used with intelligence and must be considered adjunctive tests rather than stand-alone diagnostic tools. The purpose of this article is to review the diagnostic aids and recent advances in early detection of precancerous and cancerous lesions. [Patel Y NJIRM 2015; 6(6):95-101]

Key Words: Oncology, Early detection, Vital Staining.

Author for correspondence: Dr. Yachana Vipul Patel, RH NO-7, Shreeji Villa, Hyde Park, Serve no-587, Marketyard, Gultekdi, Pune 411037. Email: yachanapatel92@gmail.com.

Introduction: Malignant neoplasms of epithelial cell origin, derived from any of the three germ layers, are called carcinomas.[1] `Oral cancer is a global health problem with increasing incidence and mortality rates in countries such as India, Sri Lanka, Brazil, South Africa and among south Asian countries.[2] Cancer develops over a period of time, going through intermediate stages of different biological significance. Early diagnosis and treatment offers the best prognosis and even the chance of cure.

Cancer can appear as a harmless red or white lesion, an ulcer or a lump showing benign characteristics but without management, will invade into adjacent structures and spread to lymph nodes leading to metastasis.[3]One of the many reasons for high mortality rate is delayed diagnosis because the patient has minimal discomfort and symptoms. Oral cancer screening should be a part of the routine dental check-up. The most common of these are squamous cell carcinomas (90%) arising from the mucosal lining of the oral cavity[4, 5] caused by tobacco use and alcohol consumption. Delayed diagnosis can also be due to incomplete understanding or awareness that a small lesion can also have a high malignant potential. Over the years, a number of diagnostic aids have been developed to aid in early diagnosis of oral premalignant and malignant lesions which would be discussed in this article.

Classification of diagnostic aids-

I. Clinical Methods
   - Vital Staining-
     i. Toluidine Blue
     ii. Lugol's Iodine
   - Vizilite

II. Photodiagnosis
   - 5-Aminolevulinic acid mediated Fluorescence Endoscopic Imaging
   - 5-Aminolevulinic acid mediated Digitised Fluorescence Endoscopic Imaging
   - Autofluorescence Spectroscopy
   - Fluorescence Photography

III. Histopathological Methods
   - Exfoliative cytology
   - Oral CDx system
   - Biopsy

IV. Molecular Methods
   - Quantification of nuclear DNA content
   - Tumour Markers
   - Microsatellite Markers

V. Others

I. CLINICAL METHODS

1. VITAL STAINING

In vivo vital staining has been used extensively in gynaecology to detect malignant changes in the cervix.[6] Richart found that toluidine blue is retained in abnormal epithelial cells while lugol's iodine is retained in normal epithelium cells.[7] Oral carcinoma in situ and early invasive carcinomas have demonstrated affinity towards toluidine blue dye. Lugol's iodine and toluidine blue have been used together in detection of oral precancerous and cancerous lesions.

i. TOLUIDINE BLUE

Toluidine blue was first used by Richart[6] in 1963 to stain uterine cervical carcinoma in situ. Strong et.al[8], Silverman S and Migliorati[9] , advocate the use of toluidine blue to detect malignant lesions.

Toluidine blue (tolonium chloride) is an acidophilic metachromatic dye of the thiazine group. It selectively stains acidic tissue components like sulphates, carboxylates and phosphate radicals, thus staining DNA and is partially soluble both in water and alcohol. As dysplastic and malignant cells have higher nucleic acid content than normal (DNA) or altered DNA content, retain the dye and aid in recognition of mucosal changes[10]. Malignant epithelium may also contain intracellular canals that are wider than normal, which facilitates penetration of the dye.[7]

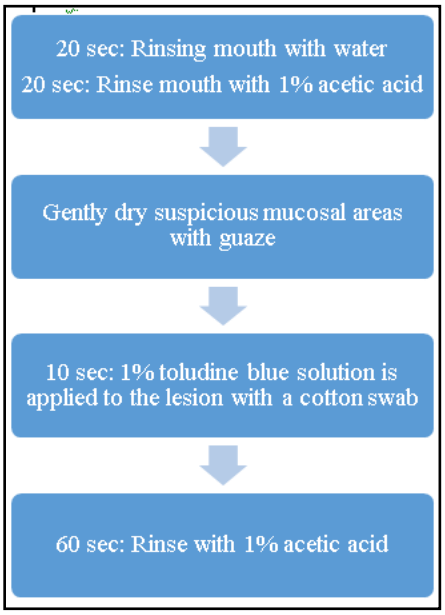
Recent studies on toluidine blue stained lesions have reported a link between carcinoma and loss of heterozygosity at 3p and 17p, while dysplasia resulted in loss of heterozygosity at 9p.[11] The presence of loss of heterozygosity has also been reported in high frequency of toluidine blue stained lesions with or without low grade dysplasia.

Toluidine blue solution composition-[6]

- Toluidine blue -1 gm
- Acetic acid -10cc
- Absolute alcohol -4.19cc
- Distilled water -86cc
- pH adjusted to 4.5

Technique[12]: Toluidine blue staining is less specific because it has high false-positive results as it stains two types of lesions i.e. squamous cell carcinoma and inflamed traumatic tissue.[13, 14] The test is repeated after 10-14 days to eliminate inflamed traumatic lesions which would have healed by then. A second positive test makes biopsy mandatory.[15] Commercially available in ready to use kit (OraScan@: Germiphene, Ontario, Canada) as a 3 component system and other preparations are OraScreen and Oratest.

Table 1:



ii. LUGOL'S IODINE

Richart[7] had proposed that malignant lesions would have limited amount of glycogen, compared to normal epithelium, and used lugol's iodine for delineation of malignant change. Lugol's iodine produces a brown-black stain which is due to the reaction of iodine with glycogen. Normal tissue stains brown and proliferating epithelium is unstained or poorly stained. In oral mucosa, glycogen content varies with the keratinisation of the area of mucosa.[16]

Lugol's iodine solution-[6]

- Iodine -2g
- Potassium iodide -4g
- Distilled water -100cc

Toluidine blue and Lugol's iodine staining is used as an adjunct to sound clinical judgement in the diagnosis of risk patients, delineating the extent of the lesion, selecting the site for biopsy and follow up of patients after treatment for cancer. They are used as additional aids in assessing high risk patients and suspicious oral lesions.[6]

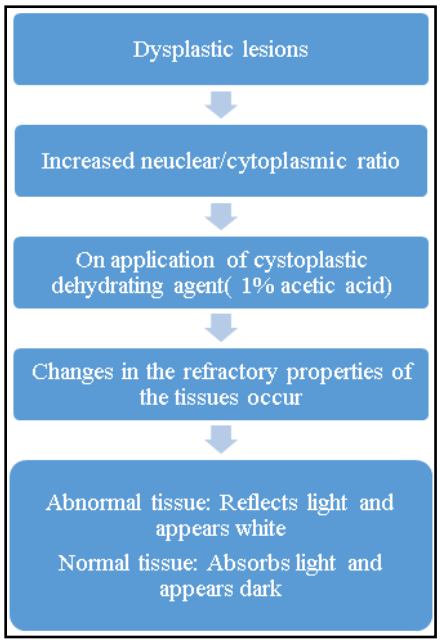
2. VIZILITE

Tissue reflectance has been used for many years as an adjunct in the examination and diagnosis of premalignant and malignant lesions. ViziLite (Zila, Inc.

In Phoenix) is a non toxic chemiluminescent light, the patient should initially rinse the mouth with 1% acetic acid which helps to remove the surface debris and may increase the visibility of epithelial cell nuclei. After rinsing the mouth with 1% acetic acid direct visual examination of the oral cavity is done using blue light.[17] Under this light abnormal epithelium glows differently than normal epithelium thus making it visible, though cannot necessarily say if they are potentially malignant. Normal epithelium absorbs light and appears dark while abnormal epithelium reflects light and appears white, as the nucleus becomes larger due to dysplasia. Lately, Toluidine blue was also added to aid in the marking of an acetowhite lesion for subsequent biopsy once the light source is removed.

The Vizilite kit contains ViziLite rinse (1% acetic acid solution), ViziLite capsule (chemiluminescent light) and a ViziLite retractor (sheath and handle) for single use. Commercially available as ViziLite system (ViziLite Plus with TBlue system; Zilla, Batesville, AR, USA) and MicroLux DL (Zilla, Batesville, AR, USA).

Table 2:



II. PHOTODIAGNOSIS

In photodynamic therapy, a non cytotoxic photosensitizer is localized in neoplastic tissue and gets toxic by light excitation. For photodiagnosis one can study either the natural autofluorescence of the background or the distribution of fluorescence for oxygen fluorochrome or a combination of both. The principle of light induced fluorescence spectroscopy is based on detection of endogenous tissue autofluorescence or exogenous fluorescence of photosensitizers selectively accumulated in tumour tissue excitation and emission of fluorescence depends on how light is scattered and absorbed in tissue. Scattering is caused by difference in the index of refraction of different tissue components and absorption is dependent on the molecular composition of the same components.[18, 19] Two photosensitizers are known to have a high specificity and sensibility for tumour diagnosis: m-THPC (Foscan) and Delta aminolevulinic acid, Levulan.[20] Normal mucosa appears pale green due to the tissue autofluorescence resulting from stimulation with blue light excitation at 400-460 nm wavelength. Dysplastic and malignant lesions appear darker than the surrounding normal tissue as they have decreased fluorescence.

1) 5-Aminolevulinic acid mediated Fluorescence Endoscopic Imaging:
Application of Aminolevulinic acid (ALA)
↓
Produces an endogenous photosensitizer proptoporphyrin (PPIX) within the dysplastic cells
(Precursor in biosynthesis of heme)
↓
These cells can be easily detected by the fluorescence of PPIX.

2) 5-Aminolevulinic acid mediated Digitised Fluorescence Endoscopic Imaging

Wei Zheng et al[22] were the first to present clinical results on diagnosis of early precancerous and cancerous lesions utilising digitised 5-ALA-PPIX fluorescence endoscopic imaging system. The digitised imaging has the capability of acquiring high quality on-line images and quantifying the fluorescence intensity of the diseased oral tissues. Biopsies can be directed by easy assessment of the resection margins.

3) Autofluorescence Spectroscopy

Autofluorescence spectroscopy is also known as laser-induced fluorescence spectroscopy/ fluorescence polarization spectroscopy/ time-resolved fluorescence spectroscopy/ fluorescence lifetime imaging.[23] The use of tissue autofluorescence (non invasive) in screening and diagnosis of precancerous and cancerous lesions have been well documented.[24, 25] This concept is used for diagnosis of dysplastic lesions with changes in the structure and metabolism of the epithelium and sub epithelial stroma when interacting with light. In dysplastic lesions there are complex mixture of alterations due to tissue remodelling such as the

breakdown of collagen matrix and elastin composition as well as decrease in flavin adenine dinucleotide (FAD) concentration, and increase in the reduction form of nicotinamide adenine dinucleotide (NADH) associated with progression of the disease. Fluorescing products in human body are numerous like tryptophan, porphyrins, collagen cross-links, elastin, NADH, FAD.[26] Commercially available are VELscope (LED Dental, Inc. White Rock, BC, Canada) and Identafi (R) 3000 Ultra (Trimira, LLC, Houston, Texas).

4) Fluorescence Photography

K. Onizawa et al[27] have investigated the usefulness of fluorescence photography for the diagnosis of oral cancer. Repeated fluorescence photography shows reduction and diminution of positive fluorescence in cancer regression during treatment. There is an increase in the fluorescence intensity and area if the cancer is progressive and enlarging. There is accumulation of porphyrin compounds in the dysplastic tissue over a period of time. So the positive fluorescence could be due to the accumulated compounds. This system is an adjunct to the diagnosis, ultimately biopsies are mandatory.

III. HISTOPATHOLOGICAL METHODS

1) Exfoliative Cytology

Exfoliative cytology is the microscopic examination of shed cells from an epithelial surface.[28] Treatment procedure, however, cannot be constituted only on the results of exfoliative cytology, biopsy is mandatory. The use of this technique is limited due to increased false negative results, as many lesions have thick keratinised surface which makes it difficult to detect dysplastic changes in a smear.[29] The sample is collected with the help of a cotton swab, a brush, a small wooden stick by gently scraping the lesion.

2) Oral CDx test

Oral CDx test is a highly specialised computer based analysis of an oral brush biopsy specimen. This system uses a specialised brush that collects transepithelial cellular samples composed of free cells and clusters. These samples are fixed onto a glass slide and stained by Papanicolaou test and then analysed microscopically by means of a computer based imaging system. The material needed to perform this is provided in the Oral CDx test kits (OralScan Laboratories Inc, USA). The analysis process is performed utilising a specially designed image processor that detects as few as two abnormal epithelial cells scattered among more than one hundred thousand normal cells.

The results are reported as “negative or benign”, “positive” or “atypical”. Positive indicates definitive cellular evidence of epithelial dysplasia or carcinoma. Atypical indicates abnormal epithelial changes.

Table 3:

Atypical Result	Positive Results	Negative Results
• indicates abnormal epithelial changes requires referal for incisional biopsy	• indicates definitive evidence of dysplasia and patient has to be referred for incisional biopsy	• Indicates no epithelial abnormality

3) Biopsy

A biopsy is the controlled and deliberate removal of tissue from a living organism for the purpose of microscopic examination.[30] Biopsy is considered as a gold standard confirmative test for the diagnosis of oral premalignant and malignant lesions.[31] Different types of biopsies used are Incision biopsy, Punch biopsy and Excision biopsy.

Biopsy is indicated for the following reasons:

- To rule out malignancy for any oral lesion that persists for more than 2 weeks after the exclusion of local irritants.
- To evaluate any mucosa showing a precancerous lesion like erythroplakia or leukoplakia.
- To confirm a clinical diagnosis.

Biopsy is contra-indicated in the following instances:

- In seriously ill patients with systemic disorders that may worsen or secondary complications arise postoperatively.
- In areas that are difficult to access.
- In cases of suspected vascular lesions or unstable coagulopathy, where an incisional biopsy should never be performed because of risk of massive and persistent bleeding.

Incisional biopsy - It is done in lesions that are large (>1.0cm), multiple, diffuse or suspected malignancy. The biopsy specimen should represent a portion of the lesion and a portion of normal healthy tissue. Incisional biopsy should be narrow and deep rather than broad

and shallow. The depth of the incision should be three times the width.[32]

Excisional biopsy- It is reserved for lesions that can be cured through complete removal, that are smaller than 1 cm, that are clinically benign and surgically accessible.[32]

Punch biopsy- Punch biopsy is a useful alternative to traditional scalpel biopsy. The Keyes biopsy punches come in sizes ranging from 1.0 to 12.0cm in 0.5 cm increments.[33]

IV. MOLECULAR METHODS

1) Quantification of nuclear DNA content-

The DNA content of a nucleus is dependent on the number of chromosomes present within it. Polyploidy refers to three or more times the haploid number of chromosomes, while aneuploidy refers to an abnormal number of chromosomes, both associated with dysplasia and malignancy.[34] Atkin and Richards[35] established that the quantitative analysis of DNA-content reflects the total chromosomal content which can be used to distinguish between malignant and normal cells. The flow cytometer is an automated, precise, reproducible, reliable and objective measuring device of cellular DNA content. All ploidy abnormalities are collectively called 'DNA aneuploidy' which independently can be used as a diagnostic and prognostic tool. Gressel-Pietrusky et al[36] investigated and concluded that only severe dysplasia can be detected. Present day flow cytometers with appropriate computer software are able to detect mild, moderate and severe dysplasia of premalignant oral lesions.

2) Tumour markers

Tumour markers are defined as biochemical substances e.g. hormone, enzymes or proteins synthesized and released by cancer cells or produced by the host in response to cancerous substances and are used to monitor or identify the presence of a cancerous growth. They can be present in blood circulation, body cavity fluids, cell membranes and cell cytoplasm.[37]

There is no single marker present in oral cancer that is not present in normal or benign mucosal lesions. The individual analysis applied on their own lack sufficient sensitivity or specificity to be of reliable use as a screening tool for oral cancer.[15]

3) Microsatellite markers:

Polymerase chain-based microsatellite analysis:

It has emerged as one of the most powerful techniques that is used for the amplification of genes and their RNA. It is used to detect mutations in cancer-associated oncogenes, tumour suppressor genes, monoclonality in T and B cell lymphomas, chromosomal translocations, chronic myelogenous leukemia and minimal residual neoplastic disease.[38] It requires only small quantities of DNA and yet yields valuable data and so is frequently used in head and neck cancers.

V. OTHERS

1) Saliva test- Saliva from patients as a diagnostic fluid meets the demands for an inexpensive, non-invasive and accessible diagnostic tool as it serves as mirror of the body. Due to lack of knowledge of disease markers and a low concentration of these markers in saliva when compared to serum, the diagnostic value of saliva is not fully realised. Nowadays, highly sensitive assays such as DNA micro-assay, mass spectrometry and RNA markers at low concentrations in saliva, thus expanding the utility of saliva as a diagnostic tool.[39, 40] Researchers are working on a fascinating new oral cancer screening- a test that can analyse saliva for early gene changes.
2) Fine Needle Aspiration Cytology (FNAC) - It was initially conceived as a means to confirm a clinical suspicion of local recurrence or metastasis of known cancer without subjecting the patient to further surgical intervention. The technique is relatively painless, produces a speedy result and is inexpensive.[41] Any information obtained by FNAC must always be correlated with clinical judgement and with other investigations.

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