Diagnostic aids of oral cancer

The World Health Organization has clearly identified prevention and early detection as major objectives in the control of the oral cancer.

At the present time, screening and early detection of oral cancer is still largely based on visual examination of the mouth.

Adjunctive clinical techniques such as;

1. Toluidine blue,
2. Exfoliative cytology,
3. Brush biopsy,
4. Chemiluminescence
5. Tissue autofluorescence.
6. Photodynamic Detection (PDD)

have been suggested to increase our ability to identify areas of dysplasia/early OSCC that are not visible to naked eye.

1. **Toluidine blue staining**

Toluidine blue (tolonium chloride), is a vital dye that is believed to stain nucleic acids. Hence, it has been used as an aid to the identification of clinically occult mucosal abnormalities and as a useful way of demarcating the extent of a potentially malignant lesion prior to excision

**To perform Toluidine blue test,**

Have the patient first rinse his mouth with water

Aspirate excess saliva with suction

Apply one percent acetic acid (a mucolytic agent) with a cotton applicator.

If there is a large deposit of fibrin or debris in an ulcer, remove this by suction.
Place a small amount of one percent toluidine blue on the entire lesion as well as on some of the surrounding oral mucosa.

Instruct the patient to rinse his mouth with water thus washing away the excess toluidine blue.

If the lesion is stained, the test is positive; biopsy immediately.

### 2. Exfoliative Cytology

Microscopic examination of cells desquamated from a tissue surface or lesion as a means of detecting malignancy and microbiologic changes.

**Indications for Oral Cytology**

- Diffused lesions
- Premalignant or malignant lesions
- Patient not indicated for biopsy
- Urgent results

** Procedures in Oral Cytology**

- Fill out the history form completely, adequately describing and identifying each lesion smeared.
- Prepare tongue blade or stainless steel spatula.
- Remove debris from the lesion and stroke the tongue blade firmly across the questionable area.
- Use a separate tongue blade soaked in its own container of water for each lesion smeared.
- Smear the collected cells evenly on a labelled glass slide.
- Immediately fix the slide by immersing it in 95 percent ethyl alcohol.
- Allow the slides to air-dry and send them to an oral or general pathologist for processing and evaluation.
- The final report should contain a general description of the types of cells present (epithelial, inflammatory, etc.) as well as an assessment of malignancy.
• A variant of exfoliative cytology are swabs that look for the presence of bacteria. For example, if a doctor suspects a patient has candida infection he/she may take a swab of the mucosa, deposit the results in a Petri dish, and recheck the dish in 24-48 hours to see if colonies have grown.

3. **brush biopsy**

the technique involves the use of a specially designed circular brush (OralCDx Brush), which is used to sample cells of the suspected epithelial lesion.

**Clinical Technique**

• The brush may be moistened with water or the patient's saliva and applied to the surface of the lesion.

• Contact between the brush and the mucosal surface may be along either the flat end or the rounded surface, with moderate pressure applied.

• The brush is then rotated until pinpoint bleeding is noted, signaling entry into the lamina propria and, thus, obtaining epithelial cells through the full-thickness of the epithelium.

• Removed cells are transferred to a glass slide by distributing the obtained material evenly over the glass surface.

• A fixation step follows immediately by flooding the slide with fixative solution (alcohol/propylene glycol).

• Allowing it to air dry.

• Stained with a modified Papanicolaou test.

• Analyzed microscopically via a computer-based imaging system.

• **Liquid based cytology**

Liquid-based cytology is a painless procedure that utilizes a cell harvester (e.g., brush) to obtain a noninvasive collection of epithelial cells from the various levels of the surface epithelium.

After the brush head is submerged and twirled within a bottle of alcohol-based fixative, the brush head is separated from its flexible plastic shaft.
This technique helps maximize the number of collected cells available for microscopic examination by a pathologist.

**Liquid-Based Brush Cytology**

**Indications:**

- Evaluation of white (leukoplakia) or red (erythroplakia) or mixed red and white (erythroleukoplakia) mucosal lesions for possible premalignant dysplastic changes. [Request PAP staining]

- Evaluation of suspected active herpes simplex infection. [Request PAP staining]

- Evaluation of suspected candidiasis. [Request periodic acid-Schiff (PAS) staining]

- Screening for the 13 different types of oncogenic human papilloma viruses (HPV) including HPV types 16 & 18 which have been shown to cause oropharyngeal and base of tongue squamous cell carcinoma when no risk factors of tobacco use and ethanol abuse are known. [Request HPV staining]

**Contraindications:**

- Proliferative, traumatic, or immune-mediated epithelial lesions (e.g., papilloma, aphthous ulcer, lichen planus, traumatic ulcer, etc.)

- Connective tissue lesions (e.g., fibroma, peripheral ossifying fibroma, pyogenic granuloma, etc.)

- Pigmented lesions (e.g., amalgam tattoo, melanotic macule, nevus, etc.)

**Chemiluminescence**

Clinical inspection of oral mucosa with the aid of chemiluminescent blue/white light (ViziLite system) was recently suggested to improve the identification of mucosal abnormalities.

**Chemiluminescence Technique**

- involves the use of an oral rinse with a 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 510nm).
• 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.

• Hence normal mucosa appears blue, whereas 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.

• More recently, the ViziLite system was modified in order to include the use of Toluidine Blue and a new chemiluminescence device (MicroLux DL) was introduced.

• **Tissue Fluorescence Spectroscopy**

  Fluorescence spectroscopy is a non-invasive technique used in the detection of the soft tissue lesions (early or premalignant changes in the tissue).

  • Every cell of the tissues contains molecules, which have a characteristic feature to become fluorescent when excited by ultraviolet or in a violet range radiation of suitable wavelength.

  • This fluorescence emission, arising from endogenous fluorophores, is an intrinsic property of cells and is called autofluorescence.

• **Tissue Fluorescence Imaging**

  The concept behind tissue autofluorescence is that

  • changes in the structure (e.g., hyperkeratosis, hyperchromatin increased cellular/nuclear pleomorphism),

  • metabolism (e.g. concentration of flavin adenine dinucleotide [FAD] nicotinamide adenine dinucleotide [NADH]) of the epithelium,

  • changes of the subepithelial stroma (e.g. composition of collagen matrix and elastin),

alter their interaction with light.

When tissue illuminated with short wavelength light (usually violet or blue) the cells become excited and then emit longer wavelength light (lower energy).
• **Normal** cells emit green light
• **Dysplastic** cells emit red light

This phenomena is utilised in the detection of cancer using fluorescence spectroscopy

6. **Photodynamic Detection (PDD)**

medical technology (diagnostic technique) involving administration of light activating chemical (photosensitizer to enhance cell fluorescence) to the targeting cells that is stimulated usually by short wavelength light then collected (different wavelength with less energy) and analysed by the spectroscope.

**Surgical biopsy**

Surgical biopsies is still considered the ‘gold standard’ for the diagnosis and can be classified as

1. Excisional biopsy
2. Incisional biopsy

**Aspiration** biopsy is only useful for deep lesions

A number of cutting instruments can be used when performing a biopsy:

• a conventional scalpel,
• a punch,
• B-forceps.

1. **Excisional biopsy**

involves total removal of the lesion, with slight peripheral and in-depth safety margins, applicable to papillomas, fibromas or granulomas.

Such biopsies has a;

• diagnostic
• therapeutic,

Role since complete removal of the lesion is carried out ensuring clear and normal peripheral margins
2. The incisional technique involves the removal of a representative portion of the target lesion and of a part of healthy tissue.

If the lesion is extensive, different samples should be obtained, placing each of them in a separate and adequately identified container.

**The oral mucosal punch;**

is a rapid, simple, safe and inexpensive technique for obtaining a representative sample of most oral zones.

- The instrument consists of a cylindrical cutting blade, tissue cylinders (2 to 8 mm in diameter) can be obtained and the most widely used calibre being 4&5 mm.
- The punch is grasped between the index and thumb, supporting the cylinder over the surface lesion.
- If a small-diameter cylinder is obtained, suturing of the residual wound is usually not necessary, and the bleeding can be contained by simply applying a piece of gauze or surgical dressing.
- The wound heals by second intention, with good aesthetic results.