the mouth with 5-10cc of 1% toluidine blue solution for 30 sec followed by 1% acetic acid solution rinse for 1 min and then rinse with water.[23] The lesion appears pale blue to dark blue in colour depending up on the DNA content present at the site as shown in figure 1. Several studies have evaluated the use of TB as an adjunct in oral cancer screening along with several limitations. Lingen et al listed the limitations as – absence of randomized controlled trials, lack of histological diagnosis as a gold standard and variability in methods of application.[12] Various researchers have putforth that only dark blue staining should be regarded as positive.[24]Studies assessing TB has shown sensitivity 97% - 93% and specificity 92.9%- 73.3%.[25-26] TB should not be used alone but along with other techniques as it can help to localize areas for biopsy or brush biopsy.


Fig.1. Toluidine Staining. A) Buccal mucosa before staining and B) Buccal mucosa after staining.

Lugols solution was introduced by Camillo Golgi. It is used for demarcation of the malignant change producing a brown black stain when iodine reacts with the glycogen content. It helps in differentiating the inflammatory epithelium and normal epithelium.[27] The test starts with 1% acetic acid rinse for 20 sec and then rinse with water followed by application of Lugols Iodine at the lesion with cotton pellet for 10-20 secs.[28] Double staining i.e. with TB and LI serves as useful adjunct in the diagnosis malignant lesions as poorly differentiated lesions do not have glycogen content and therefore do not take up Lugol's iodine stain.

**Chemiluminescence- Reflective Tissue Fluorescence**

Chemiluminiscence is production of light during a chemical reaction. It is being used in the USA since November 2001. It is easy, fast, safe, painless and non invasive system. It consists of a hand held device, disposable light sticks that emit light at wavelength of 430-580nm. The most well known device working on this principle is Vizilite. It involves the use of 1% acetic acid solution rinse for 1 min to remove surface debris followed by blue-white light illumination. The normal epithelium absorbs the light and dysplastic epithelium reflects the light as shown in figure 2. Normal epithelium appears bluish and the dysplastic epithelium appears acetowhite (distinctly white) as shown in figure 3. Early diagnosis of oral cancer can be improved using tolonium chloride.[29] New modified system MicroLux DL has been introduced which uses battery powered fiberoptic visible light source instead of Chemiluminiscence.[30]

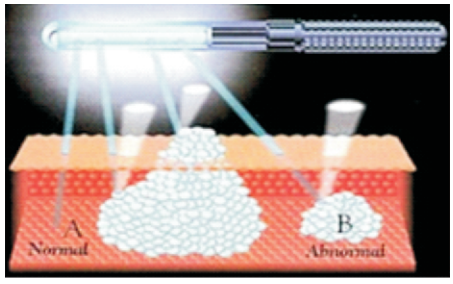


Fig 2. Mechanism of Chemiluminescence. A) Normal epithelium absorbs Vizilite illumination and appears dark. B) Dysplastic epithelium reflects Vizilite illumination and appears white

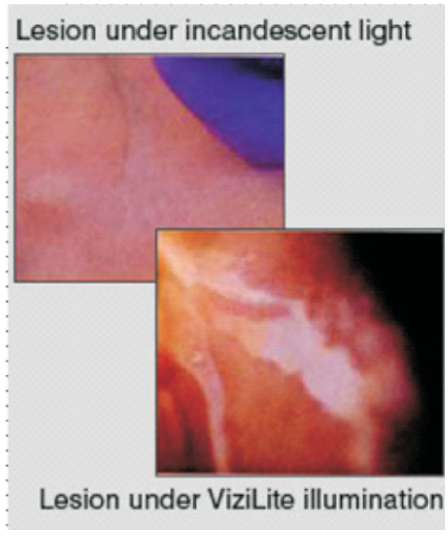


Fig 3.Chemiluminescence using vizilite. A) Lesion under incandescent light. B) Acetowhite lesion under blue-white light.

**Auto Fluorescence- Narrow Emission Tissue Fluorescence**

Auto Fluorescence is the alteration in the interaction of light and epithelium due to changes in structure and metabolism of epithelium.[31] Fluorescence involves the exposure of tissues to a variety of excitation wavelengths so that there is dissimilarity between normal and dysplastic tissues. [12]. VEL scope is the best known autofluorescence tool to aid in early finding of oral cancer.[32] It is a portable device that allows direct visualization of the lesion. The hand held device acts as a light source and the clinician can view through the protected eyepiece (Fig.4). The device provides intense blue excitation light (400-460nm) under which normal mucosa emits pale green auto fluorescence and the dysplastic area absorbs the light and appears dark (Fig 5). The sensitivity ranges from 97% to 98% and specificity ranges from 94% to 100%. [28]

Fig 4. VELscope hand held device

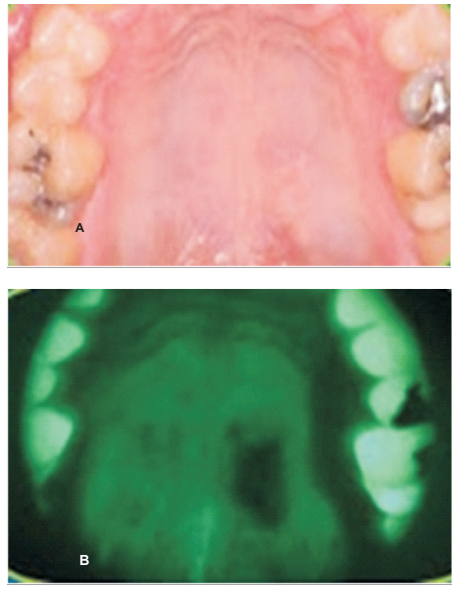


Fig 5. Auto Fluorescence using VELscope. A) Tissue under normal light. B) VELscope shows full extent of the lesion.

**Auto Fluorescence Spectroscopy**

The system consists of small optical fibre which generates a range of excitation

wavelengths and a spectrograph which receives and records on a computer and analyse spectra of reflected fluorescence from the tissue. It is very accurate in distinguishing the normal mucosa from the dysplastic mucosa but as the optical fibre is small in size therefore can analyse a small lesion with well defined boundaries. Research is required in spectroscopy for better clinical applicaition.[31][12]

## Salivary analysis

Saliva as a diagnostic tool for detection of oral cancer is the latest concept. It is a non invasive, easy to collect and inexpensive molecular marker for oral cancer detection. The principle of analysis is based on the fact that the salivary composition is altered. Analysis of salivary macromolecules, enzymes, cytokines, growth factors, metalloproteases, endothelin, telomerase, cytokeratines, mRNA and DNA transcriptase is done.[33] High salivary counts of bacteria such as Capnocytophaga gingivalis, Prevotella melaninogenica and Streptococcus mitis is present in patients with oral cancer. 23 However further studies are required in the field of salivary analysis.

## Optical Coherence Tomography

It is a non invasive method to spot areas of inflammation, dysplasia and cancer by recording surface reflections to construct a cross- sectional image of the tissue. The use of surface Plasmon resonant gold nanoparticles enhances the image.[34]A latest study confirms the viability of using OCT to recognize tissue and cellular changes in malignant cells. But it does not provide definitive diagnosis and also can not differentiate between the lesions.[35]

## DNA Ploidy

It measures the nuclear DNA content to measure cell potential to transform into malignant cell. The cytological samples after staining with Feulgen dye are evaluated with a reference set of cells and a computer assisted analysis identifies difference of cellular DNA content. Cancer succession is contribute by genomic instability and dysplastic lesions are differentiated by atypical DNA content.[36]

## Conclusion

Early detection of oral cancer is important for overall survical of the patient as generally it is diagnosed at late stage. To improve the patient outcome for screening of pre-malignancy condition, non- invasive techniques of diagnosis are very important as the patient might not be willing for any invasive technique when there are no clinical signs of dysplastic lesions that might lead to malignany. Several researches are being done to look for a better and faster non- invasive diagnostic aid for oral cancer.

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