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Review Article

CONVENTIONAL AND ADVANCED DIAGNOSTIC AIDS IN ORAL CANCER SCREENING – THE JOURNEY SO FAR

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ABSTRACT

Oral cancer accounts for a significantly higher percentage of all cancer cases. Survival rates of oral cancers are relatively low in comparison to other major cancers, although incidence rates are as low as 3%. Oral cancer is the most common cancer among Indian males and third most common cancer among Indian females. Delay in diagnosis, metastases, and the presence of secondary tumors are the major reasons for the poor prognosis of oral cancers. Innocuous potentially malignant lesions have higher chances for malignant transformation and early diagnosis of these lesions is necessary for improving survival rates. The patient's overall outcome can be enhanced through early diagnosis and management of these potentially malignant lesions, as the risk factors are well documented for oral cancers. Currently available clinical diagnostic tools developed for the early detection of oral cancers includes toluidine blue dye (TB) (tolonium chloride), Oral brush biopsy, chemiluminescence using Vizilite, salivary diagnostics, and several imaging devices such as Velscope and multispectral optical imaging systems. This paper provides a detailed review about the various diagnostic aids in the detection of oral cancers and also emphasizes a dentist's role in combating this dreadful entity.

Keywords: Oral Cancer, Toluidine Blue Staining, Oral Brush Biopsy, Chemiluminescence.

INTRODUCTION

Head and neck cancers are among the sixth most common human cancers [1], and constitute 3% of all types of cancers [2]. Oral cavity is the site of oral cancer in 48% of cases, and oral squamous cell carcinoma (SCC) accounts for 90% of these cancers. The annual incidence of oral squamous cell carcinoma is more than 300,000. Annual incidence recorded in the United States, Europe and Japan is 35,000 [2]; 40,000 and 10915 respectively [4]. The tongue is the most common site for intraoral carcinomas, constituting around 40% of all cases in the oral cavity proper with the posterior-lateral border and ventral surfaces of the tongue being the most common sites. Second-most common intraoral site is the floor of the mouth, whereas the gingival mucosa, buccal mucosa, labial mucosa, and hard palate are less frequently involved. Recent studies have shown an increasing trend of head and neck cancers, particularly tongue cancer in young adults. Suspected etiological factors include tobacco and its products, drug abuse, environmental factors, and human papilloma virus [5]. Oral carcinomas show high mortality rates with approximately 9,000 deaths every year. Oral cancers may kill one person, every hour, every day and is more dangerous than breast cancer, cervical cancer, and prostate cancer [6].

The American Cancer Society's screening protocol for all head and neck cancers (including oral cancers) states that asymptomatic individuals between the ages of 20 and 40 should be screened every three years and asymptomatic patients after the age of 40 should be screened annually. High risk individuals, such as smokers and alcohol users should be examined every year, regardless of their age [7]. The detection of oral cancer in early stages is quite difficult and any procedure that facilitates the visualization of suspicious lesions may be fruitful in early detection. Alterations in the surface texture, integrity, color, size or contour, and mobility of structures indicates a suspicion for oral leukoplakia or squamous cell carcinoma [8]. Red or white lesions or a long standing ulceration is the characteristic clinical appearances of pre-malignant lesions or malignancies. However, as only a small fraction of these lesions undergo malignant change and an oral examination unfortunately fails to distinguish between lesions that are potentially dangerous from lesions that are benign. Hence, the recent advancements in oral cancer research in the development of potentially useful diagnostic tools at the clinical and molecular levels for the early detection of oral cancers are of outmost importance. However, tissue biopsy along with pathologic assessment remains the gold standard for oral cancer diagnosis [9]. Variousclinical diagnostic tools developed for the early detection of oral cancer include the following-

1. Vital staining (toluidine blue dye, lugol's iodine staining, methylene blue staining)

2. Oral CDx brush biopsy kits

3. Chemiluminescence (vizilite)

4. Tissue fluorescence imaging (Velscope system)

5. Tissue fluorescence spectroscopy

6. Salivary biomarkers

7. Optical coherence tomography

8. DNA ploidy

9. Biopsy

Till date, the above mentioned diagnostic aids are not equivalent or superior to visual inspection and examination [10, 11].

DISCUSSION

Potentially useful diagnostic tools for the early detection of oral cancer are the result of advancements in oral cancer research. Various diagnostic tools in the clinical and molecular levels are-

Vital staining (toluidine blue)

Tolonuim chloride also known as Toluidine blue, (TB) has been used in the past for the detection of mucosal abnormalities of the cervix and the oral cavity. TB is an acidophilic metachromatic dye that has an affinity to selectively stain the acidic tissue components, sulfate, carboxylate and phosphate radicals such as DNA and RNA, but not normal mucosa. Proposed mechanism of action is-

(a) Nuclei of malignant cells have an increased uptake of the dye, and this manifests as increased DNA synthesis.

(b) Rapid dye penetration through randomly arranged tumor cells.

The patient is asked to rinse the mouth with the dye and the physician then inspects for the areas of blue staining. Depending on the degree of dysplasia, malignant lesions stain dark blue and dysplastic lesions stain different shades of blue [8]. Blue staining in a patient is indicative of biopsy. Occasionally, the normal mucosa may retain a small amount of dye, which can be wiped away with acetic acid. Rough or keratinous surfaces (e. g., the dorsum of the tongue, gingival crevices) may also retain the stain. Non-malignant areas of inflammation occasionally stain with toluidine blue. It is mandatory to re-stain all positive lesions within 14 days to decrease the false positive rate. Patients with previous carcinoma of the upper aero digestive tract can be screened with the use of TB. These patients are at high recurrence risk; therefore, clinicians may add toluidine rinses to their visual examination [8, 12]. TB is highly sensitive and moderately specific for malignant lesions, it is far less sensitive for pre-malignant lesions and a false negative rate of up to 58% has been reported. The various steps of TB staining are summarized in fig. 1a-d.

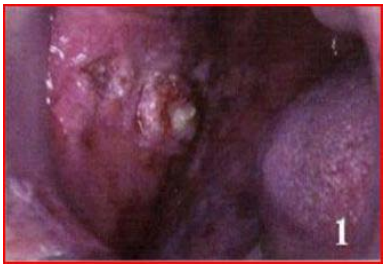

Fig. 1a: Suspected innocuous lesion

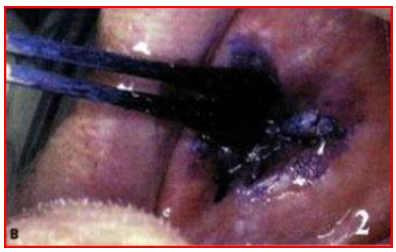

Fig. 1b: Application of 1% toluidine blue dye

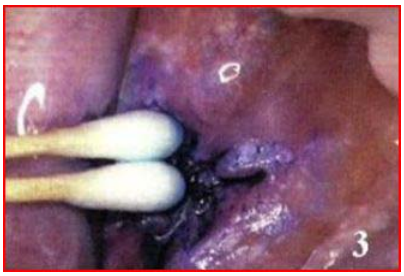

Fig. 1c: Neutralization with 1% acetic acid

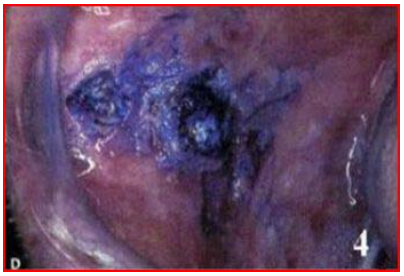

Fig. 1d: Retention of blue color, indicative of malignant lesion fig. reference 1a-1d-

Lugol's iodine staining

Italian Camillo Golgi introduced this stain. Lugol's Iodine solution is formed by two grams of iodine and four grams of potassium iodide in 100 cc of distilled water. After recording the clinical features and photography of the clinically suspicious lesions, 1% acetic acid is applied to the lesional tissue for 20 Sec and then rinsed with water. Later, another photograph is taken following the application of Lugol's iodine at the lesion with a cotton bud for 10-20s.

Lesions showing brown stain is considered as positive while lesions without any retention of stain are considered as negative [13]. The glycogen content present in the normal epithelium forms the basis of selective staining of the intact mucosa with Lugol's iodine. This selective staining helps in differentiating the inflammatory or carcinomatous epithelium from the normal epithelium where the glycogen content is low [14-16].

Staining with TB along with Lugol's iodine (Double staining technique) helps in differentiating the inflammatory lesions. This helps in the clinical determination of the degrees of differentiation of malignant lesions because poorly differentiated malignant lesions without glycogen content do not show Lugol's iodine retention. Pre therapeutic assessment of biologic aggressiveness of the disease can also be made by this double staining technique. Depending on the retention of the dyes, the biopsy site can be determined [17]. Double staining technique is also used for high risk patients and selecting biopsy sites for patients with wide field cancers [18].

Oral brush biopsy

OralCDx (OralScan Laboratories, Inc.) is a computer-assisted oral biopsy system that collects trans-epithelial cellular samples. [fig. 2a]. This system contains a specialized brush for the brush biopsy, a glass slide, a form, a fixative (alcohol/ polyethylene glycol), and a container for sending samples to the CDx laboratory [19]. [fig. 2b]. The procedure involves placement of the brush on the lesion and rotating it for 5-10 times till it produces reddening or haemorrhagic spots [fig. 2c]. The obtained cell material is placed on a dry slide, fixed, and sent for analysis [20-22]. [fig. 2d]. Highly keratinized leukoplakia is a contraindication for the use of this method, as it does not allow enough basal cells to be gathered. Also, inflammatory conditions frequently give atypical results. TB is a fast and relatively simple procedure that does not cause bleeding or require anesthesia. Currently, the technique is combined with molecular analysis. This increases its specificity and permits the identification of genetic anomalies, such as mutations of the tumor suppressing gene p53, epigenetic alterations, and genomic instability. Reported sensitivity values ranged from 71.4% to 100% and specificity from 32% to 100%.

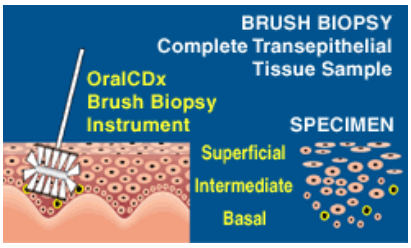

Fig. 2a: Trans-epithelial biopsy system

Fig. 2b: Oral brush biopsy brush

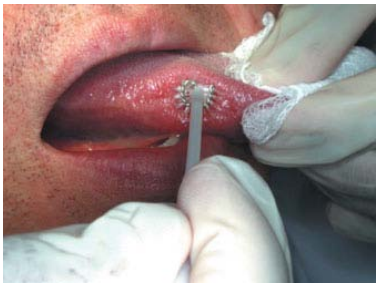

Fig. 2c: Placement of brush on the mucosa, and 5-15 times rotation to obtain trans-epithelial biopsy

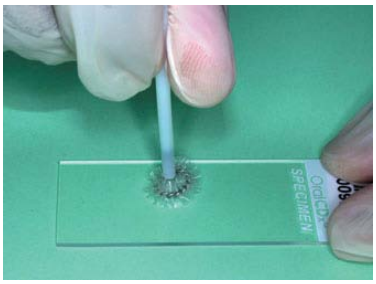

Fig. 2d: Transfer of cells onto glass slide

Chemiluminescence (Vizilite)

Chemiluminescence by definition is the emission of light from a chemical reaction [23]. Vizilite, a diagnostic tool for the early detection of oral cancer is based on the principle of chemiluminescence [fig. 3]. The kit contains 1% acetic acid solution, a capsule with an outer shell of flexible plastic and an inner vial of fragile glass, and a retractor [22]. Activation requires breakage of the glass vial by bending the capsule. This permits the chemical products to react and produce a bluish-white light with a wave length of 430-580 nm that lasts for around 10 min [24]. The procedure involves a one-minute mouthwash with 1% acetic acid solution. Under diffuse bluish-white chemiluminescent light, normal mucosa absorbs the light and appears blue, whereas the light is reflected by abnormal cells with a higher nucleus: cytoplasm ratio and by epithelium with excessive keratinization, hyperparakeratinization, and / or significant inflammatory infiltrate, which appear acetowhite with brighter, more marked, and more distinguishable borders [25-27]. ViziLite® system enhances the clinician's ability to detect oral lesions, particularly white lesions and those with white and red areas. The sharp borders between normal and abnormal oral mucosa is easily delineated by ViziLite plus. Also, the borders observed usually extended beyond than those detected in the visual examination [8, 27]. The majority of these lesions can be diagnosed with incandescent light, and that mouthwash with acetic acid allowed the additional detection of some lesions [25]. The reported sensitivity is 100% and the specificity ranges from 0%-14.2%.

Fig. 3: Chemiluminescence with vizilite

Tissue fluorescence imaging (Velscope system)

Use of tissue autofluorescence has been used for the screening and diagnosis of pre-cancerous and early cancers of the lung, uterine cervix, and skin. More recently, it has been used in the oral cavity. The changes in the structure and metabolism of the epithelium and sub-epithelial stroma alter their interaction with intense blue light (400 to 600 nm) [10, 28]. Velscope system is a tissue fluorescence imaging system used for inspection of the oral mucosa [10, 12, 29]. [fig. 4]. Under the intense blue light (400 to 600 nm), normal oral mucosa emits a green auto-fluorescence, whereas abnormal areas absorb the fluorescent light and appear dark. Hence, early detection of pathological lesions is possible by detecting the early biochemical changes even before their evident appearance. Velscope system seems to be very promising due to its ability and effectiveness in identifying the visually occult lesions and lesion margins that are occult to visual examination under white light [29]. The sensitivity values range from 97% to 98% and specificity from 94% to 100%.

Fig. 4: Autofluorescence with velscope

Tissue fluorescence spectroscopy

This system consists of a small optical fiber that produces various excitation wavelengths and a spectrograph that receives records and analyzes the spectra of reflected fluorescence from the tissue [10, 12, 29]. This technique accurately distinguishes malignant lesions from healthy oral mucosa with high sensitivity and specificity [10, 12, 29]. As the optical fiber can sample only a small mucosal area, this technique is not suitable to detect new lesions or to demarcate large lesions [29],thus limiting the use of spectroscopy for evaluating well-defined small mucosal lesions that has been already diagnosed through clinical inspection, with the attempt to clarify its benign or (pre) malignant nature [fig. 5a and b].

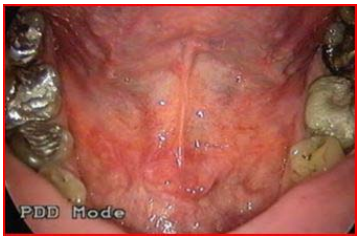

Fig. 5a: Suspected lesion

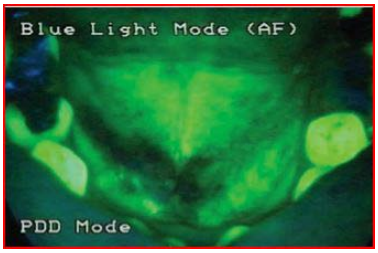

Fig. 5b: Spectroscopy revealing green color, suggestive of malignancy

Salivary biomarkers

Saliva may be used as a diagnostic tool for molecular biomarkers for oral cancer detection. Saliva is a mirror of the body and reflects normal and disease states and its use as a diagnostic fluid meets the demands of an economic, easy to collect and non-invasive diagnostic tool.

Saliva, as a diagnostic tool, has many merits over serum-

- Saliva collection is a non-invasive procedure.
- Non costly
- Large populations can be screened.

Measurement of specific salivary macromolecules and examination of proteomic or genomic targets such as enzymes, cytokines, growth factors, metalloproteinase, endothelin, telomerase, cytokeratins, mRNAs, and DNA transcripts can be done by the saliva [23-32]. Cyfra 21-1, TPS, carcino-embryonic antigen (CEA), SCC, CA125, and CA19-9 are the most studied epithelial serum circulatory tumor markers in the saliva of carcinoma patients.

Optical coherence tomography

Optical coherence tomography (OCT) is a non-invasive tomographic imaging modality. The technique detects areas of inflammation, dysplasia, and cancer by recording subsurface reflections to build a cross-sectional architectural image of the tissue. Contrast enhancement of the images may be done with the use of surface plasmon resonant gold nanoparticles [33]. The imaging range of OCT technology suitable for the oral mucosa is with a tissue penetration depth of 1 mm to 2 mm [34-36].

DNA ploidy

DNA ploidy measures the nuclear DNA content. The cytological samples after staining with Feulgen dye are compared against a reference group of cells, and a computer-assisted analysis identifies deviations of cellular DNA content. Cancer progression is contributed by genomic instability, and dysplastic lesions are distinguished by abnormal DNA content [37].

Biopsy

Scalpel or punch biopsy and histopathology remains the gold standard for diagnosis of potentially malignant disorders. The diagnosis of mild and moderate dysplasias and determination of early-stage invasion of carcinoma *in situ* (CIS) or squamous cell carcinomas (SCC) is dependent upon the variations amongst the pathologist's findings [38, 39]. Adequate sampling of oral lesions is an important factor for the histopathological diagnosis of oral SCC. Histopathological changes may be seen even when visual examination fails to detect an oral lesion clinically (e. g. "Field cancerization,") [40].

CONCLUSION

Potentially malignant disorders pose an important threat to the overall survival of an individual. Oral health professionals play a major role in early detection and treatment of these disorders, thus combating these dreadful lesions and improving the prognosis. A wide variety of diagnostic aids are currently available which are used for early detection of these disorders.

CONFLICT OF INTERESTS

Declared None

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