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Original Research

Diagnostic aids used in the early detection of oral cancers

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ABSTRACT:
Cancer of the oral cavity is the sixth most common malignancy reported worldwide, and it has one of the highest mortality rates among all cancers. In India oral cancer is the most prevalent cancer in men and the third most prevalent cancer in women, and it makes up 40% of all cancers in the country.[1] In India and Southeast Asia, the chronic use of betel quid (paan) in the mouth has been strongly associated with an increased risk for oral cancer. Early oral cancers and precancerous lesions are often subtle and asymptomatic. Historically the screening of patients for signs of oral cancer and precancerous lesions has relied upon the conventional oral examination. A variety of commercial diagnostic aids and adjunctive techniques are available to potentially assist in the screening of healthy patients for evidence of otherwise occult cancerous change or to assess the biologic potential of clinically abnormal mucosal lesions. The increased public awareness of oral cancer made possible by the marketing of recently introduced screening adjuncts is commendable. The tantalizing implication that such technologies may improve detection of oral cancers and precancers beyond conventional oral examination alone has yet to be rigorously confirmed.Therefore the objective of this review is to assess the effectiveness of different diagnostic aids in the early detection of precancerous and cancerous lesions in the oral cavity.[3,4]
Key words: Diagnostic aids, early detection, oral cancer

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INTRODUCTION

Cancer of the oral cavity is the sixth most common malignancy reported worldwide, and it has one of the highest mortality rates among all cancers. In India oral cancer is the most prevalent cancer in men and the third most prevalent cancer in women, and it makes up 40% of all cancers in the country.[1] In India and Southeast Asia, the chronic use of betel quid (paan) in the mouth has been strongly associated with an increased risk for oral cancer. Early oral cancers and precancerous lesions are often subtle and asymptomatic.

Despite advances in surgery, radiation and chemotherapy, the mortality rate associated with oral cancer has not improved in the last 40 years. Ultimately, 50% of the people who have oral cancer die as a result of malignancy. Treatment of oral cancer often produces dysfunction and distortions in speech, mastication and swallowing, and dental health. It can also affect the patient's ability to interact socially, hence it must be considered among the most debilitating and disfiguring of all cancers.[2]

Much of the contemporary oral oncology research is focused on the identification of clinical, histopathologic and molecular biologic parameter of oral precancers and cancerous lesions which might aid in the recognition of those lesions with the highest risk of transformation. Over the years numerous diagnostic aids have been suggested in oral cancer detection but none of them have proved entirely satisfactory.Therefore the objective of this review is to assess the effectiveness of different diagnostic aids

in the early detection of precancerous and cancerous lesions in the oral cavity.[3,4]

Historically the screening of patients for signs of oral cancer and precancerous lesions has relied upon the conventional oral examination. A variety of commercial diagnostic aids and adjunctive techniques are available to potentially assist in the screening of healthy patients for evidence of otherwise occult cancerous change or to assess the biologic potential of clinically abnormal mucosal lesions. The increased public awareness of oral cancer made possible by the marketing of recently introduced screening adjuncts is commendable. The tantalizing implication that such technologies may improve detection of oral cancers and precancers beyond conventional oral examination alone has yet to be rigorously confirmed.

CLASSIFICATION OF DIAGNOSTIC AIDS USED IN CANCER DETECTION[5]

1. CLINICAL METHODS

- Vital Staining
- Toluidine Blue
- Lugol' s Iodine
- Vizilite
- Velscope

2. PHOTODIAGNOSIS

- 5-Aminolevulinic acid mediated Fluorescence Endoscopic Imaging
- 5-Aminolevulinic acid mediated Digitized Fluorescence Endoscopic Imaging
- Autofluorescence Spectroscopy
- Fluorescence Photography

3. HISTOPATHOLOGICAL METHODS

- Exfoliative cytology
- Oral CDx system
- Scalpel Biopsy
- FNAC

4. MOLECULAR METHODS

- Quantification of nuclear DNA content
- Tumour markers
- Microsatellite markers

I. CLINICAL METHODS

1. VITAL STAINING

In vivo vital staining has been used extensively in gynaecology for the detection of malignant change of the cervix via colposcopy. Richart found that toluidine blue would stain areas of cervical carcinoma in situ and delineate abnormal from normal epithelium. In contrast, Lugol's iodine is retained in normal squamous epithelial cells, but not in dysplastic or malignant cells of squamous epithelium of the cervix. Oral carcinoma in situ and early invasive oral carcinoma demonstrate affinity for toluidine blue dye. Lugol's iodine and toluidine blue have been used together in the detection and the diagnosis of oral lesions.

TOLUIDINE BLUE

Toluidine blue (tolonium chloride) is an acidophilic metachromatic dye of the thiazine group which selectively stains acidic tissue components (sulfates, carboxylates and phosphateradicals), thus staining DNA and RNA. It stains mitochondria DNA, cells with greater than normal DNA content or altered DNA seen inmalignant cells. In addition, malignant epithelium may contain intracellular canals that are wider than normal epithelium, which may facilitate penetration of the dye.

TECHNIQUE FOR THE TOPICAL APPLICATION OF TOLUIDINE BLUE:[6]

- Rinse the mouth twice with water
- (20 seconds each).
- Rinse mouth well with 1% acetic acid
- (20 seconds).
- Gently dry suspicious mucosal areas with gauze (do not abrade tissues).
- Apply I % toluidine blue solution to the lesion with a cotton swab.
- Rinse again with acetic acid
- (approximately 150 ml for one minute)
- Rinse with water.
- Repeat in two weeks if positive staining occurs,
- Biopsy all sites which stain positive on two successive visits.

Toluidine blue stains two types of lesions: squamous cell carcinoma and inflamed traumatic areas. Patients with a positive test are required to be retested in 10 to 14 days to eliminate inflamed traumatic lesions which will have healed by this time. A second positive makes biopsy mandatory. Toluidine blue is commercially available in a ready-to-use kit (OraScan@: Germiphene, Ontario, Canada) as a 3 component system. One component is a flavored 1 % Toluidine blue 10 ml solution. The other two are pre- and post-rinse solutions consisting of flavored 1 % acetic acid. The purpose of the pre-rinse is to remove excess saliva and provide a consistent oral environment. The post-rinse reduces the overall background level of staining and facilitates identification of suspect lesions.Other preparations are Orascreen and Oratest.

LUGOL'S IODINE

Lugol's iodine, also known as Lugol's solution, first made in 1829, is a solution of elemental iodine and potassium iodide in water, named after the French physician J.G.A. Lugol. Lugol's iodine solution is often used as an antiseptic and disinfectant, for emergency disinfection of drinking water, and as a reagent for starch detection in routine laboratory and medcal tests.

Richart had proposed that malignant cervical lesions would have a limited amount of glycogen, compared to normal squamous epithelium, and used Lugol' s solution for delineation of malignant change. Normal tissue stains brown, but proliferating epithelium is unstained or poorly stained. Lugol's iodine solution produces a brown-black stain by reaction of the iodine with glycogen. Glycogen content is inversely related to the degree of keratosis. In the oral mucosa, the glycogen content varies with the keratinization of the area of mucosa. Thus uptake of Lugol's iodine in keratinized lesions should be reviewed with caution.

MECHANISM OF ACTION OF LUGOLS IODINE[7]

- Squamous epithelium contains glycogen, whereas precancerouslesions and invasive cancer contain little or no glycogen.
- Iodine is glycophilic and is taken up by the squamous epithelium,staining it mahogany brown or black.
- Columnar epithelium does not change colour, as it has no glycogen.
- Immature metaplasia and inflammatory lesions are at most onlypartially glycogenated and, when stained, appear as scattered, illdefineduptake areas.
- Precancerous lesions and invasive cancer do not take up iodine (asthey lack glycogen) and appear as well-defined, thick, mustard orsaffron yellow areas.

The use of toluidine blue and Lugol's iodine as an adjunct to sound clinical judgement is of value in the diagnosis of patientsat risk, selecting the site for biopsy and follow-up of patients after treatment for cancer. These tissue stains should be used as additional aids in assessing high-risk patients and suspicious oral lesions.

FORMULATION OF TISSUE STAINS

Toluidine blue solution	Lugol's iodine solution
Toluidine blue 1g	Iodine 2g
Acetic acid 10cc	Potassium iodide 4g
Absolute alcohol 4.19cc	Distilled water 100cc
Distilled water 86cc	
pH adjusted to 4.5	

2. VIZI LITE

Vizilite (Zila, Inc. in Phoenix) is a non toxicchemiluminescent light that is shined in the mouth. Under this light, abnormal tissue glows differently from normal tissue thus making it more visible, though cannot necessarily tell if they are potentially cancerous. **The Vizilite (Oral Lite)** is a chemiluminescent light source system indicated for use as an adjunct to conventional oral mucosal screening by trained health care providers for the identification, evaluation, and monitoring of oral mucosal abnormalities in a population at increased risk for oral cancer.

VIZILITE TEST KIT COMPONENTS EACH KIT CONTAINS

- Vizilite Rinse (1 % Acetic Acid Solution)
- Vizilite Capsule (chemiluminescent light stick)
- Vizilite Retractor (sheath and handle) for a single use

PHYSICS OF VIZILITE TECHNOLOGY

Normal epithelium absorbs Vizilite; appears dark. Abnormal epithelium reflects Vizilite; appears white. As a cell becomes more dysplastic the nucleus becomes larger compared to the rest of the cell. The enlarged nucleus reflects light and thus appears white.

PRINCIPLE OF ACTION

Following application of a cytoplasmic dehydration agent such as an acetic acid solution, leukoplakic lesions are seen with changes in refractile properties that occur in atypical nonkeratinized squamous epithelium due to an increased nuclear: cytoplasmic ratio. Supplementing conventional projected incandescent illumination (conventional light) with diffuse chemiluminescent light (Vizilite) has been clinically shown to increase the detection of biopsy proven squamous cell dysplasia and malignancy in squamous epithelium in the lower female genital tract when compared with both detection by the naked eye and detection with magnified visualization with incandescent light.

THE VIZILITE BLUE ORAL LESION IDENTIFICATION AND MARKING-SYSTEM

It isa three- component swab system which is indicated as an adjunct to the **ViziLite Test** for oral

mucosa lesions, for further evaluation and monitoring of lesions by providing physical marking of lesions already differentially identified with ViziLite in a population at increased risk for oral cancer.The ViziLite Blue Oral Lesion Identification and Marking System is notbeing proposed for use in the initial oral mucosal examination without initial lesion identification with ViziLite.

VELSCOPE

VELscope can be used to literally see beneath the surface to detect potentially dangerous growths we might have otherwise missed. The VEL scope utilizes the natural phenomenon of tissue fluorescence, the emission of light by healthy tissue, to locate suspect tissue that would otherwise be invisible. The VELscope system consists of a light source, light guide and a Viewing handpiece.[8]

It emits a safe, visible, blue light into the mouth, which excites healthy mucous tissue to fluoresce an apple-green colour. Since most pre-malignant cancerous changes start below the surface, they are often not apparent to the eye until they progress to the surface. However, as soon as these changes occur, they disrupt the fluorescent ability of the tissue, making their presence known early on. According to clinical studies, the VELscope system detects 98% of tumors and pre-tumors. The VEL scope exam takes about two minutes, involves no pain or inconvenience, is completely safe, and can be done as part of a regular hygiene check-up.

I. PHOTODIAGNOSIS

There are intensive researches on the use of optic methods for early diagnosis, based on fluorescence measurements. These techniques, called medical optoelectronic, come from cancer photochemotherapy and have application in optical biopsies. In photodynamic therapy (PDT) a non cytotoxic photosensitizer localizes preferentially in neoplastic tissue and gets toxic by light excitation. For the photodiagnosis one can study either the natural auto fluorescence of background of the concerned tissue, or the distribution of fluorescence for an oxygen fluorochrome, like those used in photochemotherapy, or the combination of both.

LIGHT-INDUCED FLUORESCENCE (LIF) SPECTROSCOPY

LIF spectroscopy has shown the potential to improve the diagnostic accuracy and efficacy for early cancer detection in various organ sites including the oral cavity. The principle of LIF spectroscopytechnique is based on detection of endogenous tissue autofluorescence or exogenous fluorescence of photosensitizers selectively accumulated in tumor tissue.[9]

USE OF FLUORESCENT MARKERS

Fluorescence is the re- emission of energy in the form of light by a drug at the moment of its return to the fundamental energy level after energy absorption (excitation). Several conditions have to be fulfilled for the drugs to fluorescence with good quantum efficiency. Studies are done based on photosensitizer's preferential fixation in cancer tissues. Two photo sensitizers are known to have a high specificity and sensibility for tumour diagnosis: m-THPC (Foscan) and Delta aminolevulinic acid, Levulan.

1) S-Aminolevulinic acid induced Fluorescence Endoscopic Imaging

Aminolevulinic acid (ALA), a precursor in the biosynthesis of heme, induces the production of the endogenous photosensitizer protoporphyrin. After oral administration or topical application of ALA and the synthesis of PPIX within the dysplastic cells, these cells and tumors can be easily detected by the fluorescence of PPIX. In oral cavity, ALA is used for the detection of neoplasms and dysplastic tissue.[10]

Fluorescence endoscopy system (Karl Storz Endoscope, Karl StorzGmbH& Co., Tuttlingen, Germany)is interfaced to a PC-basedimage acquisition and analysis system for fluorescence diagnosticimaging of human oral lesions in the clinic. The light source is a 100W xenon short arc lamp (Karl Storz D-light system, Karl StorzGmbH & Co.) with filter options for white light (WL) andfluorescence (FL) excitation. Fluorescence excitation light isfiltered by a band pass filter (380–440 nm), whereas the emittedfluorescence signal is filtered by a long pass observation filter(cut-on wavelength 450 nm).

2) IMAGE ANALYSIS

The fluorescence images acquired areanalysed to extract the red, green and blue (RGB) intensities, as well as the hue, saturation andintensity (HSI) values of the images. The red-to-blue (R/B) andred-to-green (R/G) intensity ratios are also calculated for each image. Among the images acquired for each site, three sharpest images are selected to obtain the mean values of R/B, R/G, H, Sand I. These mean image parameters are used to distinguish thetissue type at three levels: (1) Normal vs hyperplastic tissue (2) Normal vs SCC tissue

(3) Hyperplastic vs SCC lesions.

3) AUTOFLUORESCENCE SPECTROSCOPY

Autofluorescence spectroscopy is a promising tool for oral cancer detection, It is non-invasive and easily applicable for the detection of alterations in the structural and chemical compositions of cells, which may indicate the presence of diseased tissue. Autofluorescence of tissues is due to several endogenous fluorophores. These comprise fluorophores from tissue matrix molecules and intracellular molecules like collagen, elastin, and nicotinamide adenine dinucleotide phosphate

(NADH). Autofluorescence spectroscopy can be useful in guiding the clinician to the optimal location for biopsy. Its reliability might be improved by using a reference database of spectra from healthy mucosa.[11]

4) FLUORESCENCE PHOTOGRAPHY

K. Onizawa et al have investigated the usefulness of fluorescent photography for the diagnosis of oral cancer. Repeated fluorescence photography revealed reduction and diminution of positive fluorescence associated with cancer regression due to irradiation or antitumor agents. The intensity and area of positive fluorescence increased with progression and enlargement of carcinoma. Based on this study it is suggested that fluorescence in epithelial dysplasia might be different from that of malignant ulcerative lesions because the frequent release of blood constituents to the surface is unlikely. Accumulation of porphyrin compounds in dysplastic tissue, has been shown in experiments and positive fluorescence in dysplasias could be in part due to accumulated endogenous porphyrin compounds.

3. HISTOPATHOLOGICAL METHODS

1) Oral Exfoliative Cytology

Exfoliative cytology is the microscopic examination of shed cells from an epithelial surface. it is recommended as a diagnostic aid for studying the malignant potential especially of ulcerated and erythematous oral lesions.

THE CYTOLOGIC SMEAR IS USUALLY REPORTED BY THE CYTOLOGIST INTO ONE OF FIVE CLASSES[12]

- Class I: (Normal) indicates only normal cells.
- Class II: (Atypical) indicates the presence of minor atypia but no malignant changes.
- Class III: (Indeterminate) the cells display wider atypia that may be suggestive of cancer, precancerous lesions or carcinoma in situ. Biopsy is recommended.
- Class IV: (Suggestive of cancer) A few cells with malignant characteristics or many cells with borderline characteristics. Biopsy is mandatory.
- Class V: (Positive for cancer) obviously malignant cells. Biopsy is mandatory.

2) ORALCDX TEST

The OralCDx Test is a highly specialized, computer-assisted analysis of an oral brush biopsy performed in the dental office. The computer-assisted analysis of the brush biopsy specimen is performed at Oral Scan Laboratories. The brush biopsy instrument and all of the other materials needed to perform the procedure are included in Oral CDx test kits

THE BRUSH BIOPSY SAMPLE ANALYSIS

The Brush Biopsy (CDx Laboratories, Suffren, NY) was introduced as a potential oral cancercase-finding device in 1999.In addition to the pathologist's manual microscopic analysis, each brush biopsy specimen is scanned by the OralCDx computer. This consists of a neural-networkbased, image-processing system, specifically designed to detect oral epithelial precancerous and cancerous cells. It searches the brush biopsy specimen for a combination of abnormal cellular morphology and abnormal keratinization, which uniquely characterizes dysplasia and carcinoma of the oral epithelium. The image analysis process is performed utilizing a specially designed image processor that can detect as few as two abnormal oral epithelial cells scattered among more than one hundred thousand normal cells typically distributed on each oral brush biopsy specimen.

3) BIOPSY

A biopsy is the controlled and deliberate removal of tissue from a living organism for the purpose of microscopic examination. It is considered to be confirmative for evaluating the cancerous or precancerous nature of a lesion. The techniques used to perform a biopsy of the oral mucosal lesion are Incision biopsy, Punch biopsy and Excision biopsy**.**

FNAC

Needle aspiration biopsy (NAB), also known as fine needle aspiration cytology (FNAC), fine needle aspiration biopsy (FNAB) and fine needle aspiration (FNA), is a diagnostic procedure sometimes used to investigate superficial (just under the skin) lumps or masses. In this technique, a thin, hollow needle is inserted into the mass to extract cells that, after being stained, will be examined under a microscope. Fine needle aspiration biopsies are very safe, minor surgical procedures. Often, a major surgical (excisional or open) biopsy can be avoided by performing a needle aspiration biopsy instead.[13]

IV. MOLECULAR TECHNIQUES

1) QUANTIFICATION OF NUCLEAR DNA CONTENT

The DNA content of a nucleus is dependent upon the number of chromosomes present within it. Polyploidy refers to three or more times the haploid number of chromosomes, whilst aneuploidy refers to an abnormal number of chromosomes; both are associated with epithelial dysplasia and malignancy." Atkin & Richards established that the quantitative analysis of DNA-content reflects the total chromosomal content which can be used to distinguish between malignant and normal cells.

2) TUMOUR MARKERS

Tumor 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. Tumor markers may be present in blood circulation, body cavity fluids, cell membranes and cell cytoplasm. Biomarkers are grouped into 3 classes: Genomic markers, Proliferation markers, and Differentiation markers.[14,15]

3) MICRO SATELLITE MARKERS

- Polymerase Chain-Based Microsatillite Analysis for Allelic Loss

This is one of the more sensitive techniques available for studying clonal changes in tumors and premalignant lesions. It requires only small quantities of DNA yet yields valuable data on the loss of chromosomal regions that contain putative suppressor genes. Hence we can obtain information on critical genetic events even before the identification of the actual suppressor gene. This approach has been used frequently in head and neck cancers. However, frequent occurrence of loss of heterozygosity (LOH) is demonstrated in oral premalignant lesions, and several regions of loss common to squamous cell carcinomas have been observed in dysplastic lesions and hyperplasias. LOHs at 9p and 3p occur early and are present in hyperplastic or mild dysplasias in addition to higher-grade lesions.[16]

Screening and early detection in populations at risk have been proposed to decrease both the morbidity and mortality associated with oral cancer. However, the visual detection of premalignant oral lesions has remained problematic throughout the world. This is in stark contrast to skin lesions such as melanoma, where visual screening has been shown to have sensitivity and specificity rates of 93 and 98 percent.

Further controlled clinical studies are required to confirm the true accuracy, sensitivity and specificity of these new diagnostic methods, since they have shown very inconsistent results, especially in regard to their specificity. It is also necessary to determine whether the cost-benefit relationship of these techniques is positive and to establish their usefulness as ancillary or alternative methods to conventional biopsy.[4]

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