

# World Journal of *Stomatology*

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**EDITORIAL**

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*Sharma G*



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## Diagnostic aids in detection of oral cancer: An update

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### Abstract

Oral cancer is the sixth most common malignancy with almost 500000 new cases reported worldwide annually. The diagnosis of oral cancer at an early stage has a good prognosis as the survival rate is high (around 80%). However, the majority of oral cancer cases are diagnosed at a later stage with a considerably

poor 5-year survival rate of 50% according to World Health Organization statistics. Thus, an effective management strategy for oral cancer will depend on its early identification and intervention which would pave the way for superior prognosis. Despite the obvious advantage of earlier diagnosis of oral cancer, no approach has yet proven to be a reliably successful in diagnosis of oral cancer at an early stage. Currently, the primary line of screening of oral cancer is performed by visual inspection, which is a subjective examination. Among the screening tests or diagnostic aids now available for oral cancer, few (toluidine blue, brush biopsy, salivary and serum bio-markers) have been utilised and studied for many years while others have recently become commercially available. The authors in the present article review all the modalities of screening aids used in oral cancer detection and provide an update on the latest screening tools used in oral cancer detection.

**Key words:** Oral cancer; Diagnostic aids; Brush biopsy; Tissue fluorescence

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**Core tip:** The overall 5-year survival rates for oral cancer have remained low (50%) for the past decades and are considered among the worst of all cancer death rates. Despite the obvious advantage of earlier diagnosis of oral cancer, no approach has yet proven to be a reliably successful in diagnosis of oral cancer at an early stage. Currently, the primary line of screening of oral cancer is performed by subjective visual inspection. Recent advancements in oral cancer research have led to the development of potentially useful diagnostic tools at the clinical and molecular level for early detection of oral cancer.

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## INTRODUCTION

Oropharyngeal malignancies are the 6<sup>th</sup> most frequent category in the world. Squamous cell carcinoma of the oropharynx area and lip is typically delineated as Oral cancer. The survival rates (five-year) for oral malignancies have continued to remain poor (about 50%) for the previous few years and are contemplated amongst the poorest of all malignancy death rates including colorectal cancer, cervical cancer and breast cancer<sup>[1]</sup>. It is also correlated with excessive frequencies of second oral cancers, with nearly one-third of cases experiencing a reappearance of the occurrence of a second primary carcinoma in spite of a rigorous follow-up. Though substantial advances in management of oral cancer have occurred, the timely recognition of oral cancer and its various predecessors continues to be the most excellent strategy to safeguard survival rates of patient and better life quality of patients<sup>[1]</sup>.

The key risk reasons of oral malignancies are tobacco (both smoking and smokeless tobacco) and alcohol abuse<sup>[2]</sup>. Absence of knowledge of the various indications, clinical presentation and predisposing reasons for cancers of the oral cavity are considered to be responsible for the diagnostic delay. Lately, World Health Organization (WHO) highlighted the importance of health care professionals in reducing around 30% of a projected 15 million malignancies and moreover, successfully management of one-third by effectively organizing oral carcinoma examination and diagnostic stratagems<sup>[2]</sup>.

Currently, the primary line for cancer screening is achieved through a subjective visual examination. An improvement in the proficiency of health care workers to identify premalignant conditions and lesions at an initial stage is required to help in early detection<sup>[3]</sup>. This can be accomplished by intensifying public attentiveness concerning the relevance of routine intra-oral examination and the development of diagnostic aids that can be used by the oral health care professionals to swiftly identify oral premalignant lesions<sup>[3]</sup>. Various adjunctive screening techniques have been established to enhance the recognition and dissimilarity between an innocuous lesion and oral premalignant and malignant lesion<sup>[3]</sup>. The progress of diagnostic tools at the genetic level in the prompt and timely diagnosis of oral cancer has further provided another depth to oral cancer research<sup>[1]</sup>.

## CONVENTIONAL ORAL EXAMINATION

Conventional oral examination has various disadvantages like false positive findings, including psychological trauma, over-diagnosis, increased human and financial resources, recognition of varied clinical presentations

of premalignant lesion<sup>[4]</sup>. Only a minor fraction of leukoplakias become cancerous and there is impossibility of distinction amongst cancerous lesions and their equivalents that do not convert into malignancy<sup>[4,5]</sup>.

## BRUSH BIOPSY

In 1999, the OralCDx Brush Test System (Brush Biopsy) was presented as a probable oral malignancy detection method. The examination of clinical lesions that would typically not be subjected to biopsy because the index of suspicion for malignancy was low established upon clinical features were the primary target areas for brush biopsy. There is typically collection of an epithelial sample (including the superficial, intermediate and parabasal/basal layers) of cells from a mucosal lesion. When an atypical or positive outcome is conveyed, the practitioner should supplement with a biopsy (scalpel) of the suspicious condition, since the usage of oral CDx (brush biopsy) does not specifies conclusive finding<sup>[2,4]</sup>.

In a patient with multiple suspicious malignant areas brush biopsy would be valuable, as it is implausible that patient would swiftly comply with numerous scalpel biopsies<sup>[4]</sup>. Brush biopsy would be advantageous in a clinical scenario where patient is improbable to revisit for a continuation intra-oral examination or consents to an instantaneous recommendation to a maxillofacial surgeon<sup>[4]</sup>. Though there is uncertainty of brush biopsy as a diagnostic support in oral malignancies, the thoughtful usage of this technology may be clinically beneficial<sup>[4]</sup>.

## VITAL TISSUE STAINING

Toluidine blue (tolonium chloride) stains mitochondrial DNA, dysplastic cells which have increased DNA content or modified DNA in cancerous cells<sup>[5]</sup>. The local application of toluidine blue (a metachromatic dye) facilitates in recognizing malignant alterations and potential areas of high-grade dysplasia. Lugol's solution is used for delimitation of the cancerous alteration that generates a brownish black stain when glycogen reacts with iodine. The usage of combination of Lugol's iodine and toluidine blue provides a valuable addition for diagnosis of patients who are at an increased risk and for selecting the site for biopsy with wide field cancers prior to management<sup>[6]</sup>.

The overall sensitivity of vital tissue staining for the recognition of malignancies in oral cavity has varied (0.78-1.00) and the specificity has also ranged from 0.31 to 1.00<sup>[4]</sup>. Various disadvantages of toluidine blue staining are no randomized controlled trials, dearth of research studies organized in a primary care setting, lack of usage of gold standard (histopathological diagnosis), dissimilarity in approaches ranging from one time rinse, double time rinse, oral mucosal "painting" and uncertainty of using or defining pale staining which is subjective<sup>[4]</sup>. There is necessity to assess toluidine blue staining with histopathology, genetic and molecular



risk prognosticators and with conclusion (*i.e.*, malignant transformation)<sup>[7]</sup>.

## CHEMILUMINESCENCE

Chemiluminescence [commercially available as ViziLite (Zila, Batesville, AR, United States)] is an intraoral examination diagnostic tool to increase recognition, assessment and scrutinizing of oral mucosal aberrations in patients with increased possibility of malignant transformation<sup>[3]</sup>. Disposable chemiluminescent light packet is used in ViziLite plus whereas the MicroLux unit utilizes a light source which is battery-powered and reusable. The usage of acetic acid (1%) wash is done to eliminate superficial residues and to improve the conspicuousness of nuclei of the epithelial cells, perhaps as an outcome of slight dehydration of the cells. Normal epithelium appears lightly bluish under blue-white illumination whereas aberrant epithelium looks noticeably white in appearance (acetowhite)<sup>[4]</sup>.

The usage of a disposable chemiluminescent light stick which is conveniently hand-held for single time is done that emanates varied light at wavelengths of 430, 540 and 580 nm<sup>[8]</sup>. Light is absorbed by usual epithelium that appears dark while precancerous conditions and lesions emerge whitish. The alteration in colour is correlated to distorted thickness of epithelium and the greater nuclear substance and matrix of mitochondria that specially reflects light in the precancerous lesions and conditions<sup>[8,9]</sup>.

The distinction between cancerous, benign and inflammatory oral lesions cannot be done. Another disadvantage is its increased cost, reduced specificity, increased frequency of false positives, leading to unwarranted biopsies<sup>[10]</sup>. Vizilite has been found to be more accurate in detecting leukoplakias than erythroplakias and red lesions<sup>[11]</sup>. Future research is essential to evaluate the sensitivity and specificity of vital tissue staining with respect to histopathological and clinical attributes and to establish its accurate practicality for standard intra-oral examinations of the oral cavity<sup>[12]</sup>.

## NARROW-EMISSION TISSUE FLUORESCENCE

The usage of tissue auto fluorescence in the examination and identification of premalignant conditions in the cervix, skin and lung has been suitably verified<sup>[8]</sup>. When tissues are exposed to a light of particular wavelength, there is auto fluorescence of cellular fluorophores after excitation (Fluorescence imaging). A visual examination of variation in colours is observed due to cellular changes that modulate fluorophores' concentrations affecting the absorption of light in the cells<sup>[4]</sup>.

Visually Enhanced Lesion Scope (VELscope system; LED Dental Inc., White Rock, B.C.) comprises of light-source (wave length: 400-460 nm) and a component

(manual) to assist in detailed examination or inspection<sup>[12]</sup>. Typically oral mucosal tissues emanate a auto-fluorescence light of green colour but anomalous oral mucosal lesions absorbs the auto-fluorescent light and emerge as darker areas<sup>[12]</sup>. However its routine usage is not corroborated since there is an increased specificity, expense and the absence of scientific verification<sup>[12]</sup>. A recent research revealed that the VELscope was beneficial in substantiating oral premalignant lesions like leukoplakia and erythroplakia. However the difference between greater-risk and lower-risk lesions could not be done<sup>[13]</sup>. However, VELscope system is displaying promise due to its efficiency in identifying mucosal lesions and their borders that are covert to intra oral clinical inspection under white light<sup>[2,14]</sup>.

## CONFOCAL *IN-VIVO* MICROSCOPY

Confocal reflectance microscopy is an optical technology that delivers comprehensive descriptions of tissue structure and morphological characteristics of cell trans-epithelium in real time<sup>[15]</sup>. Confocal *in vivo* microscopy assists the compilation of pathological level high resolution imaging from the tissue for disease recognition in cell biology with an advantage of optical sectioning<sup>[16]</sup>. *In vivo* confocal images from the oral cavity show the distinctive characteristics like variability in nucleus findings that can recognize malignancy from normal oral mucosa<sup>[6,16]</sup>.

## TISSUE FLUORESCENCE SPECTROSCOPY

The illumination of oral cavity tissue with UV-Visible light region results in the absorption of photons by fluorophores. It results in the excitation of fluorophores that causes emission of lower energy photons which are perceived as fluorescence from the mucosal surface<sup>[17]</sup>. The auto fluorescence spectroscopy system contains an optical fibre which is small and similarly generates wavelengths of variable excitations and consists of a spectrograph that collects the continuums of reflected fluorescence from the cellular structures and analyses the received information on a computer<sup>[2,14]</sup>. A study revealed that 405 nm wavelength excitation best differentiates normal oral mucosa with oral premalignant lesions<sup>[18]</sup>. However, a disadvantage is the reduced specificity in recognition of potentially malignant conditions. Auto fluorescence is typically caused by protoporphyrin and the variable concentration of blood components that vacillates proportionately during cancerous progression and retrogression<sup>[19]</sup>. The addition of fluorescence-reflectance or dual digital systems, backscattered light analysis and ultraviolet spectra can overwhelm the disadvantages of auto fluorescence<sup>[17]</sup>.

## COLPOSCOPY

Colposcopy (direct microscopy) is a recognized medical

diagnostic technique used to inspect the tissues of the vagina, vulva, and cervix, carried out under illuminated light with a magnified view of the area of interest<sup>[20]</sup>. Colposcopy provides three-dimensional images of the tissue surfaces examined with portable video cameras attached and viewed on a television monitor screen. The colposcope is mounted with a green/blue filter to enable the inspection of alterations in vascularity and color quality as unfiltered white or yellow light diminishes the dissimilarity concerning the adjoining tissue and the arterioles. An optimum working distance of 200 mm for the focal length of the microscope is required. The accurateness of colposcopy was 70%-98% for the recognition of oral mucosal alterations with a study showing that colposcopy of oral premalignant lesions had benefits in choosing a representative area of biopsy<sup>[20,21]</sup>.

## SALIVARY BIOMARKERS

Greater than a hundred probable oral cancer biomarkers in saliva have been described in the English literature, established primarily on comparing the quantities obtained in oral cancer patients to the quantities acquired in persons who act as controls<sup>[22]</sup>. Various salivary proteins like  $\alpha$ -amylase, interleukin 8, tumor necrosis factor- $\alpha$ , Statherin, CA 125, Endothelin-1, CD44, Catalase, Cyclin D1, CEA, Maspin, Lactate dehydrogenase and Transthyretin have been evaluated<sup>[22]</sup>. Salivary biomarkers are a promising non-invasive approach but there are still some challenges. Absence of calibration for the method of salivary sample collection, variability in processing and storing; extensive capriciousness concentrations of probable oral cancer biomarkers in saliva of both the non-malignant individuals and oral cancer cases and a requirement for additional validation are the few challenges in usage of salivary biomarkers<sup>[22]</sup>.

## CELL AND TISSUE MARKERS

There are numerous categories of cell and tissue markers that could offer supplementary knowledge in addition to intra-oral clinical assessment and pathological analysis<sup>[23]</sup>. Tumour growth markers like epithelial growth factor (EGF), Cyclins, AgNOR, bcl2 and telomerase have been used<sup>[23]</sup>. Three angiogenic biomarkers CD105 and Eph receptor tyrosine kinases (Ephs), vascular EGF and four hypoxia biomarkers (GLUT-1, carbonic anhydrase IX, hypoxia inducible factor 1a, and erythropoietin receptor) were identified as biomarkers<sup>[24]</sup>.

Retinoblastoma protein, p53 and Cyclin-dependent kinase inhibitors are the examples of tumour suppression markers and anti-tumor response<sup>[23]</sup>. The matrix metallo proteins are proteases typically expressed by invasive cancers and the contiguous stroma and their expression has often been reviewed in various studies<sup>[25]</sup>. Cathepsins, Integrins and desmoplakin have also been found as markers of tumor invasion<sup>[23]</sup>. Cytokeratins,

filaggrins, involucrin and glutathione S-transferase have all been investigated<sup>[23]</sup>.

## ELASTOGRAPHY

Lymph node hardness (elasticity) is a major criterion to differentiate between an inflammatory enlargement and a malignant enlargement. Elastography assesses the behaviours of compliance of cellular structure. The compression to tissues generates displacement or strain in the tissue structure and hence by measuring tissue strain, hardness of the tissue can be estimated. The images obtained by elastography are evaluated before and after compression of cervical lymph nodes<sup>[26]</sup>.

## SURFACE ENHANCED RAMAN SPECTROSCOPY

Raman spectroscopy delivers a factual, great - exactitude and sensitive procurement of the molecular tissue structure due to the particular interaction of cellular molecules with photons<sup>[27]</sup>. The spectral characters of lipids, nucleic acids and proteins functions as precise Raman biomarkers to differentiate between malignant and normal oral mucosal area<sup>[27]</sup>. Raman spectroscopy brings knowledge which is corresponding or even advanced to recognized procedures in oral carcinogenesis. The disadvantages are that it is random and nonimaging, requires expensive equipment, extensive process, lack of spatial information and multifaceted algorithms to discern various categories of tissues<sup>[17]</sup>. These concerns impart trials for their forthcoming usage in the clinical setting. Recently, a multispectral optical imaging device for diagnosis of premalignant lesions was done<sup>[28]</sup>.

## OPTICAL COHERENCE TOMOGRAPHY

The recording of subsurface images to develop an overall cross-sectional tissue structural representation is optical coherence tomography. The multimodal distribution of polyethylene glycol linked gold nanoparticles that are antibody-conjugated augments the distinction in *in-vivo* images of cancerous lesions in oral cavity in a hamster model<sup>[29]</sup>. The practicality of managing optical coherence tomography to detect structural modifications in cancerous molecules was observed in a recent pilot research in 27 cancerous patients<sup>[30]</sup>.

## POSITRON EMISSION TOMOGRAPHY

Fluorodeoxyglucose-positron emission tomography (FDG-PET) examination shows proficient precision and prognostic significance in defining lymphatic condition and thus helping in assessment and timely diagnosis of oral malignancy in affected patients<sup>[6,31]</sup>. PET/computed tomography (CT) can identify and distinguish surgical

and radiation-induced variations from residual or recurrent neoplasias because cancerous cells uphold greater FDG for lengthier intervals of time as compared to infectious and inflammatory structures<sup>[32]</sup>. Recent research have revealed that PET/CT had a high (> 90%) accuracy for locating the recurrent tumour<sup>[32]</sup>.

## ROSE BENGAL STAINING

Rose Bengal stain (RB), the 4, 5, 6, 7-tetrachloro-2', 4', 5', 7'-tetraiododerivative of fluorescein, can be utilized as a screening tool to detect oral precancerous lesions<sup>[33]</sup>. Du *et al*<sup>[34]</sup> concluded in a study that RB staining may be better than toluidine blue staining. Future research can ascertain the RB stain as an effective diagnostic tool in the recognition of oral precancerous lesions.

## BIO-NANOCHIP

Recently, a novel bio-nanochip (BNC) sensor which is a fast oral-cytology test that amalgamates the power of cytological morphometric examination with quantification of neoplastic biomarkers was documented<sup>[35]</sup>. Generally, microfluidics technology (lab-on-a-chip) is the adjustment, miniaturization, amalgamation, and automation of analytical laboratory procedures into a solitary chip<sup>[29]</sup>. The conducted study on quantitative BNC method to oral cytology effectively revealed cancerous and pre-cancerous conditions in a short time duration (< 45 min)<sup>[35]</sup>. The recognition of cancerous cells in the BNC sensor utilized membrane-related cell proteins that are especially present on the cellular membrane structure of neoplastic cells<sup>[36]</sup>.

## DNA PLOIDY ANALYSIS

Recent research has described the probable use of DNA ploidy analysis to predict the character of premalignant lesions in oral cavity. Aneuploidy (chromosomal imbalance) in dysplastic cells seen in premalignant lesions, as found by high resolution flow cytometry is suggestive of high possibility of oral malignancy transformation<sup>[37]</sup>. DNA ploidy analysis helps in compensating for intra- and inter-observer irregularity in the grading of dysplasia observed in premalignant lesions and might potentially aid in directing the management of the lesion, and probably suggest more aggressive treatment<sup>[38]</sup>.

## CONCLUSION

The WHO has noticeably recognized prevention and early recognition as the chief objectives in the crusade to limit the cancerous conditions internationally<sup>[2]</sup>. Various diagnostic tests shown promising findings, but lack of their definite substantiation is the major hurdle. Limitations include low sample volumes, absence of systematically comprehensive clinical studies, deficient usage of histopathological and cellular plotting of optically modified mucosa, necessity of more com-

prehensive investigation of reasons and absolute evaluation with other recognition techniques<sup>[2]</sup>. There has been a dramatic escalation in the growth of many probable pre-cancerous investigation techniques and still superior diagnostic aids are required to combat this deadly disease. More exploration is necessitated to confirm the effectiveness of these diagnostic aids and they need to be validated and financially viable to be relevant in the developing nations.

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