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Potentially Oral Malignant Lesions & Oral Cancer- Current & Future Diagnostic Techniques: a Review

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ABSTRACT *Head and neck cancers account for 5 % of all tumors, and about 50 % of head and neck tumors occur specifically in the oral cavity. In 2000, 300,000 of the 615,000 new cases of oral cavity tumors reported worldwide were primary oral squamous cell carcinomas. Despite the recent treatment advances, oral cancer is reported as having one of the highest mortality ratios amongst other malignancies and this can much be attributed to the late diagnosis of the disease. 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. New technologies have provided an exciting new array of clinical diagnostic tools for localizing or emphasizing abnormal mucosa in the dental office, especially potentially malignant lesions. In recent years, screening technologies have become available that supplement the visual examination. The ultimate goals are to reduce mortality and morbidity, and to improve patients' quality of life. This paper emphasizes on various aids in diagnosing potentially malignant lesions or early oral cancer along with their critical evaluation.*

INTRODUCTION:

Oral cancer ranks as the fourth most frequent cancer among men and eighth for women worldwide and may affect the tongue, cheeks, peridontium or any other part of the oral pharynx. Oral cancer is a significant health problem throughout the world.[1] Oral cancer detection at an earlier stage saves lives. The Indian subcontinent accounts for one-third of the world burden.

The 90% of oral cancers are oral squamous cell carcinoma. This cancer, when found early, has an 80 to 90% survival rate[2]. Early detection and management of pre-malignant oral lesions can significantly reduce the progression of these lesions into invasive cancer, and would thus reduce morbidity and mortality.[3-5] WHO has reported oral cancer as having one of the highest mortality ratios amongst other malignancies. [6]

Historically, the screening of patients for signs of oral cancer and potentially malignant 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.[7]

Criteria for screening and for screening tests:
Wilson and Jungner summarized the various different criteria's for oral cancer which should meets at least three of these criteria, screening measures for this condition seem warranted.[8]

Criteria for the screening programme:

1. The disease must be an important health problem
2. Facilities for diagnosis and treatment must be available
3. An accepted treatment must be available for patients with recognised disease
4. There must be a recognizable latent or early symptomatic stage
5. Suitable test must be available
6. Test should be acceptable to the population
7. There should be an agreed policy on whom to treat as patients
8. The natural history of the condition should be adequately understood
9. The screening programme should be (cost)-effective
10. Screening process should be a continuing process and not a 'once and for all' project

In addition, there are a various characteristics that should be considered in the development of an ideal screening test.
Characteristics of a good screening test:[7]

1. Simple, safe and acceptable to the patient
2. Detect disease early in its natural history
3. Preferentially detect those lesions which are prone to cancer
4. Detect lesions which are treatable or where an intervention will prevent progression
5. Have a high positive predictive value and low false negatives (high sensitivity)

A variety of new diagnostic aids and adjunctive techniques are available to potentially assist in the screening of asymptomatic patients for the detection of otherwise occult oral cancerous lesions or potentially malignant disorders. Same tests have been proposed to assess the risk of potentially malignant disorders i.e. their potential to become cancer.[9,10]

Most oral cancer screening programs include the simple visual inspection. The "gold standard" for the detection of early oral cancers is the conventional oral examination (COE) using normal (incandescent) light, during which the dentist identifies oral mucosal lesions that may be cancerous or potentially malignant [11-13] Each of these techniques and devices, including the COE, has limitations and strengths which are summarized in the table 1. Screening methods include the use of toluidine blue[14,15] , brush biopsy (exfoliative cytology)[16,17], chemiluminesce and fluorescence imaging[18]which deal with the diagnosis of lesions that have already been detected by the patient, dentist or other clinician but a definitive diagnosis can only be made by a tissue biopsy & histopathology.

Toluidine blue: Toluidine blue (tolonium chloride) is a metachromatic dye used in histology laboratories to stain nuclear material, it stains DNA very well.[7,19] Its use as a screening tool for oral cancer detection, or as an adjunct to a COE for the identification of oral lesions, is more problematic because

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of significant number of both false positive and false negative reactions that have been associated with this method. Historically, it has proven valuable for demarcating the extent of a lesion prior to surgical removal[11]. In a review of 2008 the performance of visual exam with toluidine blue shows sensitivity ranged from 38 to 98%, while specificity varied from 9 to 93%.[20] In general examination, toluidine blue is associated with low specificity and this has prevented toluidine blue from becoming a standard component of early oral cancer detection efforts in the USA.

Exfoliative cytology: Exfoliative cytology is an easy, non-invasive procedure and hence could be carried out even on slightest suspicion regarding the nature of the given lesion by using PAP stain.[21]

Brush cytology: Brush cytology involves the use of a circular brush designed to obtain a complete trans-epithelial tissue sample which is then smeared onto a glass slide for the subsequent identification of abnormal or malignant cells.[13]

Advantages of this method are that it causes minimal discomfort and less bleeding. However, the accuracy, sensitivity, and specificity of brush cytology for the identification of cancerous and potentially malignant lesions is disputed and a follow-up scalpel tissue biopsy is needed in the case of an malignant cells, result as the technique does not provide a definitive diagnosis.[12]

Light-based detection systems:
Light-based detection systems use chemiluminescent light to enhance the identification of oral mucosal abnormalities. Recently, this form of tissue reflectance-based examination has been adapted for use in the oral cavity and is currently marketed under the names ViziLite Plus and MicroLux DL. ViziLite Plus uses a disposable light packet, while the MicroLux unit offers a reusable, battery-powered light source. Before the examination, the patient must first rinse with a 1% acetic acid solution, and then the oral cavity is examined using a blue-white light source. [11]

The 1% acetic acid wash is used to remove surface debris which may increase the visibility of epithelial cell nuclei, possibly as a result of mild cellular dehydration. Under blue-white illumination, normal epithelium appears lightly bluish while abnormal epithelium appears distinctly white (acetowhite). ViziLite Plus also provide a tolonium chloride solution (TBlue), which is intended to help in the marking of an acetowhite lesion for subsequent biopsy once the light source is removed[8]. In the oral environment, likewise, it makes the keratin more white and, therefore, more visible to the naked eye.[19]

Narrow emission fluorescence
It depends on the natural auto fluorescence of mucosal tissue which is visualized by using a screening device that emits a concentrated blue light (peak wavelength 405–436 nm) [12]. Fluorescence spectroscopy involves the exposure of tissues to a range of excitation wavelengths so that subtle differences between normal and abnormal tissues can be easily identified. On the other hand, fluorescence imaging involves the exposure of oral tissue to a rather specific wavelength of light, which results in the auto fluorescence of cellular fluorophores after excitation. The occurrence of cellular alterations will change the concentrations of fluorophores, which will affect the scattering and absorption of light in the tissue, thus resulting in changes in color that can be observed visually.[22]

The VELscope is a portable device that allows for direct visualization of the oral cavity and is being marketed for use in one of the oral cancer screening system. Under intense blue excitation light (400–460 nm), normal oral mucosa emits a pale green auto fluorescence when viewed through the selective (narrow-band) filter incorporated within the instrument hand piece. Proper filtration is critical, as the intensity of the reflected blue-white light makes it otherwise impossible to visualize the narrow auto fluorescent signal dark by comparison to the surrounding healthy normal tissue. Using this device, Lane et al., investigated the ability of the VELscope to identify potentially malignant lesions.[23]

Optical Biopsy –
The use of light (optical biopsy) in the diagnosis represents a leap into the future. The aim of this procedure was to develop a technique that could act as an adjunct or even a substitute to histopathology which reduce surgical trauma and the workload of pathology departments and services[24-26]

Tissue Biopsy & Histopathology:
The most reliable and popular method for the diagnosis of potentially malignant lesions or oral cancer is tissue biopsy followed by a histopathological evaluation of the tissue specimen[27-28] .

The American Academy of Oral and Maxillofacial Pathology recommends that "all abnormal tissue be submitted promptly for microscopic evaluation and analysis'.[29] In some types of pathology, histopathology is important not only in diagnosis but also to determine whether there is evidence of malignancy, provide information on the clinical behaviour of the lesion, also give prognostic information in some of the cases which directly impact on patient management. It is generally accepted that microscopic or histopathological examination of tissue is the gold standard for the diagnosis of many lesions that present in the oral cavity and surrounding regions.[30]

Biomarkers
The most predictive of the molecular markers assessed in oral Squamous cell carcinoma (OSCC) development include the chromosomal polysomy (DNA ploidy), TSG p53 protein expression, and changes (loss of heterozygozity; LOH) in chromosomes 3p or 9p.[31] The use of biomarkers as adjuncts to routine histopathological examination may help prognostication and effective management of potentially malignant lesions but their routine use is still hampered by the cost and complexity of the tests, the lack of facilities in some laboratories and limited outcome studies till date. More readily available markers, such as those of cell proliferation (Ki-67 antigen) and apoptosis (Bax, Bcl-2), may also play a diagnostic role: apoptotic Bcl-2 expression decreases significantly in dysplastic and early invasive lesions and then increases almost to normal tissue level in consequent stages while Ki-67 expression increases sharply in initial stages of OSCC, but significantly decreases in later stages.[32]

Molecular markers for the diagnosis of OSCC can be quested in 3 levels; (I) changes in the cellular DNA, which result in (II) altered mRNA transcripts, leading to (III) altered protein levels (intracellularly, on the cell surface or extracellularly) as summarized in Table 2.

Saliva as a Perfect Diagnostic Medium
Saliva has been long proposed and used as a diagnostic medium[33-35] because it is easily accessible and its collection is non-invasive, inexpensive, non time-consuming, requires minimal training and can be used for the screening of large population samples[35,36]. Whole saliva can be collected with or without stimulation. Stimulation can be performed with masticatory movements or by gustatory stimulation (citric acid)[37]. Stimulated saliva however, can be collected in larger quantities, but will be little bit altered in content[38,39]. Unstimulated saliva can be collected by merely spitting in a test tube or by leaving saliva drooling from the lower lip [39] and it is more often used for the diagnosis or follow up of systemic diseases. The concept of a saliva test to diagnose OSCC is even more appealing. Promoter [40-47]hypermethylation patterns of TSG p16, O6-methylguanine-DNA-methyltransferase, and death-associated protein kinase have been identified in the saliva of head and neck cancer patients[48]

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The other main techniques currently used in the detection of oral dysplasia are fluorescence, Raman spectroscopy, microendoscopy, elastic scattering spectroscopy and optical coherence tomography.[24-26,49-53]

Optical Coherence Tomography:
Optical coherence tomography (OCT), first applied in 1991 by Huang et al., is a non-invasive, interferometric (superimposing or interfering waves) tomographic imaging modality that allows millimeter penetration with micrometre-scale axial and lateral resolution. OCT has been applied in the head and neck in an attempt to detect areas of inflammation, dysplasia and cancer; results were promising but some studies suffered from poor-resolution images and poor penetration depth.[54-56]

Gold Nanotechnology:
It has great potential for early detection, accurate diagnosis and treatment of cancer.

This technique is simple, less invasive, provides increase contrast for diagnosis of oral cancer, is non toxic to human beings, with no photo bleaching or blinking which is inherent to many other flourophores[57]

Raman spectroscopy:

Raman spectroscopy is a spectroscopy technique based on "inelastic" light scattering of monochromatic light, usually from a laser source since the detected wavelengths are different from that of the applied light. Inelastic scattering means that the frequency of photons in monochromatic light changes upon interaction with a tissue. Fourier transform infrared (FTIR)/Raman spectroscopy has been successfully applied for the diagnosis of OSCC in the hamster cheek pouch model resulting in 100% sensitivity and 55% specificity[58]

Trimodal spectroscopy:
It uses three independent optical diagnostic techniques (fluorescent spectroscopy, diffuse scat-tering spectroscopy and elastic scattering spectroscopy) to achieve better results, reaching sensitivity and specificity of 96% in differentiating between normal oral mucosa, dysplasia and OSCC and a sensitivity of 64% and specificity of 90% in distinguishing between dysplasia and OSCC.[44]

Orthogonal polarization spectral (OPS) imaging for in vivo-visualization of the microcirculation facilitates high resolution images of the oral mucosa. By this technique OSCC are characterized by chaotic and dilated vessels accompanied by numerous areas of hemorrhage.59

Other recent Technique:
Banumathi.A et al 2009 have proposed cyst detection and severity measurement of cysts using image processing techniques and neural network methods. The suspicious cyst regions are diagnosed using Radial Basis Function Network.60

Optical Coherence Tomography (OCT): For the imaging depth of 2-3 mm, OCT is suitable for oral mucosa. They also detect oral cancer in 3-D volume images of normal and potentially malignant lesion proposed by Woonggyu Jung et al.[61]

Genetic Programming proposed by Simon Kent[62] in 1996 is relatively a new technique for the automatic discovery of computer programs which offer solutions to complex problems. This procedure is used to evolve programs which are able to diagnose oral cancer and potentially malignant lesions.

Wavelet - neural networks by Ranjan Rashmi Paul et al[63] - The wavelet coefficients of TEM images of collagen fibers from normal oral sub mucosa and oral submucous fibrosis tissues have been used in order to choose the feature vector which, in turn, used to train the Artificial Neural Network. The trained network could satisfy the normal and precancer stages. Various Other proposed future diagnostic technologies are listed in table 3: [65]

CONCLUSION:
The importance of routine screening to improve early diagnosis of oral malignancies cannot be overemphasized. Worldwide, there has been a call for early detection in at-risk populations to decrease the morbidity and mortality associated with oral cancer visual detection is a well-established and accurate diagnostic method for other types of cancer (for example skin melanoma), the visual detection of premalignant oral lesions remains problematic. Recent advances have shown that the risk of malignant transformation is associated with chromosomal aberrations. The ability to identify lesions and to predict which lesions will undergo malignant transformation would facilitate early diagnosis and subsequent disease management. These adjuncts for detection and diagnosis have the potential to assist in early detection, leading to early diagnosis and improved treatment outcomes.

Table 1: Various Diagnosing aids & their limitations

Detection Method	Limitations	Strengths
Conventional Oral Examination (COE)	• Large numbers of oral mucosal abnormalities are present (5–15%); most are benign • Some potentially malignant lesions may occur in mucosa that appears clinically normal	Gold standard" test • All dental practitioners are trained in this technique · Easy to perform
Brush Cytology	• Accuracy, sensitivity and specificity are controversial • Must be followed by surgical biopsy if abnormal cells are detected	· Minimally invasive · Easy to perform · inexpensive
Toluidine Blue Staining	High number of false positive and false negative reactions	Useful for demarcating the extent of a lesion before the removal of tissue
Light-based Detection Systems (Vizilite and Microlux)	Cannot distinguish between oral malignancy, premalignant lesions, benign keratoses, and other mucosal inflammatory conditions	Enhances proper visualization of oral white lesions
Narrow Emission Fluorescence	Does not reliably identify less severe disease	Distinguishes severe dysplasia and invasive carcinoma from normal tissue

Table 2: List of protein markers and their effects.

Altered protein markers	Changes in the cellular DNA	Altered mRNA transcripts
Elevated levels of defensin-1	Allelic loss on chromosomes 9p	Presence of IL8
Elevated CD44	Mitochondrial DNA mutations	Presence of IL1B
Inhibitors of apoptosis (IAP)	p53 gene mutations	DUSP1 (dual specificity phosphatase 1)
Squamous cell carcinoma associated antigen (SCC-Ag)	Promoter hypermethylation of genes (p16, MGMT, or DAP-K)	H3F3A (H3 histone, family 3A)

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Carcino- embryonic antigen (CEA)	Cyclin D1 gene amplification	OAZ1 (ornithine decarboxylase antizyme 1)
Carcino-antigen (CA19-9)	Increase of Ki67 markers	S100P (S100 calcium binding protein P)
CA128	Microsatellite alterations of DNA	SAT (spermidine/spermine N1-acetyltransferase)
Serum tumor marker (CA125)	Presence of HPV	
Intermediate filament protein (Cyfra 21-1)		
Tissue polypeptide specific antigen (TPS)		
Reactive nitrogen species (RNS)		
Lactate dehydrogenase (LDH)		
Immunoglobulin (IgG)		
Insulin growth factor (IGF)		
Metalloproteinases MMP-2 and MMP-11		

Table 3: Future diagnostic technologies

Laser-induced fluorescence spectroscopy
2 photon fluorescence
Elastic scattering spectroscopy
Light-induced fluorescence spectroscopy
Photoacoustic imaging
Photon fluorescence
Orthogonal polarization spectral (OPS) imaging
Quantum dots Optical coherence tomography (OCT)
Nuclear magnetic resonance spectroscopy
Doppler OCT
Chromoendoscopy
Narrow band imaging (NBI),
Immunophotodiagnostic techniques
Differential path length spectroscopy
2nd harmonic generation
Terahertz imagin

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Table 2: List of protein markers and their effects.
Table 3: Future diagnostic technologies

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