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REVIEW ARTICLE

AVENUES TO EARLY DETECTION OF ORAL PREMALIGNANT AND MALIGNANT LESIONS: A REVIEW OF THE CURRENT TECHNOLOGIES

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ABSTRACT: In spite of the advances in the therapy for management of Oral Cancer the prognosis still remains poor. The survival of these patients is highly dependent on the early detection of these lesions and subsequent, prompt treatment. Scalpel biopsy though considered to be the gold standard for diagnosis is invasive and associated with high morbidity and as such is reserved for evaluating highly suspicious lesions. There is a need to devise tests which are non-invasive, highly specific and sensitive and cost effective too. Various diagnostic tests used nowadays for early detection of malignancy include brush biopsy, toluidine blue staining, Lab On a Chip, Saliva based Oral Cancer Diagnosis, Laser Capture Micro dissection, Spectral Cytopathology, A multispectral digital microscope (MDM), Optical coherence tomography, Oral Auto fluorescence. In this review an attempt has been made to examine the role of these tests and technologies and assess their role in early detection of malignancies.

KEYWORDS: Oral cancer, brush biopsy, saliva, biomarkers, Oral Auto fluorescence, spectral cyto-pathology.

INTRODUCTION: The idea of pre cancer has been a slowly changing and often confusing concept, beginning with the 1805 suggestion by a European panel of physicians that there are benign diseases which will always develop into invasive malignancy if followed long enough[1].With today's definition, a pre cancer is considered to only hold an increased risk of cancer transformation.[2]

Oral squamous cell carcinoma (SCC) is the most common cancer of the head and neck. Each year it accounts for more than 300, 000 cases worldwide, more than 30, 000 cases in the United States and more than 3, 000 cases in Canada. The 5-year survival rate for oral SCC has remained at approximately 50% for the past several decades.[3]

Early detection of cancer is of prime Importance. It helps us to reduce morbidity and mortality. Around 300, 000 patients are annually estimated to have oral cancer worldwide and in India it constitutes 30-40% of cancer load. The rising trend of usage of pan masala, gutka owes to the cancer.[4,5]

A key factor in the lack of improvement in prognosis over the years is the fact that a significant proportion of oral SCCs are not diagnosed or treated until they reach an advanced stage. This diagnostic delay may be caused by patients (who may not report unusual oral features) or health care workers (who may not investigate observed lesions thoroughly),[6-8] and it is presumed that such delays are longer for asymptomatic lesions.

The key to early detection of Dysplasia and Oral Cancer: Early detection of dysplasia and oral cancer can be achieved by a) Screening programmes for general population which could help in

identification of asymptomatic patients with suspicious lesions b) Diagnostic tools for detection of dysplasia and oral cancer in asymptomatic patients with oral abnormality.

Oral Cancer Screening: Early detection of precancerous lesions and cancerous lesions will significantly reduce the mortality and morbidity as has been established from cancer screening programs for a variety of - including the Pap test for cervical cancer and mammography for breast cancer. However, several publications have demonstrated that oral cancer screening has limited value as a method for detecting precancerous or early cancerous lesions. In the only randomized controlled oral cancer screening trial conducted in India and involving over 130, 000 individuals, the authors concluded that visual examination was useful as a method of screening for oral cancer only in high risk cases like chronic smokers or alcoholics.[9]

Oral cancer screening is fraught with problems including the fact that approximately 5-15% of the general population may have an oral mucosal lesion. While the majority of these lesions are benign, clinical inspection alone cannot differentiate which lesions are potentially precancerous and cancerous and which ones are benign. The classic clinical presentation of a premalignant lesion or malignancy includes a red spot, white spot or persistent ulcer. However, only a small percentage of these types of lesions are cancerous and an oral examination unfortunately cannot discriminate between lesions that are potentially dangerous from lesions that are benign. A Cochrane review on this subject failed to find any evidence to confirm or refute the usefulness of screening for oral malignancies.[10]

Early Diagnosis Early detection of oral cancer is one of the most efficient ways to reduce the high mortality from this disease. Early detection can minimize the morbidity of the disease and its treatment, which is associated with a severe loss of function, disfigurement, depression and poor quality of life. However, based upon the National Cancer Institute's SEER program, which collects data on oral cancer, there has been little or no change in the past twenty years in the detection of oral cancers at early stages.[11]

Malignant transformation of dysplasia, which is quite unpredictable, occurs over years - during which time the lesion can be treated, potentially preventing oral cancer from developing. Oral precancerous lesions may also occasionally regress if the healthcare professional motivates the patient to reduce the risk factors including elimination of carcinogens including tobacco and alcohol.

DIAGNOSTIC TESTS:

Cytological Techniques: During the last few decades, oral cytology has resurfaced as the focus of scientific research. However, in contrast to the sampling of cells of the uterine cervix, analysis of surface epithelial cells of the oral cavity and oropharynx by standard exfoliative cytology has proven unreliable so far. The shape of the oral cavity makes it impossible to examine the complete mucosal surface. Without loss of minimal invasiveness, it was not possible to access the deeper cell layers of the oral cavity with conventional exfoliative cytology.[12]

The Brush Biopsy: Dysplastic or immature epithelial cells arise, of course, from the bottom of the squamous epithelium, and should not be expected to be found by scraping a thick surface layer of keratin Oral white patches have a thicker keratin layer than their cervical counterparts.[7]

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Pap smears are used effectively for oral red lesions and oral ulcers to identify infections, especially candidiasis, and atypical cells in erythroplakia, a disease in which dysplastic epithelial cells are typically near the surface. They are seldom used for white keratotic lesions.

The brush biopsy or Oral CDx test has overcome this fatal shortcoming by screwing a bristle-covered wire (the "brush") through the thick surface keratin to the basal layer of the epithelium.[8] This relatively painless procedure captures the deeper epithelial cells on the bristles and the entire brush is sent to a pathology lab, where the cells are removed and plated on a microscopic slide. From that point on, the process is the same as a routine pap smear.

A cytotechnologist, pathologist or, more recently, a computer-associated optical scanner compares the size of each individual cell with the size of its nucleus. Large, dark nuclei are found in dysplastic or immature cells, as are abnormal nuclear shapes (pleomorphism). Results are usually reported out as one of three levels of risk. Recently, liquid-based cytology (LBC) has become a principle methodology in cytopathology replacing conventional smears, owing to better cell recovery and morphologic preservation.[13]

Scalpel Biopsy: The cornerstone for diagnosing premalignant and malignant oral diseases has been tissue sampling by scalpel biopsy and subsequent histological examination. Oral biopsy is an invasive procedure and involves both psychological implications for the patient as well as possible often technical difficulties for the health practitioner. In case of extensive lesions, the most representative areas must be selected in order to avoid diagnostic errors.[14]

An incisional biopsy should be of sufficient size and depth to include part of the advancing margin of tumour as a number of histological characteristics of the primary tumour, such as the grade of malignancy and depth of invasion, have been shown to have prognostic value in terms of tumour recurrence, lymph node involvement, and cause-specific survival.[15]

Certain Experimental studies have revealed an increase in frequency of neck metastasis from stage I and stage II OSCC after incisional biopsy and the presence of tumour cells have been noticed in the peripheral blood 15 min after incisional biopsies using a conventional scalpel.[16,17]

Care should be taken to preserve the Oral biopsy specimens since it can be affected by a number of artifacts resulting from crushing, fulguration or incorrect fixation and freezing.[18] There is a long standing controversy regarding selection both of the technique (incisional versus excisional) and of the surgical instruments used to avoid artifacts; punch biopsy may have some benefits.[19]

Toluidine Blue Staining: Toluidine blue (TB) staining is claimed to be a simple, inexpensive and sensitive adjunct tool for identifying early OSCC and high-grade dysplasia's.[20] Toluidine blue is a member of thiazine group of metachromatic dyes.[21]In vivo staining may identify early lesions which could be missed on clinical examination.[22]

Supra vital stain Toluidine Blue (TB) has been used to mark the area for biopsy and to mark the full extent of premalignant lesion. It has been reported that toluidine blue stains premalignant and malignant lesions, but, not the benign lesions and normal mucosa.[21,23]

When a 1% aqueous TB solution is applied to a suspicious lesion for 30 seconds, this acidophilic metachromatic nuclear stain helps to differentiate areas of carcinoma in situ or invasive carcinoma from normal tissue. Although TB has been found to be highly sensitive and moderately specific for malignant lesions, it is far less sensitive for premalignant lesions with false negative rates of up to 58% reported for identifying mild-to-moderate dysplasia.[24,25]

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Moreover it can outline the full extent of the dysplastic epithelium or carcinoma when excisions are planned.[26] It helps in selecting the biopsy sample site in premalignant lesions. Also, it can help the follow up of patients with oral cancers. Toluidine blue has also been demonstrated to help assess the status of margins around oral cancer at the time of resection.[27]

Lab on a Chip: As we know that Oral squamous cell carcinoma (OSCC) is a disfiguring and deadly cancer. Having made many advances in therapy, still many patients continue to face a poor prognosis. The key to survival is early detection in patients with OSCC. Since there are no accurate, cost-effective, and reproducible method present to screen patients for OSCC and as such many patients are diagnosed at advanced stages of the disease.

Early detection would identify patients, facilitating timely treatment and close monitoring. Mass screening requires a rapid oral cancer diagnostic test that can be used in a clinical setting. Current diagnostic techniques for OSCC require modern laboratory facilities, sophisticated equipment, and elaborate and lengthy processing by skilled personnel. The lab-on-chip technology holds the promise of replacing these techniques with miniaturized, integrated, automated, inexpensive diagnostic devices.

Broadly, microfluidics technology also referred to as lab on a chip or micro total analysis system (TAS) is the adaption, miniaturization, integration and automaton of analytical laboratory procedures into a single device or chip. Microfluidics is often regarded as the chemistry or biotechnology equivalent of the silicon integrated circuit chip that has revolutionized electronics, computers, and communications.

The detection of oral dysplastic and cancer cells within the chip utilizes membrane-associated cell proteins that are singularly expressed on the cell membranes of dysplastic and cancer cells as well as their unique gene transcription profiles.[28]

Saliva based Oral Cancer Diagnosis: Salivary diagnostics has fascinated many researchers and has been tested as a valuable tool in the diagnosis of many systemic conditions and for drug monitoring. Advances in the field of molecular biology, salivary genomics and proteomics have led to the discovery of new molecular markers for oral cancer diagnosis, therapeutics and prognosis.

Several salivary tumor markers are found to be significantly increased in the saliva of oral cancer patients.[29,30] Molecular markers for the diagnosis of oral cancer can be quested in 3 levels;[31] changes in the cellular Deoxyribonucleic acid (DNA) which results in altered mRNA transcripts leading to altered protein levels intracellularly, on the cell surface or extra cellularly. Markopoulos et al.,[31] have summarized the molecular markers for the diagnosis of OSCC.

RNA as a Biomarker: RNA has been found to be a robust and informative marker and salivary RNA signatures have been identified for oral cancer. RNA for years was thought to degrade in saliva due to the various RNAases that is present in saliva.[32] However, cell free RNA is present in saliva both in intact and fragmented forms.[33] It has been speculated that salivary mRNA is contained in apoptotic bodies[34,35] or actively released in exosomes or micro vesicles.[36,37,38] Researchers[39] compared the clinical accuracy of saliva with that of blood RNA biomarker for oral cancer detection and found four RNA biomarkers that have a sensitivity and specificity of 91% and 71% and a collective receiver operator characteristic (roc) value of 0.95.[40]

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Protein Markers: Protein markers are differentiation antigens of corresponding normal tissue and characterize a certain stage of its maturation. They originate from live cells and show high tissue specificity. However, they may be detected in other pathologies as well.[41] Salivary protein markers have shown moderate sensitivity and specificity as prognostic markers.[31]

Alterations in host cellular DNA.

DNA markers are universal i.e., there is not a single tumor cell with in the lesion which does not contain its genetic material. They originate from dead cells, are detected in the early stage of tumorigenesis and are absolutely onco specific; showing a direct cause-and-effect relationship with tumorigenesis. However, tissue specificity of DNA markers is very low.[41]

Mitochondrial DNA mutations have also been useful to detect exfoliated OSCC cells in saliva.[31] Such mutations have been identified in 46% of head and neck cancer and in 67% of saliva samples from OSCC patients by direct sequencing.[42]

It has been observed that p53 gene which is located on chromosome 17p13.1 exhibits mutation in 50-70% of epithelial tumors[43,44] and LOH of p53 allele has been reported in 22% of pre-cancer and 20% of oral cancer. However, the prognostic significance of p53 in oral cancer is yet to be established although there are multiple studies comparing the expression of p53 in premalignant lesions and malignancies.[45,46] Boyle et al.,[44] using plaque hybridization identified tumor specific p53 mutations in 71% saliva samples from patients with head and neck cancer. Other genes such as p16, p27, p63, p73 related to p53 and cell cycle have been found to be altered in varying degrees in oral cancer.[43]

Promoter methylation, an alternate form of gene silencing, which depends on the epigenetic factor has been described to be involved in OSCC.[43] The main genes to be methylated are CDKN2A, CDH1, MGMT, DAPK1.[43,47]

Rosas, et al.,[48] identified aberrant methylation of at least one of these genes (p16, MGMT, DAP-K) in OSSC and detected promoter hyper methylation in 65% of matched saliva samples in OSCC patients. Levels of Ki67 marker were increased while 8-oxoguanine DNA glycosylase, phosphorylated-Src and mammary serine protease inhibitor (Maspin) were found decreased in the saliva of patients with OSCC.[49]

Laser Capture Micro dissection: Laser Capture Micro dissection provides an ideal method for the extraction of cells from specimens in which the exact morphology of both the captured cells and the surrounding tissue are preserved.24and as such has made the study of cancer biology more precise and has greatly boosted the efforts in defining the molecular basis of malignancy.[50]

It has been seen that when rapid immunohistochemical staining techniques are combined with LCM, more accurate micro dissection of cellular subsets can be obtained.[51] LCM may be also used to detect the biomarkers and establish protein fingerprint models for early detection of OSCC. LCM combined with SELDI-TOF-MS technology and bioinformatics approaches may not only facilitate the discovery of better and precise biomarkers but also provide a useful tool for molecular diagnosis.[52,53]

Spectral Cytopathology: SCP, is a novel approach for diagnostic differentiation of disease in individual exfoliated cells. SCP is carried out by collecting information on each cell's biochemical composition via an infrared micro spectral measurement, followed by multivariate data analysis. Deviations from a cell's natural composition produce specific spectral patterns that are exclusive to the cause of the deviation or disease.

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These unique spectral patterns are reproducible and can be identified and employed via multivariate statistical methods to detect cells compromised at the molecular level by dysplasia, neoplasia, or viral infection. In this proof of concept study, a benchmark for the sensitivity of SCP is established by classifying healthy oral squamous cells according to their anatomical origin in the oral cavity. Classification is achieved by spectrally detecting cells with unique protein expressions: for example, the squamous cells of the tongue are the only cell type in the oral cavity that have intracytoplasmic significant amounts of keratin, which allows them to be spectrally differentiated from other oral mucosa cells.[54]

A multispectral digital Microscope (MDM): A multispectral digital microscope is designed and constructed as a tool to improve detection of oral neoplasia. The MDM acquires in vivo images of oral tissue in fluorescence, narrowband (NB) reflectance, and orthogonal polarized reflectance (OPR) modes, to enable evaluation of lesions that may not exhibit high contrast under standard white light illumination. The device rapidly captures image sequences so that the diagnostic value of each modality can be qualitatively and quantitatively evaluated alone and in combination.[55]

Elastic Scattering Spectroscopy: Is an emerging technique that generates a wavelength-dependant spectrum, which reflects structural and morphological change within tissues at scattering centers like the nucleus, chromatin concentration, sub-cellular organelles, structural proteins, lipids, and erythrocytes. It is fast, reliable, cheap, non-invasive diagnosis, in situ, and real-time. It is used for the diagnosis of malignancy, monitor chemotherapy levels, free-flap oxygenation levels, and to assess surgical margins and regional lymph nodes intra-operatively.[56]

Optical coherence Tomography: is a new high-resolution optical technique that enables minimally invasive imaging of near-surface abnormalities in complex tissues, also known as confocal microscopy and Optical Doppler Tomography. It is based on low-coherence interferometry using broadband light to provide cross-sectional, high-resolution subsurface tissue images.[57]

The ViziLite – Highlighting the Keratin: Vizilite makes the keratin whiter and, therefore, more visible to the naked eye in the oral environment, even a thin leukoplakia which might otherwise have been missed could be detected after a minute of contact with acetic acid. The ViziLite (R) system takes advantage of this and adds bright blue light to even further enhance keratin detection.[58-60]

Since the technology uses reflected light solely and as such can only give us information from the most superficial cell layers. Dysplasia, of course, begins in the lowest layers of the epithelium and so reflected light will identify such cells only if they are associated with surface hyperkeratosis, e.g. leukoplakia. With this caveat, however, it does well, with a very high ability to enhance identification of keratotic patches.[58,61,62]

The ViziLite system derives light from either chemical tubes (chemiluminescence) or a laser and, recently, toluidine blue has been added to the kit (ViziLite Plus (R) for identification of superficial nuclear abnormalities. As with other adjunctive diagnostic technologies, the ViziLite (R) exam has disadvantages. It seems to have a high proportion of false positive and false negative tests, relative to identification seems to have a high proportion of false positive and false negative tests, relative to identification of dysplastic cells rather than hyperkeratosis.[60,62]

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Oral Autofluorescence: Our cells contain molecules capable of self-fluorescence, especially when activated (excited) by specific light waves. Certain Optical devices, such as the VELScope (R) (LED Dental, Inc. White Rock, BC, Canada), and the new Identafi (R) 3000 Ultra (Trimira, LLC, Houston, Texas), take advantage of the fact that we all glow to a certain degree.[63-67] Excitation and emission of fluorescence depends on how light is scattered and absorbed in tissue: scattering is caused by differences in the index of refraction of different tissue components, while absorption is dependent on the molecular composition of the same components.[64,67]

Fluorescent signaling is used to assess the metabolic state of tissues and to identify primitive/dysplastic cells. The amount of fluorescence given off from living tissues is very slight; certainly not capable of being seen under normal conditions. However, if violet or blue light is used in a darkened room and the clinician peers through an eyepiece or pair of glasses which filter out virtually all reflected light and only allows transmission of light of the wavelength (s) of the fluorescing tissues, the autofluorescence is easily seen.

The wavelengths which excite the greatest fluorescence in oral mucosa range from 400 to 460 nm, i.e. violet and blue light. The Identafi (R) 3000 Ultra shines a violet light of approximately 405 nm, which especially stimulates a blue/violet fluorescence. This device also provides two other types of light: a white light suitable for a conventional visual examination, and a green-amber light that highlights keratinized mucosa and submucosal blood vessels. The generated light is less intense or bright than that of the VELscope (R) but this does not seem to influence the amount of tissue fluorescence given off. The VELScope (R) uses a blue light with peak intensity at approximately 436 nm; this wavelength especially stimulates a green fluorescence.

An immature or dysplastic epithelial cell has much less NADH and FAD activity than a normal cell and so mucosal areas with such cells will not fluoresce, thereby appearing black (actually blackish-green or blackish-blue) through the eyepiece or glasses.[67] Additionally, data also suggests that the cross-links in sub epithelial collagen fibers beneath dysplastic cells also lose fluorescent activity, contributing to the “black spot” seen through the filter.[67]

CONCLUSION: Early detection of Oral cancer is the key for better prognosis and as such oral health care professionals can play a significant role. Early detection of oral cancer is possible even at the precancerous stage by using non-invasive, painless outpatient procedures of combined in vivo supra vital toluidine blue staining and brush biopsy. This technique increases the sensitivity and specificity in detecting pre malignant lesions and also to minimize false negatives. Light based detection systems have been claimed to improve sensitivity and specificity but so far controlled studies have failed to justify their application and may be used as an adjunct.

Brush biopsy and scalpel biopsy are effective diagnostic tests for evaluating suspicious oral lesions which may be precancerous or cancerous. With the advent of newer diagnostic modalities it may be apt to conduct controlled trials in low risk and high risk populations before integrating these newer technologies into the practice. Moreover promoting Oral cancer awareness through mass media may be beneficial as the people in general will become aware of the signs and symptoms and which in turn will lead to early detection of such cases and decrease in mortality.

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