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Review Article

Advanced Diagnostic Aids in Detection of Potentially Premalignant Oral Epithelial Lesions - A Review

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Abstract

Oral cancer is one of the most mutilating disease afflicting mankind. Precancerous lesions, conditions and early stage oral cancer cannot be adequately identified by visual inspection alone and may be easily overlooked and neglected.

Differentiation between early stage cancer, precancerous lesions and benign lesions is often difficult because of the similar appearance of the lesions.

Surgical biopsy is a gold standard for diagnosis, but this needs professional services, which are impractical at times. Alternative screening methods which are non-invasive, easily performed and highly accurate are the norms for any test to accept as an alternative for histopathology.

Early diagnosis is one of the most important single factor in combating oral cancer. Every general dental practitioner should be aware of recent advances in diagnostic oral medicine in order to provide a high level of care.

This paper provides a summary of comprehensive diagnostic modalities that can be used for early detection, which is crucial for its ultimate control and prevention.

Keywords: Diagnostic Aids; Non-invasive; Oral Cancer; Potentially Premalignant Oral Epithelial Lesions; PPOEL; PMDs

Introduction

Cancer is defined as an uncontrollable growth of cells that invade and damages even its surrounding tissues in a human body. Latinised from a Greek term named 'Karkinos', meaning a crab, cancer simply resembles like a drastic extend of a crab's claws into its adjacent tissues. Cancer considered as the 2nd most leading cause of mortality among the developed countries and the 3rd most leading cause of death in developing countries [1]. The International Agency for Research on Cancer (IARC) estimated that the burden of cancer globally has risen to 18.1 million new cases and 9.6 million deaths in the year 2018 [1].

Oral cancer ranks among the top three variants of cancer in India [2]. Oral cancer, also known as mouth cancer, is a malignant condition of the lining of the lips, mouth, or upper throat in a human body. In year 2018, oral cancer occurred globally in about 355,000 people, and resulted in 177,000 deaths. About 92-95% of all oral malignancies are oral squamous cell carcinomas (OSCC) [2]. It produces dysfunction and distortions in speech, difficulty in mas-

tication and swallowing, and affects the patient's ability to interact socially [3].

Therefore, 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 [4]. Unfortunately, most patients are diagnosed with advanced stage disease.

Oral Cancer Screening for oral cancer implicates searching for oral potentially premalignant oral epithelial lesions and cancerous lesions, typically before symptoms occur [5]. Precancerous lesions can be a major warning or an early detection sign for oral cancer as it is stated that about 80% of oral cancer progresses from precancerous lesions and about 2-12% of precancers are transformed into cancer [6]. If a precancerous lesion develops, there is more chance of malignant transformation, which is defined by the World Health Organization (WHO) as "A morphologically altered tissue which has a greater than normal risk of containing a microscopic focus of cancer at diagnosis or of transforming into a malignancy after diagnosis" [7]. It has been well by many researchers that virtually all oral cancer is preceded by visible clinical changes in the oral mucosa usually in the form of white or red patch [8]. These lesions are known as premalignant lesions and premalignant conditions.

At a workshop by the WHO Collaborating Centre for Oral Cancer and Precancer in the United Kingdom, the term 'Oral potentially malignant disorder' (OPMD) was recommended. However, A new term 'potentially premalignant oral epithelial lesion' (PPOEL) has recently been used as a broad term to define both histologic and clinical lesions that have malignant potential Premalignant disorders are usually found on the buccal mucosa, followed by gingiva, tongue and floor of the mouth [9].

Etiology

Tobacco use is the commonest pre-disposing factor for the development of an intraoral white lesion although a certain proportion of oral white patches have no known cause. In the developing world, tobacco use and areca nut use, either alone or in combination, account for the vast majority of leukoplakias.

Global Adult Tobacco Survey (GATS) 2016-17, nearly 42.4% of men, 14.2% of women and 28.6% of all adults currently use tobacco, The Indian data suggest that the relative risk of developing oral cancer is 2.82 for smokers and 5.98 for chewers, 80% of oral cancers progress from precancerous lesions and about 2-12% of precancers are transformed into cancer [10,11].

The type of tobacco usage influences the distribution of the lesions: reverse cigar smoking causes lesions on the hard palate, chewing causes lesions at the site of quid placement and smoking of cheroots is associated with floor of mouth leukoplakias [12]. Smoking causes changes at the buccal mucosa or commissures [13,14].

Support for the importance of tobacco in the aetiology of leukoplakia is given by the regression and/or disappearance of many lesions following abstinence. In a series of 138 Danes, it was noted that in those who abstained from smoking for 3 months, 56% of lesions regressed or disappeared while in those who quit permanently, 78% of lesions regressed or disappeared after a year [15]. In Californians with leukoplakia, 50 patients stopped smoking following diagnosis of leukoplakia and 44% of lesions disappeared.

Established	Strongly suggestive	Possible	Speculative
Smoking	Sunlight (lip)	Viruses, sexual practices	Mouth-washes
Chewing tobacco	Radiation	Immune deficiency	Mate drinking
Snuff dipping	Syphilis	Dentition?	Periodontal disease
Alcohol misuse	Candidial infections	Ethnicity?	Familial
Betel quid, syphilis		Iron deficiency or vitamin deficiency	Irregular teeth or restorations
Oncogenes and tumor suppressor genes		Organ transplantation	

Table 1: Causes and various risk factors for oral cancer and precancer [16,17].

Concept of malignant transformation

Factors associated with increased risk of malignant transformation [5,8,16]

Studies show that only a small fraction and not all PMDs turn malignant, and the challenge has been to identify the high-risk lesions

that could turn malignant. Researchers found that greater than normal risk for malignant phenotype are associated with:

- Red and white intermixed lesions, or presence of multiple lesions.
- Proliferative verrucous surface appearance, or presence of nodule, erosion, ulceration, or presence of candidiasis.
- Non-smoker (passive smokers have greater risk), or those with no habits (idiopathic leukoplakia).
- Lesion not regressed after habit cessation, or after the causative initiating factor is removed, or continuation of habit after initial diagnosis.
- Duration of the lesion before initial diagnosis (long duration poor prognosis).
- Lesion size greater than 200 mm^2.
- High-risk anatomic site - floor of mouth, postero- lateral border tongue, lip.
- Young age at diagnosis (30-35yrs).
- Female gender (for unknown reason 47% of women show malignant transformation).

Classification

Precancerous lesions	Precancerous conditions
Leukoplakia	Submucous fibrosis
Erythroplakia	Actinic keratosis
Palatal lesions in reverse smokers	Lichen planus
	Discoid lupus erythematosus

Table 2: Classification of precancerous lesions and conditions: [WHO 1978] [6].

High Risk	Life-style Related	Infections	Immunodeficiency	Inherited Disorders
Erythroplakia	Smokeless tobacco Keratosis	Hyperplastic Candidiasis	Solid Organ Transplantation	Xeroderma Pigmentosum
Leukoplakia	Reverse smoker's palate	Viral (HPV, HIV, EBV, HBV, HSV)	Graft Versus Host disease	Dyskeratosis congentia
Oral Submucous Fibrosis	Actinic Cheilitis	Tertiary Syphilis	Chronic cutaneous lupus erythematous	Epidermolysis bullosa
Erosive Lichen Planus				Bloom syndrome

Table 3: Another classification system classified potentially malignant disorders of the oral cavity as follows [18].

Lesion	Clinical features
Leukoplakia	White plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer (Warnakulasuriya., *et al*. 2007).
Erythroplakia	A fiery red patch that cannot be characterized clinically or pathologically as any other definable disease (Warnakulasuriya., *et al*. 2007).
Palatal lesions associated with reverse smoking	Red, white, or mixed lesions of the palate in patients who smoke with the lighted end of the tobacco (Warnakulasuriya., *et al*. 2007).
Oral lichen planus	White striations, white papules, white plaques, erythema, erosions, or blisters affecting predominantly the buccal mucosa, tongue and gingivae (Sugerman and Savage 2002)
Oral submucous fibrosis	Blanching and stiffening of the oral mucosa leading to limitation in opening of the mouth with fibrous bands in different sites of the mouth (Warnakulasuriya., *et al*. 2007)
Actinic keratosis	Solar keratosis of the lip secondary to sun exposure with dry and whitish gray scaly erosions on the lip mucosa (Zide 2008).
Discoid lupus erythematosus	White papules, central erythema, a border zone of irradiating white striae and peripheral telangiectasia (Schiodt and Pindborg 1984).

Table 4: PPOEL and their clinical features [19].

Diagnostic aids

The diagnostic phase of patient management begins with an assessment of the medical history and its potential impact on the dental history and overall management of any oral disease or condition. Health history questionnaires must include pertinent questions relative not only to general health, but also to what the practitioner must know as the oral, head and neck examination and treatment plan.

Need for advanced methods for screening

Thomson showed that 36% of patients newly diagnosed with OSCC had histologic evidence of dysplasia or carcinoma *in situ* in a biopsy from clinically normal mucosa from the corresponding, contralateral anatomic site. The detection of OSCC is further impaired by the late presentation of symptoms such as ulceration, induration, fixation, bleeding and cervical lymphadenopathy. Symptomatic lesions present at an advanced stage (III/IV) 60% of the time. Thus, any technology which highlights oral premalignant lesions in a highly sensitive and specific manner will undoubtedly aid clinicians in early diagnosis and treatment of these conditions [20].

Clinical Methods	Optical Methods	Imaging Methods	Histopathological methods	Molecular methods	Salivary diagnostic Methods
Conventional Oral Examination	Vizilite	Computed Tomography (CT).	Scalpel Biopsy	Immuno Histochemistry	Protein Electrophoresis
Vital Staining	MicroLux DL	Magnetic Resonance Imaging (MRI).	Oral CDx Brush Test	Flow Cytometry	Sialochemistry.
	Velscope	Positron Emission Tomography (PET).	Cytology	Polymerase Chain Reaction (PCR)	
	Fluorescence Spectroscopy	Thalium-201(201Tl) Scintigraphy	Laser Capture Micro Dissection	Blotting Techniques	
		Optical Coherence Tomography (OCT).		Spectral Karyotyping Fluorescent In-situ Hybridization (FISH).	
		Narrow Band Imaging (NBI).		Comparative Genomic Hybridization	
				DNA Microarray	

Table 5: Diagnostic aids for PPOEL.

Categories	Techniques		Sensitivity (%)	Specificity (%)	Citations
Vital staining	Toluidine blue staining		38-100	9-100	(Warnakulasuriya and Johnson 1996Rajmohan., *et al*. 2012)
	Methylene blue staining		90-91.4	66.6-69	Riaz., *et al*. 2013)
	Lugol's iodine staining		87.5-94.7	83.8-84.2	Chaudhari., *et al*. 2013)
Light-based detection systems	Chemiluminescence	ViziLite	71-100	0-84.6	Rashid and Warnakulasuriya 2015
		Microlux/DL	77.8-94.3	77.8-94.3	(McIntosh., *et al*. 2009; Ibrahim., *et al*. 2014).
	VELscope		30-100	15-100	Bhatia., *et al*. 2013
	Photodynamic diagnosis		79-100	50-99	Sieron., *et al*. 2008

Optical diagnostic technologie	Raman spectroscopy	97.44-100	77-100	Guze., *et al*. 2015
	Elastic scattering spectroscopy	72-98	68-75	Green., *et al*. 2014
	Diffuse reflectance spectroscopy	76-100	76-97	Stephen., *et al*. 2013
	Narrow-band imaging	84.62-96	88.2-100	Yang., *et al*. 2014
	Optical coherence tomography	73-100	78-98	Green., *et al*. 2014
	Confocal reflectance microscopy	73	88	(Olivo., *et al*. 2011)
Salivary biomarker	miRNA-184	n/a	n/a	(Zahran., *et al*. 2015)
	IL-6	n/a	n/a	(Sharma., *et al*. 2011)

Table 6: Non-invasive techniques with their sensitivity and specificity for detection of PPOEL [19].

Advanced diagnostics

Polymerase chain reaction

Polymerase chain reaction (PCR) is an extremely versatile technique used for copying of DNA. It allows a single DNA sequence to be copied millions of times or altered in a predetermined way. PCR technique is used for quantitative measurements of DNA or RNA molecules.

Polymerase chain reaction technique has been used to amplify DNA in samples from oral carcinomas and has been analyzed with restriction fragment length polymorphism. It detects the commonly implicated molecular alterations in oral cancers such as loss of heterozygosity, microsatellite instability and changes in methylation pattern.

Another interesting trend currently being investigated is the use of the PCR to determine if surgical margins obtained at the time of surgery that are histopathologically free of tumor contain a small amount of histologically undetectable tumor cells.

Limitations [21]

- Difficulties can be encountered when studying small quantities of DNA, since the ingredients necessary for PCR may be exhausted.
- Specificity of reaction may be limited and depends on many factors.
- Long DNA fragments are difficult to amplify when the starting material is degraded such as that obtained following formalin fixation
- Susceptibility of process to contamination
- Nucleotide sequence errors.

Hybridization methods

Hybridization refers to the pairing of complementary RNA or DNA strands to produce a double-stranded nucleic acid. The nucleotide base-pair relationship is so specific that strands cannot anneal unless the respective nucleotide strand sequences are complementary. All hybridization methods use a radio-or fluorescence labelled DNA or RNA probe that binds to the target DNA or RNA of interest, permitting visualization. The target nucleic acids can either be immobilized in a membrane ("blotting") or examined in tissue sections (*in situ*) [21]:

- **Southern blotting:** A widely used method for analyzing the structure of DNA. This involves the transfer or blotting of DNA fragments onto a membrane.
- **Northern blotting:** Modification of Southern blot analysis permits the study of RNA from tissues.
- **Western blotting:** The separation and identification of proteins in a similar fashion is referred to as "Western blotting". A limitation of these types of investigations is that the genetic material is removed from its topographic surroundings. The genetic material under investigation comes from a heterogeneous collection of stromal and neoplastic cells. This "contamination" with stromal cells dilutes the signal from the neoplastic cells [23].
- ***In situ* hybridization (ISH):** Chromosome *in situ* hybridization (CISH) is a cytogenic technique that is used to detect and localize the presence or absence of specific DNA sequence or chromosome. It uses fluorescent probes that bind to only those parts of the chromosomes with which they show a high degree of sequence similarity. Fluorescence microscopy is used to find out where the fluorescent probe is bound to the chromosome, thus providing an ability to directly visualize the genetic change in tissue sections or exfoliated cells.

In oral cancers, this technique is used to form the diagnosis and evaluate prognosis and remission of the disease. Analyses of normal and premalignant lesions adjacent to tumors have demonstrated that chromosome instability can be detected in the field of the tumor and the degree of chromosome instability increases with the degree of histologic progression toward cancer. Studies indicate that most leukoplakia lesions contain an abnormal number of chromosomes 7 and 17, and lesions with greater than 3% proportion of cells with trisomy 9 have a significant higher likelihood of progression to cancer.

Micro array analysis

It is commonly known as 'Gene Chip'. In general terms microarrays refers to a variety of technical platform in which high density assays are performed in parallel on a solid support. A method to study gene expression patterns at mRNA level is micro array analysis [24].

Microarrays are typically small rectangles of glass or nylon membrane to which high density DNA or protein probes are arranged in a regular grid pattern. Samples are applied to the microarray for measurement of potentially hundreds of thousands of targets simultaneously in a single experiment.

Gene profiling method has been used for comparing the normal and cancerous oral mucosa, and a distinct pattern of gene expression was observed when normal and cancerous cells were compared in genome-wide analysis of oral cancer. Gene profiling by DNA microarrays is capable of identifying up-regulated or down-regulated genes correlated with oral tumor recurrences and lymph node metastasis, thus helping clinician to plan a treatment that prevents recurrence and reduce chances of metastasis.

Loss of heterozygosity [20]

LOH is defined as a loss of genomic material (from few thousand nucleotides to a whole chromosome) in one of a pair of chromosomes.

Recent studies show that loss of specific chromosomal regions (loss of heterozygosity, LOH) that contain known or presumptive tumor suppressor genes is an early predictor of subsequent progression of oral premalignant. Incorporation of LOH findings into staging of oral premalignancy could improve our ability to identify and manage high-risk premalignant lesions, particularly those with relatively benign histology but high-risk genetic changes (high-risk LOH pattern). Some of the TSGs involved in head and neck cancers include p53, Rb (retinoblastoma), and p16INK4A.

Risk	LOH
Low risk LOH pattern	Retention of 3p and 9p
Intermediate risk pattern	LOH at 3p and/or 9p
High risk pattern	LOH at 3p and /or 9p plus loss at 4q, 8p, 11q, 13q or 17 p

Table 7: LOH.

Laser capture microdissection [22]

Laser capture microdissection (LCM) is a new and exciting technology for rapid preparation of relatively pure cell samples from tissue sections.

In oral disease investigations LCM has been used to identify immunoglobulin genes in plasma cells in salivary glands, to study the expression of differentiation and growth-related genes in oral cancer, and to determine amplification or loss of specific Oncogenes.

Other potential future diagnostic technologies [25]

- Laser induced fluorescence spectroscopy
- Light induced fluorescence spectroscopy
- Elastic scattering spectrography
- Raman spectroscopy
- Photoacoustic imaging
- Photon fluorescence
- Orthogonal polarization spectral imaging
- Quantum dots
- Optical coherence tomography (OCT)
- Trimodal spectrography
- Doppler oct
- Nuclear magnetic resonance spectrography
- Chromoendoscopy
- Narrow band imaging
- Immunophotodiagnostic techniques
- Differential length spectroscopy
- 2 photon fluorescence
- 2nd harmonic generation.

Conclusion

It is estimated that most of all cancers and cancer mortality worldwide are preventable through early detection, as it provides a greater chance of initiating early and successful treatment. Only sure way to avoid cancer is reduce our chances by a balanced approach to cancer prevention, early detection, and effective early treatment. PPOELs are often undiagnosed due to lack of public awareness and due to lack of knowledge among medical professionals.

Clinical appearance and diagnosis of a lesion is not adequate to determine its premalignant nature as not all white lesions turn malignant. Diagnostic biopsy and histopathological examination should be considered for any mucosal lesion that persists for more than 14 days after obvious irritants have been removed.

The role of oral physician is pivotal in early detection of the oral precancers and cancers. They not only form a firm basis for discontinuance of habits in educating and motivating but also help in preventing the progression from precancers to malignancy. Also, they form a crucial support system for the morbidity management. Thus, it is inevitable for the clinicians to be erudite regarding the diagnosis and further management of PPOEL.

Conflict of Interest

None.

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