Current Trends in Diagnosis of Oral Cancer and Premalignant Lesions: An Update

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ABSTRACT
The incidence of oral cancer worldwide is around 500,000 new cases every year, accounting for approximately 3% of all malignancies, thus creating a significant worldwide health problem. The annual incidence and mortality rates vary between different races, genders and age groups. Screening and an early detection are believed to decrease both the morbidity and mortality which are associated with oral squamous cell carcinoma, because unlike many anatomic sites, in the oral cavity, premalignant lesions are often visible on clinical examination. A variety of new and emerging diagnostic aids and adjunctive techniques are currently available to potentially assist in the screening of healthy asymptomatic patients for the detection of otherwise occult oral cancerous lesions or potentially malignant disorder. This paper reviews the advances in the diagnostic techniques for detecting lesions early and predicting their progression or recurrence.

Keywords: Oral cancer, Premalignant, Biopsy, Staining, Biomarkers, DNA ploidy.

INTRODUCTION
Oral cancer is the 5th most common cancer in the world, accounting for numerous deaths annually. Epidemiologic differences exist in South Asia, where oral cancer ranks 1st among all types of cancers in male patients and 3rd in female patients. Oral cancer is associated with chronic irritating factors, such as tobacco, smoking, alcohol, and betel quid (BQ) use. While cigarette smoking and alcohol drinking are the major risk factors in Western countries, BQ use and smoking are major factors in the causation of oral cancer in South Asia, Southeast Asia and Taiwan. Most cancers of the oral cavity are oral squamous cell carcinomas (OSCC), and tobacco, alcohol and betel use the main risk factors for these and many potentially malignant lesions (PMLs). The main high risk groups are older adult males who use tobacco and alcohol. It is expected that early diagnosis of PML can reduce mortality. Early diagnosis of OSCC can speed proceeding to treatment and can improve the prognosis. This requires patients to seek an oral and dental examination at an early stage. Conventional oral examination is the standard method of revealing PML and OSCC, confirming the clinical suspicion by biopsy and histopathological examination. This article discusses the different modalities utilized for detection of oral squamous cell carcinomas and potentially malignant lesions.

ORAL CANCER DIAGNOSTIC AIDS
Vital Staining
Various attempts to clinically highlight probable dysplastic areas before biopsy have, unfortunately, not proven to be absolutely reliable but may be of some help where there is widespread ‘field change’, such as seen in patients at high risk for OSCC. Toluidine blue (TB) staining is a simple and inexpensive diagnostic tool that uses a blue dye to highlight abnormal areas of mucosa. Toluidine blue is a basic metachromatic nuclear stain which stains nuclear material of malignant lesions and PML but not normal mucosa. Toluidine blue staining may identify high-risk oral PMLs with poor outcome and positive TB staining may be related to genetic changes [allelic loss or loss of heterozygozity (LOH)] associated with progression to OSCC even in histologically benign lesions and lesions with mild dysplasia.

Biopsy and Histopathological Examination
The biopsy should be sufficiently large to include suspect and apparently normal tissue to give the pathologist a chance to make a diagnosis and not to have to request a further specimen. Since red rather than white areas are
most likely to show any dysplasia present in the lesion, a biopsy should include the former. The prognostic value of histopathological features related to a primary OSCC tumor and the cervical lymph nodes have been reviewed. Emphasis is given to practical aspects of the histopathological assessment, potential inaccuracies, the importance of the partnership between surgeon and pathologist, the need for standardization throughout the histopathological assessment, and the value of accurate documentation of findings. Furthermore, even histological examination of a specimen is fraught with potential pitfalls and is subjective. A major problem in PML is to ensure that the biopsy is taken of the area most likely to contain the greatest number of cellular changes suggestive of premalignancy (dysplasia): to this end, red rather than white areas should be selected for biopsy. Vital staining may facilitate this. False negative results are still occasionally possible from incisional biopsy and, even where dysplasia has been excluded in a leukoplakia by incisional biopsy, studies have shown that the lesions, if wholly excised, may prove to contain OSCC in up to 10%. The Brush Biopsy

The Pap smear, a time-honored, effective tool for finding dysplastic cells of the uterine cervix lost popularity among dentists during the 1960s because it seemed unable to find dysplastic cells in oral leukoplaikias. This is undoubtedly due to the fact that oral white patches have a thicker keratin layer than their cervical counterparts. 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. Today 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. 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. Recently, liquid-based cytology (LBC) has become a principle methodology in cytology replacing conventional smears, owing to better cell recovery and morphologic preservation. The Food and Drug Administration (FDA) has approved two LBC methods for gynecologic and nongynecologic cytologic sample processing. One method uses a filtration process and a computer-assisted thin layer deposition of cells (Thinprep® CYTYC Corp, Boxborough, MA), while the other method involves a sedimentation process (TRiPath Imaging, Burlington, NC). Both of these methods produce an evenly distributed thin layer of epithelial cells devoid of blood and the ubiquitous inflammatory cells that may interfere with cytologic examination. Liquid-based cytology had been adopted to analyze the brush biopsy samples of oral mucosa. Liquid-based cytology methodology appears to not only increase the sensitivity and specificity of cytologic diagnosis but, significantly, also provides additional samples for immunohistochemical and other molecular studies which are not possible with conventional cytologic smears. It is a good tool in an experienced, knowledgeable hand, with very few false positive or negative results when used appropriately. Biomarkers

Since the introduction of molecular techniques, such as examination for abnormal protein expression, including tumor suppressor genes (TSGs) and other genetic changes, molecular markers have revealed neoplastic changes in PML (and furthermore may predict involvement of tumor resection margins and lymph nodes, and prognosis). The most predictive of the molecular markers thus far available and assessed in OSCC development include the TSG p53 protein expression, chromosomal polysomy (DNA ploidy), and changes (termed loss of heterozygozity; LOH) in chromosomes 3p or 9p (probably due to changes in the TSG p16). 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.

The ViziLite

The ViziLite (R) system utilizes acetic acid and adds bright blue light to even further enhance keratin detection. This technology uses reflected light solely and so 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. With this caveat, however, it does well, with a very high ability to enhance identification of keratotic patches. The light is derived 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. ViziLite Plus (R) system is capable of identifying dysplastic or immature cells when they are close enough to the surface. As an adjunctive test, this system is valuable in that it increases awareness of the oral cancer and precancer detection dilemma for both the clinician and the patient, and it should, certainly, find hyperkeratotic patches that may have been missed with routine visual inspection. Whether or not it can detect dysplastic cells without toluidine blue staining, and in the absence of surface keratin, has not been adequately proven and, certainly, there are those who considered the technology to add little or nothing to the routine visual examination.22,24

VEL Scope

VEL scope is a hand-held device which was approved by Federation Dentaire Association for direct visualization of autofluorescence in the oral cavity. Only recently, it was introduced in the market as a diagnostic adjunct for oral cancer detection. The VEL scope Vx is one of the most powerful tools available today for assisting in oral abnormalities especially oral cancer. The distinctive blue-spectrum light causes the soft tissues of the mouth to naturally fluoresce. The use of VEL scope Vx is a safe and simple technique and the entire examination can be done in about 2 minutes. However, it is a relatively new device and so far only a limited number of studies have been done on its effectiveness as a diagnostic adjunct for oral cancer.25

DNA Ploidy

DNA ploidy is the measurement of nuclear DNA content. This may provide a surrogate measure of gross genetic damage and this could act as a surrogate for individual molecular markers. Normally, a non-dividing somatic cell contains a diploid amount of DNA in 23 pairs or 46 chromosomes. Just before cell division, the DNA is doubled and in mitosis; the 23 pairs of chromosomes are evenly distributed to two daughter cells. In somatic cells, if a doubling of the DNA during S-phase occurs without a subsequent cell division, the nucleus will then contain quadruples of the DNA, making the cell tetraploid. Multiple copies of DNA in excess of diploidy is termed polyploidy. If the chromosomes are not uniformly distributed to the daughter cells or if parts of chromosomes become detached, the chromosomal segregation during mitosis is termed unbalanced, a situation termed aneuploidy and commonly observed in many cancers.26 DNA ploidy can be measured fairly simply with automated image cytometry of nuclei obtained from routinely processed tissue samples and the expertise is available in many pathology laboratories.

SALIVA—A DIAGNOSTIC AID IN DETECTION OF ORAL CANCER

Exfoliative cell samples have been used to detect genetic alterations in the oral epithelium of patients at high risk from oral cancer,27 and to detect microsatellite alterations in OSCC. The concept of a saliva test to diagnose OSCC is even more appealing.28 Promoter 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. Forensic science has since shown that saliva can contain a number of messenger ribonucleic acid (mRNA) fragments including salivary specific statherin, histatin 3, and the proline-rich proteins PRB1, PRB2 and PRB3 as well as the ubiquitously expressed spermidine N1 acetyl transferase (SAT), β-actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The mRNAs in saliva, such as β-actin, SAT and interleukin-8 are relatively stable despite the presence of salivary ribonucleases.29 mRNAs in saliva have been tested in over 300 saliva samples from OSCC patients and healthy people, and the signature was always present in higher levels in the saliva of OSCC patients than in saliva from healthy people, with an overall accuracy rate of about 85%.30 This avenue of research is thus clearly most appealing.

CD44, a multistructural and multifunctional cell surface transmembrane glycoprotein molecule involved in cell proliferation, cell differentiation, cell migration, angiogenesis, presentation of cytokines, chemokines, and growth factors to the corresponding receptors, and docking of proteases at the cell membrane, as well as in signaling for cell survival, is also detectable in saliva.31 CD44 isoforms containing the variant 3 (V3) exon include a growth factor binding site and may be involved in OSCC progression.30 Salivary soluble CD44 (SolCD44) levels were found significantly raised in head and neck cancer (HNSCC) patients compared with normal controls and detected 79% of mucosally invasive HNSCC using preliminary cutoff points. However, SolCD44 levels did not vary significantly with tumor size, stage, recurrence, history of radiation treatment, or tobacco and alcohol risk factors.32 Laser-induced fluorescence spectroscopy used to examine OSCC in the hamster buccal pouch model shows increased fluorescence in malignant areas.33 Light-induced fluorescence spectroscopy can distinguish between benign (normal and hyperkeratosis) and dysplasia with a sensitivity of 92% and a specificity of 95%.34 Laser-induced fluorescence (LIF) spectroscopy has been developed for the diagnosis of cancer using an algorithm based on nonlinear maximum representation and discrimination feature (MRDF) method.35 Raman spectroscopy looks at the vibrational changes in tissue that parallel changes in chemical composition,
and is sensitive (e.g.) to changes in DNA content. Raman spectroscopy is widely used in chemical analysis and is based on ‘inelastic’ light scattering since the detected wavelengths are different from that of the applied light. Fourier transform infrared (FTIR)/Raman spectroscopy has been successfully applied for the diagnosis of OSCC in the hamster cheek pouch model with 100% sensitivity and 55% specificity.  

PET Scan

Our oral cavity is one of the cancer sites in the head and neck which is accompanied by a high incidence of regional metastasis. Due to cervical lymph node metastasis, there is significantly reduction in the survival of the patient and which is an important prognostic factor for scientific debate today. Positron emission tomography (PET) with fluorodeoxyglucose (FDG) is increasingly day-by-day as a useful tool in preoperative staging of cancer patients.  

According to recent studies and research, FDG-PET scanning shows good accuracy and predictive value in determining lymph node status and thus helping in screening and early diagnosis of oral cancer in affected patients. Standardized uptake value (SUV) of the tumor mass helps in prognosis for overall survival of the affected patient.  

EMERGING DIAGNOSTIC ADJUNCTS LAB-ON-A-CHIP

Microfluidics technology also referred to as lab-on-a-chip or micro total analysis systems (TAS) is the adaptation, miniaturization, integration and automation of analytical laboratory procedures into a single device or ‘chip’. Microfluidics is often regarded as the chemistry or biotechnology equivalent of the silicon integrated silicon chip that has revolutionized electronics, computers and communications. Microfluidics is by definition suited for handling living cells (whose typical diameter is a few micrometers) in a three-dimensional, biologically relevant environment. This microfluidics chip accepts saliva sample, can be operated by minimally trained personnel, and can provide a diagnostic answer in an automated and timely fashion. The detection of oral pre-cancer (dysplastic) and cancer cells within the chip will take advantage of membrane-associated cell proteins that are singularly expressed on cell cancer cells. The measured profile is compared with archived gene transcription profiles to determine the cancer type and stage. As such, this system provides a means for automated, rapid detection and molecular analysis of cancers in a miniaturized format suitable for use in the clinic and/or the operating room (Table 1).  

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CONCLUSION

Although oral cancer is a fatal disease with high mortality rate, early diagnosis and effective screening can cure it in its initial stages. Current diagnostic aids help in its timely detection. But as it is said that prevention is better than cure, educating people about the ill effects of tobacco products will help in preventing and eliminating this social evil.

REFERENCES


