Diagnostic Aids of Precancerous Oral Lesions

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Abstract

Early detection of oral cancer is the key to survival. The most effective method of combating oral cancer is early detection, diagnosis and eradication of early-stage lesions and their precursors. Historically, the screening of patients for signs of oral cancer and precancerous lesions has relied upon the conventional oral examination. A variety of commercial diagnostic aids and adjunctive techniques are now 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. Advances in diagnosis and staging at the molecular level are expected to affect choice of treatment and patient outcomes. The realm of oral cancer and pre-cancerous lesions detection adjuncts and tests is an exciting and constantly progressing area of research and technology. Integration of the adjuncts and tests discussed here can help uncover hidden lesions before they have the chance to progress into malignancy, and hopefully improve patients’ chances of living a long, healthy life.

Keywords: Cytology, Diagnosis, Oral cancer, Pre-cancerous, Squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (SCC) is the most common cancer of the head and neck.1 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 either patients (who may not report unusual oral features) or health care workers (who may not investigate observed lesions thoroughly).2-4

Early evaluation of oral pre-cancerous lesions can have a dramatic impact on oral cancer mortality rates.5 Detection of oral cancer in the early asymptomatic stage dramatically improves cure rates and patient’s quality of life by minimizing extensive, debilitating treatments. The 5 years survival rate for patients with early, localized disease approximates 80%; for those with distant metastases, it is 19%.6 Unfortunately, more than 50% of patients with oral cancer display evidence of spread to regional lymph nodes and metastases at time of diagnosis, and approximately two-thirds of patients have apparent symptoms, a negative prognostic indicator.7

Pre-cancers and early-stage oral cancers cannot be adequately identified by visual inspection alone and easily may be overlooked and neglected, even by highly trained professionals with broad experience.8 The most effective method of combating oral cancer is early detection, diagnosis and eradication of early-stage lesions and their precursors.9

The diagnosis and treatment of oral premalignant lesions and SCC are currently based on histopathological features, site of involvement and stage of disease. Recent advances in techniques for detecting lesions and predicting their progression are reviewed here. Advances in diagnosis and staging at the molecular level are expected to affect the choice of treatment and patient outcomes.

EARLY DETECTION OF PRE-CANCEROUS ORAL LESIONS

Clinical Examination

Clinical examination for oral premalignant lesions and SCC should include a thorough head, neck and intraoral examination, with examination of the cervical lymph nodes and visual examination and palpation of the oral mucosal surfaces. The location, size, border, color, and surface characteristics of any lesion should be recorded, so that future changes can be recognized. When a biopsy is...
performed, site selection is critical, as the histologic features may vary in non-uniform lesions.\(^5\)

**Exfoliative Cytology**

Oral biopsy represents the gold standard for determining the nature of a mucosal lesion and for diagnosing SCC, and exfoliative cytology has, until recently, been discounted as a tool for assessing oral mucosal lesions. However, techniques have now been reported that include evaluation of exfoliated oral epithelial cells and comparisons of these methods with biopsy techniques. Exfoliative techniques have the advantage of being minimally invasive, and they do not require a local anesthetic. Use of a cytobrush reportedly allows sampling of the full thickness of stratified squamous epithelium of the oral mucosa.\(^10\)

**The Brush Biopsy**

Today pap smears are used effectively for oral red lesions and oral ulcers to identify infections, especially candidiasis, and atypical cells in erythroplakia. The brush biopsy or Oral CDx test is relatively painless procedure captures the deeper epithelial cells on the bristles and the entire brush is sent to a pathology laboratory, 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.\(^11\)

**Liquid-based Cytology (LBC)**

In recent times, LBC has become a principle methodology in cytopathology replacing conventional smears, owing to better cell recovery and morphologic preservation.

LBC had been adopted to analyze the brush biopsy samples of the oral mucosa. LBC 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. The brush biopsy and LBC, as with all adjunctive technologies for oral dysplasia, must be used with intelligence and its routine use requires that the clinician be relatively knowledgeable about the clinical features of pre-cancers and can properly identify the most “severe” area to brush.\(^11\)

**Toluidine Blue In vivo Staining of Deoxyribonucleic Acid (DNA)**

Toluidine blue (tolonium chloride) is a metachromatic dye long used in histology laboratories to stain nuclear material, since it stains DNA very well. This test is premised on the fact that mucosal cells with extra DNA, i.e. large nuclei, attract and retain the stain, even after the bulk of the stain has been washed off with acetic acid.\(^12\)

Unfortunately, this dye test is awkward to use, requiring an acetic acid (vinegar) rinse before and after, and there are high proportions of false positives and false negatives.\(^13,14\) It frequently needs to be repeated because the false positive tests are often trauma- or inflammation-related. Moreover, the dysplastic cells lying deeply in a thick keratotic lesion will likely not be stained adequately. Even with its limitations, however, the toluidine blue test can be a good adjunctive test in the hands of an experienced, knowledgeable clinician, and is especially effective with erythroplakia and carcinoma in situ.\(^13,14\) It should not be considered a standalone test and will not give a diagnosis, but it can help to localize areas to biopsy or to brush biopsy.

**The ViziLite-Highlighting the Keratin**

In the oral environment, acetic acid makes the keratin more white and, therefore, more visible to the naked eye. 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.\(^15,17\) This technology uses reflected light solely and so can only give us information from the most superficial cell layers. 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. 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 of dysplastic cells rather than hyperkeratosis.\(^17,18\) As an adjunctive test, this system is valuable in that it increases awareness of the oral cancer and pre-cancer detection dilemma for both the clinician and the patient.

**Oral AutoFluorescence-When the Mucosa Doesn’t Glow**

Two optical devices, the VELScope(R) and Identify(R) take advantage of the fact that, to a certain degree, we all glow. Each of our cells contains molecules capable of self-fluorescence, especially when activated (excited) by specific light waves.\(^19,23\) 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.\(^21,23\) In humans, these fluorescing products are numerous: tryptophan, porphyrins, collagen cross-links, elastin, nicotinamide adenine dinucleotide (NADH), and flavins, flavin adenine dinucleotide (FAD).\(^23\) This fluorescent
signaling has been 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 the 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 Identify(R) 3000 Ultra shines a violet light of approximately 405 nm, which especially stimulates a blue/violet fluorescence. The light shines from a battery-powered device roughly the size, shape and weight of a dental handpiece; the user looks through special filtering glasses. 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 VELScope(R) uses a blue light with peak intensity at approximately 436 nm; this wavelength especially stimulates a green fluorescence. The device shines light out of a handheld “gun” that is tethered to a light source which typically remains on a cart or countertop plugged to the wall, and the user looks through a filtered eyepiece that disallows reflective and ambient light.

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 (blackish-green or blackish-blue) through the eyepiece or glasses. In addition, data were also suggested 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.

The beauty of the self-fluorescence test is that the light used to excite the oral cells penetrates to the deepest part of the epithelium and so easily reaches dysplastic cells in the lower regions of the epithelium, as well as the subepithelial collagen fibers. This deep penetration can, however, prove to be a bit of a disadvantage in certain settings, since several nondysplastic tissue changes are also positive with this test.

**CONCLUSION**

Since the oral cavity is the only region of the aero digestive tract that can be effectively screened, dentists should continue to be encouraged to perform oral cancer examinations of all patients. Public education that stresses the importance of yearly oral cancer examinations, identification of the warning signs of oral cancer, and recognition of the hazards associated with tobacco and alcohol use is necessary to reverse the high morbidity and mortality rates associated with this disease.

Molecular techniques are expected to aid in diagnosis and staging of the disease, and in providing intermediate markers to assess treatment interventions. In addition, advances in knowledge may lead to new therapies, ultimately improving the management of at-risk lesions once they are identified, as well as improving the prevention and management of SCC. Combining information from molecular markers with exfoliative techniques may overcome some of the current limitations of exfoliative cytology. These combined techniques may prove to be sensitive and specific procedures that can be performed sequentially over time and perhaps as screening methods for at-risk lesions already identified.

Molecular markers are expected to become essential in the diagnosis and management of patients with oral cancer; they will guide future study and clinical care and will ultimately lead to new interventions directed at the molecular changes of cancer. Use of these technologies allows earlier diagnosis and staging of tissue change, before changes in cell morphology occur and certainly before tissue changes become clinically visible. Ultimately, the use of various diagnostic aids may lead to better survival and less treatment-associated morbidity through early recognition of and intervention for at-risk oral lesions.

**REFERENCES**


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