Review Article

Adjunctive Techniques and Diagnostic Aids in the Early Detection of Oral Premalignant Disorders and Cancer: An Update for the General Dental Practitioners

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Abstract

Oral cancer (OC), a potentially fatal disease, is a major health concern across the world. It is reported to be the sixth most common cancer in the world with a disappointingly low 5-year survival rate, despite major advances in onco-medicine in the past three decades. The low 5-year-survival rate is associated with late diagnosis of the disease, while OC diagnosed at early stages enjoy a much higher 5-year-survival rate, comparatively. Although the oral cavity is one of the most easily accessible parts of the body for cancer screening, OC is typically diagnosed at later stages. The delay in diagnosis is one of the factors for the poor 5-year survival rate and high mortality and morbidity among patients. Therefore, an early diagnosis is of utmost importance. Visual and tactile examination and scalpel biopsy are still considered the gold standard for definitive diagnosis of oral potentially malignant disorder (OPMD) and OC. Nevertheless, adjunctive techniques could be employed to increase the ability to distinguish benign abnormalities from dysplastic/malignant changes. These would also aid in identifying areas of dysplasia/early OC that are not visible to the naked eye and tackle the delay in diagnosing OPMD/OC. These adjunctive tools are not a replacement for visual and tactile examination but are supplementary aids. They could be used to screen healthy patients for the presence of any occult cancerous change and evaluate the biological potential of clinically abnormal mucosal lesions, thus enabling early recognition and diagnosis which might increase survival rate and reduce mortality and treatment-associated morbidity.

Keywords: *Adjunctive techniques, early diagnosis, oral cancer, oral potentially malignant disorder*

Introduction

Among the cancers of the human body, cancers of the head and neck rank as the sixth most common. Oral cancer (OC) is part of the head and neck cancers and comprises about 85% of this category.[1] Squamous cell carcinomas account for 95% of OC with adenocarcinomas, adenoid cystic tumors, lymphomas, and melanomas comprising the remaining 5%.[2] The World Health Organization (WHO) predicts about 657,000 diagnoses of new cases of OC globally each year, with a death rate of more than 330,000 patients. OC occurs in males twice more frequently than in females. Although historically OC occurred after 40 years of age, it is now occurring in individuals younger than 40. OC is a disease associated with lifestyle. Lifestyle behavioral risk factors include tobacco usage, betel quid chewing, alcohol misuse, and dietary micronutrient deficiencies.[3]

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Some of the emerging risk factors being investigated are human papillomavirus infections, immunosuppression, mate drinking, khat chewing, poor oral hygiene, and alcohol content in mouthwashes.[4]

In general, the 5-year-survival rate for OC is estimated to be about 81% for patients with localized disease, and <50% for late-stage OC. The patient's 5-year rate of survival is about 42% when regional nodes are involved, and the rate drops to a dismal 17% when distant metastasis occurs.[5] This low 5-year rate of survival has been relatively constant for the last few decades and the cost of treatment of late-stage OCs is estimated to be a staggering USD 3.2 billion. Of all the major cancers, OC has the lowest 5-year-survival rate, probably because they are usually diagnosed at the advanced stages.[6] However, the survival rate improves drastically if detected early, compared to most other cancers.[7] Early detection, diagnosis and treatment are the best interventions for reducing the high morbidity and mortality associated with OC. However, OCs are detected at late stages, usually stage III and IV, which results in treatment complications, recurrences, poor prognosis, and a financial burden. This delay in diagnosis could be due both to the patients reporting late to the clinician and professional delay when clinicians miss detecting the lesions owing to lack of expertise. Therefore, an empirical emphasis must be given to the importance of routine oral mucosal screening as part of general health screening to both the patients and the general dental practitioners.

Usually, visible changes in the oral mucosa in the form of white, red, and/or speckled lesions precede the development of OC. These oral potentially malignant disorders (OPMDs) go through varying degrees of dysplasia, classified as mild, moderate, and severe, and carcinoma *in situ*, before developing into invasive carcinoma.[8] Most of these disorders appear asymptomatic in their early stages and may be detected by dental practitioners on routine oral examination.[9] However, often, an apparently normal-looking mucosa could harbor an OPMD or OC lesion, which could pose as a diagnostic challenge even for an oral medicine specialist, let alone a general dental practitioner.[10] This is where the adjunctive techniques in the diagnosis of these lesions may prove useful. It is vital, therefore, that general dental practitioners are knowledgeable about the various adjunctive techniques that are available for routine screening of the oral mucosa to further investigate or, where necessary, make referrals to specialists for treatment.

It is one of the major objectives of the WHO to lighten the burden of OC globally through interventions that can help prevent or detect the disease at an early stage.[11] To attain this WHO objective, routine screening for OPMD and OC needs to be carried out. Screening is defined as "the application of a test or tests to people who are apparently free from the disease in question in order to separate those who probably have the disease from those who probably do not." Basically, screening detects the presence of disease in people who are symptom-free.[12] For the detection of oral premalignant disorders and cancer, screening is still carried out through systematic visual and tactile clinical examination of the oral cavity, to a large extent. It is a well-known fact that the limitation of visual examination is the subjective interpretation.[13] Although visual and tactile examination are the screenings tests recommended by the OC Foundation and the WHO, OPMD, and early OC, within oral mucosal areas that appear normal could pose diagnostic challenges.[14]

Visual and tactile examination and scalpel biopsy are still considered the gold standard for definitive diagnosis of OPMD and OC.[15] Nevertheless, adjunctive techniques could increase the ability to differentiate between benign abnormalities and dysplastic/malignant changes. These would also aid in identifying areas of dysplasia/early OC that are not visible to the naked eye and tackle the delay in the diagnosis of OPMD or OC.[13] These adjunctive tools are not a replacement for visual and tactile examination but are supplementary aids. They could be used for the presence of any occult cancerous change and/or evaluate the biological nature of lesions that appear clinically abnormal.[15] Before embarking on the use of any adjunctive diagnostic tool, features such as positive and negative predictor values, sensitivity, and specificity of these tools must be considered. When a person with the target disease churns a positive result for a test, it corroborates the sensitivity of the test/tool. Specificity of a test or tool is when a person without the target disease produces a negative result. An inaccurate positive result is termed a false positive result. Positive predictive value is the percentage of patients with a positive test who have the disease [Table 1]. Negative predictive value is the percentage of patients with a negative test who do not have the disease. There is a myriad of diagnostic tools out there and covering them all is not within the scope of this review. This article will review the most widely used adjunctive techniques that could be used by the general dental practitioners in their everyday practice, with a brief mention of the newer ones.

Oral Cytology

One of the earliest and most cost-effective of the diagnostic tools is oral cytology. Ever since papanicolaou

Table 1: Types of diagnostic techniques with their specificities and sensitivities

Technique	Mode	Sensitivity and specificity (%)
Conventional oral examination	Visual inspection	95/95
Vital staining	Tissue staining	38-98/9-93
Oral cytology	Conventional exfoliative cytology	76.8-100/88.9-100
Oral cytology	Brush biopsy (OralCdx)	92/94
Light based system - vizilite	Chemiluminescence	100/14
Light based system - velscope	Autofluorescence	98/100
Salivary biomarkers	Salivary analysis	71/75

and Traut substantiated the use of exfoliative cytology for diagnosing cervical cancers, it has been applied to diagnose oral diseases.[16] Exfoliative cytology is a technique which is less time-consuming and could be performed in-office. It is minimally invasive causing little discomfort if any and the results could be obtained almost immediately. The accuracy of the results has been debated but generally is considered unreliable. The basis of oral cytology is that the cells that are dysplastic and cancerous possess lesser and weaker desmosomes. Hence, these dysplastic and cancerous cells exfoliate easily and can be sampled from the surface of the lesions. These cells are then viewed under the microscope for cellular atypia if any. Clinically suspicious oral lesions should be biopsied regardless of exfoliative cytology results. However, negative findings need to undergo meticulous clinical examination. When exfoliative cytology churns out findings such as increased nuclear area, increased nuclear: Cytoplasmic ratio, increased keratinization, nuclear hyperchromatism, nuclear pleomorphism, or chromatin clumping, it most definitely is indicated for biopsy and further microscopic examination.[17] The most common and utmost disadvantage with this technique is the presence of disaggregated cells, which poses diagnostic challenges. In the detection of OC, exfoliative cytology has shown a sensitivity rate in the range of 76.8% to 100%, while the specificity ranged between 88.9% and 100%.[18]

In 1999, oral cytology evolved leaps and bounds with the introduction of the so-called brush biopsy. Since then, brush biopsy has become a popular oral cytology tool which is simple to use, minimally invasive, and painless to the patient. It is relatively inexpensive and has a good psychological acceptance by the patients.[19] Brush biopsy is a technique which samples the trans-epithelial cells from a part of the mucosa, while exfoliative cytology samples the superficial layer cells. Cells from the superficial, intermediate, and parabasal/basal layers of the epithelium can be easily sampled with the innovative brush biopsy. This technique could be an adjunctive tool to diagnose early dysplasia and early OC in those patients who present with no symptoms, with occult lesions, and in those who do not mandate expeditious biopsy. With this tool, we could eliminate guesswork about which lesion requires surgical biopsy and lessen the delay in referring patients for scalpel biopsy and microscopic evaluation.[13,20,21]

A computer-assisted analysis of the oral brush biopsy, also known as OralCDx® system (OralCDx, OralScan Laboratories Inc., Suffern, N. Y.) was introduced in 2000 which received the American Dental Association "Seal of Acceptance" in the same year.[22] Results from CDx would be interpreted as negative, positive, or atypical. Negative results indicate no cellular abnormalities and warrant no further treatment. Positive result denotes signs of epithelial dysplasia or OC, and results that are atypical indicate abnormal epithelial changes warranting further investigation.[22,23] Nonetheless, when the brush biopsy computed system generates a positive result, the lesion must be subjected to a conventional scalpel biopsy and histological examination to arrive at a confirmatory diagnosis.[13,23,24] In a study by Hohlweg-Majert *et al*., the sensitivity for the detection of abnormal cells by means of OralCDx was 52%, specificity 29%, and the positive predictive value 63%.[25]

Vital Staining

Toluidine blue (TB) or tolonium chloride has been used as an adjunctive screening tool for over 40 years. It is an expeditious, economic, and effectual diagnostic tool. TB is an acidophilic, basic, metachromatic dye that differentially stains malignant cells but not normal epithelial cells.[26] The intensity of the staining of the malignant cells perhaps could be attributed to the increased DNA and RNA content within them and the broader intercellular junctions between them, when compared to normal epithelial cells.[27] It is useful in detecting occult, asymptomatic lesions and affirm suspicious lesions detected clinically. It could be used as an adjunctive screening tool on a routine basis for high-risk patients, or as periodic check. TB could also be used for teaching purposes in academic settings, or to reassure a concerned patient.[11] Limitations of TB are that inflammatory lesions could also stain positively

with TB. To bring down the false positives, local etiological factors must be considered and eliminated before the procedure. Patients with a positive stain are retested after 14 days and a second positive stain makes biopsy mandatory.[28] However, if a clinically sinister-looking lesion does not stain positively then it is recommended to be sent for scalpel biopsy and histological examination.[28,29] Some studies have pointed out that since TB reacts with RNA, mutagenic effect is a possibility especially when stained cells are exposed to light or other high-energy radiations.[30] False-negative results have also been a concern in the past and some studies have reported false-negative results in about 6.7%.[7] Few researchers have urged to use TB with caution and pointed out that TB generating a higher percentage of false-negative results could be a concern for physicians and patients.[31] However, the overall sensitivity of TB staining for the detection of OPMD and OC has ranged from 0.78 to 1.00 and the specificity from 0.31 to 1.00.[15]

Light Based Diagnostic Tools

Chemiluminescence

Luminescence is a phenomenon of light emission, which happens when an excited molecule relaxes to its ground state. Luminescence can be categorized based on the source of energy to obtain the excited state.[32] Chemiluminescence is the property of light emission or energy emission due to a chemical reaction. During the process of carcinogenesis, there are abnormal structural and metabolic changes taking place within the oral mucosa. These abnormalities can cause a difference in their light-absorbing and reflecting properties.[33] The technique involves the clinical inspection of oral mucosa with chemiluminescent blue/white light which has wavelengths of 430, 540, and 580 nm. Light gets absorbed by the normal epithelium, which will appear dark following light absorption. Nonetheless, hyper keratinized or dysplastic mucosa appear white which is related to the altered epithelial thickness, higher density of nuclear content and mitochondrial matrix. The tissue allows the dysplastic areas to preferentially reflect the low energy blue-white light emitted by the device and appear as "aceto-white." It helps to visually distinguish between normal mucosa and oral white lesions, which would appear with brighter, sharper, and distinct margins. Light systems namely ViziLite, ViziLite Plus (Zila, Fort Collins, CO, USA), DIFOTI™ (Electro-Optical Sciences, Irvington, NY, USA), and MicroLux DL systems (AdDent, Inc., Danbury, CT, USA) adapt this technology, in order to enhance the visibility of occult oral mucosal lesions, that may be overlooked during conventional oral examination. The ViziLite system uses disposable chemiluminescent sticks, whereas the MicroLux DL uses rechargeable battery-operated light system. Both these systems require a prerinsing of the oral cavity with 1% acetic acid which removes the surface debris of cells thereby increase the visibility of epithelial cell nuclei and coagulates the cellular surface proteins, which reduces the transparency of the epithelium. ViziLite plus has an additional TB staining kit, to demarcate the "aceto-white" lesion for subsequent biopsy.[15,34,35] Although there is inadequate evidence to support the use of chemiluminescence as an adjunctive aid to detect PMD or OC, some studies have suggested that they may be helpful in identifying lesions that cannot be visualized under incandescent light.[35,36]

Autofluorescence

The phenomenon of fluorescence occurs in certain atoms and molecules which can absorb light at a particular wavelength and eventually emit light of a longer wavelength after a short interval of time. The molecules that are capable of exhibiting this phenomenon are called fluorophores. Oral cavity tissues contain fluorophores such as nicotinamide adenine dinucleotide and flavin adenine dinucleotide and the cross-links between collagen bundles. Fluorophores are also seen abundantly in keratin, elastin, fibrin, etc. Amino acids such as tryptophan, tyrosine, and phenylalanine also act as fluorophores. Ultraviolet photons are absorbed by these molecules which then emit lower energy, longer wavelength photons that are visualized clinically as fluorescence.[33] These devices use blue light excitation between 400 and 460 nm wavelength. Mucosal abnormalities can alter the absorption and scattering of fluorophores. Normal oral mucosa emits a pale green fluorescence when viewed through a filter and abnormal tissue is associated with a loss of autofluorescence and appears dark.[14] Various autofluorescence devices are being marketed namely VELscope (LED Medical Diagnostics Inc., Barnaby Canada) which is an acronym for Visually enhanced lesion scope (VELscope), OralID®(Forward Science™, Houston, Texas, USA), Bio Screen® (AdDent Inc., Danbury, Connecticut, USA). All these systems use an extrinsic light source to excite endogenous fluorophores within the oral mucosa.[14,37] Autofluorescence and reflectance properties can be combined in screening tools. Since OC is also associated with increased angiogenesis, it has an effect on both autofluorescence and reflectance properties. The angiogenic vasculature is different from that of healthy tissue. Increase in the production of pro-angiogenic factors in OC leads to uncontrolled development of new blood vessels. As a result, the

number of blood vessels within a given microscopic area, known as microvessel density (MVD) is usually high and irregular. Identafi® (StarDental-DentalEZ, Lancaster, PA, USA) is an autofluorescence system that combines autofluorescence with reflectance. It has three different wavelengths of light such as white, violet (405 nm), and green-amber (545 nm). The white light provides classical visualization of oral mucosa whereas the violet light excites endogen fluorophores, enabling autofluorescence. The green-amber light excites hemoglobin molecules in the blood and causes reflectance, to detect changes in angiogenesis and MVD and visualize the vasculature underlying the mucosa. A mirror is attached to the probe.[38-40]

Salivary biomarkers

A biomarker is an indicator of normal and pathologic activities or pharmacologic responses to therapeutic intervention which can be measured and assessed.[41] Biomarkers can provide unbiased information of the current physiologic state of any living organism.[42] In recent times, using saliva as a medium of "liquid biopsy" and biomarker to diagnose and predict risk factors for OPMDs has gained popularity.[43,44] It is a cost-effective and noninvasive technique. Genomic profiling is a technique that can be used to test for OC salivary biomarkers such as growth factors, cytokines, and epithelial tumor factors.[45] This can be done from a simple oral swab. In the saliva of OC patients, the epithelial serum circulatory tumor markers most widely researched are Cyfra 21-1, tissue polypeptide-specific antigen (TPS), carcinoembryonic antigen, squamous cell carcinoma, CA125, and CA19-9.[46]

Summary

To attain the WHO objective of controlling the burden of OC worldwide, an increase in the awareness of regular oral mucosal screening must be achieved among the public and the general dental practitioner (GDP). Diagnosis by a conventional oral examination with digital palpation can be subjective and although scalpel biopsy and histopathological examination are the gold standards, adjunctive techniques can be supplementary tools for routine screening which can assist the GDPs to accelerate referrals and reduce unnecessary biopsies. It is recommended that these adjunctive tools be encouraged to be used in routine dental practice. However, comprehensive knowledge on the limitations, constraints, sensitivity, and specificity of the selected technique is advocated.

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Conflicts of interest

There are no conflicts of interest.

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