A review of diagnostic aids for oral cancer screenings in general practice dentistry

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Abstract
Early detection of oral cancer is a public health priority in which oral health professionals must play a leading role. Numerous diagnostic aids have been developed as adjuncts to the routine oral cancer screening examination to improve in this endeavor. This paper reviews the principles and current evidence behind these techniques and provides recommendations for their use in general practice dentistry. Among the modalities examined are toluidine blue staining, light-based detection systems (including chemiluminescence, tissue fluorescence imaging, and tissue fluorescent spectroscopy), brush biopsy, and salivary analysis. We conclude that despite encouraging results, strong evidence to support these diagnostic aids is still lacking. Further well-designed studies in low-risk populations and with sound methodology are required before use of these adjuncts can be recommended without reservation.

Introduction
Oral and oropharyngeal cancer is the sixth most commonly reported cancer worldwide, with an estimated annual incidence of over 400,000 cases [1]. Unlike many other cancers, however, its incidence is increasing [2], making it a pressing public health issue now and in the coming years. Early detection of malignant and potentially malignant oral lesions is recognized as one of the most efficient ways to reduce the high mortality rates of oral cancer and the morbidity associated with its treatment, which can leave patients with significant disturbances in speech, mastication, and dental health. If diagnosed early, oral cancer is one of the most curable cancers, with a survival rate exceeding 80% [3]. Yet most are detected in their advanced stages [4,5], which is one of the reasons that the 5-year survival rate for has remained relatively stable at 50% for the last few decades [2]. This is especially troubling considering the oral cavity is one of the most accessible and visible anatomical regions of the human body.

One approach to improving early detection of oral cancer is to improve the ability of oral health professionals in detecting these lesions. Currently, a conventional oral examination that includes visual inspection under incandescent light and palpation of the head and neck region constitutes the gold standard screening test for oral cancer. Similarly, the reference diagnostic standard is tissue biopsy and histopathological diagnosis. Therefore, any visually detected lesion(s) that raises suspicion requires biopsy for definitive diagnosis. However, conventional oral examination is not without its limitations. For instance, early cancerous and precancerous lesions are often subtle and rarely demonstrate the clinical characteristics of more advanced cases such as ulceration, induration, pain, or cervical lymphadenopathy. In addition, premalignant lesions can be clinically heterogeneous and may mimic a variety of benign or reactive conditions. There is also a growing realization that some premalignant and early cancerous lesions are not readily visible to the naked eye [6]. To this end, a variety of oral cancer diagnostic aids have been developed as adjuncts to the standard visual and tactile oral examination with the aim of improving early detection oral lesions (Table 1). These adjuncts implement different principles of detection and have been evaluated in a variety of clinical settings. The aim of this paper is to review the principles and current
evidence behind these diagnostic aids and to provide recommendations for their use in general practice dentistry.

**Oral Cancer Diagnostic Aids**

**Toluidine blue staining**

Toluidine blue (TB), also known as tolonium chloride, is a vital dye with a high affinity for nucleic acids. It has a long history of use as an aid in the identification of mucosal abnormalities of the cervix and oral cavity but it has seen a resurgence of interest in recent years. TB is used to detect sites undergoing rapid cellular proliferation, such as areas of carcinoma in situ or high grade dysplasia, which theoretically have higher nucleic acid content than normal sites. The dye is applied using a topical swab or as a 1% aqueous mouth rinse, with lesions staining blue considered to be positive. It is claimed to be a simple, inexpensive, and sensitive tool for identifying early squamous cell carcinoma (SCC) and high-grade dysplasias and many surgeons also favor its use to demarcate the margins around a lesion prior to excision \(^7\).

A large number of studies have evaluated the use of TB as an adjunct in oral cancer screenings but many of these studies have significant limitations, including variability in methodology and interpretation of test results, such as what constitutes a positive test result (e.g. pale blue versus dark blue). The largest drawback of these studies as pertaining to dentistry is that they do not assess TB’s role as a diagnostic adjunct in lower-risk populations, such as patients in general dental practices, but rather have been carried out in secondary and tertiary care institutions in the hands of specialists, often on patients at high-risk or with a previous history of oral cancer. Overall, the sensitivity of TB staining for the detection of oral carcinomas varies between 78% and 100% and the specificity varies between 31% and 100% \(^8\). In detecting dysplasia, however, the sensitivity appears to be significantly lower \(^9\). A systematic review by Gray et al. \(^10\) concluded that there is no evidence that TB is effective as a screening test in primary care settings, citing the high rate of false positives and low specificity in staining dysplastic lesions. However, this does not preclude its usefulness as an adjunct to clinical examination and experienced clinicians may find the test useful in evaluating the presence oral lesions in high-risk individuals, such as patients at risk for a second primary lesion \(^9\).

**Light-based adjunctive aids**

Several light-based oral cancer diagnostic aids have been developed with the aim of assisting clinicians in identifying malignant and premalignant lesions. These aids operate on the assumption that the structural and metabolic changes that take place in the mucosa during carcinogenesis give rise to distinct profiles of absorption and refraction when exposed to different types of light or energy. The most common methods employed by these aids include chemiluminescence, tissue fluorescence imaging, and tissue fluorescent spectroscopy.

**Chemiluminescence**

Chemiluminescence refers to the emission of light during a chemical reaction. The most well known diagnostic aid using this principle is the ViziLite\(^\text{®}\) system (Zila Pharmaceuticals, Phoenix, AZ, USA) introduced in 2002, which is aimed at potentiating certain features of a lesion, such as brightness and sharpness of its borders. The system involves an oral rinse with a 1% acetic acid solution for 1 minute to remove the glycoprotein layer and partially desiccate the cells of the oral mucosa, thereby improving the visibility the nuclei. A handheld device emitting blue/white light of 490-510 nm in wavelength is then used to examine the oral tissues. Theoretically, abnormal cells have a higher nuclear to cytoplasmic ratio and reflect the light to appear distinctly white (or acetowhite) with brighter, sharper, and more distinct margins, compared to normal cells that absorb the light and appear blue \(^11\).

The literature reporting on the efficacy of the ViziLite system in detecting premalignant oral lesions is sparse and most of the existing studies have been cross-sectional and in patients with known oral lesions rather than the general population \(^12-16\). Therefore, the reported sensitivities in many of these studies approaches 100% but the specificities are significantly lower (0 to 14.2%). To reduce the number of false positives, the system has been recently been combined with TB stain (ViziLite Plus \(^9\)). While the specificity may be slightly improved with this combination, very little scientific data has been published on this system. It is important to note that no study has demonstrated the ability of the ViziLite system to differentiate dysplasia/carcinoma from benign lesions. Hence, the majority of studies have investigated how the system enhances subjective clinical evaluation of intra-oral lesions including brightness, sharpness, and texture with respect to routine examination under incandescent light. While some authors have reported that this technique can improve detection of intra-oral abnormalities \(^14\), others have suggested that it does not help and can even make visualization more difficult because of distracting highlights on the oral mucosa \(^15\). Based on this current evidence, it is unclear what added benefit chemiluminescence provides to practicing dentists. As pointed out by Lingen et al. \(^8\), if a clinician identifies a suspicious lesion, they are obligated to obtain a definitive diagnosis via biopsy. Thus, subjective
improvement in the ability to see a lesion would provide minimal diagnostic advantage. What are needed are more clinical trials that specifically investigate whether this system can reliably detect premalignant lesions that are not detected by clinical examination alone.

**Tissue fluorescence imaging**

The concept behind tissue autofluorescence is that changes in epithelial cell structure (e.g. hyperchromatosis, increased cellular/nuclear polymorphism) and metabolism (e.g. concentration of flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH)), as well as changes in the subepithelial stroma (e.g. composition of collagen matrix and elastin), alter the interaction of oral mucosa with light. Specifically, these epithelial and stromal changes can alter the distribution of tissue fluorophores and their ability to emit fluorescence after stimulation with an intense light. The VELscope® (Visually Enhanced Lesion Scope; LED Dental Inc., White Rock, BC, Canada), which was introduced in 2006, operates on this principle. The device works by emitting a cone of light in the blue spectrum (400-460 nm) into the oral cavity, causing fluorophores in the oral tissue to excite and fluoresce. The fluorescence can then be viewed directly through a selective (narrow-band) filter embedded within the instrument. According to the manufacturer, normal mucosa emits a pale green autofluorescence, whereas areas suspicious for epithelial dysplasia or SCC produce decreased levels of autofluorescence and appear darker compared to the surrounding healthy tissue.

Early studies on the VELscope were encouraging, with sensitivities of 97% to 100% and specificities of 94% to 100% being reported \[17-18\]. However, the majority of these studies had serious limitations, most notably the inclusion of patients with a known history of dysplasia or carcinoma in situ. In addition, some of these studies were pilot studies while others were case studies, neither of which is an acceptable level of evidence to change clinical practice \[19\]. More recent studies have evaluated the clinical utility of the VELscope more rigorously and as expected, reported sensitivity and specificity values are significantly lower \[20-25\], ranging from 30% to 92% and 15.3% to 77%, respectively. However, these studies also have limitations, as the majority were conducted by specialists on a referral basis. The only study examining use of the VELscope in low-risk patients concluded that clinical examination is more valid than use of the VELscope for discriminating benign mucosal alterations from premalignancy and did not support its use as an oral cancer screening adjunct \[24\]. In general, there is concern about the potential for false negative findings, which may cause clinicians to overlook premalignant or malignant lesions, and false-positive findings, which could lead to undue patient anxiety and increased health care costs. The manufacturers themselves caution that loss of autofluorescence is not only limited to epithelial abnormalities but can also occur with prominent surface vascularity, including areas of inflammation, and hyperpigmentation.

At the current time, most authors currently do not support the use of this device in routine screening assessments of asymptomatic dental patients. At most, it can be used to help identify lesions that may have been overlooked with a conventional oral examination but even then, interpretation of results requires skill and training, making it best suited for oral medicine specialists.

**Tissue fluorescence spectroscopy**

In addition to visual autofluorescence, a technique called autofluorescence spectroscopy has recently emerged in oral oncology research. This system consists of a small optical fiber that produces various excitation wavelengths and a spectrograph that receives the data and records it on a computer \[25\]. The computer software then analyzes the spectra of reflected fluorescence from the tissue. This technique has the advantage of eliminating the subjective interpretation of tissue fluorescence changes. However, a disadvantage is more variables (e.g. combination of wavelengths, methodology of fluorescence analysis) have to be tested and considered, which has led to unclear results. Overall, autofluorescence spectroscopy seems to be very accurate for distinguishing lesions from healthy oral mucosa, with high sensitivity and specificity, especially when malignant tumors are compared to healthy mucosa \[28\]. However, the ability of the technique to distinguish and classify different types of lesions has been reported to be low. Moreover, autofluorescence spectroscopy is for practical reasons not suitable to detect new lesions or to demarcate large lesions as the optical fiber can sample only a small mucosal area \[9\]. This limits the use of spectroscopy to the evaluation of well-defined and small mucosal lesions that have already been identified through clinical examination. Further research is still needed to support the clinical application of this diagnostic aid.

**Brush Biopsy**

The oral brush biopsy, also known as the OralCDx® Brush Test (ODx Laboratories, Inc., Suffren, NY, USA), was introduced in 1999 as a way to investigate innocuous-looking oral lesions for dysplasia or cancer that would not otherwise have been biopsied because of low-risk clinical features. The kit consists of a specially designed stiff bristle brush, a glass slide, a form, a fixative
(alcohol/polyethylene glycol), and a container for sending samples to the OralCDx laboratory. The test works by placing the brush on the lesion and rotating it in one spot until it produces reddening or hemorrhagic spots. This allows for collection of a sample that is representative of the entire epithelium, including the superficial, intermediate, and basal layers. The sample is then placed on the glass slide, fixed, and sent to the OralDx laboratory where it is analyzed via a computer-based imaging system. Results can be reported as “negative” (without epithelial abnormalities), “atypical” (epithelial changes of uncertain diagnostic meaning), “positive” (evidence of dysplasia or carcinoma), or “inadequate” (incomplete transepithelial specimen), with “atypical” or “positive” results requiring incisional biopsy for definitive diagnosis.

The OralCDx Brush Test has been the subject of many studies and a strong body of evidence exists supporting its accuracy. In every study in which the same lesion was simultaneously tested with both a brush and scalpel biopsy, OralCDx was shown to have a sensitivity and specificity exceeding 90% [28]. As some authors have pointed out, however, in some of these studies, scalpel biopsy was performed after brush biopsy of lesions with high-risk clinical features, but not after brush biopsy of innocuous-looking lesions [9]. Therefore, the sensitivity and specificity values must be interpreted with a degree of caution. Additionally, more studies need to be completed in low-risk populations with benign-appearing oral lesions, as there is concern with reduced accuracy and increased rate of false positives [25, 29]. One of the largest drawbacks of this test appears to be the time delay before the incisional biopsy, as authors have found an average delay of over 100 days before the diagnosis of a malignant lesion [10-31]. Nevertheless, brush biopsy may be useful in certain clinical scenarios. For example, it may be beneficial in the patient with multiple oral lesions where it is unlikely that the patient would readily consent to multiple scalpel biopsies. Similarly, it may be useful in the non-compliant patient who is unlikely to come back for a follow-up exam or accept an immediate referral to an oral surgeon [8]. There has been an effort to improve the technique and increase its sensitivity by combining it with molecular analyses [32]. This permits the identification of genomic anomalies such as mutations of the tumor suppressing gene p53, epigenetic alterations, genomic instability, and microsatellite instability (MSI), among others [33]. However, these methods are in their infancy and few clinical studies exist to support any added benefit to the brush biopsy.

**Salivary Analysis**

There is an attractive possibility that saliva testing may be an effective, non-invasive, screening test for oral cancer. The principle is based on the fact that salivary composition is altered in patients with oral cancer. The approach relies on measuring specific salivary macromolecules, enzymes, cytokines, growth factors, metalloproteases, endothelin, telomerase, cytokeratines, messenger ribonucleic acid (mRNA) and DNA transcripts. It has been shown that the salivary levels of total sugar, protein-bound sialic acid, free sialic acid, sodium, calcium, immunoglobulin G, albumin and lactate dehydrogenase are significantly higher in patients with oral cancer than those with healthy mucosa [34-36]. In addition, patients with oral cancer have been found to have significant alterations of various oxidative stress-related salivary parameters, epithelial tumor markers CYFRA 21-1, tissue polypeptide-specific (TPS) antigen, and various RNA transcripts (e.g. insulin-like growth factor, matrix metalloproteinases (MMP-2 and MMP-9), interleukin-8 and 1B) [34-40]. These biomarkers may be used as a tool for the diagnosis, prognosis, and post-operative monitoring of oral cancer. However, well-designed studies are still required to investigate whether salivary analysis could prove to be a feasible and cost-effective tool for these purposes.

**Conclusion**

Oral health professionals must play a leading role in improving the early detection of oral cancer. Simple visual and tactile examination is limited by subjective interpretation and the potential for occurrence of dysplasia and SCC in areas of normal-looking oral mucosa. To overcome these limitations, numerous diagnostic aids have been developed as adjuncts to the standard oral examination. Some of these aids, such as TB staining, have been studied extensively, while others, such as salivary analysis, are just beginning to be explored. Unfortunately, the evidence behind these diagnostic aids as a whole is limited and none should be used in place of a standard clinical examination and biopsy. At the present time, the utilization of these techniques in clinical practice is largely anecdotal and has proven to be most useful for experienced clinicians and specialists by improving their ability to detect dysplasia and SCC in high-risk individuals attending secondary and tertiary care centers. General practitioners must weigh the current evidence behind these diagnostic aids and determine what added benefit, if any, they provide to their patients. In the meantime, further well-designed studies with sound methodology and in low-risk populations are needed to help assess these new and evolving modalities of oral cancer detection.
References


20. Farah CS, McIntosh L, Georgiou A, McCullough MJ. The efficacy of autofluorescence


Table 1: Diagnostic aids for oral cancer screenings.

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