Advances in Diagnostic Adjuncts for Oral Squamous Cell Carcinoma

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Abstract: Oral cancer is the 8th most common cancer in males and the 15th most common in females in the United States. Each year, it affects approximately 22,000 Americans and results in approximately 5300 deaths. The five-year survival rate of oral cancer remains low (53% to 60%) for the past three decades and delayed diagnosis has been suggested to be one of the major reasons. The detection and diagnosis of oral cancer is currently based on clinical visual examination and histopathological evaluation of the biopsy material. In responding to the need for early detection of oral cancer, several diagnostic adjuncts have been developed over the years. The purpose of this article is to review the current knowledge about the commercially available diagnostic adjuncts as well as to review the research on the development of the promising tools for the early detection of oral cancer.

Keywords: Oral cancer, diagnostic adjuncts, salivary diagnostics.

INTRODUCTION

Oral cancer is the 11th most common cancer in the world [1]. Each year approximately 22,000 Americans are diagnosed with it and about 5300 die. More than 90% of the oral cancers are squamous cell carcinoma (OSCC), which arises from the epithelial lining of the oral cavity. For the past three decades, the five-year survival rate of oral cancer has been improved some but remains in the range of 53% to 60%, which is one of the lowest five-year survival rates of all the major cancer types [1, 2]. Despite the fact that oral cavity is easily accessible for direct visual examination, most OSCC is not diagnosed until an advanced stage, which has been suggested to be one of the major reasons for a minimally improved survival rate [3]. This fact underscores the importance of patient education and the need to improve clinicians' ability to recognize early malignant and pre-malignant changes in the oral cavity.

In responding to the need for improving the clinicians' ability in detecting early malignant changes, various diagnostic adjuncts have been developed over the past few decades. The currently available diagnostic adjuncts for OSCC can be classified into the following three major categories: 1) Vital tissue staining (e.g. toludine blue or tulonium chloride), 2) Brush cytology (e.g., Oral CDx; from CDx Laboratories in Suffern, New York); and 3) Visualization adjunct (e.g., the ViziLite system, from Zila, Inc. of Phoenix, Arizona; MicroLux DL, from AdDent Inc., Danbury, CT; VELscope, from LED Dental Inc., of Vancouver, B.C. in Canada and Identafi 3000, from Trimima Remicalm of Houston, Texas) [4,5]. Some claimed to assist the differentiation between clinically benign and pre-malignant or malignant lesions (Oral CDx). Others aimed to detect pre-malignant or malignant changes that were not well recognized by clinical visual examination (toludine blue, ViziLite, MicroLux DL, VELscope and Indentafi 3000).

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Although many studies have investigated sensitivity and specificity of these diagnostic adjuncts, a survey and a recent critical evaluation of the published studies both concluded that there is still no convincing or adequate data to support their efficacy specifically for early detection or for reducing the death rate from OSCC [3-6]. Readers are strongly encouraged to read the recently published excellent reviews about these diagnostic adjuncts [3-5]. This mini review does not intend to repeat the content of the recent reviews on this subject and therefore, will only briefly discuss these currently available diagnostic adjuncts but extend the scope to the promising diagnostic adjuncts that are currently under development for early detection of oral cancer.

CURRENTLY AVAILABLE DIAGNOSTIC ADJUNCTS

Vital Tissue Staining – Toludine Blue Staining

Toludine blue is a metachromatic dye that binds to DNA in vital tissues. It is not cancer specific, but it has been reported to stain cells with relatively increased amounts of DNA and possibly altered DNA in premalignant and malignant epithelial lesions [4]. Toludine blue has been used for decades for screening abnormal epithelium in the cervix. Although it has not been approved by the FDA for use in the US as an oral cancer screening technique, it has been used as such for decades in other parts of the world [3].

Many studies have investigated sensitivity and specificity of toludine blue for detecting pre-malignant and malignant lesions, however, the results varied and were inconsistent [7-10]. Toludine blue staining appears to be useful as an adjunct to clinical examination in most studies, with a sensitivity ranging from 77-100% and a specificity ranging from 62-100% [4, 7, 8, 10]. However, a low positive predictive value of 43.5% for potentially malignant lesions [10] and a false-negative rate as high as 20.5% for pre-malignant lesions [8] also have been reported.

Brush Cytology

Brush cytology (Oral CDX, Fig. **1**), developed in 1999, has become popular in dental offices in recent years [11-14].

This technique requires the clinician to collect epithelial cells by rotating a provided circular brush against the lesion until pinpoint bleeding is seen clinically, indicating penetration of the basement membrane and a collection of cells from all layers of the epithelium [11]. The cells are then fixed and sent to the company for a computer-assisted specimen analysis. The results are interpreted as falling into one of four categories: inadequate, negative, atypical, or positive; and the company recommends a follow-up scalpel biopsy for patients with an atypical or a positive result.

Fig. (1). The kit for Oral CDx from CDx Laboratories in Suffern, New York. It contains a cytobrush, a blank microscopic slide, a plastic container, two packages of fixative, a plastic bag and a test requisition form.

In a few studies comparing results of brush cytology to scalpel biopsies, sensitivity for detecting oral epithelial dysplasia or OSCC was reported high; ranging from 71.4% to 92% [15-17], although cases with false negative results have also been reported [15,18]. However, the specificity was reported as low, around 32% [15,16]; and a high false positive rate (meaning initial "atypical" results from brush cytology, followed by scalpel biopsy that revealed no dysplasia or malignancy) was reported as ranging from 43 to 89% [16,19]. In addition, a significant weakness of CDX is its inability to detect non-dysplastic leukoplakias which have a malignant transformation rate of approximately 1% [20].

Visualization Adjuncts

Adjunct based on tissue reflectance- ViziLite, ViziLite Plus, MicroLux

This category includes ViziLite (Fig. **2**) and ViziLite Plus with TBlue from Zila Pharmaceuticals, Phoenix, and MicroLux DL from AdDent Inc., Danbury, Connecticut.

The ViziLite system and MicroLux are derived from an adjunct for diagnosing cervical lesions (speculoscopy) [4]. These products have been approved by the FDA to enhance the identification of oral mucosal lesions. For both systems, the patient is asked to rinse with a 1% acetic acid solution and then the oral cavity is examined by the clinician under the illumination of an activated chemiluminescent light (ViziLite) or a battery-operated light source (MicroLux DL). The differentiation between normal and abnormal oral epithelium is based on color appearance under the blue-white light generated by those two light sources. The normal epithelium appears light bluish whereas the abnormal epithelium appears distinctly white (aceto-white). The

ViziLite Plus also contains a tolonium chloride solution (TBlue) for marking the aceto-white lesion for subsequent biopsy once the light source is removed.

Fig. (2). The ViziLite System from Zila Pharmaceuticals, Phoenix. It consists of a brown bottle of 1% acetic acid solution and the disposable chemiluminescence light source package.

Several studies have investigated the benefit of using the ViziLite compared to conventional visual examination alone [21-26]. The ViziLite system was reported to highlight lesions of leukoplakia as well as oral benign lesions such as leukoedema, frictional keratosis, traumatic ulcer and lichen planus [21, 23, 24]. Most of these studies either did not include comparison to the diagnostic gold standard i.e. histopathologic diagnosis based on a scalpel biopsy, or contained only a limited number of cases subsequently biopsied, which made it difficult to assess specificity and sensitivity [21-24, 27]. There was only one study that correlated the ViziLite findings to the histopathologic diagnosis and the sensitivity, specificity and accuracy were reported to be 100%, 0% and 18.2%, respectively [25]. Therefore, the system may provide little or very limited benefit over conventional visual examination in assessing clinically suspicious oral lesions [3, 25, 26].

Adjunct Based on Tissue Autofluorescence - VELscope

The study of autofluorescence in normal and abnormal oral tissues has been an ongoing research interest in various regions of the world for the past four decades [28-34] and DeVeld *et al.* [35] provide an excellent review. Tissue autofluorescence is produced by fluorophores that exist in living tissue upon excitation with a suitable wavelength. The fluorophores can be in the extracellular matrix (collagen and elastin), in the blood (hemoglobin) or in the cells (keratin, the reduced form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD)). The concentration of fluorophores changes in pathological conditions that alter the blood concentration, nuclear size distribution, collagen content and epithelial thickness [35]. Alterations in tissue autofluorescence have been observed in OSCC and *in vivo* autofluorescence imaging and spectroscopy have been applied for early detection of premalignant and malignant lesions in the oral cavity [30-34].

The VELscope is a hand-held device that was approved by FDA for the direct visualization of autofluorescence in the oral cavity. It was recently introduced to the market as a

diagnostic adjunct for oral cancer detection. The VELscope consists of a light source which emits an intense excitation blue light (400-460 nm) and a handpiece with a selective filter for direct visualization (Fig. **3**) [3]. Under the illumination of this blue light, normal oral mucosa shows a pale green autofluorescence while abnormal tissue is proposed to show loss of autofluorescence and appears dark.

Fig. (3). The VELscope system from LED Dental Inc., White Rock, British Columbia, Canada. It consists of a light source which emits an intense excitation blue light (400-460 nm) and a handpiece with a selective filter for direct visualization.

The VELscope is a relative new device and so far only a limited number of studies have investigated its effectiveness as a diagnostic adjunct for oral cancer [36-39]. In the study reported by Lane *et al*. [36], clinical suspicious lesions (based on visual examination) in 44 patients were illuminated by VELscope followed by the biopsy. The sensitivity and specificity was reported to reach 98% and 100%, respectively. Poh *et al*. [38] demonstrated the potential application of VELscope in the operating room in assisting the determination of the surgical margins for oral cancer. They examined the cancerous lesion with VELscope and recorded their finding before the patients underwent surgery. They found that the loss of autofluorescence extended beyond the clinically visible tumor margin in 19 of the 20 tumors and the extension varied from 4-25 mm. Eighty-nine percent of these sampled areas of loss of autofluorescence showed the presence of carcinoma or epithelial dysplasia histologically. Poh *et al*. [37] also describe three cases where clinically normal mucosa showed loss of autofluorescence under VELscope examination. The biopsy of these three "lesions" revealed epithelial dysplasia or carcinoma-in-situ. This case report suggested the utility of VELscope in assisting clinical examination for screening purpose. Of note, these three studies all originated from British Columbia Cancer Agency [36-38]. Recently, Huber examined the oral cavity of 130 smokers with VELscope and compared the findings with conventional visual examination in an observational pilot study [39]. A total of 10 suspicious lesions were identified by conventional examination and no additional lesions were identified by VELscope. VELscope examination did not appear to enhance the detection of suspected lesions. In addition, Huber also noted that common oral inflammatory conditions, such as mucosal

pigmentation, cheek biting, gingivitis, oral ulcerations and viral eruptions also frequently demonstrated loss of autofluorescence on VELscope examination. Therefore, the utilization of the VELscope as a general screening adjunct was also questioned by the author.

More studies are required in assessing the usefulness of VELscope for oral cancer screening.

Adjunct Based on Tissue Autofluorescence and Reflectance-Identafi 3000

Identafi 3000, from Trimima® Remicalm of Houston, Texas, is the most recently developed device which was introduced to the market in early 2009. It uses white, violet and amber light to excite oral tissue and allows the clinicians to perform the conventional examination with the white light, to observe the changes in tissue autofluorescence with the violet light and to examine the areas of loss of autofluorrescence with the amber light [40]. The amber light illumination is proposed to enhance the reflectance property of normal tissue which will assist differentiation in the vasculature between normal and abnormal tissue. The vasculature in normal tissue is well defined in contrast to the abnormal tissue which was reported to show a diffuse vasculature. It is designed to identify lesions that may not be apparent to the naked eye. No research study has been reported to investigate the effectiveness of this new device yet.

DEVELOPING DIAGNOSTIC ADJUNCTS

Salivary Diagnostics

Salivary diagnostics, using saliva for disease diagnosis and health surveillance, for OSCC is a newly developing and ongoing area of research that shows considerable promise as a non-invasive method for early detection of oral cancer [41,42]. Several proteins, mRNAs, enzymes, and chemicals in the saliva are found sufficiently different in OSCC and normal controls to suggest that they might be potential biomarkers for OSCC. These potential salivary biomarkers for OSCC include 1) proteins of nuclear factor-kappa B (NF-κB) dependent cytokines (tumor necrosis factor-α (TNF-α), interleukin-1, interleukin-6, interleukin-8), basic fibroblast growth factor, Cyfra 21-1, cancer antigen- 125 (CA-125), tissue polypeptide antigen, endothelin; 2) mRNA of interleukin-8, interleukin-1, DUSP1 (dual specificity phosphatase 1), H3F3A (H3 histone family 3A), OAZ1 (ornithin decarboxylase antizyme 1), S100P (S100 calcium binding protein P), SAT (spermidine/spermine N1-acetyltransferase EST); and 3) reactive nitrogen species (nitric oxide and nitrates), GST (glutathione S-transferase), SOD (superoxide dismutase) and 8-OHdG (8-hydroxy-deoxyguanosine) [27, 43-48]. Recently, microRNAs (miRNA) also have been found in human saliva and two miRNAs, miR-125a and miR200a, were found to be present in significantly lower levels in the saliva of OSCC patients than in healthy controls [49]. This new finding makes miRNAs a new set of potential biomarkers for OSCC.

Salivary diagnostics has been one of the major funding focus for The National Institute of Dental and Craniofacial Research (NIDCR) for the past several years. In 2002, the NIDCR initiated a concerted research effort by giving seven U01 awards to develop microfluidics and microelectrico-

mechanical systems (MEMS) for salivary diagnostics and now the progress is moving towards commercialization [50]. A handheld, automated device for rapid measuring multiple salivary biomarkers for oral cancer, the "Oral Fluidic NanoSensor Test", is currently under development by the University of California at Los Angels (UCLA) research team. It is estimated to be available for research and patient application in 3 years [50].

In Vivo Confocal Microscopy

Confocal microscopy is a well established imaging technique for research in cell biology. It provides the advantages of optical sectioning and high resolution imaging, achieved by blocking the light originating from tissue layers above and below the focal plane [51,52]. Confocal microscopy was originally used for study *ex vivo* specimens but has been applied to *in vivo* human tissues in recent years. *In vivo* confocal reflectance microscopy has demonstrated the ability to obtain images of tissue architecture and cell morphology with resolution similar to histology. In addition, because of the optical sectioning ability of the instrument, no surgical procedure, sectioning or staining would be required for this procedure. *In vivo* confocal images have been successfully obtained from the oral cavity [53-55]. A recent pilot study also showed that distinctive features such as nuclear irregularity and spacing in the images obtained by a fiber optic confocal reflectance microscope could be used to differentiate OSCC from normal oral mucosa [56]. Although further optimization of the instrument and validation of the data is still needed, the promising results provide preliminary optimism for another non-invasive tool for the early detection of oral cancer and premalignant lesions.

CONCLUSION

Several diagnostic adjuncts for detecting early changes for OSCC are currently available. Although many studies have investigated sensitivity and specificity of these diagnostic adjuncts, there is still no convincing or adequate data to support their efficacy specifically for early detection or for reducing the death rate from OSCC. A few new techniques, including salivary diagnostics and *in vivo* confocal microscopy, are in the development stage. Extensive research efforts have been devoted in discovering salivary biomarkers for the early detection of OSCC. An automated device for rapidly measuring multiple salivary biomarkers for oral cancer may be available in the near future.

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