Analysis of new diagnostic methods in suspicious lesions of the oral mucosa

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Abstract

Objective: The objective of this study was to analyse publications related to examination techniques that might improve the visualisation of suspicious lesions of the oral mucosa (ViziLite® system and VELscope® system) or that might facilitate the cytological identification of suspicious lesions (OralCDx®).

Methods: A literature search was performed, using the PubMed database and the key words "brush biopsy", "Oral-CDx", "ViziLite" and "Velscope", limiting the search to papers in English or Spanish published from 2002 to 2008.

Results: According to the results of studies identified, the ViziLite® system has a sensitivity of 100% and specificity ranging from 0-14.2%, the VELscope® system has a sensitivity of 98-100% and specificity of 94-100% and the Oral CDx® system has a sensitivity of 71.4-100% and specificity of 32-100%.

Conclusion: Clinical examination and histopathological confirmation with biopsy remain the gold standard for the detection of oral cancer. More randomised controlled studies are needed to confirm the positive cost-benefit relationship and the true usefulness of these "new diagnostic methods" in oral mucosal pathology.

***Key words:** ViziLite, VELscope, OralCDx, brush biopsy, oral cancer, precancer, diagnosis.*

Introduction

Oral cancer is the sixth most frequent malignant tumour (1), with around 500,000 cases worldwide (2). Although the morbidity and mortality of other types of cancer have decreased over the past few decades, the same is not true for oral cancer. Its treatment can be easy and unaggressive when the diagnosis is early, with a survival rate of around 80% (3). Nevertheless, around 50% of diagnosed patients die within five years (4).

One-third of patients diagnosed with a malignant oral neoplasm report that they were examined during the three years before the diagnosis (5). Consequently, oral health professionals play an important role in the early detection of malignant and premalignant conditions and could make a considerable contribution to a decrease in its incidence by identifying high risk patients and educating them in healthy habits (6,7).

At present, the main approach to detect epithelial changes in oral mucosa is a combination of visual examination and palpation (6,8,9). Unfortunately, routine examination for the detection of oral cancer is not practiced as frequently as would be desirable (7,9,10).

Screening for oral cancer and precancerous lesions may reduce the incidence and mortality rates associated with oral cancer (11). It has been suggested that visual oral screening could avoid approximately 40,000 deaths from oral cancer worldwide (12), indicating that screening programmes could be associated with a reduction in public health costs (11). On the other hand, screening can be associated with problems related to false positives, including psychological trauma, overdiagnosis and overtreatment. These aspects must be considered before any "screening" programme is undertaken, ensuring its benefits exceed the risks (11).

Sankaranarayanan et al. (13) conducted a clinical trial and concluded that routine screening in high-risk groups (tobacco and/or alcohol consumers) produced a significant decrease in mortality from oral cancer.

Given the difficulty of detecting oral cancer in early stages (6), any procedure that facilitates visualisation of suspicious lesions could help the clinician in its detection (8). Therefore, new diagnostic techniques and instruments have been developed for use in routine examinations, including ViziLite® and VELscope® mucosa visualisation systems and new cytological analysis techniques, such as OralCDx®.

The objective of this review was to analyse the most recent information published on these three complementary examination systems.

Material and Methods

A literature search was conducted in the PubMed database between February and March 2008, using the key words: "brush biopsy", "OralCDx", "ViziLite" and "VELscope" and limiting the search to papers in English or Spanish published between 2002 and February 2008. Other studies were selected from references cited by articles found in the literature search.

Results

The three techniques are described below.

A. ViziLite®:

In 2002, the ViziLite® system (Zila Pharmaceuticals, Phoenix, AZ) became the first system approved by the FDA to improve the visualisation of early cancer lesions in head and neck examinations.

The kit consists of a 1% acetic acid solution, a capsule (which emits light), a retractor and manufacturer's instructions (6). The capsule is formed by an outer shell of flexible plastic and an inner vial of fragile glass. Although the company has not provided data on its precise composition, some authors (6) reported that the outer capsule may contain acetylsalicylic acid and the inner vial hydrogen peroxide. For its activation, the capsule is bent, breaking the glass vial so that the chemical products react and produce a bluish-white light with a wave length of 430-580 nm that lasts for around 10 min (6).

The patient performs a one-minute mouthwash with the acetic acid solution to remove the glycoprotein barrier and slightly dry the mucosa. The intensity of ambient light is then dimmed and a diffuse bluish-white chemiluminescent light is applied. Normal cells absorb the light and have a bluish colour, whereas the light is reflected by abnormal cells with a higher nucleus:cytoplasm ratio and by epithelium with excessive keratinisation, hyperparakeratinisation and/or significant inflammatory infiltrate, which appear acetowhite with brighter, more marked and more distinguishable borders (5,7,8,10).

Six articles were selected for the evaluation of this system (5-10). Their main characteristics are shown in Table 1. The reported sensitivity was 100% and the specificity ranged from 0%-14.2% (6,8).

B. VELscope®

The VELscope® system (Visually Enhanced Lesion Scope; LED Dental Inc., White Rock, B.C.) is a simple manual device developed by LED Medical Diagnostics in association with scientists of the British Columbia Cancer Agency (BCCA). It detects the loss of fluorescence in visible and non-visible high-risk oral lesions by applying direct fluorescence. The loss of fluorescence reflects a complex mixture of alterations to the intrinsic tissue distribution of fluorophores (14,15).

It consists of a source of light that emits a wave length of 400 to 460 nm and a manual unit for direct visualisation. Under this light, normal oral mucosa emits a green auto-fluorescence, whereas abnormal areas absorb the fluorescent light and appear dark (15-17). Hence, early biochemical changes are detected before their more evident appearance, permitting the early detection of pathological lesions (14).

Four studies (14-17) were selected for the evaluation of this system. Their main characteristics are shown in Table 1. Reported sensitivity values ranged from 97% to 98% and specificity from 94% to 100% (14-16).

C. OralCDx

OralCDx (OralScan Laboratories, Inc.) is an oral transepithelial biopsy system that uses computer-assisted brushing. The kit consists of a special brush for the brush biopsy, a glass slide, a form, a fixative (alcohol/polyethylene glycol) and a container for sending samples to the CDx laboratory (18).

The brush is placed on the lesion surface and rotated 5-10 times until it produces a reddening or haemorrhagic spots. The procedure does not require topical or local anaesthesia. The cell material obtained is transferred to the slide and fixed, and it is then placed in the receptacle with the corresponding bar-code and sent for analysis (18). The sample is analysed by a specialised pathologist in a CDx laboratory, classifying the sample as: "Negative" (without epithelial abnormalities); "Atypical" (epithelial changes of uncertain diagnostic meaning, specifying whether it is atypical in favour of inflammation

Table 1. Summary of analysed studies referring to visualisation systems (Vizilite ® and VELscope ®).

Author, year and reference	Type of article	Sample	Sensitivity	Specificity	Main conclusions
ViziLite					
Huber et al., 2004 (7)	Pilot study	150	-	-	Epithelium behaviour similar to that of the uterine cervix under chemiluminescent illumination
Ram and Siar , 2005 (6)	Cross-sectional study	40	100%	14,2%	Diagnostic aid and follow-up of patients with precancerous lesions and cancer
Epstein et al., 2006 (10)	Cross-sectional study	134	-	-	Facilitates the detection of lesions of the oral mucosa, mainly white ones
Epstein et al., 2007 (9)	Cross-sectional study	84	-	-	It may improve the visual identification of malignant and premalignant oral lesions
Farah and McCullough, 2007 (8)	Cross-sectional study	55	100%	0%	It does not help in the identification of malignant and premalignant lesions of the oral mucosa
Oh and Laskin, 2007 (5)	Cross-sectional study	100	-	-	Acetic acid mouthwash may be useful but not chemiluminescent light
VELscope					
Poh et al., 2006 (15)	Cross-sectional study	20	97%	94%	It may help to establish safer secure surgical margins in tumour excision
Kois and Truelove, 2006 (14)	Case series	4	98% (BCCA* data)	100% (BCCA* data)	It permits the diagnosis of lesions that would not otherwise have been diagnosed
Balevi, 2007 (16)	Opinion article	-	98% (BCCA* data)	100% (BCCA* data)	The evidence is inadequate for its routine use. It may be useful in specialised clinics
Westra and Sidransky, 2006 (17)	Opinion article	-	-	-	Factors that may affect optical qualities of the oral mucosa must be analysed
Studies are grouped by affinity of results					

or atypical in favour of dysplasia); "Positive" (evidence of dysplasia or carcinoma) or "Inadequate" (incomplete transepithelial specimen) (18-22).
Fifteen studies were selected (18-32) for the assessment of this system. Their main characteristics are shown in Table 2. Reported sensitivity values ranged from 71.4% to 100% and specificity from 32% to 100% (18,20,21,31).

Discussion
Epithelial changes in oral mucosa are detected mainly by direct visualisation and palpation of the soft tissue, and instruments to facilitate visualisation would be useful. The diagnosis is established by means of traditional biopsy, the most widely used method, with some ancillary techniques.

Regular evaluation is crucial for patients with dysplastic alterations in oral mucosa that have the potential for malignant transformation. The failure to identify alterations can allow lesions to progress to stages with a worse prognosis and higher morbidity. Therefore, ancillary detection methods are required for the follow-up so that decisions made are more likely to be correct (14).
A. ViziLite®: Use of the ViziLite® system may increase the ability of clinicians to detect oral lesions, mainly white lesions and those with white and red areas (7,9,10). Although some studies (10) claim that only a small percentage of lesions are visualised with Vizilite®, no significant differences have been found in lesion detection (5,8,10).
Some authors (6) reported that the borders observed were usually more extensive than those detected in the

Table 2. Summary of studies on Oral CDx ®.

Author and reference	Type of article	Sample	Sensitivity	Specificity	Main conclusions
Sciubba et al., 1999 (18)	Multicentre study	945	100%	Positive results 100% and atypical 92.9%	It may be a useful ancillary instrument for the early detection of oral cancer
Christian, 2002 (19)	Cross-sectional study	930	-	-	It could be a valuable ancillary instrument for oral cancer screening
Scheifele et al., 2004 (21)	Retrospective study	80	92,3%	94,3%	It may be a useful instrument for oral cancer screening
Bench, 2006 (23)	Opinion letter	-	-	-	It is not an invasive technique. It could relieve patients and professionals of stress
Eisen and Frist, 2003 (24)	Letter to Editor	-	-	-	CDx laboratories have only confirmed a percentage of false negatives below 1%
Greenberg, 2002 (25)	Editorial	-	-	-	Controversial. It may be useful in patients with biopsied lesions and high-risk groups
Potter et al., 2003 (26)	Case series	4	-	-	False negatives delay diagnosis by a mean of 117.25 days
Porter et al., 2005 (27)	Letter to Editor	-	-	-	Refer lesions and excise them in case of doubt
Eisen and Frist, 2005 (22)	Letter to Editor	-	-	-	New categories are added to the classification of lesions in CDx laboratory reports
Rick and Slater , 2003(28)	Letter to Editor	-	-	-	High sensitivity, very low specificity
Poate et al., 2004 (20)	Retrospective study	120	71,4%	32%	Additional markers of abnormal proliferation and differentiation are necessary to improve it
Acha et al., 2005 (29)	Literature review	-	-	-	Molecular analysis of RNA extracted from cells obtained by scraping can be performed
Mehrotra et al., 2007(30)	Literature review	-	-	-	It permits analysis by techniques such as cytomorphometry, DNA cytometry and molecular analysis
Driemel et al., 2007 (31)	Cross-sectional study	159	78% (without immunocytochemistry) and 95% (with immunocytochemistry)	96% (without immunocytochemistry) and 99% (with immunocytochemistry)	It can be complemented with DNA cytometry, AgNOR analysis and immunocytochemistry
Hirshberg et al., 2007 (32)	Retrospective study	46	-	-	The addition of morphological analysis and FISH to samples obtained by brushing increases specificity and improves prognosis
Studies are grouped by affinity of results					

visual examination. However, others concluded that the majority of these lesions can be diagnosed with incandescent light, and that mouthwash with acetic acid allowed the additional detection of some lesions (5).

Farah and McCollough (8) considered that ViziLite® cannot discriminate between malignant, benign and inflammatory oral lesions. In fact, the main drawback of this technique is its low specificity and the high rate of false positives (See Table 1), which could give rise to unnecessary biopsies (6). Its combination with toluidine blue has been proposed (ViziLite Plus®) in order to reduce the number of false positives without increasing

the rate of false negatives (9), but very little scientific evidence on this combination has been published to date.

Other limitations are its high cost and its inability to indicate the appropriate site for a biopsy (6). It has also been pointed out that there is no clinical evidence to justify the additional cost of the system and that detection by an expert clinician remains essential (8).

Hence, we can conclude that further studies are necessary to assess the sensitivity and specificity of this system in relation to clinical, cytological and histological characteristics of the oral epithelium and to determine

its true usefulness for routine examinations of the oral cavity.

Furthermore, because all studies have been conducted by specialists in oral disease, their results cannot be extrapolated to dental patient populations (9). Given that general dentists are the professionals who may experience greater difficulties in diagnosing suspicious lesions of the oral mucosa, studies are required to evaluate the diagnosis of lesions by general dentists with and without the help of this system.

B. VELscope®: Supporters of this system affirm that it may help to detect cases that would otherwise go unnoticed, although it cannot ensure that the clinical decision on the potential for malignant transformation is correct (14).

According to the BCCA (14), this system has a sensitivity of 98% sensitivity and specificity of 100% in discriminating between normal tissue and severe dysplasia, in situ carcinoma or invasive carcinoma. However, false positives have been reported, for instance in cases of inflammation, and it does not detect all areas of dysplasia (14,16). Therefore, Velscope® cannot be used as a diagnostic tool but rather as complementary to a thorough visual inspection and palpation.

A further use claimed for the VELscope® system is related to Slaughter´s concept of field cancerisation (15). According to this hypothesis, genetically altered cells extend widely throughout the epithelium in oral cancer patients. For this reason, oral carcinomas are excised along with apparently normal adjacent oral mucosa. Despite this approach, there is still a high recurrence rate of primary carcinomas (10-30%), even occurring in cases where margins appear microscopically free of alterations. It has been observed that this recurrence may be related to genetic and epigenetic alterations that can be detected by means of microsatellite analyses (15,17). Taking these data into account, an intraoperative biopsy would not be adequate, and the time (5 h) required for genetic assessment of the margin has led some authors to call for objective and simple methods that offer satisfactory real-time intraoperative results (17).

Poh et al. (15) used the VELscope® system to detect field cancerisation and determine surgical margins. Analysis of biopsies taken from these margins confirmed that they were areas of carcinoma or dysplasia or risk areas according to the microsatellite analysis, with loss of molecular heterozygosity. In 19 out of 20 tumours studied, the area with fluorescence loss was larger than that of the clinically apparent lesion. Among the 102 margin biopsies taken, fluorescence loss identified 32 out of the 33 biopsies as cancer or dysplasia, and a significant correlation was found between a high degree of dysplasia and loss of fluorescence. These authors therefore consider that this system may be useful in the intraoperative identification of high-risk fields (15).

However, we consider that further studies with larger samples are required that take account of factors and variables that may influence the optical properties of mucosa, such as inflammation. Although it appears that this instrument may be useful in specialised centres, its routine use does not appear to be justifiable due to the high risk of false positives, the high cost and the lack of scientific evidence.

C. OralCDx®: Oral cancer is usually asymptomatic in its early stages and does not show the classical clinical characteristics associated with advanced oral cancer (ulcer, induration, tumour, bleeding and cervical adenopathies). It can therefore go undiagnosed, even by specialists. Moreover, there is a high prevalence of epithelial abnormalities (5-15%) in the general population, and the histological study of them all is not a practical proposition (18,19).

At present, histopathological examination is the only reliable method to detect the presence and degree of dysplasia. However, an incisional biopsy is an aggressive invasive procedure that poses a technical challenge to some professionals and has negative psychological implications for some patients. It is of limited value in extensive lesions and, according to some authors, it offers a less than perfect sensitivity because of the subjectivity of the pathologist's report (18,20,29,33). Some authors have questioned the reliability of biopsies because of the high inter- and intra-observer variability in the interpretation of the degree of epithelial dysplasia, and because they may not always be representative of the whole lesion (33). This has very important implications, given that the histopathological diagnosis usually determines the treatment of this lesion.

Use of the OralCDx system has been supported for the diagnosis of oral lesions with epithelial abnormalities in order to confirm their benign nature or reveal precancerous or cancerous lesions when there is no clinical suspicion, identifying those that require histological study with incisional biopsy for their full characterisation. However, when there is a high suspicion of malignity, lesions must be biopsied directly with scalpel (18,22,24).

Advanced carcinomas frequently present necrosis and/or overinfection, making transepithelial access impossible. Likewise, leukoplakia with a high degree of keratinisation does not allow enough basal cells to be gathered and is therefore a contraindication for the use of this method. In addition, inflammatory conditions frequently give atypical results. Consequently, the clinical judgement of the professional is indispensable, and, if this technique is to be used, it is very important to identify the lesions in which this procedure can be performed (20-22).

According to Sciubba (1999), brush biopsy presents several advantages, being a fast and relatively simple pro-

cedure that does not cause bleeding or require anaesthesia (18).

Different sensitivity and specificity values have been reported for this technique according to the design of the studies (See Table 2). The calculation of false negative results is of great importance, since they are the main shortcoming of brush biopsy, and rates ranging from 1 to 4.1% have been reported (24,26,28). Some researchers (21) identified possible causes of false negatives, including topographic errors, time delay between brush and incisional biopsy and intra- and inter-observer variability in the histological study. Some authors (25,26) have described the time delay before the incisional biopsy as a major drawback, describing a mean delay of 117.25 days before the diagnosis of malignant lesion (26).

Scheifele et al. (21) calculated the likelihood ratios to evaluate whether the brush biopsy could be recommended. They found a positive likelihood ratio of 16.2, indicating that a positive result is 16.2-fold more likely in a lesion with than without dysplasia or carcinoma. The negative likelihood ratio was 0.08. These results are within acceptable limits and the authors therefore felt able to recommend brush biopsy (21).

Efforts are currently being made to improve the technique and increase its specificity by combining it with molecular analyses. These permit the identification of genetic anomalies, such as mutations of the tumour suppressing gene p53, epigenetic alterations, genomic instability (e.g., loss of heterozygosity), and instability of microsatellites, morphological analysis and FISH (29,30-32,34).

Analysis of the literature indicates that it may be a good ancillary system for oral cancer screening but generates considerable controversy and requires further testing in controlled clinical trials. It is important to bear in mind that dentists must rely on their clinical judgement to evaluate suspicious lesions, regardless of whether the brush or incisional biopsy is negative, and that an incisional biopsy with cold scalpel is always mandatory in lesions with a high suspicion of malignity for their histopathological analysis.

Conclusion

It can be concluded from this review that clinical examination and histopathological confirmation remain the "gold standard" for the detection of oral cancer, despite the development of new diagnostic techniques or instruments. Further controlled clinical studies are required to confirm the true accuracy, sensitivity and specificity of these new diagnostic methods, since they have shown very inconsistent results, especially in regard to their specificity. It is also necessary to determine whether the cost-benefit relationship of these techniques is positive and to establish their usefulness as ancillary or alternative methods to conventional biopsy.

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